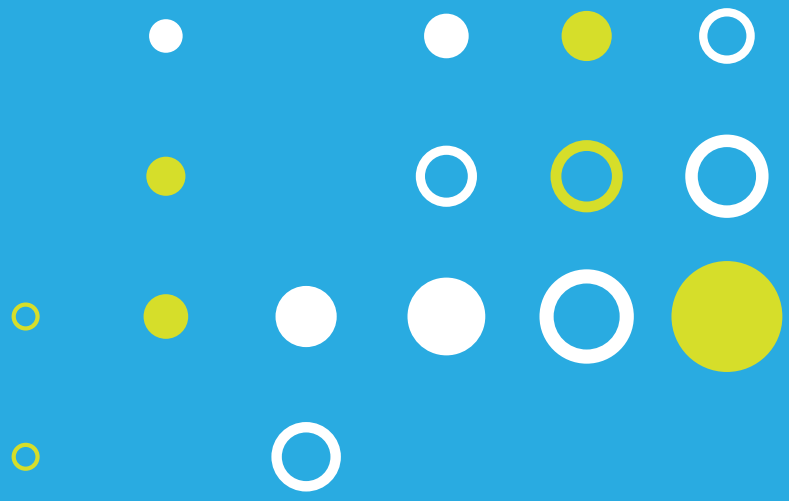
BIOCELL

nº 32

ISSN: 0327-9545 (print)
ISSN: 1667-5746 (online)

November 2008



SAIB
Sociedad Argentina de
Investigaciones en Bioquímica
y Biología Molecular

B I O C E L L

formerly ELECTRON MICROSCOPY AND CELL BIOLOGY

Official journal of the Sociedades Latinoamericanas de Microscopía Electrónica (SLAME), Iberoamericana de Biología Celular (SIABC), Federación Iberoamericana de Biología Celular y Molecular, and Sociedad Argentina de Investigaciones en Bioquímica y Biología Molecular (SAIB).

BIOCELL - Volume 32 - Supplement - November 2008 - Mendoza, Argentina.

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ISSN 0327 - 9545 (print) - ISSN 1667 - 5746 (electronic)

This journal is included in the Life Sciences Edition Contents, the Science Citation Index, Scisearch and Research Alert databases of Current Content; ISI/BIOMED, Index Medicus and MEDLINE available on the NLM's on line MED'LARS systems; EMBASE/Excerpta Medica; Chemical Abstracts, Biological Abstracts (BIOSIS), Index Medicus for Latin America and LATINDEX, SciELO Argentina.

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Editorial office: biocell.journal@gmail.com Production office: biocell@fcm.uncu.edu.ar http://www.cricyt.edu.ar/biocell

SUBSCRIPTIONS: Check or pay order to BIOCELL should be sent to Secretaría Editorial at the Journal's address. Year subscription including mailing: Individual U$S 90. Institutions U$S 110.

BIOCELL
2008, Vol. 32 (Suppl.): 1-

ISSN 0327 - 9545
PRINTED IN ARGENTINA

- SAIB -

44th Annual Meeting
Argentine Society for Biochemistry and Molecular Biology Research

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

November 8-11, 2008

Villa Carlos Paz, Córdoba
República Argentina

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DELEGATES OF SCIENTIFIC SECTIONS

-Cell Biology-
María Elena Teresa Damiani
IHEM-CONICET, Facultad de Ciencias Médicas
Universidad Nacional de Cuyo

-Lipids-
María del Rosario González Baró
INIBIOLP-CONICET, Facultad de Ciencias Médicas
Universidad Nacional de la Plata

-Microbiology-
Raúl Raya
CERELA-CONICET
Universidad Nacional de Tucumán

-Plants-
Claudia Casalongué
IIB-CONICET, Facultad de Ciencias Exactas y Naturales
Universidad de Mar del Plata

ACKNOWLEDGMENTS

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

Agencia Nacional de Promoción Científica y Tecnológica
(ANPCyT)

Consejo Nacional de Investigaciones Científicas y Técnicas
(CONICET)

European Molecular Biology Organization
(EMBO)

International Union of Biochemistry and Molecular Biology
(IUBMB)

Universidad Nacional de Córdoba
(UNC)

SAIB 2008 CONGRESS OVERVIEW

Saturday, November 8th	Sunday, November 9th	Monday, November 10th	Tuesday, November 11th
	8:30-10:30 **Symposia** Room A: Plant Bioch. & Mol. Biology Room B: Signal Transduction	8:30-10:30 **Symposia** Room A: Plant Bioch. & Mol. Biol II Room B: Cell Biology	8:30-10:30 **Symposia** Room A: Lipids Room B: Microbiology
	10:30-11:00 Coffee break	10:30-11:00 Coffee break	10:30-11:00 Coffee break
	11:00-13:30 **Oral Communications** Room A: CB (C01/08) Room B: MI (C01/10) Room C: LI (C01/10)	11:00-12:45 **Oral Communications** Room A: CB (C09/15) Room B: ST (C01/07) Room C: EN (C01/04) and SB (C01/03)	11:00-13:30 **Oral Communications** Room A: CB (C16/22) Room B: PL (C01/10) Room C: MI (C11/19)
	13:30-15:30 Lunch	13:30-15:30 Lunch	13:30-15:30 Lunch
14:00-18:00 Registration	15:30-16:30 Room C **Plenary Lecture** Dr. Robert V. Farese, Jr	15:30-16:30 Room C **Plenary Lecture** Dr. James R. Alfano	15:30-16:30 Room C **Plenary Lecture** Dr. Andrés Aguilera.
18:00-18:15 Room A **Opening Ceremony** 18:15-19:15 Room A **Opening Lecture** Dr. Nestor Carrillo	16:45-18:45 **Posters** – coffee MI (P01/34) CB (P01/30) PL (P01/25) LI (P01/15) BT (P01/20)	16:45-18:45 **Posters** - coffee MI (P35/60) CB (P31/61) PL (P26/50) LI (P16/25) BT (P21/33) ST (P01/12)	16:45-18:45 **Posters** - coffee MI (P61/88) CB (P62/90) SB (P01/10) NS (P01/6) ST (P13/33) EN (P01/13)
19:15-20:15 Room A **EMBO Lecture** Dr. Félix Rey	19:00- 20:00 Room A **Plenary Lecture** Dr. Enrique J. Rodriguez-Boulan	19:00- 20:30 Room A **Short Lectures "Junior Faculty"** Dr. Paula Casati (19:00-19:30) Dr. Javier Valdéz (19:30- 20:00) Dr. Guillermo Lanuza (20:00-20:30)	19:00- 20:00 Room A **SOLS Lecture** Dr. Sonsoles Campuzano
21:00 Cocktail	20:00-21:00 Room A **Plenary Lecture** Dr. Barañao	20:30 Room A SAIB Assembly	21:00 Closing Dinner

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

PROGRAM

SATURDAY, November 8th, 2008

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

18:00-18:15 Room A
OPENING CEREMONY

18:15-19:15 Room A
OPENING LECTURE
IUBMB Symposium: Plant Biochemistry and Molecular Biology
Néstor Carrillo
Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET)
Facultad de Ciencias Bioquímicas y Farmacéuticas
Universidad Nacional de Rosario, Santa Fe, Argentina
"Whith a little help from the (old) friends: use of Cyanobacterial flavodoxin for the development of stress tolerance in plants"
Chairperson: Ricardo Wolosiuk, Fundación Instituto Leloir, Buenos Aires

19:15-20:15 Room A
"EMBO" LECTURE
Félix A. Rey
Structural Virology Unit
Virology Department
Institut Pasteur. Paris, France
"Structural studies of viral envelope proteins: insights to understand the mechanism of membrane fusion induced during virus entry"
Chairperson: Luis Mayorga, IHEM-CONICET, Universidad Nacional de Cuyo

21:00 **COCKTAIL**

SUNDAY, November 9th, 2008

08:30-10:30 Room A
SYMPOSIUM
"Plant Biochemistry and Molecular Biology I"
Chairpersons: María E. Alvarez, CIQUIBIC-CONICET, Universidad Nacional de Córdoba
Raquel Chan. Universidad Nacional del Litoral

08:30 - 09:10 ***Alan Jones***
Department of Biology and Pharmacology, School of Medicine
University of North Carolina Chapel Hill, North Carolina, USA
"Novel sugar signaling through a putative receptor GAP (GTPase-accelerating protein) in Arabidopsis"

09:10-09:50 ***Anna Amtmann***
FBLS, University of Glasgow, Glasgow, UK,
Max-Planck Institute of Molecular Plant Physiology, Golm, Germany
"Effects of potassium deficiency on primary metabolism in Arabidopsis thaliana"

09:50-10:30 ***Natasha Raikel***

Director, Institute for Integrative Genome Biology and Center for Plant Cell Biology,
Department of Botany and Plant Sciences, University of California. Riverside, California, USA.
"Molecular and physiological connections between light and defense signaling mechanisms"

08:30-10:30 Room B

SYMPOSIUM

"Signal Transduction"

Chairpersons: Omar Coso, IFIBYNE-CONICET, Universidad de Buenos Aires
Eduardo Cánepa, FCEN-CONICET, Universidad de Buenos Aires

08:30-09:10 ***Piero Crespo***

Instituto de Biomedicina y Biotecnología de Cantabria (IBBTEC),
Consejo Superior de Investigaciones Científicas (CSIC) - IDICAN - Universidad de Cantabria.
Departamento de Biología Molecular, Facultad de Medicina, Santander. España.
"Space as a regulator of signalling pathways: The Ras-ERK paradigm"

09:10-09:50 ***Silvana Rosso***

CIQUIBIC-CONICET. Dpto. Química Biológica. Facultad de Ciencias Químicas.
Universidad Nacional de Córdoba. Córdoba. Argentina.
"WNT signalling regulates neuronal development"

09:50-10:30 ***Lucio H Castilla***

Program of Gene Function and Expression,
University of Massachusetts Medical School. Worcester, Massachusetts. USA.
"The transcription factor PLAGL2 role in leukemia development is dependent of AKT/mTOR pathway"

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

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

Room A

Cell Biology (CB-C01 / CB-C08)

Chairpersons: José L. Daniotti, CIBICI-CONICET, Universidad Nacional de Córdoba
Ana Russo de Boland, Universidad Nacional del Sur

11:00-11:15
CB-C01
OSMOREGULATION AND ENDOCYTIC SIGNALING IN *Trypanosoma cruzi*
Schoijet AC[1]; Docampo R[2]; Miranda K[3]; De Souza W[3]; Torres HN[1]; Flawiá MM[1]; Alonso GD[1].
[1]INGEBI-CONICET, Bs As, Argentina; [2]U. of Georgia, USA; [3]U. Federal do Rio de Janeiro, Brazil

11:15-11:30
CB-C02
CHARACTERIZATION OF THE COMPLETE SET OF SNARES IN THE GOLGI-LACKING EUKARYOTE *Giardia lamblia*
Elias EV; Quiroga R; Gottig N; Lujan HD.
Facultad de Medicina. Universidad Católica de Córdoba, Córdoba.

11:30-11:45
CB-C03
THE PATHWAYS OF CATHEPSIN D IN RAT EPIDIDYMIS MAY BE REGULATED BY STEROID HORMONES
Carvelli L[1]; Aguilera C[1]; Bannoud N[1]; Barrera P[1]; Morales CR[2]; Sosa MA[1].
[1]IHEMCONICET. UNCuyo, Mza, Argentina. [2]McGill University, Montreal, Canadá.

11:45-12:00
CB-C04
DIFFERENTIAL SORTING OF ANTIBODY TO GM1 AND CHOLERA TOXIN AFTER INTERNALIZATION IN EPITHELIAL CELLS
Iglesias-Bartolomé R; Comin R; Moyano AL; Daniotti JL..
CIQUIBIC (UNC-CONICET), Fac. de Cs. Químicas, Universidad Nacional de Córdoba, Córdoba.

12:00-12:15
CB-C05
MOLECULAR COMPONENTS INVOLVED IN SPECIFIC STEPS OF THE AUTOPHAGY/MULTIVESICULAR BODY (MVB) PATHWAYS
Fader CM; Sanchez DG; Mestre MB; Colombo MI.
IHEM-CONICET, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza.

12:15-12:30
CB-C06
PHYSIOLOGICAL MODIFICATION OF *Bufo arenarum* SPERM DURING MOTILITY ACTIVATION
O´Brien ED; Krapf D; Cabada MO; Arranz SE.
IBRCONICET. Área Biología, Facultad de Cs. Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Santa Fe.

12:30-12:45
CB-C07
HYPERTONICITY INDUCES CCTα REDISTRIBUTION IN NUCLEOPLASMIC LAMIN A/C SPECKLES IN MDCK CELLS
Favale NO; Fernandez-Tome MC; Sterin-Speziale B.
Biología Celular, FFYB-UBA, IQUIFIB-CONICET, Buenos Aires, Argentina.

12:45-13:00
CB-C08
THE FIFTH CYTOPLASMIC DOMAIN OF NA^+/K^+-ATPASE MEDIATES ITS INTERACTION WITH ACETYLATED TUBULIN
Zampar GG; Carbajal A; Chesta ME; Díaz NM; Chanaday NL; Casale CH; Arce CA.
CIQUIBIC (Conicet), Dpto. Química Biológica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba.

11:00-13:30 **ORAL COMMUNICATIONS**

Room C

Lipids (LI-C01 / LI-C10)

Chairpersons: Norma Giusto, INIBIBB-CONICET, Universidad Nacional del Sur
María J. Tacconi de Alaniz, INIBIOLP-CONICET, Universidad Nacional de La Plata

11:00-11:15
LI-C01
EFFECT OF PESTICIDES ON APOPTOSIS BIOMARKERS IN VARIOUS RAT TISSUES
Astiz M; Tacconi de Alaniz MJ; Marra CA
INIBIOLP, CCT-CONICET, Fac. Ciencias Médicas-UNLP.

11:15-11:30
LI-C02
EFFECT OF APOAI LIPIDATION STATE ON CHOLESTEROL AND PHOSPHOLIPIDS MOBILIZATION AND EFFLUX FROM CELLS
Gonzalez MC; Cabaleiro L; Toledo JD; Garda HA
INIBIOLP, Fac. de Ciencias Médicas. Universidad Nacional de La Plata.

11:30-11:45
LI-C03
THE RECYCLING CERAMIDE IS A PRECURSOR OF HYPERTONICITY INDUCED INCREASE OF GLUCOSYLCER SYNTHESIS
Pescio LG; Sterin-Speziale NB
Biología Celular. FFyB, UBA. IQUIFIB-CONICET. Buenos Aires, Argentina.

11:45-12:00
LI-C04
MALONYL-ACP AS A PUTATIVE SIGNAL FOR THE GLOBAL FATTY ACID REGULATOR FAPR OF *Bacillus subtilis*
Martinez MM[1]; Zaballa ME[1]; Schaeffer F[2]; Alzari P[2]; Schujman GE[1]; Vila A[1]; De Mendoza D[1]
[1]Instituto de Biología Molecular y Celular de Rosario IBR-CONICET-UNR; [2]Institut Pasteur, Paris

12:00-12:15
LI-C05
TRANSCRIPTIONAL REGULATION OF CCTα GENE PROMOTER BY E2F AND CELL PROLIFERATION
Elena C; Banchio C
Instituto de Biologia Molecular y Celular de Rosario IBR-CONICET. UNR. Rosario, Argentina.

12:15-12:30
LI-C06
GLYCEROL-3-P-ACYLTRANSFERASE 2 IS EXPRESSED IN TESTIS AND INVOLVED IN CELLULAR PROLIFERATION
Cattaneo ER; Pellon Maison M; Coleman RA; Gonzalez Baro MR
INIBIOLP- CCT La Plata- CONICET-UNLP.

12:30-12:45
LI-C07
ROLE OF INTRAMITOCHONDRIAL ARACHIDONIC ACID IN BREAST CANCER CELLS
Duarte A[1]; Orlando U[1]; Castillo AF[1]; Delettieres D[1]; Solano AR[1]; Pasqualini ME[2]; Maloberti P[1]; Podestá EJ[1]
[1]IIMHNO, Depto Bioquimica Humana, Facultad de Medicina, UBA. [2]1ra Cat.Biol.Cel.EMbr.His, IBC-FCM-UNC.

12:45-13:00
LI-C08
CLONING AND EXPRESSION ANALYSIS OF SEVERAL *Beauveria bassiana* GENES INVOLVED IN ALKANE DEGRADATION
Pedrini N[1,2]; Keyhani NO[2]; Juarez MP[1]
[1] INIBIOLP (CONICET-UNLP), Argentina. [2] University of Florida, USA.

13:00-13:15
LI-C09
CLONING AND FUNCTIONAL CHARACTERIZATION OF DELTA 4- SPHINGOLIPID DESATURASES FROM TRYPANOSOMATIDS
Vacchina P; Rosso C; Uttaro AD; Tripodi KEJ
IBR-CONICET- FCByF- Universidad Nacional de Rosario.

13:00-13:30
LI-C10
BIOSYNTHESIS OF VERY LONG CHAIN FATTY ACIDS IN *Trypanosoma cruzi*
Livore VI; Uttaro AD
IBR-CONICET, Dpto. Microbiología, FCByF, UNR

11:00-13:30 **ORAL COMMUNICATIONS**

Room B

Microbiology (MI-C01 / MI-C10)

Chairpersons: José R. Echenique, CIBICI-CONICET, Universidad Nacional de Córdoba
Christian Magni, IBR-CONICET, Universidad Nacional de Rosario

11:00-11:15
MI-C01
PLANT FLAVONOID-INDUCED GENES AND NOVEL TYPE III SECRETED PROTEINS IN *Mesorhizobium loti* MAFF303099
Sanchez C[1], Iannino F[1], Deakin W[2], Ugalde R[1], Lepek V[1]
[1] IIB-UNSAM, Buenos Aires, Argentina; [2] Université de Geneve, Switzerland.

11:15-11:30
MI-C02
***Xanthomonas* MANIPULATES HOST DEFENSE RESPONSE TO PRODUCE DISEASE**
Rigano LA[1], Bouarab K[2], Vojnov AA[1]
[1]Fundación Pablo Cassará, Centro Milstein, Bs As, Argentina; [2]Université de Sherbrooke, Qc, Canadá.

11:30-11:45
MI-C03
CHARACTERIZATION OF THE INVASION MECHANISM OF *Serratia marcescens* IN EPITHELIAL CELLS
Fedrigo GV[1], Campoy EM[2], Colombo MI[2], García Véscovi E[1]
[1]IBR-CONICET, U.N.R., Rosario; [2]IHEM- CONICET, U.N.Cuyo, Mendoza.

11:45-12:00
MI-C04
HLA SECRETED BY THE ASPDE SYSTEM, BELONGS TO AN IRON RESPONSIVE OPERON IN *A. tumefaciens*
Haurigot L[1], Ferella M[1], Russo DM[1], Downie JA[2], Zorreguieta A[1]
[1]Fundación Instituto Leloir, IIBBA-CONICET, FCEyN-UBA;. [2]John Innes Centre Norwich, UK

12:00-12:15
MI-C05
BIOFILM FORMATION IN *Leptospira*: STUDY IN A SWINE FIELD STRAIN FROM ARGENTINA
Brihuega B[1], Samartino L[1], Auteri C[1], Gomez R[3], Cataldi A[2], Caimi K[2]
[1]Instituto de Patobiologia INTA; [2]Instituto de Biotecnologia INTA, [3]IBBM UNLP La Plata.

12:15-12:30
MI-C06
MICROBIAL ECOLOGY OF AMMONIA-OXIDIZING BACTERIA IN AN ACTIVATED SLUDGE WITH UNSTABLE NITRIFICATION
Figuerola ELM, Erijman L
Instituto de Investigaciones en Ingeniería Genética y Biología Molecular (INGEBI-CONICET).

12:30-12:45
MI-C07
GENOTYPIC AND FENOTYPIC DIFFERENCES AMONG *Staphylococcus aureus* FROM PATIENTS WITH OSTEOMYELITIS
Lattar SM[1], Tuchscherr L[3], Centron D[1], Barberis C[2], Buzzola F[1], Von Eiff C[3], Cerquetti M[1], Sordelli D[1]
[1]University of Buenos Aires; [2]San Martín Hospital; [3]University of Münster, Germany.

12:45-13:00
MI-C08
ESTIMATION OF MICROBIAL DIVERSITY MEASURED BY DNA REASSOCIATION USING A FLUOROMETRIC METHOD
Guerrero LD, Erijman L
Instituto de Investigaciones en Ingeniería Genética y Biología Molecular (INGEBI-CONICET).

13:00-13:15
MI-C09
MOLECULAR BASES OF CIPROFLOXACIN RESISTANCE IN *P. aeruginosa* DEFICIENT IN 8-OXO-G REPAIR SYSTEM
Morero NR, Argaraña CE
CIQUIBIC-CONICET, Dpto. de Química Biológica, Fac. de Ciencias Químicas, UNC, Córdoba, Argentina.

13:00-13:30
MI-C10
UNCOVERING THE ANAEROBIC COPPER-RESISTANCE PATHWAY IN *Salmonella*
Pontel LB, Soncini FC
Instituto de Biología Molecular y Celular de Rosario, CONICET, and Fac. de Cs. Bioq. y Farm., UNR.

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

15:30-16:30 Room C

LECTURE

Robert V. Farese, Jr

Gladstone Institute of Cardiovascular Disease
University of California. San Francisco, California, USA
"Mechanisms of neutral lipid synthesis and storage"
Chairperson: Diego de Mendoza, IBR-CONICET, Universidad Nacional de Rosario

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

Microbiology (MI-P01/MI-P34)
Cell Biology (CB-P01/CB-P30)
Plant Biochemistry and Molecular Biology (PL-P01/PL-P25)
Lipids (LI-P01/LI-P15)
Biotechnology (BT-P01/BT-P20)

19:00-20:00 Room A

LECTURE

Enrique J. Rodriguez-Boulan

Department of Ophthalmology. Dyson Vision Research Institute.
Weill Medical College of Cornell University. New York, USA.

"Generation and maintenance of cell polarity"

Chairperson: Hugo Maccioni, CIQUIBIC-CONICET, Universidad Nacional de Córdoba.

20:00-21:00 Room A

LECTURE

Lino Barañao

Ministro de Ciencia, Tecnología e Innovación Productiva
Presidencia de la Nación. Argentina

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

MONDAY, November 10th, 2008

08:30-10:30 Room A

SYMPOSIUM

"Plant Biochemistry and Molecular Biology II"

Chairpersons: Claudia Casalongué, IIB-CONICET, Universidad de Mar del Plata
Estela Valle, IBR-CONICET, Universidad Nacional de Rosario

08:30-09:10 ***Gary Stacey***

National Center for Soybean Biotechnology,
University of Missouri, Columbia, Missouri, USA

"Soybean functional genomics: root hair biology as a model system"

09:10-09:50 ***Jean T. Greenberg***

Molecular Genetics and Cell Biology Department,
University of Chicago, Chicago, Illinois, USA

"Local and systemic interactions between Pseudomonas syringae and their plant hosts"

09:50-10:30 ***Ken Shirasu***

RIKEN Plant Science Center,
Tsurumi-ku, Yokohama, Japan

"The Sgt1-Hsp90 chaperone complex for Nlr-protein dependent innate immunity"

08:30-10:30 Room B

SYMPOSIUM

"Cell Biology"

Chairpersons: María Teresa Damiani, IHEM-CONICET, Universidad Nacional de Cuyo, Mendoza
Claudia Tomes, IHEM-CONICET, Universidad Nacional de Cuyo, Mendoza

08:30-09:10 ***Enrique A. Mesri***

Department of Microbiology and Immunology. Leonard M. Miller School of Medicine
University of Miami. Miami, USA.

"Animal models of KSHV-induced Kaposi's sarcoma"

09:10-09:50 ***Andrew Quest***

Deputy Director Center for Molecular Studies of the Cell (CEMC)
Laboratory of Cellular Communication
ICBM Faculty of Medicine, University of Chile, Santiago de Chile, Chile
"Caveolin-1, a scaffolding protein with ambiguous roles in cancer"

09:50-10:30 ***Thomas Surrey***

Cell Biology and Biophysics Unit
European Molecular Biology Laboratory, EMBL
Heidelberg, Germany
"Molecular mechanism of microtubule plus-end tracking revealed by fluorescence microscopy imaging of in vitro reconstitutions"

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

11:00-12:45 **ORAL COMMUNICATIONS**

Room A

Cell Biology (CB-C09 / CB-C15)

Chairpersons: José L. Bocco, CIBICI-CONICET, Universidad Nacional de Córdoba
Paula Cramer, IFIByME-CONICET, Universidad de Buenos Aires

11:00-11:15
CB-C09
REGULATION OF P-ATPASES BY ACETYLATED TUBULIN: *S. cerevisiae* H^+-ATPase REGULATION MECHANISM
Campetelli AN[1]; Monesterolo NE[1]; Santander VS[1]; Previtali G[1]; Arce CA[2]; Casale CH[1].
[1]Dpto. de Biologia Molecular-UNRC. [2]CIQUIBIC-UNC.

11:15-11:30
CB-C10
P19INK4D FUNCTION RELIES ON SEQUENTIAL PHOSPHORYLATION
Marazita M[1,2]; Martí M[1]; Pignataro OP[2]; Cánepa ET[1].
[1]Departamento. Química Biológica, FCEyN, UBA. [2]Lab. de Endocrinología Molecular, IBYME-CONICET.

11:30-11:45
CB-C11
E2F MEDIATED INDUCTION OF P19INK4D PLAYS AN IMPORTANT ROLE IN CELL CYCLE AND DNA DAMAGE RESPONSE
Carcagno AL; Sonzogni SV; Ceruti JM; Giono LE; Canepa ET.
Laboratorio de Biología Molecular - Departamento de Química Biológica FCEN UBA Buenos Aires.

11.45-12:00
CB-C12
GLUCOCORTICOID DIFFERENTIAL EFFECTS ON P21CIP1 EXPRESSION, CELL CYCLE AND SURVIVAL OF MAMMARY CELLS
Hoijman E; Pecci A.
Departamento de Química Biológica, IFIBYNE-CONICET, FCEN-UBA.

12:00-12:15
CB-C13
DIFFERENTIAL BEHAVIOUR OF CYSTEINE CATHEPSINS IN THE *INTRINSIC* AND *EXTRINSIC* PATHWAYS OF APOPTOSIS
V. Stoka, L. Bojic, G. Droga-Mazovec, A. Petelin, S. Ivanova, U. Repnik, M. Klaric, V. Turk, B. Turk
Department of Biochemistry and Molecular Biology, J. Stefan Institute, Ljubljana, Slovenia.

12:15-12:30
CB-C14
NOCTURNIN IS A DEADENYLASE INVOLVED IN THE POSTTRANSCRIPTIONAL CONTROL OF CIRCADIAN GENE EXPRESSION
Garbarino Pico E[1]; Green CB[2].
[1]CIQUIBIC-DQB, FCQ, Universidad Nacional de Córdoba. [2]Department of Biology, University of Virginia.

12:30-12:45
CB-C15
DEPOLARIZATION-INDUCED CHANGES IN INTRAGENIC CHROMATIN REGULATE ALTERNATIVE SPLICING IN NEURAL CELLS
Schor IE; Rascovan N; Kornblihtt AR.
LFBM, IFIByNE-CONICET, FCEN, Universidad de Buenos Aires. Argentina.

11:00-12:45 **ORAL COMMUNICATIONS**

Room B

Signal Transduction (ST-C01 / ST-C07)

Chairpersons: Estela Machado, Universidad Nacional de Rio Cuarto
María Teresa Tellez-Iñón, INGEBI-CONICET, Buenos Aires

11:00-11:15
ST-C01
FUNCTIONAL CHARACTERIZATION OF DesK, THE *Bacillus subtilis* THERMOSENSOR
Martín M[1]; Albanesi D[1]; Alzari P[2]; De Mendoza D[1]
[1]IBR- CONICET- UNR. Suipacha 531, 2000 Rosario, Argentina. [2]Institut Pasteur, Paris, France.

11:15-11:30
ST-C02
MECHANISM OF SIGNAL TRANSDUCTION BY THE THERMOSENSOR DesK FROM *Bacillus subtilis*
Albanesi D[1]; Martín M1; Buschiazzo A[2]; Trajtenberg F[2]; Mansilla MC1; Haouz A[3]; Alzari P[3]; De Mendoza D[1]
[1]IBR- CONICET, Rosario, Argentina. [2]Institute Pasteur, Uruguay. [3]Institute Pasteur, Paris, France.

11:30-11:45
ST-C03
ROLE OF THE TRANSCRIPTION FACTOR KLF6 IN JNK-MEDIATED APOPTOSIS
Andreoli V; Bocco JL
Dpto. Bioquímica Clínica. CIBICI CONICET. Fac. Cs. Químicas, Universidad Nacional de Córdoba.

11:45-12:00
ST-C04
MARCKS PHOSPHORYLATION PLAYS A REGULATORY ROLE ON PIP2 CONCENTRATION IN ACROSOMAL EXOCYTOSIS
Rodriguez Peña MJ; Mayorga LS; Michaut MA
Lab. Biología Celular y Molecular. IHEM-CONICET, Universidad Nacional De Cuyo, Mendoza, Argentina.

12:00-12:15
ST-C05
A MITOCHONDRIAL KINASE CASCADE INDUCES ERK PHOSPHORYLATION OF A KEY CHOLESTEROL TRANSPORT PROTEIN
Poderoso C[1]; Converso DP[2]; Duarte A[1]; Neuman I[1]; Maloberti P[1]; Carreras MC[2]; Poderoso JJ[2]; Podesta EJ[1]
[2]IIMHNO, Depto. Bioquímica Humana, Fac. de Medicina, UBA. [2]Met. del Oxígeno, Htal. de Clínicas, UBA.

12:15-12:30
ST-C06
REGULATION OF STARD7 EXPRESSION BY LITHIUM AND HIGH GLUCOSE IN JEG-3 CELLS
Rena VC; Flores-Martín J; Angeletti S; Panzetta-Dutari G; Genti-Raimondi S
Dpto. Bioq. Clínica, Facultad de Ciencias Químicas-UNC. CIBICI-CONICET. Argentina.

12:30-12:45
ST-C07
α2M/LRP-1 INDUCES ERK1/2 PHOSPHORYLATION BY PKC-αβI ACTIVATION IN J774 CELLS
Cáceres LC; Sánchez MC; Chiabrando GA
CIBICI (CONICET). Dpto. Bioq. Clín. F.C.Q., U.N.C., C. Universitaria, Córdoba, Argentina.

11:00-12:45 **ORAL COMMUNICATIONS**

Room C

Enzymology (EN-C01 / EN-C04) and Structural Biology (SB-C01 / SB-C03)

Chairpersons: Silvia Moreno de Colonna, Universidad de Buenos Aires
Mirtha Biscoglio, IQUIFIB-CONICET, Universidad de Buenos Aires.

11:00-11:15
EN-C01
INTERACTION OF ALKYLAMMONIUM IONS WITH THE ACTIVE SITE OF *P. aeruginosa* PHOSPHOCHOLINE PHOSPHATASE
Beassoni PR; Otero LH; Boetsch C; Domenech CE
Departamento de Biología Molecular, FCEFQyN, Universidad Nacional de Río Cuarto.

11:15-11:30
EN-C02
INTERACTION OF MG^{2+}, ZN^{2+} AND CU^{2+} IN THE ACTIVE SITE OF *P. aeruginosa* PHOSPHOCHOLINE PHOSPHATASE
Otero LH; Beassoni PR; Lisa AT; Domenech CE
Departamento de Biología Molecular, FCEFQyN, Universidad Nacional de Río Cuarto.

11:30-11:45
EN-C03
SWAPPING STRUCTURAL-FUNCTIONAL DETERMINANTS BETWEEN BACTERIAL AND PLANT FERREDOXIN $NADP^+$ REDUCTASES
Musumeci MA; Ceccarelli EA
Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET UNR

11.45-12:00
EN-C04
ROLE OF THE INVARIANT GLUTAMIC ACID IN THE ACTIVE SITE OF FERREDOXIN-NADP(H) REDUCTASES
Dumit V[1]; Essigke T[2]; Ullmann GM[2]; Cortez N[1]
[1]IBR-CONICET, Univ. Nac. De Rosario, Argentina. [2]Bayreuth University, Bayreuth, Germany.

12:00-12:15
SB-C01
STRUCTURAL AND BIOLOGICAL ASPECTS OF THE *Micrurus pyrrhocryptus* VENOM
Dokmetjian JC; Del Canto SG; Vinzón S; Biscoglio de Jiménez Bonino MJ.
ANLIS "Dr. Carlos G. Malbran"; Química Biológica, FFyB, UBA. IQUIFIB (UBA-CONICET).

12:15-12:30
SB-C02
METAL SELECTIVITY OF THE SINGLE SUPEROXIDE DISMUTASE OF *Rhodobacter capsulatus*
Di Capua C; Cortez N
Inst. de Biología Molecular y Celular de Rosario IBR (CONICET & UNR), Suipacha 531, S2002LRK Rosario.

12:30-12:45
SB-C03
CRYSTAL STRUCTURE OF THE REGULATORY SUBUNIT OF PKA FROM *Saccharomyces cerevisiae*
Rinaldi J[1]; Wu J[2]; Yang J[2]; Rossi S[1]; Moreno S[1]; Taylor SS[2]
[1]Departamento de Química Biológica, FCEyN-UBA.[2]Chemistry & Biochemistry Department, UCSD.CA, USA.

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

15:30-16:30 Room C

LECTURE

James R. Alfano

Plant Science Initiative and Department of Plant Pathology
University of Nebraska. Lincoln, Nebraska, USA

"The molecular basis of plant immunity suppression by the Pseudomonas syringae type III effector HopU1"

Chairperson: Fernando Soncini, IBR-CONICET, Universidad Nacional de Rosario

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

Microbiology (MI-P35 / MI-P60)
Cell Biology (CB-P31 / CB-P61)
Plant Biochemistry and Molecular Biology (PL-P26 / PL-P50)
Lipids (LI-P16 / LI-P25)
Biotechnology (BT-P21 / BT-P33)
Signal Transduction (ST-P01/ ST-P12)

19:00-20:30 Room A

SHORT LECTURES "JUNIOR FACULTY"

Chairperson: Eduardo Ceccarelli, IBR-CONICET, Universidad Nacional de Rosario

19:00-19:30 ***Paula Casati***

Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI-CONICET).
Facultad de Ciencias Bioquímicas y Farmacéuticas
Universidad Nacional de Rosario. Rosario, Santa Fe, Argentina

"Impact of UV-B radiation on Plants"

19:30-20:00 ***Javier Valdéz***

Centro de Investigaciones en Química. Biológica de Córdoba (CIQUIBIC-CONICET)
Facultad de Ciencias Químicas. Universidad Nacional de Córdoba. Córdoba, Argentina.
"Palmitoylation of Type II transmembrane proteins (SNARES) in yeast"

20:00-20:30 ***Guillermo Lanuza***

Fundación Instituto Leloir, Buenos Aires.
"Functional analysis of neuronal development in the spinal cord"

20:30 **SAIB General Assembly**

TUESDAY, November 11th, 2008

08:30-10:30 Room A

SYMPOSIUM
"Lipids"

Chairpersons: María González Baro. INIBIOLP CONICET-UNLP, La Plata
Gabriela Salvador. INIBIBB CONICET-UNS, Bahia Blanca

08:30-09:00 ***Claudia Banchio***

Instituto de Biología Molecular y Celular de Rosario IBR-CONICET.
Universidad Nacional de Rosario. Rosario, Santa Fe, Argentina
"Phosphatidylcholine biosynthesis and neuronal differentiation"

09:00-09:30 ***Steven Farber***

Carnegie Institution for Science, Embryology Dept., Baltimore, Maryland, USA
"Visualizing intestinal lipid metabolism in live animals: Studies with guts"

09:30-10:00 ***Marta Aveldaño***

Instituto de Investigaciones Bioquímicas de Bahía Blanca (CONICET-UNS)
Bahía Blanca, Argentina
"Lipids with long and very long chain (VLC) polyenoic fatty acids: from cell localization to function"

10:00-10:30 ***Patricia Torres Bozza***

Laboratório de Imunofarmacologia
Instituto Oswaldo Cruz. Rio de Janeiro, Brasil
"Biogenesis and functions of lipid bodies in inflammation and cancer"

08:30-10:30 Room B

SYMPOSIUM
"Microbiology"

Chairpersons: Raúl Raya. CERELA-CONICET, Universidad Nacional de Tucumán.
Rosana E. De Castro, IIB-CONICET, Universidad Nacional de Mar del Plata.

08:30-09:00 ***Alejandra Gruss***

Lactic Acid Bacteria and Opportunistic Pathogens Lab (UBLO)
INRA, Centre de Recherches de Jouy-en-Josas. Domaine de Vilvert, Jouy-en-Josas, France
"How respiration of lactic acid bacteria makes life easier in a crowded space"

09:00-09:30 ***Graciela Lorca***

Department of Microbiology and Cell Science. IFAS, University of Florida.
Gainesville, Florida, USA
"Chemical biology of transcription factors: high throughput approach to unveil signal molecules"

09:30-10:00 ***Mya Breitbart***

Biological Oceanography. College of Marine Science. University of South Florida
Saint Petersburg, Florida, USA
"Metagenomics for viral discovery"

10:00-10:30 ***Bénédicte Michel***

Centre de Génétique Moléculaire
Département Génétique des Fonctions Cellulaires, CNRS
Gif-sur-Yvette, France
"Recombinational rescue of blocked replication forks in Escherichia coli"

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

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

Room A

Cell Biology (CB-C16 /CB-C22)

Chairpersons: Guillermo Alonso, INGEBI-CONICET, Buenos Aires
Gustavo Schujman, IBR-CONICET, Universidad Nacional de Rosario

11:00-11:15
CB-C16
DNA DAMAGE REGULATES ALTERNATIVE SPLICING THROUGH CHANGES IN POL II ELONGATION
Muñoz MJ; Perez Santangelo MS; De la Mata M; Kornblihtt AR.
Laboratorio de Fisiología y Biología Molecular, IFIBYNE-CONICET, FCEyN, Universidad de Buenos Aires.

11:15-11:30
CB-C17
DISRUPTION OF THE RNAI PATHWAY ABOLISHES SURFACE ANTIGEN VARIATION IN THE INTESTINAL PARASITE *Giardia*
Prucca CG; Slavin I; Quiroga R; Elias EV; Rivero FD; Saura A; Carranza PG; Lujan HD.
Facultad de Medicina. Universidad Católica de Córdoba. Córdoba. Argentina.

11:30-11:45
CB-C18
NOVEL FUNCTION OF P21CIP$_1$/WAF$_1$ AS A MODULATOR OF TRANSLESION DNA SYNTHESIS (TLS)
Soria G1; Speroni J[1]; Belluscio L[1]; Podhajcer OL[2]; Gottifredi V[1].
[1]Cell Cycle and Genomic Stability Laboratory. [2]Laboratory of Gene Therapy. Fundación Leloir.

11.45-12:00
CB-C19
INVOLVEMENT OF hnRNP A1 IN THE REGULATION OF RAC1 ALTERNATIVE SPLICING IN MAMMARY EPITHELIAL CELLS
Pelisch F[1]; Risso G[1]; Radisky D[2]; Srebrow A[1].
[1]IFIBYNE-CONICET, FCEyN-UBA, Bs As, Argentina. [2]Mayo Clinic Cancer Center, Jacksonville, FL, USA.

12:00-12:15
CB-C20
THE NUCLEIC ACID CHAPERONE CNBP MAY REGULATE THE STABILITY OF G-QUADRUPLEX DNA STRUCTURES
Borgognone M; Armas P; Calcaterra NB.
IBR-CONICET, FCByF-UNR, Suipacha 531, S2002LRK, Rosario, Argentina.

12:15-12:30
CB-C21
IDENTIFICATION AND ANALYSIS OF THE ZEBRAFISH CNBP PROMOTER AND REGULATORY SEQUENCES
Weiner AMJ; Calcaterra NB.
IBR-CONICET. FCByFUNR. Suipacha 531, S2002LRK. Rosario, Argentina.

12:30-12:45
CB-C22
ALPHA-2 MACROGLOBULIN/LRP1 SYSTEM INDUCES MULLER GLIAL CELL MIGRATION
Barcelona PF; Chiabrando GA; Sánchez MC
CIBICI (CONICET). Dpto. Bioq. Clín. F.C.Q., U.N.C., C. Universitaria, Córdoba, Argentina.

11:00-13:30 **ORAL COMMUNICATIONS**

Room B

Plant Biochemistry and Molecular Biology (PL-C01 / PL-C10)

Chairpersons: Ariel Goldraij, CIQUIBIC-CONICET, Universidad Nacional de Córdoba
Rita Ulloa, INGEBI-CONICET, Universidad de Buenos Aires

11:00-11:15
PL-C01
INTERACTION OF THE N- AND C-TERMINAL DOMAINS OF THE STARCH SYNTHASE III FROM *Arabidopsis thaliana*
Wayllace NZ; Valdez HA; Ugalde RA; Gomez-Casati DF; Busi MV
IIB-INTECH, UNSAM-CONICET, Chascomús, Argentina.

11:15-11:30
PL-C02
STRUCTURAL AND FUNCTIONAL ANALYSES OF HSP100 MOLECULAR CHAPERONES FROM VASCULAR PLANTS AND BACTERIA
Rosano GL; Bruch EM; Colombo CV; Ceccarelli EA
Instituto de Biología Molecular y Celular de Rosario, CONICET, UNR

11:30-11:45
PL-C03
UNRAVELING THE REGULATION MECHANISM FOR THE POTATO TUBER ADP-GLUCOSE PYROPHOSPHORYLASE
Figueroa CM[1]; Kuhn ML[2]; Ballicora MA[2]; Iglesias AA[1]
[1]Laboratorio de Enzimología Molecular, FBCB, UNL, Argentina & [2]Department of Chemistry, LUC, USA

11.45-12:00
PL-C04
CHLOROPLAST DEPENDENT ALTERNATIVE SPLICING REGULATION BY LIGHT
Petrillo E; Kornblihtt AR
IFIBYNE-CONICET; Laboratorio de Fisiología y Biología Molecular, FCEyN, UBA

12:00-12:15
PL-C05
CHARACTERIZATION OF HIGH MOLECULAR FORMS OF THE REVERSIBLE GLYCOSYLATED POLYPEPTIDE
DePino V; Grinman D; Moreno S
Fundación Instituto Leloir, IIBBA-CONICET.

12:15-12:30
PL-C06
FUNCTIONAL AND BIOCHEMICAL CHARACTERISATION OF THE TRANSCRIPTION FACTOR MTHB1 FROM *M. truncatula*
Ariel FD[1];Diet A[2]; Gruber V[2]; Frugier F[2];Chan RL[1]; Crespi M[2]
[1]LBV, FBCB, UNLitoral, Santa Fe, Argentina. [2]ISV-CNRS, Gif-sur-Yvette, France.

12:30-12:45
PL-C07
CROSS-TALK BETWEEN FLOWERING TIME AND THE DEFENSE RESPONSE MEDIATED BY THE SUN FLOWER TRANSCRIPTION FACTOR HAHB10
Dezar CA; Giacomelli JI; Bonaventure G; Manavella PA; Re D; Alves-Ferreira M; Baldwin I; Chan RL
Lab. de Biotecnología Vegetal FBCB UNL Ciudad Universitaria Santa Fe Argentina.

12:45-13:00
PL-C08
AN ACQUIRED BACTERIAL PLANT NATRIURETIC PEPTIDE-LIKE PROTEIN MODIFIES HOST HOMEOSTASIS
Gottig N; Garavaglia BS; Daurelio LD; Orellano EG; Ottado J
IBR-CONICET, Fac. Cs. Bioquímicas y Farmacéuticas-UNR, Rosario, Argentina.

13:00-13:15
PL-C09
IDENTIFICATION OF PROTEINS INVOLVED IN FREEZING TOLERANCE IN *Arabidopsis thaliana*
Cabello JV; Arce AL; Chan RL
Laboratorio de Biotecnología Vegetal, Fac. de Bioq. y Cs. Biol., UNL. Santa Fe, Argentina.

13:15-13:30
PL-C10
EMERGING PROPERTIES OF OXIDATIVE METABOLISM MODIFICATION IN TRANSGENIC POTATO PLANTS
Llorente BE; Bravo-Almonacid FF; Rodríguez V; Torres HN; Flawiá MM; Alonso GD.
INGEBI-CONICET, Buenos Aires, Argentina

11:00-13:30 **ORAL COMMUNICATIONS**

Room C

Microbiology (MI-C11 / MI-C19)

Chairpersons: Paula Vincent, INSIBIO-CONICET, Universidad Nacional de Tucumán
Carlos Argaraña, CIQUIBIC-CONICET, Universidad Nacional de Córdoba

11:00-11:15
MI-C011
REGULATION OF THE BIOSYNTHESIS OF THE IRON-MOLYBDENUM COFACTOR OF NITROGENASE
Curatti L[1], Hernandez JA[2], Rubio LM[2]
[1]CEBB-MDP-FIBA; [2]PMB, University of Califormia at Berkeley

11:15-11:30
MI-C12
O CHAIN PARTICIPATES IN CELLULAR INTERACTIONS DURING BIOFILM FORMATION IN *Rhizobium leguminosarum*
Russo DM[1], Williams A[2], Posadas DM[1], Vozza N[1], Abdián PL[1], Downie JA[2], Zorreguieta A[1]
[1]Fundación Instituto Leloir, IIBBA CONICET, Bs. As; [2]John Innes Centre, Norwich, U.K.

11:30-11:45
MI-C13
INTERACTION OF FAPR, A GLOBAL LIPID BIOSYNTHESIS REGULATOR, WITH ITS OPERATOR SEQUENCES
Reh G[1], Albanesi D[1], Schaeffer F[2], Schujman GE[1], Alzari PM[2], De Mendoza D[1]
[1]IBR-Conicet, Dpto. Microbiología, Facultad de Cs. Bioq. y Farm., UNR; [2]Instituto Pasteur, Paris.

11.45-12:00
MI-C14
ROLE OF LIPIDS IN THE ACTIVATION OF SIGMA E FACTOR DURING SPORULATION IN *Bacillus subtilis*
Diez V, Schujman GE, De Mendoza D
IBR - CONICET, Facultad de Cs. Bioq. y Farm., UNR. Suipacha 531, 2000-Rosario, Argentina.

12:00-12:15
MI-C15
A SELECTIVE BACTERIAL BIOSENSOR FOR MONITORING GOLD METAL IONS IN THE ENVIRONMENT
Cerminati S; Soncini FC; Checa SK
Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET-UNR, Rosario, Argentina

12:15-12:30
MI-C16
FUNCTIONAL AND PROTEOMIC ANALYSIS OF *Trypanosoma brucei* SKP1
Rojas F, Burchmore R[1], Mottram JC1, Téllez-Iñón MT
[1]Wellcome Centre for Mol Parasitol, Univ Glasgow, UK;, INGEBI-CONICET, Buenos Aires, RA.

12:30-12:45
MI-C17
NEW INSIGHTS ON PROTEIN LIPOYLATION PATHWAYS IN *Bacillus subtilis*
Martin N, De Mendoza D, Mansilla MC
Instituto de Biología Molecular y Celular de Rosario (CONICET) y Fac. de Cs. Bioq. y Farm., UNR.

12:45-13:00
MI-C18
METABOLIC FLUX ANALYSIS OF *E. coli* ARCA AND CREB MUTANTS BASED ON LABELING EXPERIMENTS
Nikel P[1,3], Zhu J[2], Bennett GN[2], San KY[2], Galvagno MA[1], Méndez Bs[3]
[1]IIB-UNSAM/CONICET; [2]Dpto. Qca. Biológica, FCEyN; [3]Dept. Biochemistry and Cell Biology, Rice U.

13:00-13:15
MI-C19
LOW OXIDATIVE DAMAGE IN *E. COLI* STATIONARY PHASE BY A PHOSPHATE-DEPENDENT GENES EXPRESSION
Schurig Briccio LA, Rintoul MR, Rapisarda VA
INSIBIO e Inst. de Química Biológica "Dr B Bloj"(CONICET-UNT) Chacabuco 461, 4000, Tucumán-Argentina.

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

15:30-16:30 Room C
LECTURE
Andrés Aguilera
Centro Andaluz de Biología Molecular y Medicina Regenerativa (CABIMER)
Sevilla, España.
"Genomic Instability and mRNP Biogenesis"
Chairperson: Alberto Kornblihtt, IFIBYNE-CONICET, Universidad de Buenos Aires

16:30 19:00 **POSTERS with coffee**
Microbiology (MI-P61/ MI-P88)
Cell Biology (CB-P62 / CB-P90)
Structural Biology (SB-P01 / SB-P10)
Neuroscience (NS-P01 / NS-P06)
Signal Transduction (ST-P13 / ST-P33)
Enzymology (EN-P01/ EN-P13)

19:00-20:00 Room A
"ALBERTO SOLS" LECTURE
Sonsoles Campuzano
Centro de Biología Molecular Severo Ochoa
Universidad Autónoma de Madrid. Madrid, España
"Territorial specification and pattern formation in Drosophila melanogaster: Role of the Iroquois-complex genes"
Chairperson: Héctor Torres, INGEBI-CONICET, Universidad de Buenos Aires

21:00 **Farewell Dinner**

PL-Conference.

THE MOLECULAR BASIS OF PLANT IMMUNITY SUPPRESSION BY THE *Pseudomonas syringae* TYPE III EFFECTOR HopU1

Alfano JR.
Center for Plant Science Innovation and the Department of Plant Pathology, University of Nebraska, Lincoln, NE 68588-0660 USA.

The bacterial pathogen *Pseudomonas syringae* is dependent on a type III protein secretion system and the effector proteins it injects into host cells to cause disease. Many *P. syringae* effectors have been shown to suppress plant innate immunity. However, the majority of their enzymatic activities and their plant targets remain unknown. The *P. syringae* effector HopU1 was purified and we demonstrated that it was a mono-ADP-ribosyltransferase (ADP-RT) active on the artificial substrate poly-arginine as well as plant proteins. HopU1 suppressed plant innate immunity in a manner dependent on its ADP-RT active site. Using ADP-RT assays coupled with mass spectrometry we identified the major HopU1 substrates in *Arabidopsis thaliana* extracts to be several RNA-binding proteins that possess RNA-recognition motifs (RRMs). *A. thaliana* knock-out lines defective in the glycine-rich RNA-binding protein *At*GRP7, a HopU1 substrate, were more susceptible than wild type plants to *P. syringae* infection suggesting that this protein plays a role in innate immunity. The ADP-ribosylation of *At*GRP7 by HopU1 required two arginine residues within the RRM of *At*GRP7 suggesting ADP-ribosylation may interfere with the ability of *At*GRP7 to bind RNA. Our results suggest bacterial pathogens can ADP-ribosylate plant RNA-binding proteins to suppress host innate immunity by affecting immunity-related RNA.

PL-S01.

NOVEL SUGAR SIGNALING THROUGH A PUTATIVE RECEPTOR GAP (GTPase-ACCELERATING PROTEIN) IN *Arabidopsis*

Jones AM, and coworkers
Departments of Biology and Pharmacology, School of Medicine, University of North Carolina, Chapell Hill, NC USA 27599

Adaptation to changes in sugar availability is critical for most organisms. Plants use sugars as signaling molecules and possess mechanisms to detect and respond to changes in sugar availability, ranging from the level secondary signaling molecules to altered gene transcription. Heterotrimeric G-protein coupled pathways are involved in sugar signaling in plants, and G-protein mutants display sugar-related phenotypes. Regulator of G Signaling (RGS) proteins turn off signaling by accelerating the intrinisic GTPase activity of the Gá subunits of the heterotrimeric G-protein complex. The *Arabidopsis thaliana* Regulator of G-protein Signaling protein 1 (*At*RGS1) combines a receptor-like seven transmembrane domain with an RGS domain. AtRGS1 interacts with the *Arabidopsis* Gá subunit (AtGPA1) of the heterotrimeric G protein complex in a D-glucose-dependent manner and stimulates AtGPA1 GTPase activity. Furthermore, AtRGS1 regulates the activation of AtGPA1 in a D-glucose-dependent manner. Thus, AtRGS1 is a D-glucose receptor-GTPase Accelerating Protein (GAP), thus defining the prototype for this new receptor class. It is well-known that D-glucose controls the rate of cell proliferation in meristems. Evidence is provided to show that the mechanism of this glucose control is through regulation of the activation state of AtGPA1 through D-glucose control of GAP activity of AtRGS1.

PL-S02.

EFFECTS OF POTASSIUM DEFICIENCY ON PRIMARY METABOLISM IN *Arabidopsis thaliana*

Armengaud P, Sulpice R, Miller A, Stitt M, Gibon Y, Amtmann A
FBLS, University of Glasgow, Glasgow, UK, Max-Planck Institute of Molecular Plant Physiology, Golm, Germany

K is an essential macronutrient in plants but unlike N, P andS, it is not assimilated into organic matters. Nevertheless an important role of K in metabolism is evident in the fact that K-deficiency in crops impacts on contents in primary and secondary metabolites with important consequences for both mechanical stability and pathogen/pest resistance. There is no lack of mechanisms by which K could affect primary metabolism, the most obvious ones being membrane potential, pH gradient, long distance transport, ribosomal function and enzyme activity. While all of these processes depend on K and potentially affect biosynthesis and allocation of metabolites, the exact causal relationships remain unidentified. We have adopted a multi-level approach combining transcript and metabolite profiles with enzyme activities from *Arabidopsis thaliana* plants grown under different K conditions. The study uncovered that K deficiency leads to accumulation of soluble sugars accompanied by depletion in organic acids, which could be assigned to inhibition of glycolysis in roots. N-metabolism exhibited properties of both C-starvation and C-abundance indicating that individual reactions were controlled by different signaling pathways residing either downstream or upstream of glycolysis. The comprehensive dataset provides an essential source of information for future genetic studies in this area.

PL-S03.

PLANT ENDOMEMBRANE SYSTEM AND CHEMICAL GENOMICS

Raikhel N
Director, Institute for Integrative Genome Biology and Center for Plant Cell Biology, Department of Botany and Plant Sciences, University of California Riverside, California 92521.

Chemical genomics is an exciting new technology for studying gene functions in the context of living organisms or cell systems. The approach complements existing molecular and genetics tools (e.g. mutagenesis, RNAi) by allowing fine-tunable in vivo modulations of protein functions and cellular processes. For example, lethality and redundancy are common and challenging descriptors in genetic studies of the endomembrane system. Chemical genomics can be used to overcome these challenges and study protein trafficking mechanisms. We have performed a few chemical genomics screens and identified several useful compounds. Effect of these compounds on various markers of the endomembrane system was assessed. Analogs of these chemicals were tested to identify the chemical structures that are responsible for bioactivity of these molecules. Screens for resistant and hypersensitive mutants were carried out with the goal of identifying putative targets or to identify components of the pathway. An information about these targets and pathways will be discussed.

PL-S04.
SOYBEAN FUNCTIONAL GENOMICS: ROOT HAIR BIOLOGY AS A MODEL SYSTEM
Stacey G
National Center for Soybean Biotechnology, University of Missouri, Columbia, MO, USA

The expected release of the full soybean genome sequence is already having a major impact on the ability to do biology with this important crop plant. A variety of functional genomic methods are among the tools that are enabled by access to the genome sequence. In our lab, we are applying these tools to investigate the biology of soybean root hairs, which are single cell extensions of the root epidermis. A key problem with doing functional genomics with plants is the 'cell problem'. By this I mean that usually we are measuring responses in whole tissues or the whole plant and it is difficult to know whether, for example, a gene is expressed at a low level or is indeed expressed in at a high level but only in a few cells. Therefore, our work on soybean root hairs is unique as it allows us to examine the functional genomics of a single, differentiated plant cell type. We have specifically focused on the early events in rhizobial infection of soybean root hairs using transcriptomics, proteomics, and metabolomics, as well as examining the small RNA content of this tissue. These data are being integrated using the platform of the soybean genome sequence. Various new insights have arisen from this thorough analysis and will be presented.

PL-S05.
LOCAL AND SYSTEMIC INTERACTIONS BETWEEN *Pseudomonas syringae* AND THEIR PLANT HOSTS
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Plant responses to bacterial infection are shaped by the genetics of both the host and the pathogen. Bacteria such as *Pseudomonas syringae* provoke different host responses due in large part to their ability to inject effector molecules that can trigger or suppress defenses depending on the host genotype. The host responses are complex, involving both local reactions and systemic signaling. Progress in understanding the local host interactions with effectors and systemic responses to infection will be discussed.

PL-S06.
THE SGT1-HSP90 CHAPERONE COMPLEX FOR NLR-PROTEIN DEPENDENT INNATE IMMUNITY
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The nucleotide-binding domain and leucine-rich repeat containing (NLR) proteins function as immune sensors in both plants and animals. The NLR proteins recognize pathogen-derived molecules and trigger immune responses. To function as a sensor, NLR proteins have to be correctly folded and maintained in a recognition competent state in a proper cellular location. Upon pathogen recognition, conformational change and/or translocation of the sensors would activate the downstream immunity signaling pathways. Misfolded or used sensors should be immediately discarded to avoid an inappropriate activation of the pathway, which is often destructive to the cell. Such maintenance of NLR-type sensors requires the SGT1-HSP90 pair, a chaperone machine that is structurally and functionally conserved in eukaryotes. Unveiling the cross-talks between SGT1 and other cochaperones is a major step toward the understanding of the HSP90 machinery. NMR spectroscopy, X-ray crystallography and mutational analyses of HSP90 revealed the nature of its binding with the CS domain of SGT1. We build a structural model of the HSP90-SGT1 complex, which is capable of conferring resistance via immune receptor stabilization. Deciphering how the chaperone machinery works would facilitate to understand the mechanism of pathogen recognition and signal transduction by NLR proteins in both plants and animals.

ST-S01.
SPACE AS A REGULATOR OF SIGNALLING PATHWAYS: THE Ras-ERK PARADIGM
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Subcellular localization influences the transduction of intracellular signals by unknown mechanisms. For the past years we have been investigating how Ras/ERK signals are propagated depending on the subcellular microenvironment in which they are generated. Herein, we show that the microenvironment from which Ras signals emanate, determines which substrates will be preferentially phosphorylated by the activated ERKs. We show that phosphorylation of EGFr and $cPLA_2$ is most prominent when ERKs are activated from lipid rafts, whereas RSK1 is mainly activated by Ras signals from the disordered membrane. We present evidence indicating that the mechanism underlying in this substrate selectivity, is governed by the participation of different scaffold proteins that distinctively couple ERKs, activated at defined microlocalizations, to specific substrates. As such, we show that for $cPLA_2$ activation, ERKs activated at lipid rafts interact with KSR1, whereas ERKs activated at the endoplasmic reticulum utilize Sef-1. To phosphorylate the EGF receptor, ERKs activated at lipid rafts require the participation of IQGAP1. Furthermore, we demonstrate that scaffold usage markedly influences the biological outcome of Ras site-specific signals. These results disclose an unprecedented spatial regulation of ERKs substrate specificity, dictated by the microlocalization from which Ras signals originate and by the selection of specific scaffold proteins

ST-S02.
WNT SIGNALLING REGULATES NEURONAL DEVELOPMENT
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The development and functions of the nervous system depend on the establishment of specific neuronal connections. Both intrinsic programs and extrinsic factors modulate neuronal development. Neuroblasts perceive the presence of neurotrophic factors present in the extracellular media (such as IGF-1, BDNF, NGF and Wnts), which regulate neuronal differentiation, polarization and migration. Wnt factors are secreted glycoproteins that play a crucial role on neuronal maturation as they have been implicated in axon guidance and behaviour, dendritogenesis and synapses formation. Wnts signalling through Frizzled (Fz) receptor activates Dishevelled (DVL), a first intracellular effector. Wnt-DVL signalling can activate three different pathways: the canonical or -catenin pathway, the planar cell polarity pathway and the calcium pathway. In this work, we show the roles of Wnt-DVL on neuronal differentiation and maturation. Wnt7b regulates dendrite development and complexity in hippocampal neurons. Particularly, Wnt and DVL increase dendrite arborisation through the activation of Rac and JNK. Consistent with these findings, hippocampal neurons from *Dvl1* mutant mice exhibit reduced dendritic arborisation and dominant-negative forms of Rac or JNK block Dvl-mediated dendritic growth. These findings suggest that Wnt pathway regulates dendritic maturation. However, little is known about the role of WNT signalling earlier, particularly on the initial axonal outgrowth and the establishment of neuronal polarity. Currently, we are investigating the role of Wnt factors, their Frizzled receptors and DVL on these phenomena. We found that undifferentiated neurons challenged with Wnt3a or overexpressing DVL develop multiple and more complex axons. Wnt3a seems to regulate axon formation through a non-canonical pathway. Importantly, Wnt3a activates PI3K in cultured neurons and in purified growth cones particles suggesting that Wnt3a may act through the same pathway as IGF-1 (previously defined as an essential factor for the establishment of neuronal polarity). In addition, Wnt3a cross-activates IGF-1 receptors in neurons and the Wnt effects on axons are blocked by an IGF-1 receptor blocking antibody. These findings suggest a possible parallelism between the two signalling systems: Wnt-Fz-DVL and IGF-1- IGF receptor-PI3K on axon formation. We conclude that the Wnt-DVL pathway is essential for neuronal development from axon formation to dendrite maturation by activating different secondary signalling cascades.

ST-S03.
THE TRANSCRIPTION FACTOR PLAGL2 ROLE IN LEUKEMIA DEVELOPMENT IS DEPENDENT OF AKT/mTOR PATHWAY
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The activation of cytokine signaling pathways by oncogenic mutations is a frequent event in acute myeloid leukemia (AML). Such activation is particularly frequent in core-binding factor (CBF) leukemias, and synergizing with the fusion oncogenes *CBFb-MYH11* and *RUNX1-ETO*. We have previously shown that transcription factor PLAGL2 induces myeloid leukemia in cooperation with CBF -SMMHC, the protein encoded by *CBFb-MYH11*. Here we show that PLAGL2 directly increases levels of the thrombopoietin receptor MPL in the cell surface of hematopoietic progenitors and leukemia blasts expressing CBF -SMMHC or RUNX1-ETO. This upregulation induces cytokine hypersensitivity to activating Jak2 and downstream effectors Stat3/5, Ras/Erk, and Akt/mTOR. Using bone marrow transplantation assays, we validated that PLAGL2 and Mpl can cooperate with RUNX1-ETO in leukemia development. Furthermore, the latency of leukemia was significantly delayed by treatment with the mTOR-inhibitor rapamycin. Finally, MPL levels and TPO responsiveness correlate with RUNX1-ETO in human AML. These results show that upregulation of wild type MPL receptor by PLAGL2 is an oncogenic response that participates in CBF leukemogenesis, and suggest that targeting MPL/AKT pathway may provide therapeutic benefit in AML treatment.

CB-S01.
ANIMAL MODELS OF KSHV-INDUCED KAPOSI'S SARCOMA
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Kaposi¹s sarcoma (KS) is a viral malignancy characterized by angiogenesis and proliferation of KSHV-infected spindle cells. We described a new animal model of KS (Mutlu et al. 2007. Cancer Cell 11:245-258): KSHVBac36-tranfected mouse endothelial lineage cells (mECK36) induce KSHV-infected KS-like tumors in nude mice, while mECK36 that lose the KSHV episome lose their tumorigenicity. shRNA silencing showed that vGPCR, a KSHV angiogenic gene upregulated in mECK36 tumors, is essential for angiogenesis and tumorigenicity. We found that mECK36 explanted from tumors that lose the KSHV episome are tumorigenic, indicating that, as KSHV tumorigenesis progresses in vivo, the malignant phenotype becomes irreversible and KSHV-independent. Explanted mECK36 displayed more foci of DNA repair than cells never grown in tumors indicating that DNA damage is increased in vivo. We now find that expression of constitutively activated Rac1, a mediator of vGPCR pathogenesis over-expressed in KS lesions, is sufficient to induce KS-like tumors in transgenic mice by a mechanism involving PTEN inactivation and R.O.S.-mediated oxidative DNA damage. These results points to a key role for Rac1 induced R.O.S. production in KS pathogenesis. They indicate that in addition to angiogenesis, vGPCR activation of Rac1 can increase mutation rate to foster a multi-step carcinogenesis process driven by oxidative genetic damage.

CB-S02.
CAVEOLIN-1, A SCAFFOLDING PROTEIN WITH AMBIGUOUS ROLES IN CANCER
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Caveolin-1 functions as a tumor suppressor in a variety of cellular backgrounds. More recently, we identified the inhibitor of apoptosis protein (IAP) survivin as a transcriptional target downstream of caveolin-1, whose expression is controlled via the á -catenin/Tcf-Lef pathway. Caveolin-1 recruits á-catenin to the plasma membrane, thereby reducing the cytosolic pool available for translocation to the nucleus and enhanced transcription of tumor cell survival genes like survivin. Caveolin-1 may also promote malignant tumor cell traits, including metastasis. How such dramatic switches in function are achieved remains an enigma. However, we recently observed that caveolin-1 is only able to suppress survivin expression in cancer cells when E-cadherin is present. Thus, caveolin-1 is a conditional tumor suppressor protein whose function in part depends on the presence of E-cadherin. Currently work in the laboratory focuses on developing in vivo models that will permit the identification of molecular traits in caveolin-1 associated with its role either as a tumor suppressor or promotor of metastasis.
Acknowledgements: FONDAP #15010006

CB-S03.
MOLECULAR MECHANISM OF MICROTUBULE PLUS-END TRACKING REVEALED BY FLUORESCENCE MICROSCOPY IMAGING OF *IN VITRO* RECONSTITUTIONS
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The microtubule cytoskeleton is essential to cell morphogenesis. Growing microtubule plus ends have emerged as dynamic regulatory sites in which specialized proteins, called plus-end-binding proteins (+TIPs), bind and regulate the proper functioning of microtubules. However, the molecular mechanism of plus-end association by +TIPs and their ability to dynamically track the growing end are not well understood. Here we report the in vitro reconstitution of minimal plus-end tracking systems consisting of several proteins. Using time-lapse total internal reflection fluorescence microscopy and single molecule imaging, we elucidate the molecular mechanism of how EB1 and Clip170 track growing microtubule ends. We compare the behavior of these vertebrate proteins to their orthologs from fission yeast. Our results dissect the collective interactions of the constituents of this plus-end tracking system and show how these interactions lead to the emergence of its dynamic behavior. We expect that such in vitro reconstitutions will also be essential for the mechanistic dissection of other plus-end tracking systems.

LI-S02.
VISUALIZING INTESTINAL LIPID METABOLISM IN LIVE ANIMALS: STUDIES WITH GUTS
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The larval zebrafish is an ideal model of vertebrate intestinal physiology because the optical clarity of the intestine with all its complexity (e.g. multiple cell types, symbiotic organisms, and bile) can be viewed in live animals. Data from a variety of vertebrates indicates that dietary lipids are initially absorbed by intestinal enterocytes, sorted, processed and packaged into chylomicrons that enter the lymphatic system. While several studies implicate various subcellular compartments and proteins in the absorptive process, many questions remain. We have developed a feeding assay that employs fluorescent lipids to visualize lipid uptake from the intestinal lumen in live animals. Following a high-fat meal, we observe prodigious lipid globules at the apical surface of enterocytes. Our electron microscopy studies reveal that neutral lipid accumulations are surrounded by a phospholipid monolayer, suggesting that the globules are lipid droplet organelles. We have created transgenic zebrafish lines that express well-characterized mammalian lipid droplet associated proteins expressed under heat-shock promoter control to determine if these fluorescent fusion proteins co-localize with the large lipid accumulations. These results demonstrate the feasibility of our overall goal to characterize the specific molecular mechanisms required for intestinal lipid absorption.

LI-S01.
PHOSPHATIDYLCHOLINE BIOSYNTHESIS AND NEURONAL DIFFERENTIATION
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Neuronal differentiation is a complex process characterized by a halt in proliferation and extension of neurites from the cell body. Changes in gene expression mediate the metabolic 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. Our results indicate that membrane biogenesis is driven by the elevated expression of a subset of lipid biosynthetic genes following retinoic acid (RA)-induced differentiation of Neuro2A cells. The transcription of genes encoding key enzymes in the CDP-choline pathway of phospholipids biosynthesis is stimulated, including the Chka gene for choline kinase (CK) and the Pcyt1a/b genes for the CTP:phosphocholine cytidylyltransferase (CCT). The promoter regions for all the genes indicated common DNA sequences which are proposed to be binding sites for transcription factors (TFs) that coordinately activate expression in a RA-dependent manner. Alteration of CK or CCT expression by siRNA or overexpression regulated PtdCho synthesis and neurite extension following RA treatment. These results indicate that PtdCho synthesis is necessary for RA-dependent differentiation of Neuro2A cells.

LI-S03.
LIPIDS WITH LONG AND VERY LONG CHAIN (VLC) POLYENOIC FATTY ACIDS: FROM CELL LOCALIZATION TO FUNCTION
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In seminiferous tubules, spermatogenic cells proliferate and differentiate to produce spermatozoa, supported by Sertoli cells. Among their multiple functions, the latter phagocytize lipid-rich residual bodies that are normally formed in spermiogenesis as well as apoptotic bodies from germ cells that die from any cause. Glycerophospholipids (GPL) rich in PUFA (22:5n-6) and sphingomyelins (SM) rich in very long chain PUFA (28:4n-6 to 32:5n-6) belong to germ cells, as these lipids disappeared from the testis of fertile adult rats after these cells died as a result of irradiation, cryptorchidism, or doxorubicin. Surviving Sertoli cells efficiently maintained lipid homeostasis in the testis. They processed and disposed of materials arising from former germ cells, including free cholesterol and GPL, to make cholesterol esters with VLCPUFA and 22:5n-6. PUFA-GPL and VLCPUFA-SM (and ceramides) evolved with germ cell differentiation, part of the VLCPUFA becoming a-hydroxylated, to eventually compose spermatozoa. In the latter, GPL and SM species were distributed unevenly between tail and head, part of these lipids being hydrolyzed differentially after sperm capacitation and acrosomal exocytosis. Thus, definite lipids with unique fatty acids are created in the testis with the teleological objective of eventually producing the lipid metabolites that govern these male gamete-specific reactions.

LI-S04.
BIOGENESIS AND FUNCTIONS OF LIPID BODIES IN INFLAMMATION AND CANCER
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Lipid body accumulation within leukocytes and other cells is a common feature in both clinical and experimental infectious, neoplasic and other inflammatory conditions. We will discuss the contemporary evidence related to the biogenesis and structure of lipid bodies (lipid droplets) as inflammatory organelles. Lipid body biogenesis is a highly regulated process, that culminate in the compartmentalization of a specific set of proteins and lipids, that place lipid bodies as inducible organelles with roles in cell signaling, regulation of lipid metabolism, membrane trafficking and control of the synthesis and secretion of inflammatory mediators. Pertinent to the roles of lipid bodies in inflammation and cell signaling, enzymes involved in eicosanoid synthesis are localized at lipid bodies and lipid bodies are sites for eicosanoid generation in cells during pathogen-infection and cancer. Moreover, inhibition of lipid body formation has roles in the modulation of host response to infection and in the inhibition of PGE2 synthesis in colon cancer cells with implications to cancer cell growth. Collectively, lipid bodies are emerging as dynamic and functional active organelles, critical regulators of different inflammatory diseases, and attractive targets for novel anti-inflammatory therapies.

MI-S01.
HOW RESPIRATION OF LACTIC ACID BACTERIA MAKES LIFE EASIER IN A CROWDED SPACE
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Lactic acid bacteria (LAB) comprise the diverse group of bacteria used in foods for their fermentative metabolism. We are exploring a second metabolic option available to certain LAB, as illustrated for *Lactococcus lactis*: When placed in an aerobic environment containing a heme source, *L. lactis* activates a respiration metabolism, which results in decreased lactic acid production, and a strong growth and survival advantage. Respiration metabolism thus leads to a better quality of life for LAB in the presence of oxygen. Respiration metabolism has such a strong impact on bacterial life that both the bacterium and its environment are changed. Numerous LAB, including those used for probiotic applications, may benefit from respiration when the proper cofactors (heme, and in some cases Vitamin K2) are available. The bacterial environment is strongly affected by respiration growth: For example, in aeration, some non-respiring LAB release superoxides (e.g., *Enterococcus faecalis*) or H_2O_2 (e.g., *L. lactis* and Lactobacilli) into the medium. When respiration is activated by heme (and in some cases menaquinone) addition, radicals production is nearly abolished. Surprisingly, LAB or other bacteria may help their neighbors respire: for example, by releasing menaquinones during growth, *L. lactis* can stimulate respiration of a *Streptococcus* that requires cofactors for respiration.

MI-S02.
CHEMICAL BIOLOGY OF TRANSCRIPTION FACTORS: HIGH THROUGHPUT APPROACH TO UNVEIL SIGNAL MOLECULES
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One-component systems dominate signal transduction in prokaryotes. The availability of many microbial genome sequences provides an invaluable opportunity to understand how the activity and responses of the cell are quickly modulated as result of the changing environment. Fine tuning of gene expression requires a high diversity in the small molecule binding modules of these transcriptional regulators (TFs) and relay on their ability to bind a wide variety of small molecules. The MarR, MerR, TetR, IclR and LysR families of TFs are good examples since they have distinctive small molecule binding domains and have been reported to modulate resistance to drugs or aromatic compounds. However, in most cases, the native regulatory ligand is unknown. We took a high throughput approach to identify native or synthetic small molecules and to determine its specificity. To this end, we have cloned, purified and screened in a high throughput manner TFs against a library of chemical scaffolds. Our assay is directed to follow the kinetics of protein unfolding in presence or absence of a small molecule. The effects of the new small molecules identified by this technique are being studied on specific promotors *in vitro* by EMSA and *in vivo* by qRT-PCR.

MI-S03.
METAGENOMICS FOR VIRAL DISCOVERY
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Viruses are the most abundant biological entities on the planet and play important roles in biogeochemical cycling, horizontal gene transfer, and structuring bacterial communities. Despite their abundance and importance, methodological limitations have prevented us from characterizing viral communities. Viral metagenomics, which involves purification of viral particles and shotgun sequencing of the complete viral community, has yielded unprecedented insight into the identity and diversity of viruses. This talk describes the use of viral metagenomics to characterize the DNA and RNA viruses present in human fecal samples. While the DNA viral community consisted mostly of phage (viruses that infect bacteria), the human fecal RNA viral community was dominated by plant viruses. In particular, pepper mild mottle virus, was extremely abundant in the fecal samples, and has been subsequently identified in raw sewage from throughout the United States. Pepper mild mottle virus of dietary origin was still infectious to plants after passage through the human gut. This suggests that humans may be a novel dissemination mechanism for plant viruses. Further examination of reclaimed water (the endproduct of wastewater treatment which can be used for agricultural irrigation) demonstrated the presence of these plant viruses, as well as phage and pathogens of humans, animals, and insects.

MI-S04.

RECOMBINATIONAL RESCUE OF BLOCKED REPLICATION FORKS IN *Escherichia coli*

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Replication impairment is one of the major causes of genetic instability in several different organisms. In prokaryotes, inactivated replication forks can restart by the re-assembly of the replication machinery. In addition, several strategies of processing of the inactivated replication fork can take place prior to the replisome re-assembly. Most of these strategies imply the action of recombination proteins, which is not necessarily accompanied by a strand-exchange reaction. Depending on the origin of replication arrest, different Rec proteins are required for efficient replication restart which may reflect different structures of stalled forks. The involvement of recombination proteins in replication fork restart results in part from the formation upon replication impairment of single-stranded DNA regions and double-stranded DNA ends that are substrates for recombination enzymes. An additional link between replication and recombination results from the capacity of certain enzymes (PriA, RuvAB) to recognize two types of targets, inactivated replication forks and recombination intermediates. Actually, we have recently described that, in addition to its action on Holliday junctions, the RuvAB complex can act on inactivated replication forks to reverse them. We will describe *ruvAB* mutants that are compromised for fork reversal but remain fully capable of homologous recombination.

LI-C01.
EFFECT OF PESTICIDES ON APOPTOSIS BIOMARKERS IN VARIOUS RAT TISSUES
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We previously demonstrated that the administration (i.p. 1/50 to 1/250 LD50, three times a week for 5 weeks) of dimethoate, glyphosate and zineb to rats, alone or in combination, provokes oxidative stress in liver (L) and brain (B) tissues. Now, we investigated the status of the apoptotic cell death pathway under these conditions. Caspase-3 activity was apparently unaltered in L and in cerebral cortex or substantia nigra. However we found higher activities of milli- and micro-calpains in treated groups compared with controls in both B regions. This proteolytic activity increased with pesticide associations. The inner mitochondrial membrane integrity (MMI) was decreased in treated animals between 20 to 60% compared with control samples from L or B preparations; nevertheless there were no differences in the outer MMI. We also observed a decrease in mitochondrial cardiolipin (CL) content which is usually associated with cytochrome C release. An apoptotic DNA fragmentation pattern was also observed in L and in B regions. We concluded that relative low doses of pesticides were able to induce an oxidative stress condition which is potentially enough to induce cellular apoptosis. This scenario is aggravated by the administration of pesticide mixtures. Results could contribute to the understanding of the etiological role that pesticides should play in neurodegenerative human illnesses.

LI-C02.
EFFECT OF APOAI LIPIDATION STATE ON CHOLESTEROL AND PHOSPHOLIPIDS MOBILIZATION AND EFFLUX FROM CELLS
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Apolipoprotein AI (apoAI) conformational flexibility permit to adopt different conformations in discoidal high density lipoprotein (HDL), depending on cholesterol (Co) content and disc size.These alternative conformations, specially those concerning to central domain, seem to be important factors determining the membrane affinity and the Co exchange between discs and lipid bilayers. In order to know if alternative protein conformations result in different cellular responses, we have prepared reconstituted high density lipoproteins (rHDL) of different size and Co-content.They were used to compare cellular Co and phospholipids remotion and mobilization of intracellular Co pools, as the one available to be esterified by acyl CoA cholesterol acyl transferase (ACAT). We have observed that all sized rHDLs significantly promoted cellular Co removing, when tested at 3 and 12 hs of treatment.This ability was independent of Co content of particles. When we studied intracellular mobilization of Co pools, we observed that only apoAI, and Co-free rHDL particles of 96Å and 120Å generate Co mobilization from ACAT specific pools. Phospholipid remotion was only promoted by apoAI and rHDL without Co of 78 Å, after 12-hr incubation. In CHO cells phospholipid remotion is slower than Co remotion.

LI-C03.
THE RECYCLING CERAMIDE IS A PRECURSOR OF HYPERTONICITY INDUCED INCREASE OF GLUCOSYLCER SYNTHESIS
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We have previously reported that hypertonicity induces MDCK cell differentiation by a GlucosylCeramide Synthase (GCS) dependent mechanism. GCS catalizes the conversion of Ceramide (Cer) to Glucosylceramide (GlcCer), and it can use as a substrate de novo synthetized Cer or Cer produced by the recycling pathway. In this study we evaluate the involvement of both pathways in the hypertonicity induced increase of Glycosphingolipid (GSL) synthesis. Confluent MDCK cells were submitted to high NaCl concentration for 24 h or kept in isotonicity as control cultures. GSL metabolism was determined by using ^{14}C-Galactose (^{14}C-Gal) and ^{14}C-Palmitic Acid (^{14}C-PA) as radioactive precursors to radiolabel total GlcCer or GlcCer formed from de novo synthesis pathway, respectively. Under isotonic conditions, ^{14}C-Gal/GlcCer accounted for 23% of total GSLs formed, and the rest was equitatively distributed among LactosylCer, TrihexosylCer and GM3; while ^{14}C-GlcCer accounted for 44% of total GSLs formed from ^{14}C-PA. Over 24h of hypertonicity, GlcCer synthesis increased to 43% in the cultures incubated with ^{14}C-Gal, while ^{14}C-PA/GlcCer remained constant. The results suggest that the pool of Cer involved in hypertonicity induced MDCK cell differentiation comes from the recycling but not from de novo synthesis pathway.

LI-C04.
MALONYL-ACP AS A PUTATIVE SIGNAL FOR THE GLOBAL FATTY ACID REGULATOR FAPR OF *Bacillus subtilis*
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Fatty acid biosynthesis is essential for bacterial growth, and its homeostasis is critical for survival. In many Gram positive bacteria, this accurate regulation is established trough the action of the global transcriptional repressor FapR. We have shown that the signal sensed by FapR is malonyl-CoA, a key precursor of fatty acid biosynthesis. Another key component of this biosynthetic pathway is the acyl carrier protein (ACP), which binds all the acyl intermediates and presents them to the different enzymes of this metabolic route. The structural characterization of the malonyl-CoA–FapR complex indicated that all the atoms of malonyl-CoA involved in the interaction are also present in malonyl-ACP, which contains a phosphopantetheine prosthetic group. The objective of this work was to test in vitro if malonyl-ACP is able to interact with FapR, and to act as an effector of this regulator. To characterize the nature of the interaction, we performed isothermal titration microcalorimetry of FapR with malonyl-ACP. To identify the aminoacidic residues of malonyl-ACP involved in the interaction we carried out NMR titrations of ^{15}N-malonyl-ACP with FapR. Finally, we confirmed that malonyl-ACP releases FapR from its operator sequences by in vitro transcription. Our results indicate that malonyl-ACP is also a signal for FapR regulation.

LI-C05.
TRANSCRIPTIONAL REGULATION OF CCT*a* GENE PROMOTER BY E2F AND CELL PROLIFERATION
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Phosphatidylcholine (PC) is the major component in mammalian membranes and its biosynthesis occurs via the Kennedy pathway in which CTP:phosphocholine cytidylyltransferase (CCTa) catalyzes the rate limiting step. PC biosynthesis is an important component of cell cycle because its mass/cell doubles prior to mitosis. Previous studies showed that CCTa mRNA levels increase during S phase. As E2F family factors regulate the cell cycle by controlling the coordinate transcription of a large number of genes involved in DNA replication and cell cycle progression, we investigated the sequence of the proximal promoter of CCTa gene. By in silico analysis, we found a putative binding site for E2F at the position -212 upstream of the transcription start site. This information was further confirmed by EMSA and ChIP assays. To test how E2F regulates CCTa expression we analyzed the activity of CCTa promoter-luciferase reporter plasmids and one containing mutations that alter the E2F binding site. Furthermore we study how different levels of E2F may affect CCTa expression by overexpressing this transcription factor or by decreasing its levels using siRNA. These results suggest that E2F plays an important role in the activation of CCTa promoter during S phase showing that the expression of this gene shares regulatory mechanisms with genes involved in cell division and proliferation.

LI-C06.
GLYCEROL-3-P-ACYLTRANSFERASE 2 IS EXPRESSED IN TESTIS AND INVOLVED IN CELLULAR PROLIFERATION
Cattaneo ER, Pellon Maison M, Coleman RA, Gonzalez Baro MR.
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Glycerol-3-phosphate acyltransferase (GPAT) catalyzes the first and committed step in de novo glycerolipid synthesis. In mammals, several isoforms have been described with different cellular localization and kinetic properties. The mitochondrial isoform GPAT2 is highly expressed in rat testis and has no preference for saturated acyl-CoA substrates, so we hypothesized that it would be involved in the synthesis of triacylglycerols (TAG) rich in unsaturated fatty acids, required for the proper testicular function. Northern blot analysis showed that GPAT2 mRNA is expressed in seminiferous tubules, specifically in germ-line cells. The expression increases with age, and it is consistent with spermatogenesis.
Transient GPAT2 overexpression in CHOK1 cells increased the TAG content respecting GPAT1-overexpressing and control cells. Lipid droplets were larger and similar in number. CHO-K1 cell stably overexpressing GPAT2 showed increased proliferation compared to GPAT1 and control cells. Crystal violet proliferation assays showed that, after 65 hr, cell number was ~30% higher in GPAT2-overexpressing cells.
Our results indicate that GPAT2 is involved in TAG synthesis related to spermatic cells, and some metabolite/s derived from its activity may be involved in the testis' processes of cellular proliferation.

LI-C07.
ROLE OF INTRAMITOCHONDRIAL ARACHIDONIC ACID IN BREAST CANCER CELLS
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The inducible isoform of cycloxygenase COX-2 and the 5-, 12- and 15-lipoxygenases (LOX) are highly expressed in aggressive metastatic breast cancer. The acyl-CoA sinthetase (ACS4), a key enzyme in the regulation of intramitochondrial arachidonic acid (AA) release, has been implicated in colon carcinoma and hepatocellular cancer. The mechanism proposed for the role of ACS4 is the reduction of free AA levels and apoptosis. Since ACS4 is working in AA release and its metabolism to the LOX pathway, an important question is whether this mechanism is also operating in breast cancer cells. We measured the levels of 5-, 12-,15-HETE by HPLC in different human breast cancer cells line: MCF-7 (non aggressive phenotype) and in the MDA-MB-231 (aggressive phenotype). In MDA-MB-231 the levels of 5-,12-,15-HETE were 70, 5 and 2-fold higher than MCF7 cells. Inhibition of ACS4 expression in the aggressive cell line by siRNA reduced the LOX products to the levels observed in the non aggressive cell line. Overexpression of ACS4 in the non aggressive cell line produced an increase in COX2 expression that was blocked by the inhibition of LOX. The overexpression of ACS4 and COX2 correlates with the high aggressive phenotype. These results indicate that ACS4 is a key enzyme in the regulation of the synthesis of LOX products and COX-2 induction, a key enzyme in cancer progression.

LI-C08.
CLONING AND EXPRESSION ANALYSIS OF SEVERAL *Beauveria bassiana* GENES INVOLVED IN ALKANE DEGRADATION
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Entomopathogenic fungi have the ability to degrade insect cuticular hydrocarbons and to utilize them for energy production and incorporation into cellular components. The first oxidation round is carried out by a cytochrome P450 (P450alk), after successive steps the corresponding metabolites will eventually provide the appropriate fatty acyl-CoA for complete degradation in the peroxisomes, the site of -oxidation in fungi. Eight gene fragments displaying high homology to cytochrome P450alk as well as the peroxisomal enzymes catalase and acyl CoA oxidase were identified in a *B. bassiana* expressed sequence tagged (EST) collection. Full-length sequences for each gene were isolated by 5' and 3'-rapid amplification of cDNA ends (RACE). Expression analysis of the genes by quantitative real-time RT PCR using fungal cells grown on n-hexadecane (C16), n-eicosane (C20), n-tetracosane (C24) or n-octacosane (C28) revealed overlapping but differential expression of the isolated genes. These data indicate that *B. bassiana* is likely to contain multiple hydrocarbon degradative pathways with overlapping substrate specificities.

LI-C09.

CLONING AND FUNCTIONAL CHARACTERIZATION OF DELTA 4- SPHINGOLIPID DESATURASES FROM TRYPANOSOMATIDS

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Sphingolipids (Spl) are ubiquitous compounds present in membrane of eukaryotic cells. They comprise a long chain base (LCB) with and amide linked fatty acid. C1 OH may be substituted and LCB may be saturated (Dihydrosphingosine, DHS), unsaturated (Sphingosine, Sph) or hydroxylated (Phytosphingosine, PSph) in position 4. This desaturation is carried out by a Ä4-SphDes.

In Tritryp database, we found 3 sequences in *T. cruzi* (1 is entire), 1 in *T. brucei* and 4 in *Leishmania major* (3 are identical). A phylogenetic analysis unveiled that trypanosomatid proteins define a cluster related with fungi's group, where a few enzymes have desaturase/ hydroxylase activity.

Percentages of identities of *T. brucei* protein are 54, 52 and 37% to the enzymes of *T. cruzi*, *L. major* and *Schyzosaccaromyces pombe* (a Ä4-SphDes), respectively.

We cloned the enzymes of *T. brucei* and *T. cruzi* in pYES2, and analyzed the enzymatic activity in a yeast mutant for LCB hydroxylase (sur2). We detected desaturase but no hydroxylase activity for the enzyme of *T. brucei*, reason why we classify it as Ä4-SphDes.

T. cruzi and *T. brucei* cultures showed mainly 18C DHS, followed by C18 Sph, low level of 20C and 16C DHS, and no PSph. Since some reports depict the presence of minimal levels of PSph in *L. major*, we are currently investigating these enzymes, as some of them might be hydroxylases or bifunctional enzymes.

LI-C10.

BIOSYNTHESIS OF VERY LONG CHAIN FATTY ACIDS IN *Trypanosoma cruzi*

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Cytosolic fatty acid synthases (FAS I – II) catalyze de novo synthesis of fatty acids (FAs) up to 16 and 18 carbons. These products can be further elongated to very long chain FAs by a microsomal enzyme system called elongase (ELO). Both enzymatic systems (FAS and ELO) catalyze similar reactions but they differ markedly in their structure.

In trypanosomatids, FA synthesis has been described recently. These parasites use an ELO system instead of FAS, to supply themselves FAs de novo. *T .brucei* genome encodes 4 ELO. Three of them (TbELO1-3) lead to the synthesis of FAs from 4 to 18 carbons. The remaining ELO, TbELO5, is involved in polyunsaturated FA synthesis. *T.cruzi* and *L. major* genomes encode the four orthologues described in *T. brucei* but *T.cruzi* has 1 additional ELO (predicted ORF), while *L. major* presents 10 putative extra enzymes.

It has been reported that membranes of these last two parasites presents a variety of FA, reason why we speculate that the extra ELO (TcELO4) is involved in the synthesis of saturated FAs up to 26 carbons.

We show here the cloning and functional characterization of the additional enzyme of *T. cruzi* which is responsible at least, for the elongation of FAs from 22 to 26 carbons when expressed in a ÄElo3 yeast mutant. Currently, we are establishing the substrate specificity, using a ÄElo2/ÄElo3 yeast double mutant.

MI-C01.
PLANT FLAVONOID-INDUCED GENES AND NOVEL TYPE III SECRETED PROTEINS IN *Mesorhizobium loti* MAFF303099

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Type III secretion systems (T3SS), present in numerous plant and animal pathogens, deliver effector proteins into eukaryotic cells to enhance host colonization and initiate disease. Certain plants however have evolved a hypersensitive response against some of these effectors to resist infection. Rhizobia also have functional T3SS. Effectors translocated by this system were described to favor the nodulation efficiency in some legumes or conversely affect negatively the symbiosis in others. *M. loti* MAFF303099 has in its symbiotic island a cluster of genes necessary for the assembly of a T3SS. Mutation of this system affects competitiveness on *Lotus* spp. and improves the nodulation of *Leucaena leucocephala*. We have determined that this system is induced in response to flavonoids through NodD and the subsequent induction of TtsI, as described for other rhizobia. By a bioinformatics approach we identified other putative genes induced by the same pathway, and confirmed this transcriptional regulation for some of them by an analysis of *lacZ* fusions to the promoter regions. Translational fusions to a reporter peptide were used to analyze the T3SS-dependent secretion of the proteins encoded by these flavonoid-induced genes. We identified two new proteins whose amino-terminal domains have the capacity to direct secretion of the reporter peptide to the external media through the T3SS.

MI-C02.
***Xanthomonas* MANIPULATES HOST DEFENSE RESPONSE TO PRODUCE DISEASE**

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Xanthomonas campestris is a causal agent of disease in cruciferous plants worldwide. In the biosphere, plants are able to defend themselves through the deployment of both constitutive and induced defenses. Different signaling pathways are effective in mounting defense responses to different pathogens. The salicylic acid pathway is commonly involved in the defense against biotrophic pathogens like bacteria. In opposition the jasmonic acid pathway is involved in the defense against necrotrophic pathogens like fungi. These two pathways are antagonist, the activation of one of these pathways leads the inactivation of the other, so it is important to the plant to recognize the pathogen and activate the more effective defense pathway. To cause disease, a successful pathogen must counter or evade these defenses. In a previous work, we characterized a compound of *Xanthomonas*, the cyclic beta-(1,2)-glucan, as a suppressor of plant immune responses (Rigano et al, Plant Cell, 2007). The glucan is able to suppress expression of PR1 and callose deposition. Here the possible molecular mechanism of suppression by glucan is presented. Our results suggest that the cyclic glucan exerts its virulence effects by activating the host´s jasmonate signaling pathway.

MI-C03.
CHARACTERIZATION OF THE INVASION MECHANISM OF *Serratia marcescens* IN EPITHELIAL CELLS

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Serratia marcescens is an opportunistic human pathogen associated with urinary and respiratory tract infections, endocarditis, osteomyelitis, meningitis and septicemia. This microorganism produces numerous extracellular proteins that are predicted to play a role in the bacterial environmental adaptive capacity and pathogenic potential. Previous reports have shown that the ShlA haemolysin and the PrtA protease are able to act as virulence factors; however, the mechanisms that *Serratia* employs to invade and disseminate in eukaryotic cells are presently unknown. In this work, we demonstrate that a clinical *S. marcescens* isolate is able to invade, survive and multiply in the CHO epithelial cells. Confocal microscopy analysis revealed that intracellular bacteria colocalize with markers of late endosomes Rab7 and Lamp1. Two hours post-infection motile bacteria localized into acidic compartments. In addition, at earlier stages after entry, *S. marcescens* also colocalizes with the autophagosomal marker LC3. Inhibiting *Serratia* de novo protein synthesis with chloramphenicol blocked this process. Based on these findings, we propose that *S. marcescens* is able to transit through the endocytic pathway while it actively induces an autophagy-like process that might favour the intracellular survival and proliferation of the bacteria inside the host.

MI-C04.
HLA SECRETED BY THE ASPDE SYSTEM, BELONGS TO AN IRON RESPONSIVE OPERON IN *A. Tumefaciens*

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We have previously identified a locus encoding a Type I Secretion System in *A. tumefaciens* (AspDE), which restored protein secretion in the *prsDE* mutant of *R. leguminosarum*. An extracellular protein (Hla) encoded by an *aspDE* upstream gene cloned into the pIJ7760 cosmid was overproduced when expressed in *R. leguminosarum* A34. However, Hla is not detected in the extracellular medium of *Agrobacterium*. A34 turned out to behave as an iron responsive mutant. We noted that other genes encoded by pIJ7760 (including *hla*) might be iron responsive since their products are also overproduced in A34. Analysis of the region cloned in pIJ7760 supports the hypothesis that the *hla aspDE locus* belongs to an operon that is involved in iron acquisition. Furthermore this operon contains an upstream RirA regulatory sequence. Analysis of the Hla showed no homology with known proteins but the *hla* gene is syntenic with the HasA hemophore gene from *S. marcescens*. An *aspD* mutant showed increased cell clumping and reduced growth rate compared with the WT. Under low iron conditions, the *aspD* mutant showed increased motility, which was restored by normal iron. The secretion of several proteins encoded by the iron-responsive operon in *A. tumefaciens* was induced by hemoglobin and dependent on a functional AspDE system. AspDE might be involved in heme uptake through Hla, influencing other physiological functions.

MI-C05.

BIOFILM FORMATION IN *Leptospira*: STUDY IN A SWINE FIELD STRAIN FROM ARGENTINA

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Leptospirosis is a zoonosis of ubiquitous distribution caused by Spirochetes. Leptospires exist as saprophytic water-associate organisms or as animal pathogens that can survive in water. Previous work has demonstrated that both saprophytic and pathogenic *Leptospira* were able to produce functional biofilm, a community of bacteria embedded in an extracellular matrix attached to a surface. This structure is believed to provide protection from environmental aggressiveness. In this study we have analyzed the capability of bioflm formation in a field isolate of *L. interrogans* serovar Pomona isolated from an aborted pig compared with saprophytic *L. biflexa*. We have used light scanning electron microscopic examinations on glass and polystyrene plate models. On the other hand we have tested the possibility of biofilm formation in an *in vivo* model, using pregnant guinea pigs infected with both strains. Biofilms were formed preferentially on glass surfaces and bacteria aggregations were observed in placenta tissues by light microscopic. Scanning electron microscopic images showed cells embedded in an extracellular matrix. The formation of biofilm is consistent with the life of saprophytic strains in water and could help pathogenic strains to survive in the environment and to colonize the host.

MI-C06.

MICROBIAL ECOLOGY OF AMMONIA-OXIDIZING BACTERIA IN AN ACTIVATED SLUDGE WITH UNSTABLE NITRIFICATION

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Ammonia removal via nitrification is an important service provided by many wastewater treatment plants. It is a two-step process, usually rate-limited by the oxidation of ammonia to nitrite performed by ammonia-oxidizing bacteria (AOB). We have investigated this process during a period of full nitrification in a full-scale activated sludge from an oil refinery, which receives a high load of free ammonia, hydrocarbons and phenol and suffers from repeated periods of nitrification failure. A clone library of ammonia monooxygenase (*amoA*) gene was constructed. A total of 110 clones were divided into two OTUs separated by a genetic distance of 0.06. Dominant OTU was related to *N. europaea*, whereas a minor OTU was affiliated with the *N. nitrosa* linage. Novel primer sets were designed for quantification of *amoA* gene of the two detected taxons by real time PCR. The proportion of cells belonging to both OTUs was estimated as 0.6% and 0.06% of total bacteria. Similar abundance of the dominant taxon was obtained using a specific 16S rRNA-based assay. According to these results, AOB cell numbers are approximately three fold lower than the values predicted by current theoretical models. We propose that AOB adapted to perform under harsh conditions have very low yield, providing one possible explanation for why nitrification is unstable in the presence of toxic compounds such as phenol.

MI-C07.

GENOTYPIC AND FENOTYPIC DIFFERENCES AMONG *Staphylococcus aureus* FROM PATIENTS WITH OSTEOMYELITIS

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This study was addressed to investigate whether multiple *S. aureus* isolates from patients with chronic osteomyelitis display phenotypic and genotypic differences compared with those from patients with acute osteomyelitis. *S. aureus* was confirmed by PCR amplifications of species-specific sequences. Production of capsular polysaccharide (CP), -hemolysin, -hemolysin and slime was assessed by phenotypic procedures. Presence of the *cap*, *eap* and *pvl* genes was assessed by PCR. *S. aureus* from four patients (3, 4, 6 and 7 isolates, respectively) exhibited phenotypic and/or genotypic differences. In three of these groups of isolates differences in CP expression within each group were noted and included strains producing and not producing CP. Another patient with chronic osteomyelitis had 4 isolates exhibiting identical phenotypic features with one of them exhibiting a similitude of only ca. 78% by *Sma*I PFGE analysis. Two patients with acute osteomyelitis exhibited 4 and 10 isolates respectively, indistinguishable from each other within each group. Our results are consistent with our previous findings indicating that a higher number of CP-negative strains are isolates from patients with chronic osteomyelitis when compared with those with acute infection. Our results suggest that *S. aureus* evolves during chronic infection revealing both genotypic and phenotypic differences.

MI-C08.

ESTIMATION OF MICROBIAL DIVERSITY MEASURED BY DNA REASSOCIATION USING A FLUOROMETRIC METHOD

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The DNA heterogeneity of a microbial community reflects its diversity. This heterogeneity can be determined by thermal denaturation and reassociation. Under defined conditions, half-completed reassociation is proportional to the complexity of DNA. Therefore, this value can be used to estimate the genetic diversity of a mixed bacterial community. The advantage of this method is that allows the estimation of diversity without bias, as opposed to molecular methods based on PCR or bacteria isolation. The fraction of reassociated DNA, determined from the changes in optical density, requires the use of a thermally controlled UV-spectrophotometer. We propose the measurement of fluorescence emitted by the dye SYBR Green I bound to dsDNA, which can be readily achieved using a standard real time PCR instrument. Total DNA extracted from pure cultures and mixtures of bacteria were used to set up the optimal experimental conditions. Tested variables were DNA fragment size and sharing method, buffer and concentration of DMSO. Reassociation was adequately observed using buffers SSC 1x plus 30% DMSO. Fragment size uniformity, rather than average size, was critical to obtain clear reassociation patterns. Our long-term goal is to apply this technique for natural and engineered complex environments.

MI-C09.

MOLECULAR BASES OF CIPROFLOXACIN RESISTANCE IN *P. AERUGINOSA* DEFICIENT IN 8-OXO-G REPAIR SYSTEM

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The 8-oxodeoxyguanine (8-oxo-G) repair system participates in the prevention and correction of mutations generated by oxidative DNA damage in prokaryotes and eukaryotes. We have reported that *Pseudomonas aeruginosa* strains deficient in this repair mechanism by inactivation of the *mutT, mutM* or *mutY* genes show high frequency of resistance to Ciprofloxacin (CP). For the *mutT*-deficient strain, the CP resistance frequency was increased ~1400-fold over the wild type level, similar to the frequency achieved by the mismatch repair deficient *mutS* strain. In this work, we show that while all the CP-resistant clones derived from the *mutS* strain were mutated in GyrA (one of the mayor targets of the drug), the majority of those derived from the *mutT* strain were only mutated in *nfxB*, coding for the transcriptional repressor of the efflux pump MexCD-OprJ. The mutations in *nfxB* were mainly the A>C transversions characteristic of the incorporation of oxidized guanine into DNA, and resulted in important changes in the protein sequence. Finally, we show that CP resistance frequency of wild type and repair deficient strains increased considerably after cell exposure to the oxidizing agent paraquat. Thus, oxidative stress is strongly implicated in the emergence of CP resistant mutants in *P. aeruginosa*, and the 8-oxoG-repair pathway plays an important role in the prevention of these mutations.

MI-C10.

UNCOVERING THE ANAEROBIC COPPER-RESISTANCE PATHWAY IN *Salmonella*

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Copper resistance in Gram-negative bacteria is primarily controlled by the *cue* regulon. This regulon is composed by the Cu(I) sensor/regulator CueR that induces the expression of two target genes, *copA* and *cueO*, coding for an integral inner-membrane Cu-transporting P-type ATPase, and a periplasmic multicopper-Cu(I) oxidase, respectively. *Escherichia coli* also relies on the *cus* system to increase copper resistance under anaerobic conditions. Interestingly, *Salmonella* harbours all the *cue* components but lacks the *cus* locus. Despite of this, *Salmonella* displays higher resistance to copper than *E. coli* in anaerobic conditions. We have uncovered a novel CueR-regulated gene, named *cueP*, coding for a protein that increases resistance to copper both in aerobic and anaerobic conditions. CueP overexpression partially restored resistance to copper in a *cueO* deleted strain. To test whether CueP could functionally substitute the *E. coli cus* system for copper resistance, we replaced the entire *E. coli* chromosomal *cus* locus for the wild-type copy of the *Salmonella cueP*, including its own promoter. CueR-dependent expression of *cueP* increased resistance to copper in this engineered strain. Our results indicate that in contrast to other enterobacterial species *Salmonella* has evolved a single pathway to respond to copper excess both in aerobic and anaerobic conditions.

MI-C11.

REGULATION OF THE BIOSYNTHESIS OF THE IRON-MOLYBDENUM COFACTOR OF NITROGENASE

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Biological nitrogen fixation accounts for the major part of the global conversion of the N2 of the air into ammonium and is an essential process of the biogeochemical cycle of nitrogen that supports life on Earth. This reaction is catalyzed by the iron-molybdenum nitrogenase (MoFe nitrogenase) and alternative nitrogenases. The MoFe nitrogenase carries at its active site the unique MoFe cofactor. The synthesis of FeMo-co is a very complex process that employs the critical functions of the Nif proteins NifB, NifEN and NifH, and the participation of several other accessory factors. The NifB protein has been recently purified and shown to catalyze the synthesis of the FeMo-co precursor named NifB-co. In this work, to study regulatory features of FeMo-co biosynthesis, we uncoupled the transcription of *Azotobacter vinelandii* nifB gene from that of the nitrogen fixation (nif) regulon, which is derepressed when ammonium is not available and requires the transcription factor NifA. The results show the operation of a nitrogen source-dependent pathway for the degradation of NifB that might involve a duplicated copy of the ClpX protein (ClpX2) in *A. vinelandii*. It is also apparent that the integrity of the FeMo-co biosynthesis pathway might be important for the generation of forms of NifB that are more prone to degradation by the proposed ClpX2-dependent pathway.

MI-C12.

O-CHAIN PARTICIPATES IN CELLULAR INTERACTIONS DURING BIOFILM FORMATION IN *Rhizobium leguminosarum*

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Rhizobium leguminosarum bv. viciae develops a biofilm made of organized structures attached to a surface and consisting in multiple layers of interacting cells and water channels. The EPS and PrsDE secreted proteins are involved in the biofilm formation. The role of the O-chain LPS in these communities was studied in four *Tn5*-LPS mutants of *R.l.v.* in either 3841 or A34 backgrounds. Disrupted genes were related with the synthesis of core or O- components. All mutants showed O-chain deficiency by SDS-PAGE. Crystal violet assays indicated that the O-chain defects may affect attachment to the surface but this is highly dependent on culture conditions. Confocal microscopy showed that three of these mutants (*lpcA, lpsD* and *lpcB*) form aberrant structures, in which cellular interactions are exclusively between bacterial poles. However, the *lpsR* mutant resembled the wild type. In this mutant, overproduction of cellulose and as a consequence cell aggregation was observed. An additional mutation in the cellulose synthase gene prevented cellular aggregation and induced aberrant interactions indicating that cellulose could mask the O deficiency. Taking together these results indicate that the O-chain LPS is crucial for cell-cell interactions and that cellulose could also contribute to them during biofilm formation in *Rhizobium*.

MI-C13.

INTERACTION OF FAPR, A GLOBAL LIPID BIOSYNTHESIS REGULATOR, WITH ITS OPERATOR SEQUENCES

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FapR is a transcriptional regulator that negatively controls the expression of several genes of fatty acid and phospholipid biosynthesis in *Bacillus subtilis* (the *fap* regulon). It is the first global regulator of lipid synthesis discovered in bacteria and is largely conserved in Gram positive organisms, including several human pathogens. Malonyl-CoA acts as a negative effector of FapR promoting its release from DNA and the concomitant induction of the regulated promoters. A 17 bp consensus palindromic sequence is located in, or close to, the core promoter elements of all the transcriptional units that are members of the *fap* regulon. Upon binding, FapR protects approximately a 40 bp DNA region, which includes the palindromic sequence. Physiological and biochemical evidences indicate that in *B. subtilis* the 6 operons of the *fap* regulon are regulated by FapR to different extent. In order to understand how FapR differentially regulates the expression of these genes we decided to characterize in detail the FapR-DNA interaction through biochemical and structural approaches. Band shift assays, hydroxyl radical footprinting and isothermal titration calorimetry experiments indicate that FapR binds to DNA as a dimer and that two dimers of FapR bind to the protected sequence contacting the same side of the DNA double-strand.

MI-C14.

ROLE OF LIPIDS IN THE ACTIVATION OF SIGMA E FACTOR DURING SPORULATION IN *Bacillus subtilis*

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During sporulation in *B. subtilis* an asymmetrical division creates two compartments, a larger one (mother cell) and a smaller one (prespore). The ^{E} factor is proteolytically activated only in the mother cell by the membrane protease SpoIIGA. For this process is required the prespore protein SpoIIR. We have previously shown that inhibition of fatty acid biosynthesis precludes ^{E} activation. To establish the role of lipids in ^{E} activation, we analyzed the effect of cerulenin (a fatty acid biosynthesis inhibitor) in sporulation frequency of mixed cultures of *spoIIR* and *spoIIGA* mutants, finding that lipids are required for SpoIIR translocation or its interaction with SpoIIGA. Using a mutant version of SpoIIR (IIR Sec) we found that translocation of SpoIIR is essential for SpoIIGA activity during exponential growth. Expression of the transcription factor Spo0A (the master sporulation regulator) in exponential phase prevents IIR Sec-mediated ^{E} activation, suggesting the existence of a Spo0A-dependent inhibitor mechanism. Conversely, in the presence of SpoIIR, induction of Spo0A bypassed the lipid synthesis requirement. Finally, we analyzed ^{E} activation in a conditional mutant for phospholipid biosynthesis and found that fatty acid but not phospholipid biosynthesis is essential for ^{E} activation. Together, our results highlight the role of lipids in bacterial differentiation.

MI-C15.

A SELECTIVE BACTERIAL BIOSENSOR FOR MONITORING GOLD METAL IONS IN THE ENVIRONMENT

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Whole-cell bacterial biosensors are simple and highly accurate devices to detect toxic compounds in the environment. They consist of genetically engineered bacteria that couple a specific sensor protein with a reporter gene that produces a quantitative response. We have previously characterized a *Salmonella enterica* serovar. Typhimurium Au^{+}-sensor, GolS, that transcriptionally controls the expression of factors required for gold resistance. Unlike related proteins, GolS is able to discriminate Au^{+} from similar monovalent metal ions, such as Cu^{+} or Ag^{+}. This feature allowed us to design a bacterial biosensor based on the GolS regulon to detect Au ions in complex environments. We constructed a broad host-range reporter plasmid which carries the *gfp* gene under the control of the GolS-regulated *golB* promoter. The reporter system was tested in *S.* Typhimurium or introduced into GolS-expressing *Escherichia coli* cells. The fluorescence bacterial biosensor was functionally evaluated under laboratory conditions and calibrated regarding metal sensitivity and selectivity. The sensor bacteria detect concentration of gold ions in the range 10-1000 nM, even in samples containing in addition Ag^{+} and Cu^{+} ions. This gold-specific biosensor can be used as a technological tool to enhance recovery of this precious metal from ores and lixiviates, contributing to reduce contamination during mining.

MI-C16.

FUNCTIONAL AND PROTEOMIC ANALYSIS OF *Trypanosoma brucei* SKP1

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SKP1 encodes a conserved kinetochore protein that is essential for both the G1-S and G2-M transitions of the cell cycle in the budding yeast *Saccharomyces cerevisiae*. Skp1 also forms a component of the SCF (Skp1-cullin-F-box protein) complex, which functions as an E3 ubiquitin ligase for the ubiquitin-mediated proteolysis of cell cycle regulators at the G1/S transition of the cell cycle. The temperature sensitive mutant alleles of SKP1, skp1-11 and skp1-3, produce non-functional Skp1 as a component of the SCF complex and they are unable to accomplish the G1/S transition. We analyzed the functional role of TbSKP1 using RNA interference and overexpression in procyclics and bloodstream form cells. We show that RNAi of TbSKP1 in bloodstream and procyclic form trypanosomes results in an *in vitro* growth defect, with cells enriched in G1/S phase of the cell cycle. With the objective of identifying possible proteins regulated by SKP1-SCF, we performed 2-D DIGE with protein samples of induced vs. non-induced cells of RNAi. Differential spots were subjected to MALDI-TOF for its identification. Putative regulators of cell cycle were identified. These results suggest a role of SKP1 in the regulation of *T.brucei* cell cycle.

MI-C17.

NEW INSIGHTS ON PROTEIN LIPOYLATION PATHWAYS IN *Bacillus subtilis*

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Lipoic acid (LA), a covalently bound cofactor, is essential for the function of several key enzymes involved in oxidative metabolism. The model for protein lipoylation in *E. coli* involves two pathways: one in which exogenous LA is transferred to apoproteins in a process mediated by LA ligase (LplA), and an endogenous one, that involves LipB, which transfers octanoate to target proteins. These octanoylated domains are converted into lipoylated derivatives by lipoyl synthase (LipA). We have previously demonstrated that LipL is essential for the endogenous lipoylation pathway in *Bacillus subtilis*. Besides, this organism has two ORFs that encode products homologous to LplAs (*yhfJ* and *yqhM*). Since YqhM is homologous to an exogenous ligase, no growth or sporulation defects would be expected in an *yqhM* mutant. Surprisingly, such mutant was impaired to grow in minimal media. Adding LA or the products of the lipoylated enzymes restored mutant´s growth. This strain also showed a different membrane lipid composition than the observed in the wild type. In addition, it sporulates poorly in SM medium and this phenotype was partially reverted by the addition of LA. Due to these results we renamed *yqhM* as *lipM*. Notably, in *B. subtilis* three proteins, LipL, LipM and LipA, are essential for the endogenous protein lipoylation pathway, instead of the two-protein model of *E. coli*.

MI-C18.

METABOLIC FLUX ANALYSIS OF *E. Coli ARCA* AND *CREB* MUTANTS BASED ON LABELING EXPERIMENTS

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Escherichia coli has several complex sensing mechanisms for response to carbon availability and oxygen levels. Among them, the two-component signal transduction systems CreBC and ArcAB are responsible for regulation of carbon catabolism and transition from oxidative to fermentative metabolism, respectively. To systematically investigate the contribution of CreB- and ArcA-dependent regulation in catabolism, glucose-limited chemostat cultures were conducted for a wild-type strain, *creB* and *arcA* single mutants, and a *creB arcA* double mutant under a well-defined microaerobic condition. Metabolic flux distributions of the cultures of these strains were estimated based on ^{13}C labeling experiments. It was shown that the oxidative pentose phosphate pathway was functioning at low level under semi-aerobic conditions for the mutant strains. Fluxes through pyruvate dehydrogenase and the tricarboxylic acid cycle were found to be lower in the mutants, while that of the glyoxylate shunt was higher for the *arcA* strain. Strains bearing the *creB* mutation achieved a higher biomass yield on glucose than that of the wild-type strain or the *arcA* single mutant. Accordingly, the fluxes towards biomass were higher for those strains. Taken together, these results show that the CreBC and ArcAB systems operate in a concerted fashion to provide a fine-tuning in the central metabolic pathways of *E. coli*.

MI-C19.

LOW OXIDATIVE DAMAGE IN *E. Coli* STATIONARY PHASE BY A PHOSPHATE-DEPENDENT GENE EXPRESSION

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Bacteria have developed antioxidant defense systems for the protection against reactive oxygen species (ROS). However, these systems may be insufficient and ROS can produce oxidative damage. *E. coli* capacity to resist oxidative stress gradually declines during stationary phase. We have previously reported that *E. coli* cells grown in media with phosphate above a critical concentration (>37 mM) had high viability and low $NADH/NAD^+$ ratio in stationary phase. Here, we analyse the relationship between oxidative stress and the defense-gene expression in stationary phase cells. The endogenous stress (as cellular ROS production) and the oxidative damage to lipids (as TBARS) and proteins (as carbonyl groups generation) were determined. ROS production was minimal and consequently the levels of TBARS and protein carbonylation were lower than expected for stationary phase. In addition, cells were highly resistant to exogenous stress induced by H_2O_2. Expression of *sodA*, *katG* and *ahpC* was also assayed. Interestingly, *katG* and *ahpC* expressions were maintained high in late stationary phase. The presence of catalase and alkyl hydroperoxide reductase may explain the high viability and the low oxidative damage. Together, cells grown with high phosphate display advantages against oxidative stress in stationary phase.

CB-C01.

OSMOREGULATION AND ENDOCYTIC SIGNALING IN *Trypanosoma cruzi*

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Trypanosoma cruzi, the etiological agent of Chagas' disease, has a regulatory volume decrease (RVD) mechanism which reverses cell swelling under hyposmotic stress and allows it to respond to several environmental changes. Here we report the contribution of TcrPDEC2, a cAMP phosphodiesterase, and TcVps34, a phosphatidylinositol 3-kinase, to osmoregulation and membrane trafficking in *T. cruzi*. Inhibitors of TcrPDEC2 induced an increase in RVD in wild type cells exposed to hyposmotic stress. In addition, TcrPDEC2 localizes in the spongiome around the contractile vacuole in epimastigote cells, supporting its role in osmoregulation. Since TcrPDEC2 possesses a FYVE domain able to bind to phosphatidylinositol 3-phosphate (PI 3-P), we hypothesized that PI 3-P production might also have a role in osmoregulation. Consequently we characterized TcVps34, the first class III PI 3-kinase from *T. cruzi*. TcVps34 overexpressing cells showed enlarged contractile vacuoles and defects in the region of the flagellar pocket. Furthermore, these cells were more resistant to severe hyposmotic stress than wild type cells. In addition, TcVps34 overexpressing parasites showed alterations in vesicular acidification and receptor-mediated endocytosis. Finally, TcVps34 interacts with TcVps15, a Ser-Thr protein kinase that regulates Vps34 in yeast, suggesting the presence of a complex between these proteins.

CB-C02.

CHARACTERIZATION OF THE COMPLETE SET OF SNARES IN THE GOLGI-LACKING EUKARYOTE *Giardia lamblia*

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Giardia is an eukaryotic protozoan with unusual characteristics, such as the absence of a morphologically evident Golgi apparatus. Although both constitutive and regulated pathways for protein secretion exist in *Giardia*, little is known about the molecules and mechanisms involved in vesicular docking and fusion. In higher eukaryotes, soluble N-ethylmaleimide-sensitive factor-attachment protein receptors (SNAREs) of the vesicle-associated membrane protein (VAMP) and Syntaxin families play essential roles in these processes. An exhaustive search of the complete *Giardia* genome identified genes for 17 SNARE proteins, some of which possess atypical amino acids at the zero layer of the SNARE domain. The expression and localization of all SNAREs demonstrate that specific SNAREs localize to distinct subcellular organelles. Our results suggest that these compartments represent the extent of the endomembrane system in eukaryotes. Comparison of *Giardia* SNAREs homologous to Golgi SNAREs from other organisms does not allow the detection of a typical Golgi apparatus in trophozoites. Moreover, depletion of individual genes demonstrated that several SNAREs are essential for viability. Thus, *Giardia* requires a smaller number of SNAREs, compared to other eukaryotes, to accomplish all the vesicle trafficking events that are critical for growth and differentiation of this important human pathogen.

CB-C03.

THE PATHWAYS OF CATHEPSIN D IN RAT EPIDIDYMIS MAY BE REGULATED BY STEROID HORMONES

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Mammalian sperm acquire their fertilizing ability as they pass through the epididymal duct. Among several proteins secreted by the epithelium, high amounts of acid hydrolases are found in the lumen of the organ, although their function is still unclear. We have previously demonstrated that expression of mannose-6-P receptors is increased in epididymis of castrated rats, together with an increase of procathepsin D(pcD) secretion, probably as a response to hormonal changes. As the transport and secretion of pcD in other cell types may follow alternative routes, we proposed to study the possible relationship of pcD with expression and transport of prosaposin (pSAP) and its receptor sortilin (Sor) when epididymal cells are submitted to hormonal changes. Castrated or normal rats and RCE-1 cell line were used, and the expression and distribution of epididymal proteins were analyzed by different methods. We corrobored that secretion of pcD is increased in castrated rats or RCE-1 cells treated with estradiol and interacts with pSap. Likewise, a redistribution of pcD and Sor to the apical membranes was observed in the epithelium of castrated rats. Treatment of RCE-1 cells with NH4Cl induced retention of pcD but not pSAP indicating that they may reside in different compartments previous to be secreted. We concluded that pcD sorting is influenced by estrogens by regulating different pathways.

CB-C04.

DIFFERENTIAL SORTING OF ANTIBODY TO GM1 AND CHOLERA TOXIN AFTER INTERNALIZATION IN EPITHELIAL CELLS

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Gangliosides are glycolipids mostly located in the plasma membrane (PM). Antibodies to GM1 ganglioside are present in patients with Guillain-Barré neuropathy syndrome (GBS) and in a rabbit model of this disease. In this work, we investigated in different cell lines the binding and intracellular fate of affinity purified IgG and IgM antibodies to GM1 (GM1Ab) obtained from a rabbit model of GBS in comparing with the transport of cholera toxin (CT), which binds with a high affinity GM1. We demonstrated that in GM1-expressing CHO-K1 cells, GM1Ab is endocytosed and accumulated at the endocytic recycling compartment. Then, internalized GM1Ab is recycled back to the PM. Endocytosed GM1Ab colocalized to some extent with co-endocytosed CT at early and recycling endosomes, but not in Golgi and endoplasmic reticulum, where CT was also located. GM1Ab, in contraposition to CT, showed a reduced internalization in COS-7 cells and neuronal cell lines AH-SY5Y and Neuro2a. Results from photobleaching experiments revealed an inverse correlation between the efficiency of endocytosis and lateral mobility of GM1Ab in PM from these cell lines. Results indicate that GM1Ab and CT are differentially endocytosed and sorted, providing the basis to gain further insight into the mechanisms that operates in the intracellular trafficking and pathological effect of CT and neuropathy-associated antibodies.

CB-C05.
MOLECULAR COMPONENTS INVOLVED IN SPECIFIC STEPS OF THE AUTOPHAGY/MULTIVESICULAR BODY (MVB) PATHWAYS.
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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. In mammalian cells, different extracellular signals can trigger autophagy such as nutrient starvation, stress, or treatment with hormones. Morphological and biochemical studies have shown that autophagosomes fuse with endosomes (e.g. MVBs) forming a so called amphisome. We have analyzed at the molecular level the interaction of MVBs with the autophagic pathway in K562 cells. Our results indicate that a functional Rab11 is required for the interaction between MVBs and autophagosomes, whereas Rab7 was essential for fusion with lysosomes. Moreover, knocking down Beclin-1, to specifically affect the PI3K involved in autophagy, also alters MVB formation indicating that both pathways share molecular components. SNAREs are key molecules of the vesicle fusion machinery. We present evidence indicating that VAMP3/cellubrevin, a v-SNARE protein involved in the endocytic pathway, is necessary for fusion between MVBs and autophagosomes to generate the amphisome. In contrast, VAMP3 does not seem to be required for fusion with lysosomes, which is a microtubule-dependent event, supporting the existence of an alternative direct fusion between autophagosomes and lysosomes.

CB-C06.
PHYSIOLOGICAL MODIFICATION OF *Bufo arenarum* SPERM DURING MOTILITY ACTIVATION
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In animals with external fertilization as amphibians, gamete interactions are first established between sperm and molecules of the egg jelly coat released into the water. Hypotonic solution containing these substances is called egg water (EW). Immotile in the seminal tract, sperm initiate their motility upon spawning in hypotonic medium. The aim of present work is to identify physiological modifications during toad sperm motility activation guided by osmolarity changes and EW components. Osmolarity and ionic composition of *Bufo* seminal plasma (SP) were determined. Membrane potential (Em) changes were analyzed using fluorescent dye DiSC3 (5) and sperm motility was studied using a light microscopy. Sperm protein phosphorilation was studied in western blot analyses. Sperm was immotile in artificial SP solution (ASP: 303 mOsm/Kg, $[K^+]$=39 mM, $[Na^+]$=105 mM) and they presented a membrane depolarizated state. Sperms became motile in solutions with osmolarities below 200 mOsm/Kg. Were detected Em hyperpolarization and ser/thr phosphorilation proteins only in sperms preincubated in hypotonic solutions (10% ASP and EW). Fast and lineally swimming pathway was identified in EW preincubated sperms suggesting that jelly components trigger sperm "hipermotility". Our results suggest that toad sperm motility is correlated with changes in Em and protein phosphorylation.

CB-C07.
HYPERTONICITY INDUCES CCTα REDISTRIBUTION IN NUCLEOPLASMIC LAMIN A/C SPECKLES IN MDCK CELLS
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Papillary collecting ducts have to work in the highest renal interstitial osmolarity, and increase their phosphatidylcholine (PC) synthesis as a protective mechanism. PC synthesis occurs by Kennedy pathway where CCTα is the regulatory enzyme. CCTα is an amphitropic enzyme, but in most cell lines it is predominantly localized in the nucleus. On the other hand we have demonstrated that hypertonicity induces the formation of intranuclear lamin A/C-speckle. In the present work we examined how hypertonic medium affects the nuclear distribution of CCTα in MDCK cells. We also evaluated the relationship between the lamin A/C speckles and intranuclear CCTα. Confocal microscopy showed that under hypertonic conditions CCTα colocalize with speckles. To evaluate if this colocalization involves protein interaction, ClNa-TritonX-100 extraction assay was performed. Experiment demonstrated CCTa interaction with Lamin A/C. Silencing of Lamin A/C caused a decrease in lamin A/C speckle number and CCTα redistribution to soluble nuclear matrix. Results herein demonstrated that hypertonicity induced CCTα redistribution from soluble nuclear matrix to nuclear speckles, and this distribution depends on lamin A/C scaffold. We propose that under hypertonic condition CCTα is recruited to lamin A/C-speckles thus establishing a new intranuclear enzyme pool.

CB-C08.
THE FIFTH CYTOPLASMIC DOMAIN OF Na^+/K^+-ATPASE MEDIATES ITS INTERACTION WITH ACETYLATED TUBULIN
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Na^+/K^+-ATPase is an integral membrane protein that transports 3 Na+ out of the cell in exchange for 2 K+ by using the energy of ATP hydrolysis. We had found that this enzyme interacts with acetylated tubulin inhibiting its catalytic activity. In the present work we demonstrate that this interaction is mediated by the fifth cytoplasmic domain (CD5) of the alpha subunit of Na^+/K^+-ATPase. Three observations support this conclusion: 1) A 17-amino acid peptide corresponding to the sequence of CD5 associates with tubulin in pull-down assays; 2) retained tubulin is mainly (if not exclusively) of the acetylated isotype; and 3) the interaction is inhibited by solubilized membranes that contain full-length Na^+/K^+-ATPase. Furthermore, we developed an antibody specific to CD5 and performed cross-linking experiments between CD5 and tubulin in order to determine whether the interaction is direct (i.e. without any intermediary compound). After the covalent cross-linking of CD5 with the tubulin preparation and subsequent SDS-PAGE, we found that CD5 migrated in a single band located slightly above the acetylated tubulin band. We also characterized the cross-linked aduct by acetic acid/urea-PAGE confirming our results. Taken together, these evidences strongly support the idea that CD5 mediates the interaction between Na^+/K^+-ATPase and acetylated tubulin in a direct manner.

CB-C09.

REGULATION OF P-ATPases BY ACETYLATED TUBULIN: *S. Cerevisiae* H^+-ATPase REGULATION MECHANISM

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Acetylated tubulin interacts with Na^+/K^+ATPase and as results of this interaction the ATPase results inhibited. We have been studying the regulation of several P-ATPases by tubulin. When a $Ca2^+$ATPase enriched microsomal preparation is stimulated with calmodulin or ethanol, the $Ca2^+$ATPase-Tubulin complex is dissociated and the ATPase is activated, similarly, when S. cerevisiae are incubated with glucose, the Tubulin-H^+ATPase complex is dissociated. In all cases, when the stimuli are eliminated, the ATPase-tubulin complexes are restored and the enzymes are inhibited again. In the H^+ATPase activation, tubulin degradation has been observed. The aim of this work is to demonstrate a protease involvement in this activation mechanism. In in vivo experiments using several protease inhibitors we found that tubulin proteolisys is mediated by a metalloprotease. Affinity chromatography showed that tubulin is able to retain a protease from a yeast citosolic extract and in a tubulin degradation assay using this tubulin retained protease, we obtained significative results. These yeast citosolic proteins retained by tubulin were identified by MALDI TOF, but any protease like protein was identified. Probably, this enzyme is not a citosolic protein, so next experiments are focalized in the screening of proteins from other sub cellular compartment.

CB-C10.

P19INK4D FUNCTION RELIES ON SEQUENTIAL PHOSPHORYLATION

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We have reported that p19INK4d(p19), a member of INK4 cell cycle inhibitors, has a role in DNA repair and becomes phosphorylated upon DNA damage. In this work, we aimed to study first the involvement of predicted phosphorylation sites in p19 DNA repair activity, second to demonstrate the phosphorylation event in those sites related to the repair function and last to establish the subcellular localization of the phosphorylation process. p19 mutants were made replacing serine or threonine by alanine in predicted sites. For all of them, cell cycle arrest ability by timidine-^{3}H incorporation, DNA repair by UDS and apoptosis by caspase activity were assayed.We found that although none of the mutated sites are involved in cell cycle arrest, two of them are strictly necessary to the DNA repair function.To evaluate in vivo phosphorylation of these two mutants we performed metabolic labeling with ^{32}P. The results have shown that while one of them entirely supressed phosphorylation, the other one did not completely abrogated. It is known p19 translocates into the nucleus after genotoxic treatment.We assayed the subcellular localization of phosphorylated p19 at different time points finding that this process at least begins in the citoplasmic space. These results shows p19 is sequentially phosphorylated beginning in the cytoplasm and being necessary for exerting an effective DNA repair function.

CB-C11.

E2F MEDIATED INDUCTION OF P19INK4D PLAYS AN IMPORTANT ROLE IN CELL CYCLE AND DNA DAMAGE RESPONSE

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p19INK4 is a member of the INK4 family of proteins that regulate G1/S cell cycle transition. INK4s inhibit pRb phosphorylation by CDK 4/6 and consequently reduce the transcriptional activity of E2F. p19 is expressed periodically during the cell cycle, showing the highest level at the G1/S phase. *In silico* analysis of the p19 promoter sequence revealed potential E2F binding sites. Moreover, the overexpression of E2F in asynchronic fibroblasts led to an increase in p19 mRNA at all cell cycle phases. However, the physiological significance of p19 regulation by E2F remained to be explored. The aim of this work was to study the role of E2F in two aspects of p19 expression: its periodic regulation during the cell cycle and its induction during the DNA damage response. To achieve this, we developed a strategy based on triple helix-forming oligonucleotides (TFOs) in order to prevent E2F binding to its response elements in the p19 promoter. Proof of principle experiments showed that TFOs were able to specifically prevent p19 induction by E2F during normal cell cycle as well as following DNA damage. In subsequent experiments, TFOs transfected cells showed alterations in their cell cycle kinetics, proliferation and ability to repair damaged DNA (clonogenicity). These results highlight the significance of the regulation of p19 by E2F in different physiological situations.

CB-C12.

GLUCOCORTICOID DIFFERENTIAL EFFECTS ON P21CIP1 EXPRESSION, CELL CYCLE, AND SURVIVAL OF MAMMARY CELLS

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Glucocorticoid administration prevents post-lactation mammary gland involution, including apoptosis of mammary epithelial cells. By microarray analysis, we previously identified glucocorticoid regulated genes involved in the in vivo antiapoptotic action. Several genes are downregulated upon the hormone treatment, as the cell cycle inhibitors p21CIP1 and p18INK4c. In order to evaluate wether glucocorticoids regulate these genes by acting directly on mammary epithelial cells, we analyzed their mRNA levels by real-time PCR in mammary gland whole organ culture and in the normal mammary epithelial cell line, HC11. Dexamethasone treatment regulated the expression levels of these genes in both experimental systems. Interestingly, while glucocorticoids repress p21CIP1 and p18INK4c expression in HC11 differentiated cells, they increase the mRNA levels of these genes when the cells are undifferentiated. This differential effect was also observed at the cellular level: dexamethasone prevents apoptosis (Caspase-3 activity) induced by hormone removal of HC11 differentiated cells, but it induce cell cycle arrest (DNA content by flow cytometry) on undifferentiated cells. The differential response was also observed on mammary cells in vivo. Thus, gene expression regulation by glucocorticoids in mammary gland is dependent on cellular context, and could in turn determines the cellular outcome.

CB-C13.

DIFFERENTIAL BEHAVIOUR OF CYSTEINE CATHEPSINS IN THE *INTRINSIC* AND *EXTRINSIC* PATHWAYS OF APOPTOSIS

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The role of cysteine cathepsins in apoptosis[1] was studied in various cell lines using LeuLeuOMe to induce lysosomal membrane permeabilization. The released cathepsins cleave the proapoptotic Bcl-2 family member Bid and degrade the antiapoptotic members Bcl-2, Bcl-xL and/or Mcl-1. The papain-like cysteine protease inhibitor E-64d prevented apoptosis, Bid cleavage, and Bcl-2/Bcl-xL/Mcl-1 degradation. The pancaspase inhibitor Z-VAD-fmk failed to prevent Bid cleavage and degradation of anti-apoptotic Bcl-2 homologues but substantially decreased cell death[2].
In addition, Fas/CD95-induced apoptosis was investigated using wild-type and cathepsin $B^{-/-}$ primary skin fibroblasts. Cells with damaged mitochondria were observed 3h post apoptosis induction, whereas cells with damaged lysosomes were only seen after 15h with no difference between the two genotypes[3].
Therefore, we conclude that cysteine cathepsins trigger apoptosis in a caspase-dependent manner in the *intrinsic* (mitochondrial) pathway whereas they have no active role in the *extrinsic* (Fas/CD95) pathway.

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CB-C14.

NOCTURNIN IS A DEADENYLASE INVOLVED IN THE POSTTRANSCRIPTIONAL CONTROL OF CIRCADIAN GENE EXPRESSION

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Five to ten % of the transcripts expressed in a given tissue show daily oscillations. Presumably, this is largely generated by changes in the transcription of those genes. However, the relative importance of mRNA decay has not been established. We have characterized in mouse the unique ribonuclease reported which expression is circadian regulated, *Nocturnin* (*mNoc*). We showed that recombinant GST-mNOC is a deadenylase, a poly(A)-specific ribonuclease. Deadenylation triggers degradation or translational silencing of mRNAs. We then studied *mNoc* expression in NIH3T3 cells in response to stimuli that synchronize circadian clocks in cultures. *mNoc* was acutely induced by FBS and TPA, whereas forskolin and dexamethasone had no effect. This induction exhibited a typical immediately-early gene response. We generated an antibody and found that mNOC has a robust circadian expression pattern in mouse liver and kidney peaking at night. We then studied the subcellular localization and showed that mNOC is present in the cytoplasm and did not co-localize with P-bodies or stress granules, subcellular domains where a number of factors involved in mRNA cytoplasmic processing accumulate. Polysome analysis showed that mNOC is not associated with ribosomes or polyribosomes. Our data suggest that mNOC is involved in the posttranscriptional regulation of mRNAs whose levels decline at night or in response to stimuli.

CB-C15.

DEPOLARIZATION-INDUCED CHANGES IN INTRAGENIC CHROMATIN REGULATE ALTERNATIVE SPLICING IN NEURAL CELLS

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Changes in transcriptional elongation rate modulate alternative splicing choices. We investigated a physiological role for this mechanism analyzing how chromatin changes associated to neuronal activity affect alternative splicing of the NCAM exon 18. We determined that E18 inclusion decreases upon high K^+-induced depolarization in both cultured neurons and N2a neuroblastoma cells. Depolarization also causes general histone hyperacetylation, paralleled with increased levels of histone acetyl-transferases such as CREB-Binding Protein (CBP). Analysis of intragenic chromatin across the NCAM locus revealed low levels of marks associated with active transcription, such as H3K36 tri-methylation and H3K9 acetylation, in the neighbourhood of exon 18. Depolarization causes increased acetylation in that region, chromatin relaxation and increased RNA pol processivity. Using mutant polymerases, we determined that E18 inclusion is responsive to elongation rate, which hints at a influence of transcription in the alternative splicing regulation. Furthermore, histone hyperactetylation induced by Trichostatin A potentiated and even duplicated the depolarization effect, suggesting that changes in the chromatin structure can either facilitate or directly modulate alternative splicing regulation. These experiments point at a physiological role of intragenic chromatin modulation in mRNA biogenesis.

CB-C16.

DNA DAMAGE REGULATES ALTERNATIVE SPLICING THROUGH CHANGES IN POL II ELONGATION

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Many apoptotic genes are regulated via alternative splicing (AS) but little is known about the mechanisms controlling AS in stress situations derived from DNA damage. Here we show that ultraviolet (UV) radiation affects co-transcriptional, but not post-transcriptional, AS through a systemic mechanism involving a CDK-9-dependent hyperphosphorylation of RNA polymerase II carboxy terminal domain (CTD) and a subsequent and unprecedented inhibition of transcriptional elongation, estimated *in vivo* and in real time by FRAP. To mimic this hyperphosphorylation we used CTD mutants with serines 2 or 5 substituted by glutamic acids and found that they not only display lower elongation rates but duplicate the effects of UV light on AS in the absence of irradiation. Consistently, substitution of the serines with alanines prevents the UV effect on splicing. These results represent the first in vivo proof of modulation of elongation in response to an environmental signal, affecting in turn the kinetic coupling between transcription and splicing.

CB-C17.

DISRUPTION OF THE RNAI PATHWAY ABOLISHES SURFACE ANTIGEN VARIATION IN THE INTESTINAL PARASITE *Giardia*

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Giardia is one of the most common parasites of humans and a major cause of diarrhoea worldwide. To evade the immune response, *Giardia* undergoes antigenic variation; a process that allows the parasite to develop chronic and recurrent infections. From a repertoire of 190 variant-specific surface proteins (VSPs) coding genes, *Giardia* expresses only one VSP on the surface of each trophozoite at a particular time, but switches to the expression of a different VSP by unknown mechanisms. Here we show that regulation of VSP expression involves a system comprising homologues of RNA-dependent RNA-polymerase (RdRP), Dicer, and Argonaute (Ago), known components of the RNA interference (RNAi) machinery. In *Giardia*, clones expressing a single surface antigen efficiently transcribe several other vsps but only accumulate transcripts encoding thce actual VSP. Detection of antisense RNAs corresponding to the silenced vsps and cleavage of dsRNAs into 22-25 nt siRNA from the silenced but not for the expressed vsp implicate the RNAi pathway on *Giardia*'s antigenic variation. Remarkably, knock-down of Dicer and RdRP leads to a change from single to multiple VSP expression in individual trophozoites, while loss of Ago shows more drastic effect on *Giardia* viability. Our results suggest the involvement of a PTGS mechanism in regulating the expression of surface antigens in this important human pathogen.

CB-C18.

NOVEL FUNCTION OF p21$^{CIP1/WAF1}$ AS A MODULATOR OF TRANSLESION DNA SYNTHESIS (TLS)

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P21 up-regulation is required to block cell cycle progression after many types of genotoxic insults. In contrast, UV irradiation triggers p21 proteolysis. The biological significance of this increase in p21 turnover is unclear and might be linked to DNA repair-related processes. A few recent reports have addressed its effect on Translesion DNA Synthesis (TLS), a process that avoids prolonged replication blockage during S-phase. Interestingly, we have recently showed that efficient PCNA ubiquitination, a modification of PCNA relevant for TLS, requires p21 degradation. Moreover, p21 was shown to negatively modulate excessive TLS, thus avoiding mutagenesis. In this work we utilize different single cell analysis approaches to study the effect of p21 on TLS. We found that through its PCNA binding domain, p21 inhibited the interaction of the TLS-polymerase, polη (pol eta) with PCNA and the assembly of polη foci after UV. Moreover, stable expression of a p21 mutant that interferes with polη recruitment, without affecting normal DNA replication, induces the accumulation of the damaged-DNA marker, γH2AX, and increases cell death after UV irradiation. This work presents p21 as the first reported factor capable of negatively modulating polη recruitment to DNA-bound PCNA, which indicates that the increased UV- induced degradation of p21 might be critical for efficient TLS.

CB-C19.

INVOLVEMENT OF hnRNP A1 IN THE REGULATION OF RAC1 ALTERNATIVE SPLICING IN MAMMARY EPITHELIAL CELLS

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Rac1 is a Rho-GTPase involved in cell division, survival, motility and adhesion. Inclusion of an alternative exon "3b" gives rise to Rac1b splice variant, which is constitutively active, is found in breast and colorectal tumors and promotes cellular transformation. The mechanism controlling its induction has not been elucidated. Matrix metalloproteinase-3 (MMP-3) causes epithelial-mesenchymal transition (EMT) and malignant transformation in cultured cells. Exposure of mammary epithelial cells to MMP-3 induces Rac1b expression, and siRNA-mediated knockdown of this isoform prevents MMP-3-induced EMT. We proposed to investigate cis-acting elements and trans-acting factors responsible for the regulation of Rac1 splicing by MMP-3 and to define how its regulation impacts on cellular transformation. RNA affinity chromatography/mass spec revealed that hnRNP A1 and A2, two splicing inhibitory factors, interact with Rac1 exon 3b. RNA mobility shift assays indicated that less protein complex is assembled on 3b RNA when cells are treated with MMP-3. UV-crosslinking/immunoprecipitation suggest that MMP-3 treatment decreases the amount of hnRNP A1 bound to exon 3b. Knocking down hnRNP A1 by siRNA is not enough to induce 3b inclusion but potentiates MMP-3 stimulatory effect. We are mapping hnRNP A1 binding site within exon 3b and unraveling other factor/s involved in Rac1 splicing regulation.

CB-C20.

THE NUCLEIC ACID CHAPERONE CNBP MAY REGULATE THE STABILITY OF G-QUADRUPLEX DNA STRUCTURES

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The nucleic acid chaperone Cellular Nucleic acid-Binding Protein (CNBP) plays an essential role in vertebrate head organogenesis by controlling neural crest cell (NCC) development. CNBP binds to single-stranded G-rich nucleic acids able to adopt stable G-quadruplex structures. G-quadruplexes are characterized by the stacking of planar nucleic acid tetrads. They were described as regulative structures within the genome of several animal species. In the present study, the effect of CNBP on G-quadruplex structures was explored using Electrophoretic Mobility Shift Assay, Circular Dichroism and Polymerase Chain Reaction Stop Assay. The probes used included a sequence previously reported as CNBP target, the Nuclear Hypersensitivity Element III1 from the c-myc gene. Results suggest that at low protein:nucleic acid ratios CNBP stabilizes the G-quadruplex structure whereas at higher ratios it induces the G-quadruplex unwinding. The effect of N-terminal CNBP mutants, which lose the biochemical activities and biological function, on G-quadruplex stability was analyzed. In silico analysis of several NCC marker gene sequences found putative G-quadruplex structures in foxD3, crestin, c-myc and cnbp genes. The obtained data suggest that CNBP may regulate the stability of G-quadruplexes and, consequently, the expression of specific NCC genes during vertebrate head development.

CB-C21.
IDENTIFICATION AND ANALYSIS OF THE ZEBRAFISH CNBP PROMOTER AND REGULATORY SEQUENCES
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Cellular Nucleic acid Binding Protein (CNBP) is required for rostral head development. It is involved in the neural crest cells (NCC) proliferation/survival balance. Its embryonic gene expression is temporally and spatially restricted to NCC and some derivatives. To identify and elucidate zebrafish cnbp promoter as well as its regulatory mechanism, different genomic DNA fragments 5' upstream the gene were cloned into the Tol2 transposon vector system. The characterization of cnbp minimal promoter was examined by following EGFP expression in live embryos and comparing it with the reported embryonic gene expression pattern. As a result, two different regions were able to promote EGFP expression. One is located 800 bp upstream the putative start transcription site and displays a ubiquitous and low expression in embryos at different developmental stages. The other one, of 2100 bp, is surrounded by the intron 1 sequence and promotes a high and specific expression. Using different algorithms, we identified several putative promoter sequences and transcription factor binding sites (TFBS). Seven TFBS highly conserved among vertebrates were found. Five of them are not involved in embryonic development while the other two bind FOXD3 and PAX6, which are proteins responsible for normal NCC development. A hierarchical position of CNBP in the neural crest signalling pathway is proposed.

CB-C22.
ALPHA-2 MACROGLOBULIN/LRP1 SYSTEM INDUCES MULLER GLIAL CELL MIGRATION
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Müller cells (MC) are known to promote cellular migration during retinal proliferative diseases, which involve matrix metalloproteinases (MMPs) production. In previous studies we have demonstrated that MC express LDL receptor-related protein 1 (LRP1), and its ligand alpha 2-macroglobulin (á2M) is able to induce MMP-2 activity, suggesting that á2M/LRP1 system is implicated in cell migration during these retinal disorders. However, the biochemical mechanisms by which á2M/LRP1 system control this cellular event is, at present, unknown. Herein, we investigate the putative mechanism of á2M/LRP1 system on the MMPs regulation and MC migration using the human MIO-M1 cell line. Cell migration was determined by wound-healing assay. MIO-M1 cells were seeded in laminin- or collagen-coated dishes, and after 18 h of quiescence, a wound was created across the surface with a tip. The cells that move into the scraped area were quantified by light microscopy. á2M-induced cell migration was increased on laminin- and collagen-matrix substrate respect to control. These results were associated with the effects of á2M on: i) intracellular signal activation; ii) increased MMP-2 activity; and iii) expression and membrane focal localization of MT1-MMP and TIMP-2. Our data, taken togheter, demonstrate that á2M/LRP1 system is implicated in Müller glial cell migration.

ST-C01.

FUNCTIONAL CHARACTERIZATION OF DesK, THE *Bacillus subtilis* THERMOSENSOR

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The ability of bacteria to control the biophysical properties of their membranes allows them to thrive in a wide range of environments. When bacteria are exposed to temperatures lower than usual conditions, their membranes rigidify and as a consequence, cellular activities are suboptimal. One mechanism of adaptation involves the desaturation of acyl chains of membrane phospholipids. As a result, membrane fluidity is restored and normal cellular activities can take place at the lower temperature imposed.

In *Bacillus subtillis*, there is only one Desaturase, a 5- acyl lipid desaturase, whose expression is stringently controlled by DesR/K two component system. By means of its membrane component DesK, this system senses changes in membrane properties due to abrupt temperature changes.

We undertook *in vitro* biochemical studies on DesK to answer the question of how this membrane fluidity sensors perceive and transmit the signal. To this end, we first set up a one-step procedure to obtain functional DesK proteoliposomes. Then, we proceed to fully characterize DesK activities assaying DesK proteoliposomes´s autokinase, phosphotransferase and phosphatase activities at different temperatures. As a consequence, a biochemical-inspired hypothesis for signal perception and transduction through the membrane to the cytoplasmatic domain is discussed.

ST-C02.

MECHANISM OF SIGNAL TRANSDUCTION BY THE THERMOSENSOR DesK FROM *Bacillus subtilis*

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The *Bacillus subtilis* Des pathway is composed of the membrane 5-acyl lipid desaturase and the two component system DesK/DesR. DesK is a histidine kinase located in the membrane and DesR is a cytoplasmatic response regulator that binds specifically to the P*des* promoter. Induction of the Des pathway is brought about by the ability of DesK to assume different signaling states in response to changes in membrane fluidity. An increase in the proportion of ordered membrane lipids favors a kinase-dominant state of DesK leading to phosphorylation of DesR and activation of *des* transcription while a decrease in the phase transition temperature of the phospholipids favors the phosphatase activity of DesK promoting DesR-P dephosphorylation and turning off the expression of *des*. We undertook structural studies in order to characterize the signaling states corresponding to the different activities associated with the cytoplasmic region of DesK and to gain further insights into the mechanism by which this sensor protein can adjust its signaling state in response to changes in membrane lipid fluidity. Here, we describe the crystal structure of the complete soluble domain of DesK in two conformational states. Structure-inspired hypotheses for the distinct catalytic mechanisms and for signal transduction through the membrane to the cytoplasmic domain will be discussed.

ST-C03.

ROLE OF THE TRANSCRIPTION FACTOR KLF6 IN JNK-MEDIATED APOPTOSIS

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The function of KLF6 in cell death has been reported in different cell types, demonstrating either pro- or anti-apoptotic functions. However the mechanisms involved in these physiologically opposite outcomes are largely unknown. Environmental signals are transduced within cells by MAP kinase-dependent pathways which in turn modify the function of key transcription factors such as c-Jun. We demonstrated that KLF6 interacts with the c-Jun oncoprotein whose function can be regulated by JNK1 or JNK2 to induce differentially proliferation or apoptosis. This work aimed to investigate the role of KLF6 in the context of *jnk1-/-* and *jnk2-/-* cells which are resistant or sensitive, respectively, to UV-induced cell death. Endogenous KLF6 protein level was induced after UV radiation in *jnk1-/-* cells correlating with increased nuclear translocation. Under these conditions, the percentage of living cells marked with Annexin V was substantially increased by KLF6 indicating that cells are committed to UV-induced apoptosis. This effect was abolished upon p38 inhibition and also substantially reverted by a JNK inhibitor. In contrast, KLF6 did not modify the apoptosis induced by JNK1.

These data support that KLF6 promotes apoptosis in cells relatively resistant to UV-radiation (*jnk1-/-*), and also suggest that KLF6 function on cell death is regulated by a combination of both p38 and JNK2 activities.

ST-C04.

MARCKS PHOSPHORYLATION PLAYS A REGULATORY ROLE ON PIP2 CONCENTRATION IN ACROSOMAL EXOCYTOSIS

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MARCKS is a prominent substrate of PKC in many cell types; nevertheless the presence of MARCKS in sperm, as a possible PKC substrate has not been investigated. Using a specific antibody against MARCKS, Western blot analysis revealed the presence of MARCKS in human sperm and immunocytochemistry assays showed that MARCKS localized at the acrosomal region in human sperm. This localization prompted us to investigate if MARCKS might participate in acrosomal exocytosis (AE). We expressed MARCKS effector domain (ED) and assayed on the AE stimulated either by a PKC activator, PMA, or by calcium in a permeabilized sperm model. MARCKS ED inhibited specifically AE stimulated by PMA and calcium suggesting a role for MARCKS in AE. MARCKS ED has a high affinity for PIP2, and a model has emerged in which PKC-mediated regulation of MARCKS could control spatial availability of PIP2. We tested this model in our system by adding PIP2, which reverted MARCKS inhibitory effect in calcium-stimulated exocytosis. In addition, Western blot analysis using a specific phospho-MARCKS antibody shown that MARCKS phosphorylation increased after PMA treatment, and a N-terminal MARCKS antibody revealed that MARCKS was released from membrane following PMA treatment. Altogether, these results suggest that MARCKS phosphorylation might play a regulatory role on PIP2 concentration in acrosome exocytosis in human sperm.

ST-C05.

A MITOCHONDRIAL KINASE CASCADE INDUCES ERK PHOSPHORYLATION OF A KEY CHOLESTEROL TRANSPORT PROTEIN

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ERK1/2 is known to be involved in hormone-stimulated steroid synthesis, but its exact roles and the underlying mechanisms remain elusive. ERK1/2 activation by cAMP results in a maximal steroidogenic rate. We have showed that temporal mitochondrial ERK1/2 activation is obligatory for PKA-mediated steroidogenesis through MEK1/2 phosphorylation in the murine Leydig MA-10 cell line. This cascade of events is strictly dependent on stimuli, hCG/cAMP/PKA leads to the phosphorylation of a constitutive mitochondrial MEK1/2 and ERK1/2 pool. ERK1 specifically co-precipitates with a 30kDa mitochondrial form of StAR which presents a basic-hydrophobic motif shared in several ERK substrates and mitochondrial 30kDa StAR interacts with VDAC (voltage-dependent anionic channel). ERK1/2 phosphorylates StAR at Ser232 only in the presence of cholesterol. Mutagenesis of Ser232 to Ala (S232A) inhibited *in vitro* StAR phosphorylation by active ERK1/2. Transient transfection of MA-10 cells with S232A reduced the yield of steroids. We show that StAR is a novel substrate of mitochondrial ERK that is part of a multimeric kinase complex that regulates cholesterol transport. StAR phosphorylated at Ser232 may acquire additional negative charges and associate with VDAC promoting the retention of the mature form of 30kDa StAR in the outer mitochondrial membrane and to the formation of the multiprotein complex.

ST-C06.

REGULATION OF StarD7 EXPRESSION BY LITHIUM AND HIGH GLUCOSE IN JEG-3 CELLS

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StarD7 belongs to the START domain proteins involved in intracellular transport and lipid metabolism. We previously reported that StarD7 promoter activity, as well as endogenous JEG-3 mRNA levels were increased by ß-catenin S33Y overexpression. In addition, an increase in StarD7 mRNA was observed when JEG-3 cells were cultured in a high glucose medium compared with low glucose one. Here we demonstrate that lithium chloride, an inhibitor of glycogen synthase kinase-3ß, increased the nuclear ß-catenin level, the phosphorylated GSK-3ß amount and StarD7 protein expression in JEG-3. Similar data were obtained when this cell line was cultured in glycemic conditions. These changes were correlated with an increase in the mRNA levels of the inducible oxide nitric synthase. Furthermore, the transcript levels of a list of factors involved in transcriptional control of glucose and lipid metabolism were evaluated. These findings corroborate ours previous results indicating that StarD7 is regulated by Wnt-ß-catenin signaling associated to glucose and lipid metabolism suggesting that StarD7 could fulfill an important role in proliferation, inflammation, and cancer.

Supported by CONICET, FONCyT and SECyT-UNC

ST-C07.

2M/LRP-1 INDUCES ERK1/2 PHOSPHORYLATION BY PKC- I ACTIVATION IN J774 CELLS

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LDL receptor-related protein (LRP1) is a LDL receptor gene family member synthesized and processed into 515-kDa extracellular a chain and 85-kDa trans-membrane and intracellular ß chain. LRP-1 chain contains multiple ligand recognition sites and ß chain harbor motifs for endocytosis and intracellular signaling events. 2-macroglobulin-protease complex (2M*) is recognized by LRP1. Previously we demonstrated that 2M* induces cell proliferation and intracellular MAPK activation. However, the molecular mechanisms that promote this intracellular signaling activation are, at the present, unknown. Herein, we evaluate the putative participation of PKC enzymes and intracellular calcium rise on the 2M*-induced MAPK activation in two macrophage-derived cell lines, J774 and RAW 264.7. By Western blot we observed that calphostin-C blocks ERK1/2 phosphorylation in J774 and RAW 264.7 cells. To know the type of PKC involved, different inhibitors of PKC isoenzymes were used, such as RO-32-0432, GÖ-6976 and rottlerin. We demonstrated that the 2M*-induced ERK1/2 phosphorylation was inhibited by PKCa and PKC ßI inhibitors, respectively. When J774 and RAW 264.7 were incubated with BAPTA-AM, the 2M*-induced ERK1/2 phosphorylation was also fully blocked. Our data demonstrate that 2M*-induced MAPK phosphorylation is mediated by PKC / ßI activation and intracellular calcium mobilization.

PL-C01.
INTERACTION OF THE N- AND C-TERMINAL DOMAINS OF THE STARCH SYNTHASE III FROM *Arabidopsis thaliana*
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One of the isoforms of starch-synthase (SSIII) has an N-terminal transit peptide followed by three internal starch-binding domains (SBD) and a C-terminal catalytic domain (CD). Recently, we have characterized this isoform from *A. thaliana* and demonstrated that the SBDs have starch binding capacity and modulate the catalytic activity of the enzyme. In this work, we examined possible intramolecular interactions between the N- and C-domains which may contribute to the regulation of SSIII. We divided the enzyme in different polypeptides corresponding to the N-terminal SBDs containing three, two or one SBD (D123, D23, D3, D2 and D1) and the CD. We cloned, co-expressed, purified, performed kinetic characterization of the recombinant proteins and carried out protein-protein interaction analysis. Pull down and far western blotting assays show that D123 and D23 proteins (but not the individual domains D1, D2 or D3) physically interact with the CD. The presence of SBDs completely restore the kinetic parameters for glycogen, but partially restore those for ADPGlc. Our data show that the D23 region is critical for the interaction with the catalytic domain. The results presented here suggest that the N- and C-domains interaction has an important role in the modulation of *A. thaliana* SSIII activity.

PL-C02.
STRUCTURAL AND FUNCTIONAL ANALYSES OF HSP100 MOLECULAR CHAPERONES FROM VASCULAR PLANTS AND BACTERIA
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Molecular chaperones of the Hsp100 family have been identified in all kingdoms of life. Our work focuses on the Hsp100 family from chloroplasts of *Arabidopsis thaliana* (ClpC1, ClpC2 and ClpD or atHsp100s), ClpA from *Escherichia coli* and their proteolytic and regulatory partners. It has been proposed that these proteins may have roles in protein folding assistance, disaggregation, proteolysis and precursor import into chloroplasts. While some of these activities have been confirmed for ClpA, little is known about its chloroplastic counterparts mainly because they have not been purified. By means of affinity and gel filtration chromatography we were able to recover the atHsp100s as properly folded dimers with their expected molecular weight. They have basal Mg^{2+}-dependent ATPase activity. The influence of temperature, pH, ionic strength and divalent cations on ATPase activity was also assessed. We also analysed the oligomerization status of the atHsp100s and their interaction with molecular partners (ClpS1). We then explored the functional homology between ClpA and atHsp100s. The latter were expressed in an *E. coli* ClpA deficient strain and the resulting phenotype was studied using the green fluorescent protein as a probe for protein aggregation and degradation. Our results represent the first step to elucidate the poorly known protein quality control system in chloroplasts.

PL-C03.
UNRAVELING THE REGULATION MECHANISM FOR THE POTATO TUBER ADP-GLUCOSE PYROPHOSPHORYLASE
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ADPGlcPPase is the regulatory enzyme of glycogen and starch synthesis in bacteria and plants, respectively. The enzyme from plants is a heterotetramer comprised of two small (S, catalytic) and two large (L, regulatory) subunits. Most of the plant ADPGlcPPases are allosterically regulated by 3PGA and Pi. Previous studies on ADPGlcPPase from bacteria pointed out a region critical for activation in the N-term domain. To evaluate if conserved residues in such region play a role in the potato tuber enzyme, the mutants S_{Q75A}, S_{W116A}, L_{Q86A} and L_{W128A} were constructed, and the different heterotetramers were characterized. Results showed that the mutant enzymes exhibited essentially the same substrate kinetics than the wild type (S/L) enzyme; but the formers were altered in their response to 3PGA. The S/L enzyme was activated 68-fold by 3PGA and inhibited by Pi in a way reverted by the activator. Conversely, for the mutant enzymes S/L_{Q86A}, S/L_{W128A}, S_{Q75A}/L, S_{W116A}/L and S_{W116A}/L_{W128A} activation folds were calculated in 57, 22, 12, 7 and 7, respectively; and Pi inhibition was not modified by 3PGA. This is the first time that the N-term domain of a plant ADPGlcPPase is associated with 3PGA activation. Results agree with a model where the modified residues are located in loops that are responsible for propagating the allosteric activation of the enzyme.

PL-C04.
CHLOROPLAST DEPENDENT ALTERNATIVE SPLICING REGULATION BY LIGHT
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With the aim at understanding the mechanisms that regulate alternative splicing in plants, we studied the RUBISCO Activase and the RSp31 (SR protein) genes of Arabidopsis, whose alternative splicing patterns vary depending on light conditions. In both genes, light exposure causes an increase in the proportion of the shorter mRNA isoforms. We found that light intensity (but not quality) seems to be the factor that elicits changes in splicing patterns. Blue or red light produce similar effects as white light and different photoreceptor mutant plants behave as the WT in the alternative splicing response to white light. Drugs that inhibit the electronic photosynthetic transport abolish the effects of light on alternative splicing. The involvement of a chloroplast signal was explored by using chloroplast signaling mutants. Furthermore, H_2O_2 treatment of seedlings in the dark mimics the effects of light on alternative splicing, which suggests that peroxides (or ROS) might act as signal intermediates. A correlation between the proportions of splicing isoforms with gene expression suggests a transcriptional regulation of alternative splicing. Now, we are investigating whether the ROS produced by the chloroplast could be the signal that triggers changes in transcription and alternative splicing patterns and if a possible coupling could exist between both processes.

PL-C05.
CHARACTERIZATION OF HIGH MOLECULAR FORMS OF THE REVERSIBLE GLYCOSYLATED POLYPEPTIDE
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Reversible glycosylated polypeptides (RGPs) are highly conserved plant specific proteins. They perform self-glycosylation using uridine sugars as substrate. Its precise function remains unknown. To establish what factors are involved in the regulation of the RGP activity, we studied the relation between oligomerization and activity. We already published a model for the regulation of the RGP activity involving the occurrence of RGP complexes and the correlation with a decreased ability of those complexes to release glucose to UDP. Here, we found that complex formation depends on the plant development stage. These complexes are partially stabilized by disulfide bonds and the reduction of these bonds modulates the size and the activity of the protein. In addition, different native forms differing in charge and/or in size are visualized after Mono Q or Superose 12 chromatography, respectively. When the more active fractions eluted from Mono Q column was purified by UDP-hexanolamine Agarose, the pure RGP protein showed a complex UV spectrum signal, compatible with an interaction of the manganese, used as a cofactor of the RGP, with the UDP. To summarize, all data suggest that the behavior exhibited by RGP is quite unique and it can provide us a clue regarding to the function involved in polysaccharide biosynthesis in plants.

PL-C06.
FUNCTIONAL AND BIOCHEMICAL CHARACTERISATION OF THE TRANSCRIPTION FACTOR MTHB1 FROM *M. Truncatula*
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MTHB1 belongs to the HD-Zip family of transcription factors (TFs) from the model legume *Medicago truncatula* (MT). Its expression in roots increases in response to high NaCl concentrations. The full length cDNA of *MTHB1* was isolated and the codified protein was expressed in bacteria. EMSAs revealed that MTHB1 recognises in vitro the sequence CAAT(N)ATTG with the highest affinity, as other characterised members of the HD-Zip I subfamily. This DNA core was used to identify direct target genes of this TF in alternative transcriptional approaches. The validation of this box as a functional cis-acting element in the promoter regions of putative target genes is being carried out.The *MTHB1* expression pattern in separate tissues and organs from the whole plant was determined by quantitative RT-PCR, both in normal conditions and in response to alternative environmental factors. Besides, the promoter region of *MTHB1* was also isolated and cloned in a binary vector fused to the reporter gene *GUS*. A detailed expression pattern was then unravelled in roots of MT composite plants. *MTHB1* overexpression in MT roots resulted in a phenotype distinguishable from the wild type. Further functional analyses are now being undertaken also with *mthb1* TILLING mutants. The information obtained from the different approaches is helping to understand how this TF participates in root architecture determination.

PL-C07.
CROSS-TALK BETWEEN FLOWERING TIME AND THE DEFENSE RESPONSE MEDIATED BY THE *Sun flower* TRANSCRIPTION FACTOR HAHB10
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HAHB10 is a member of the sunflower subfamily II of HD-Zip transcription factors. Transgenic plants ectopically expressing this gene were previously obtained showing an early flowering and a shorter life cycle. In this work we show that these plants exhibit higher susceptibility to herbivorous attack compared to control plants and increased SA levels suggesting a major tolerance to pathogens. On the other hand, the promoter region of this gene was isolated and fused to the *GUS* reporter in order to transform *Arabidopsis thaliana* plants. In normal growth conditions, this promoter directs the expression in leaves, roots and particularly in stems and anthers during the reproductive stage. When the transformed plants were subjected to wounding or chewing the expression of the reporter was inhibited while when the plants were subjected to *P. syringae* attack, it was notably increased. Concomitant results were obtained in sunflower leaves either WT or transiently transformed. Transcriptome analysis carried out with the plants expressing the HAHB10 cDNA indicates that several genes involved in the transition from the vegetative to the reproductive stage are strongly induced. Among them, *FT*, *AGL9* and *AGL8* showed significant differences. We propose that HAHB10 plays a role in the coordination of flowering time and defense responses through a cross talk between SA and other signaling pathways.

PL-C08.
AN ACQUIRED BACTERIAL PLANT NATRIURETIC PEPTIDE-LIKE PROTEIN MODIFIES HOST HOMEOSTASIS
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Plant natriuretic peptides (PNPs) are a class of extracellular, systemically mobile peptides that elicit a number of plant responses important in homeostasis and growth. The bacterial citrus pathogen *Xanthomonas axonopodis pv. citri* (Xac) also contains a gene encoding a PNP-like protein, XacPNP, that shares significant sequence similarity and identical domain organization with PNPs but has no homologues in other bacteria. We have expressed and purified XacPNP and demonstrated that the bacterial protein, like PNPs, could alter plant physiological responses. We also observed that XacPNP transcription can respond to the host environment suggesting a role during bacterial infection. To characterize the role of XacPNP in citrus canker, we constructed a XacPNP deletion mutant. The lesions caused by this mutant were more necrotic than those observed with the wild-type and bacterial cell death occurred earlier in the mutant. Moreover, when we expressed XacPNP in *Xanthomonas axonopodis pv. vesicatoria*, the transgenic bacteria caused less necrotic lesions in the host than the wild-type. We have evidence to conclude that XacPNP enables Xac to modify host homeostasis in order to create conditions favorable to its own survival.

PL-C09.

IDENTIFICATION OF PROTEINS INVOLVED IN FREEZING TOLERANCE IN *Arabidopsis thaliana*

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Plants normally show a limited freezing tolerance occurring via different transduction signal pathways. Pathogenesis related proteins including chitinases, ß-glucanases and thaumatin like proteins as well as cell wall modifying ones are involved in such pathways.

A sunflower transcription factor was identified as playing a clue role in the freezing tolerance. TF was constitutively expressed in Arabidopsis and showed a significant tolerance in chilling and freezing conditions. A microarray analysis was performed with RNA from transgenic plants compared with non transformed ones indicating that several transcripts encoding PR and cell wall modifying proteins are induced in the transgenic genotype. It was reported that these proteins exhibit antifreeze activity in winter rye plants. However, no reports on functional analysis of these proteins related to antifreeze activity in *Arabidopsis*, are available. These transgenic plants exhibit morphological and developmental differences with their WT ones, that are abolished when transgenic plants with their own TF promoter, instead a constitutive one, directing the expression were obtained.

Apoplastic proteins differentially expressed in both genotypes were isolated. We propose that this transcription factor confers cold tolerance to plants via the induction of proteins that modify ice growth.

PL-C10.

EMERGING PROPERTIES OF OXIDATIVE METABOLISM MODIFICATION IN TRANSGENIC POTATO PLANTS

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The main goal of this study was to generate potato plants with modified antioxidant capabilities by decreasing polyphenol oxidase (ppo) levels. To accomplish this, a vector carrying a hairpin construct designed to silence ppo gene family was introduced into the Spunta variety of potato by *Agrobacterium* transformation and several transgenic plants were generated, most of them with reduced ppo activity.

The *ppo* gene silencing resulted in an inhibition of the enzymatic browning, thus transgenic potato tubers don't turn black once cut or peeled. Unexpectedly, modified tubers also present improved aroma and were consumed significantly more often than wild type (wt) tubers in mouse feeding experiments. Modified potato plants show no growth or tuber yield differences compared to wt in green house experiments.

Interestingly, transgenic potato plants possess an enhanced resistance to *Pytophthora infestans*, the causal agent of potato and tomato late blight, when compared to wt plants. Furthermore, transgenic plants accumulate higher amounts of defensive phenolic compounds during pathogen infection. A possible mechanism for the observed resistance could be that *ppo* silenced plants possess an elevated metabolic flux of non-oxidized antimicrobial phenolics. This higher availability of free phenolics could also operate in advanced steps of the infection by cross-linking cell wall components.

SB-C01.
STRUCTURAL AND BIOLOGICAL ASPECTS OF THE *Micrurus pyrrhocryptus* VENOM

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Snake venom composition varies between families, from genus to genus and even between species. Alpha-neurotoxins are capable of reversibly blocking the nerve transmission by competitively binding to the nicotinic acethylcoline receptor (AchR) located at the neuromuscular endplate leading, in severe situations, to the death by respiratory arrest. Postsynaptic neurotoxins, mainly those belonging to the three-finger group, are widely distributed in Elapidae snake venoms and that is why envenoming shows a highly neurotoxic strategy. Among the *Elapidae* subfamilies, *Elapinae* includes coral snakes, being *Micrurus* the most representative genus as regards abundance and diversity. However, the structure-function relationship of the venom of only a few species has been investigated. We herein report biological and structural aspects of the *Micrurus pyrrhocryptus* venom such as the determination of the N-terminal sequence of 11 venom proteins including PLA2s, short, long and weak neurotoxins. The complete primary structure of one of the short neurotoxins has also been determined, this being the first sequence of an alpha-neurotoxin from *Micrurus pyrrhocryptus* venom and one of the few fully determined in members of the *Micrurus* genus. This is a contribution to the snake venom basic research and to the antivenom production strategies.

SB-C02.
METAL SELECTIVITY OF THE SINGLE SUPEROXIDE DISMUTASE OF *Rhodobacter capsulatus*

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Superoxide dismutases (SODs) are metalloenzymes involved in the defense against oxidative stress. Fe/Mn family exhibited high sequence conservation. FeSODs and MnSODs usually attain significant activity with only one of the metal cofactors, although they can frequently bind both. A small group termed cambialistic is able to display significant activity with either Fe or Mn as cofactor.
The phototrophic bacterium *R. capsulatus* encodes a single cambialistic SOD (RcSOD) that preferentially binds Mn. However, metal discrimination depends on the culture conditions, with Fe fractions increasing from 7% in aerobic cultures up to 40% in photosynthetic cultures.
The aim of this work is to analyze structural factors involved in metal selectivity of the RcSOD. To reach this goal we performed heterologous expression of this protein in *E. coli* finding a correlation between metal availability in culture media and Fe-Mn composition of recombinant RcSOD. Secondly, structural stability was studied using CD spectroscopy. Fe-RcSOD showed more thermal stability than Mn-RcSOD. Finally, studies of in vitro metal capture revealed that this process is thermally triggered, suggesting that activation barriers to metal uptake are overcomed by a thermal transition in the apoprotein. As a conclusion, RcSOD metal selectivity is not only influenced by Fe and Mn availability but also by kinetic factors

SB-C03.
CRYSTAL STRUCTURE OF THE REGULATORY SUBUNIT OF PKA FROM *Saccharomyces cerevisiae*

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In mammals, the PKA holoenzyme exists as a complex of two catalytic subunits and a regulatory (R) subunit dimer. R subunits have a dimerization and docking domain at the N terminus; at the C terminus, two tandem cAMP-binding domains and in between a flexible hinge region, including a substrate-like inhibitor sequence that docks to the active site cleft of the C subunit. Much effort has been put in studying structure-function relationships in mammalian PKAs. The aim of this work is to solve the structure of the R subunit from *Saccharomyces cerevisiae*, one of the most intensively studied eukaryotic model organism in signal transduction. Unlike mammals, that have been shown to have two major classes of R subunits (I and II), *S. cerevisiae* has only one R gene. We over-expressed and purified different deletion mutants of the protein. The mutant lacking the first 167 residues was crystallized. We report the crystal structure of the protein bound to cAMP, which we determined to 2.20 Å resolution and refined to an R factor of 0,200 with an R free factor of 0,263. The new structure shows two tandem cAMP binding domains, which relative orientation is different from the one of mammalian R subunits. The two domains are more tightly bound to each other. The R subunit from *S.cerevisiae* represent a new type of R subunit, as it has different features from RI and RII.

EN-C01.

INTERACTION OF ALKYLAMMONIUM IONS WITH THE ACTIVE SITE OF *P. Aeruginosa* PHOSPHOCHOLINE PHOSPHATASE

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Pseudomonas aeruginosa phosphocholine phosphatase (PchP) and hemolytic phospholipase C (PlcH) both catalyze the hydrolysis of compounds containing choline. PlcH on phosphatidylcholine or sphingomyelin produces phosphocholine, substrate of PchP, which is hydrolyzed to choline and inorganic phosphate. Both enzymes recognize the alkylammonium moiety in their respective substrates. Therefore, a parallel study of these two enzymes might help us to find this recognition site in both proteins. To achieve this objective, we started with the study of PchP based in a molecular model which involves the catalytic site formed by motifs I, II, and III (^{31}DMDNT^{35}, ^{166}S, and ^{261}GDTPDSD^{267}, respectively). Kinetic studies were performed with wild type and site directed mutated variants using *p*-NPP as substrate and Mg^{2+} as cofactor at pH 5. All the alkylammonium ions tested (simple ones as trimethylamine, choline, betaine, or complex ones as atropine, gallamine, decamethonium, neostigmine, etc.) were inhibitors of PchP. In general, the inhibition contained competitive and noncompetitive components. From motifs I, II and III, only the seryl residue of motif II, S166, seems to be involved in the recognition of the ammonium quaternary moiety. After bioinformatics studies, we concluded that the choline binding domain found in gram positive bacteria or in higher organisms is not present in PchP

EN-C02.

INTERACTION OF MG^{2+}, ZN^{2+} AND CU^{2+} IN THE ACTIVE SITE OF *P. Aeruginosa* PHOSPHOCHOLINE PHOSPHATASE

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Phosphorylcholine phosphatase (PchP) of *Pseudomonas aeruginosa*, a product of the PA5292 gene, catalyzes the hydrolysis of phosphocholine to choline and inorganic phosphate (Pi). Phosphocholine is produced after hemolytic phospholipase C (PlcH) acts upon phosphatidylcholine or sphingomyelin. Therefore, PlcH and PchP are involved in the pathogenesis of *P. aeruginosa*. PchP belongs to the HAD superfamily as it contains three conserved sequences motifs. In mature PchP, the motifs I, II, and III are ^{31}DMDNT^{35}, ^{166}S, and ^{261}GDTPDSD^{267}, respectively. Kinetic characterization of wild type and mutated proteins, obtained by site directed mutagenesis, in addition to a molecular model of PchP helped us to understand the contribution of key residues in the conserved motifs I, II and III that are involved in the catalysis of p-nitrophenylphosphate processing after the addition of Mg^{2+}, Zn^{2+} and Cu^{2+} (these are activators of PchP activity). Our results are explained by invoking the concept of chemical hardness and softness introduced by Pearson in 1963 and its extension that "hard acids prefer to coordinate to hard bases and soft acids to soft bases" [Parr and Pearson, J. Am. Chem. Soc., 105, 7512-7516 (1983)].

EN-C03.

SWAPPING STRUCTURAL-FUNCTIONAL DETERMINANTS BETWEEN BACTERIAL AND PLANT FERREDOXIN $NADP^+$ REDUCTASES

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FNRs are flavoenzymes that participate in a broad range of redox metabolic pathways. It has been hypothesized that Y308 (in pea FNR) must be displaced for the substrate to interact with the active site. In some bacterial FNRs (i.e. *Escherichia coli*), a W follows the Y residue, probably restricting its displacement. Moreover, in plastidic FNRs, a loop maintains the FAD in an extended conformation. The absence of this loop in bacterial FNRs and, the lack of movement of the Y residue, have been pointed to be responsible for the low catalytic efficiency of this type of reductases. In order to shed light into this mechanism, we engineered a set of plastidic and bacterial FNRs in sakes of exchanging structural characteristics among them. By means of site-directed mutagenesis we deleted the loop and/or added a W residue next to Y308. Surprisingly, the kinetic behavior did not differ significantly from the wild type enzyme. In *E. coli* FNR we included the loop and deleted the terminal W obtaining a mutant enzyme which showed similar kinetics parameters to those of pea FNR. The measurements of FAD Kd revealed a decrease in FAD affinity in the FNRs that lacked the loop. Taken together, these observations leaded us to conclude that the loop in pea FNR may not be essential for high catalytic efficiency and that it is involved in FAD affinity improving the structural stability of the enzyme.

EN-C04.

ROLE OF THE INVARIANT GLUTAMIC ACID IN THE ACTIVE SITE OF FERREDOXIN-NADP(H) REDUCTASES

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Plant-type ferredoxin-NADP(H) reductases (FNRs) are favoenzymes harboring one molecule of non-covalently bound FAD that catalyses reversible reactions between obligatory one- and two-electron carriers. Members of the FNR family exhibit a very low sequence identity, defining two distinct groups: plant and bacterial FNRs. However, all FNR members contain a glutamate next to the C-terminus that is strictly conserved. FNR crystallographic structures show that the glutamate is part of the active site. Because of its position, it has been proposed to transfer protons from the external medium to the cofactor FAD. This plausible function has been tested experimentally, drawing contradictory conclusions about its role in catalysis. In this work we study the titration behaviour of the glutamate present in the FNR active site by theoretical methods. For the maize reductase, protonation probabilities are computed for all steps of the catalytic cycle by Poisson-Boltzmann electrostatic and Metropolis Monte Carlo titration calculations. The titration behaviour of the highly conserved glutamate varies for each step of the reaction cycle, depending on the substrates bound, as well as their redox state. A model is proposed rationalizing the role of the glutamate in the reaction cycle, which is consistent with the computational findings and allows a reinterpretation of previous experimental results

BT-P01.

SOLUBILITY OF RARE CODON CONTENT RECOMBINANT PROTEINS IN A CODON BIAS-ADJUSTED *E. Coli* STRAIN

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The expression of heterologous proteins in *E. coli* is strongly affected by codon bias. This phenomenon occurs when the codon usage of the mRNA coding for the foreign protein differs from that of *E. coli* resulting in frequent ribosome pausing. To overcome this effect, *E. coli* strains engineered to provide high levels of rare tRNAs are available (CodonPlus, CP). However, the increased speed of translation could cause aggregation of slow folding domains. To test this possibility, we have studied the expression of eight proteins from plants in *E. coli* BL21(DE3) pLysS´ and CP. First, we sorted them in two groups according to the percentage of rare codon content (RCC) (group L, RCC<5%; group H, RCC>5%). We then assessed the solubility of these proteins after expression in *E. coli* and found that the group L proteins were highly soluble in both strains. However, the group H proteins were localized in the insoluble fraction in the CP strain, while a portion could be recovered in the soluble fraction in the pLysS´ strain. Moreover, the expression of group H proteins in the CP strain caused retarded growth and low cell yield due to massive accumulation of inclusion bodies. Our results show that the expression of high RCC proteins in the CP strain is detrimental for protein solubility. We propose that the RCC could be a useful predictor of protein solubility in codon bias-adjusted strains.

BT-P02.

EXPRESSION OF MEGOSAMINE BIOSYNTHETIC PATHWAY IN *E. Coli:* GENERATION OF MEGALOMICIN ANALOGUES

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Megalomicins are terapeutically important compounds which have antiparasitic, antiviral and antibacterial properties. They are produced by *Micromonospora megalomicea* and differ from erythromycin by the addition of a unique deoxyamino sugar, megosamine, to the C6 hydroxyl.

In this work we identified the genes involved in the megosamine pathway and validated it by the expression of the genes in *Escherichia coli*. LC/MS/MS analysis of the dTDP-sugar intermediates produced by operons containing different sets of genes showed that production of dTDP-L-megosamine from dTDP-4-keto-6-deoxy-D-glucose required only five biosynthetic steps; the first three step were common with others TDP-sugar pathways while the last two were specific from this pathway. The validation of this pathway was demonstrated by conversion of EryC to Megalomicin A.

In addition, we feed different erythromycin C analogues, erythromicin A and azithromycin to generate new compounds, 6-megosaminil-erithromycin A and 6-megosaminil-azithromycin, respectively. These results indicate a significant flexibility of proteins which are MegDI-MegDVI, involved in the L-megosamine transference to alternative macrolactone. The antiparasitic and antibacterial activity of these new compounds is being evaluated.

BT-P03.

DEVELOPMENT OF SEED AND LEAF INOCULANTS OF THE BIOCONTROLAGENT *Bacillus amyloliquefaciens*

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Bacillus amylolyquefaciens BNM122 showed antifungal activity against several phytopathogenic fungi. To effectively use this strain as a biocontrol agent for soybean, a formulation that ensures an adequate shelf-life and maximal controlling capabilities is needed. Since *B. amyloliquefaciens* is a spore-forming species, one of the first design decisions is whether to use living cells or spores. Soybean seeds produce exudates that negatively affect the survival of other microbial inoculants. We tested the survival of exponentially growing cells and a mixture of stationary-phase cells and spores on seeds. Older cultures were little affected by seed exudates, while younger cells were sensitive. Another requirement that inoculants must meet is the tolerance to the sudden dehydration that occurs when the product contacts its target. Again, stationary-phase cultures were more resistant. Additives that protect from osmotic shock (alginate, guar and xanthans gums) increased 10 times the survival of spores, but had no effect on younger cells. Bacilli spores are tolerant to UV-B light, but it was necessary to demonstrate that they could tolerate the higher doses of radiation recorded in the summer months of the Southern hemisphere. We observed that the equivalent titre of cells on soybean leaves increased from $3x10^7$ to $4x10^7$ cfu.ml^{-1} after six days of exposition to UV-B in a culture chamber.

BT-P04.

SCALING-UP AND OPTIMIZATION OF THE PURIFICATION PROCESS OF FLAGELLIN FROM *Salmonella enterica*

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Flagellin is the ligand of the innate receptors TLR5, NAIP5 and Ipaf, being a candidate to enhance efficacy of vaccination. Usually is recovered by ultracentrifugation of sheared flagellum obtained from bacterial pellets. Our aim was to develop a pilot-scale purification process maximizing the yield in bioactive flagellin.

Several parameters were evaluated: culture conditions (static, fermentor aerated (FA), fermentor aerated and agitated (FAA), 10L volume); bacterial inactivation (tindalization, 0.1% sodium azide, 0,6% formaldehyde) and downstream steps to generate the bacterial pellet: centrifugation and tangential filtration (TF). Purity was evaluated by SDS-PAGE and biological activity using the cell line Caco-2-CCL20-luciferase, that reports TLR5-stimulating capacity.

Among all conditions, TF simplified the downstream processing and improved the yield, probably due to increase in shearing. Yields of different culture conditions combined with TF are: static/TF: 12,0 +/- 0,5 mg/L, FA/TF:20+/- 1 mg/L, FAA/TF:29,9 +/-0,9 mg/L. In all cases comparable biological activity of flagellin was observed and a single band of 48 kD was detected by SDS-PAGE.

In summary, a simple protocol to obtain several hundred milligrams of flagellin in batch condition was established. This protocol allowed to produce flagellin in enough quantity and quality to be used as adjuvant or innate agonist agent.

BT-P05.
PARAMETERS AFFECTING GROWTH AND VIABILITY OF BACTERIA IMMOBILIZED IN SOL-GEL SILICA MATRICES

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The aim of this work was to evaluate the effect of various parameters on bacteria immobilized in sol-gel-derived silica matrices. With this purpose, we evaluated the stress of immobilization over bacteria cultures obtained from different growing states including exponential phase, stationary phase and growing colonies in solid agar. We also studied the effect of cell density during immobilization and the capability of cells to proliferate inside matrices. The effect of complete medium, a carbon and nitrogen source and solely a carbon source incorporation, was studied on bacteria viability. In order to induce osmotic stress and the consequent endogenous trehalose synthesis and accumulation, an *E. coli* culture was exposed to high salt concentration, previous to immobilization. In parallel, some compatible solutes including glycerol, mannitol and trehalose were added during the gelation step. Furthermore, the effect of citric acid as a gelation agent was compared to the use of inorganic acids on bacteria viability preservation over 550 days. Best results to attain longer preservation times were obtained when we immobilized suspensions with an optimized bacterial number of 1 x 10^7 cfu/gel in the presence of LB medium using aqueous silica precursors.

BT-P06.
HYBRID ORGANIC- INORGANIC MATRICES FOR BACTERIA IMMOBILIZATION: EFFECT OF STORAGE RELATIVE HUMIDITY

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Hybrid materials used for bacteria immobilization can be defined as nanocomposites with organic and inorganic components. The aim of this work was to combine those components in order to obtain materials that could find a compromise between different properties such as mechanical strength, permeability, moisture and immobilized bacteria viability.
In this respect, we used sodium silicate as the inorganic component and sodium alginate, polyvinylalcohol (PVA), polyethyleneglycol (PEG), polyoxyethylene 20 cetyl ether (Brij 58) or carboxymethylcellulose (CMC) as the organic part. We also exposed every type of hybrid material to different storage relative humidities ranging from 15% to 58%. Viability of entrapped *Escherichia coli* was evaluated as well as the percentage of residual water and physical integrity of materials.
When nutrients were added together with bacteria, no significant differences were found among the five hybrid and the inorganic silicate matrices. High bacteria counts (10^7/10^8) were observed for humidities above 15% but for bacteria stored at 15% of relative humidity there was a decreased in the viability of approximately four orders of magnitude (10^4).
When no nutrients were added, bacteria count was around 10^4 for every humidity condition evaluated and some differences were found among matrices.

BT-P07.
STRATEGIES FOR THE ANISODAMINE PRODUCTION BY A RECOMBINANT *Saccharomyces cerevisiae* STRAIN

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Brugmansia candida is a South American native plant that produces anisodamine, scopolamine, and hyosciamine. The last ones are widely used as pharmaceuticals. Recently, alternative medical applications for anisodamine were described. The medical use of this alkaloid has many advantages since it appears to be less toxic than atropine and it has less negative effects over CNS than scopolamine. Hyoscyamine 6ß-hydroxylase (H6H, EC 1.14.11.11) catalyzes the conversion of hyoscyamine into anisodamine and scopolamine. The use of an immobilized system in biotransformation processes has many advantages comparing to the use of free cells. The aim of this work was to analyze the ability of the recombinant *Saccharomyces cerevisiae* strain carrying the *h6h*DNAc to produce anisodamine by the free and immobilized cell system. Also, the free and immobilized crude protein extract from this strain was evaluated. Alginate gel 1% was used for the entrapment. The effect of the immobilization process and different treatments of cell permeabilization were also compared. According to the results obtained in this work, the immobilization process was disadvantageous. The conversion of hyoscyamine by the free protein extract was more efficient (reaching an 83% of anisodamine) than the conversion of the alkaloid by the immobilized protein extract and the free and immobilized transformed *S. cerevisiae* cells.

BT-P08.
EXPRESSION OF *Toxoplasma gondii* ANTIGENS IN TOBACCO LEAVES AND EVALUATION OF IMMUNO RESPONSE IN MICE

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T. gondii $GRA4_{163-345}$ and $SAG1_{78-322}$ were transiently expressed in tobacco leaves by agro-infiltration. Here, we explored different approaches to improve transgene expression in plants, like subcellular targeting and codon optimization. GRA4 and SAG1were targeted to the cytoplasm, endoplasmic reticulum (ER) or to the apoplastic space by adding different targeting signals. Both sequences were modified for plant codon optimization. The expression was confirmed by Western blot and the level of recombinant proteins in leaf extracts was estimated using the Gel Pro Analizer 4.0 program. GRA4 accumulation was increased when the protein was targeted to the apoplastic space, while SAG1 accumulation was improved when the protein was targeted to the ER. However, no difference in yields was observed for GRA4 and SAG1 optimized versions. Finally, the C57/BL6 mice were immunized with SAG1, GRA4 or both -expressing leaf extracts and orally challenged with a non-lethal dose of the *T. gondii*. Mice vaccinated with SAG1, GRA4 or both showed significantly lower brain cyst burdens compared to those from the control group (SAG1 62%, GRA4 66%, SAG1+GRA4 59%). Our results showed that protective immunity could be established in mice injected with transgenic leaf extracts.

BT-P09.
EXPRESSION AND PURIFICATION OF *Leptospira interrogans* ANTIGENS. APPLICATION IN SERODIAGNOSIS

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Leptospirosis is a worldwide re-emerging zoonotic disease. The early and accurate detection of this infection is relevant in sanitary management. LipL32 is one of the most antigenic outer membrane proteins from pathogenic leptospiras. Another immunogen, Lp29, was reported as reactive since early stages of the illness. The aim of this work was the design of new antigens to develop ELISA tests for the detection of specific anti-leptospiral antibodies. We have cloned, expressed and purified the antigenic fraction of LipL32 protein and a quimeric protein consisting in the Lp29 antigen fused to the previously mentioned protein. These two recombinant proteins were evaluated by dot-blot and Western blot assays. Both assays were carried out by using specific hyperimmune rabbit sera and microaglutination (MAT) positive-negative human serum samples. LipL32 and the quimeric protein showed a good capacity to separate positive and negative samples by both immunoblot tests. The preliminary ELISA test results evidenced positive-negative differentiation capacity too. These results show a potential application of this recombinant designed proteins in the development of ELISA tests for detection of acute leptospirosis.
Granted by UNL, CAI+D 2006; ANPCyT, PICTO'04 15-22427.

BT-P10.
CHAIN-LENGTH SELECTIVITY OF AN EXTRACELLULAR LIPASE ACTIVITY FROM *Aspergillus niger* MYA 135

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The lipases (EC 3.1.1.3) have attracted the great interest of chemical and pharmaceutical industries due to their useful in both hydrolysis and synthesis reactions. Chain-length specificity has been related to structural features of lipases, but up to now, no lipase has been described as strictly specific for a given chain length. The objective of this work was to determine the selectivity of an extracellular lipase towards different chain-length substrates during hydrolysis, esterification and transesterifications reactions. All tests were carried out with 0.01 g of freeze-dried supernatant. Transesterifications and esterifications were performed in n-hexane using p-nitrophenyl alkyl esters (C10, C12, C16, C18) and acids (caprilic, caproic, linoleic, palmitic), respectively. Alcohols with chain length from C1 to C7 were also used as substrates. Hydrolysis was determined using á and ß-naphtyl acetate, myristate, palmitate, laurate and estearate. The results were expressed using the specificity constant 1/a described by Rangheard. In hydrolysis reaction the lipase presents a clear preference for a-naphtyl laurate (1/a=0.78). Concerning esterification, the maximum rate (1/a=1) was reached in the presence of linoleic acid and butanol. For transesterifications, there were preference for C12 (1/a=1) and C10 (1/a=0.9) using methanol.
This work was supported by grants PIP 6062

BT-P11.
PEPTIDE SYNTHESIS CATALYZED BY A HALOALKALIPHILIC PROTEASE FROM THE ARCHAEON *Natrial bamagadii*

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Microbial proteases are widely used in biotechnology and industry. They catalyze the hydrolysis/synthesis of peptide bonds. Most proteases used in biotechnology inactivate under the harsh conditions required for synthesis reactions such as the presence of organic solvents. Haloarchaeal proteases offer an advantage over their mesohalic counterparts as they function optimally under high salt, meaning low water activity, a feature in common with aqueous-organic solvent mixtures. We have isolated and characterized a solvent-tolerant protease from the haloalkaliphilic archaeon *Natrialba magadii* (Nep). In this investigation, the potential application of Nep as biocatalyst in peptide synthesis reactions was examined. Nep catalyzed the synthesis of Ac-Phe-Gly-Phe-NH_2 from Ac-Phe-OEt ester and Gly-Phe-NH_2 amide substrates in the presence of 30% (v/v) DMSO, 1.5 M or 0.5 M NaCl at room temperature. The purification and identification of the peptide product were assessed by RP-HPLC and ESI-MS analysis, respectively. In the presence of 1.5 M NaCl the tripeptide yield was 54% after 1 h and reached the maximum after 24 h (67%). Nep preincubated with PMSF catalyzed the synthesis of the tripeptide and prevented hydrolysis of the product at longer reaction times. The native and recombinant enzymes were similarly effective as biocatalysts in peptide synthesis.
Supported by ANPCyT, UNMDP, and CONICET.

BT-P12.
EXPRESSION OF AN ACTIVE POLYGALACTURONASE FROM *Aspergillus kawachii* IN A HETEROLOGOUS SYSTEM

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Pectinases catalyze the hydrolysis of pectin and/or pectic acid. Among pectinases, endopolygalacturonases (PGase; E.C. 3.2.1.15.) are the most important biocatalysts. *A. kawachii* expresses a PGase, namely PG1, active at acidic pH values. Low expression levels of wild type PG1 requires cloning and over expression for its industrial applications. PG1 ORF was cloned in a pYES2 (INVITROGEN ®) vector, generating the pYES2:PG1 construction. Since PG1 primary transcript has an intron and *S. cerevisiae* is not able to remove it during maturation, the enzyme couldn´t be expressed when cloned with this intron. So, it was deleted using partial PCR and digestion with restriction enzyme from pYES2:PG1; generating the pYES2:PG1Ä construction. *E. coli* Top10 cells were then transformed, and plasmid-containing cells were tested by PCR using specific primers for Pg1. Nine positive clones showed no mutations and correct ORF. The pYES2:PG1Ä was used to transform *S. cerevisiae* INVSc1. Transformed yeast clones were analyzed by colony PCR. Two positive clones were obtained and tested in terms of PG1 expression. Both clones expressed and exported an active PGase after 21 hours of induction with galactose. Expressed recombinant protein was recovered as an unique extracellular protein, has a MW of ~60 kDa and was identified as PG1 by hydrolysis of polygalacturonic acid at pH 2.5 but no at pH 5.

BT-P13.

BIOEMULSIFIER-PRODUCING *Aspergillus niger* MYA 135: EFFECT OF CULTURE CONDITIONS

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Bioemulsifiers are surface-active molecules synthetized by microorganisms, which play an important physiological role in hydrocarbon degradation. Few reports have shown that the bioemulsifier production by filamentous fungi are amount, quality and nature dependent not only on the microorganism but also on the culture conditions. Bioemulsifiers have the advantages of biodegradability, low toxicity, effectiveness and these properties enable their wide application on bioremediation, food, cosmetic and pharmaceutical industries. The aim of this work was to study the bioemulsifier production by *Aspergillus niger* MYA 135 under different culture conditions. Methods: The bioemulsifier production was conducted in mineral medium during 96 hours, at different initial pH and with the addition of $CaCl_2$ or $FeCl_3$. The emulsification index was determinated after 24 h (E-24) in supernatants using kerosene as immiscible liquid. E-24 was estimated as the height of the emulsion layer divided by the total height and multiplied by 100. Results and conclusions: Bioemulsifier production reached maximum levels at 2 days of cultivation suggesting that its accumulation was growth-associated. Although all the emulsions were stable, the maximum values of E-24 were obtained at initial pH 2 (60.0 ± 0.71 %) and with the addition of $FeCl_3$ (50.5 ± 0.70%).

This work was supported by grants PIP 6062 & PICTO 761

BT-P14.

SELECTION AND CHARACTERIZATION OF HYDROLASES SECRETION MUTANTS IN *Tetrahymena thermophila*

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The non-pathogenic ciliate *T. thermophila* converts cholesterol from food stuffs into pro-vitamin D derivatives, increasing the nutritional value of milk and egg products. Unfortunately, the process is accompanied by the release of hydrolytic enzymes that impair food's sensorial properties, i.e., taste and odor. Hydrolytic enzymes are present in lysosomes, which are secreted after fusion with the cellular membrane. Our strategy was to block the secretion process by mutation with the mutagen N-methyl-N'-nitro-N'-nitrosoguanidine followed by conjugation, for the isolation of stable genotypes. Following this protocol, 20 sec- mutants were obtained, based on decreased (acid) phosphatase activity (70%). These clones were further characterized with respect to several other lysosomal enzymes, such as protease, ß-N-acetylglucosaminidase, galactosidase and ß-glucosidase. Only two of the mutants showed similar decrease in all tested enzymatic activities. In spite of these results, the mutants showed a high level of secretion upon stimulation with Dibucaine, approximately 60% more than the wild type and other sec^- mutants (MS-1). Assuming that this drug increases the intracellular level of free Ca^{2+}, the mutants must be affected in a pathway that has not been targeted in previously characterized sec- mutants.

BT-P15.

ISOLATION AND CHARACTERIZATION OF PLASMIDS FROM *Shewanella* CSQ2. A SHUTTLE VECTOR CONSTRUCTION

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Marine microorganisms present biotechnological and industrial interest becuase of its capability to develop cold-active biocatalizers, among others. Cold-active enzymes offer economic profits due to incremented reaction rates, high stereospecificity, meager undesirable chemical reactions, lack of heating costs and easy inactivation by its thermal lability. *Shewanella* is a marine bacterial genus cold-active enzyme producer that presents a big biotechnological potential and grows using a variety of substrates.
The aim of present work was to isolate and characterize by restriction profiles, the plasmids present in *Shewanella* C_sQ_2 strain, isolated in our laboratory from Beagle Channel samples, and to build a bridge vector capable of replication in *E. coli*. For that, plasmidic DNA extractions and conventional and pulsed field gel electrophoresis analyzes were performed. Restriction profile and cloning of selected plasmid were carried out in the pBluescript vector.
Results and conclusions: two plasmids of ~3,000 and ~6,000 respectively and a megaplasmid were isolated. Restriction profile of P1 plasmid (3,000 pb) was carried out and obtained fragments were cloned into pBluescript vector, subsequently used in *E.coli* XL1 blue transformation reactions. The shuttle vector obtained is a valuable tool that would allow to study and express genes of interest of *Shewanella*' strains into *E. Coli*.

BT-P16.

BEHAVIOUR OF TWO RECOMBINANT BACULOVIRUSES FOR PEROXIDASE EXPRESSION IN LEPIDOPTERA

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Baculovirus-insect cell system is a popular choice for heterologous gene expression when an eukaryotic environment is required. *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) is by far the most widely used baculovirus expression vector. In this work two recombinant viruses were constructed in order to compare the performance of HRPC expression in Lepidopteran hosts. The first one (AcMNPVHRPC occ-) was conventionally formed by introducing the HRPC gene in locus polyhedrin. The second construction (AcMNPVHRPC occ+) was identical except for polyhedrin gene presence under p10 promoter. *Spodoptera frugiperda* larvae (approximately 200 mg each) were infected by intrahaemocoelical injection with 50 µl of 6.10^6 ufp.ml^{-1} of both viral suspensions. At different days post-infection (dpi) HRPC expression in haemolymph was measured by its enzyme activity. HRPC levels achieved with both type of virions were not significantly different at any dpi. Thus, polyhedrin gene presence under p10 promotor does not affect the HRPC expression. AcMNPVHRPC occ+ showed to be as effective as AcMNPVHRPC occ- with the great advantage of being suitable for oral infection of permissive lepidopteran species.

BT-P17.
DIFFERENT PURIFICATION STRATEGIES FOR HORSERADISH PEROXIDASE RECOVERY FROM INSECT CELL MEDIA

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Insect cells infected with recombinant baculovirus represent an interesting system to achieve high level expression of commercially attractive proteins like immunogens and diagnostic reagents. The aim of this work was to study different downstream processing strategies for the recovery of a recombinant biocatalyst (horseradish peroxidase, HRPr) directly from the Sf9 culture cell. We constructed a baculovirus vector by cotransfection of transfer vector and viral DNA BaculoGoldTM brigth in Sf9 cell line. The cDNA of HRP supports two tags (6xHis and 6xArg) and was cloned under the polyhedrin promoter and the signal peptide sequence GP67. A viral stock of 9.2 $x10^7$ $pfu.ml^{-1}$ was used to infect the suspension cell cultures. HRPr was secreted into the culture medium where it was accumulated to 11.6 $mg.l^{-1}$ at 5 days postinfection with MOI 2 and the supplementation of the medium with 7.2 µM hemin. HRPr was purified by nickel affinity chromatography (IMAC) and by ion exchange chromatography (IEC) directly from the clarified supernatant cultures. In the first case, HRPr yield was 96% with a purification factor of 60.4 and in IEC at pH 8.5 the yield was 89.9% with a purification factor of 24. High yields of HRPr were achieved by both strategies but IEC has lower cost and therefore is more suitable for scaling up the process.

BT-P18.
INFLUENCE OF DISSOLVED OXYGEN ON SAUFL-AG-286 CELL CULTURES GROWTH AND BACULOVIRUS PRODUCTION

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Despite the number of publications reporting insect cell cultivation under controlled dissolved oxygen concentration (DOc), the effects of different DOc on cell growth and polyhedral inclusion bodies (PIBs) production kinetics have been evaluated in a limited number of bioreactor studies. saUFL-Ag-286 insect cells were seeded at 3.5 x 10^5 viable cells/ml using eight identical 0.5 L concentric airlift reactors, where the DOc was set-up at 5, 15, 20, 35, 50, 70, 85 and 95% respectively. Cultures maintained in a range of 50 to 85% DOc produced the largest number of cells, with a maximum yield of 3.08 x 10^6 viable cells/ml obtained at 70% DOc. To investigate the effects of DOc on baculovirus replication, cultures were grown at 15, 35, 70 and 85% DOc in the same reactors and then infected with *Anticarsia gemmatalis* multiple nucleopolyhedovirus (AgMNPV) at a multiplicity of infection of 5 at their early exponential cell growth phase. Cells containing PIBs where first detected in 35 and 70% DOc (36 HPI), and only 12 hours later in the culture where the DOc was 15%. The yield of PIBs was also affected by DOc: infected cultures maintained at 70% DOc produced 2.5 and 1.7 more PIBs than those controlled at 35 and 15%, respectively. The results achieved in this study show the key role of DOC on both the growth of saUFL-AG-286 cell cultures and the production of AgMNPV PIBs.

BT-P19.
CHARACTERIZATION OF TRANSPOSONS FROM INSECT CELL LINES

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Insect cells are the ordinary system to study and produce baculoviruses, a pathogen used as bioinsecticide, protein expression system and gene therapy or vaccine vectors. These viruses have big dsDNA genomes and structural mutations (insertion, deletion, inversion or rearregments) could be the main force to their evolution. On the other hand, transposons are moving genetic elements spread in most organisms and probably they could be associated with that process. So that, we initiate this work with the study of the transposon influence in virus genotype stability. Thus we test the mobility of sequences between cell genome and transfected plasmids or infected baculovirus. In particular we test Sf21, Sf9, Hi5 and UFL-Ag-286 cells and AgMNPV and AcMNPV (*Anticarsia gemmatalis* and *Autographa californica* Multiple Nuclearpolyhedrosis viruses). We also characterize an insect transposon isolated from Hi5 cells called PiggyBac-Hi5. Its genetic constitution is very simple and its moving capacity was reported with success in different species. In this work we present the results associated to the genotype modification of exogen dsDNA in insect cells and a complete characterization of PiggyBac-Hi5 activity. With all this information we suggest that transposons are a crucial factor in baculovirus evolution and that PiggyBac-Hi5 is a good candidate to develope new cloning and transgenic systems.

BT-P20.
CHARACTERIZATION OF SYNCHRONIZED UFL-AG-286 CELL CULTURE TO OPTIMIZE THE BACULOVIRUS PRODUCTION

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A. gemmatalis is the main pest in soy cultures of South America. This insect could be selectively and specifically controlled by a baculovirus, the multiple Nucleopolyhedrovirus of *A. gemmatalis (*AgMNPV). Currently, the commercial production of AgMNPV is based on infected larvaes. However, the possibility of introducing genetically modified baculovirus that do not propagate in larvae has stimulated the interest to develop alternative processes of production based on in vitro spread in insects cells. The cell line UFL-Ag-286, that was established from embryos of *A. gemmatalis*, has great susceptibility and high levels of viral production. Therefore, it is the best way for in vitro production of AgMNPV. The goal of this work was to evaluate if the synchronization of cell cultures improves the production of recombinant baculoviruses. These process, applied on UFL-Ag 286 cells, was performed using cultures with low FBS concentration, generating a significant decrease of the proliferation factors. The cultures were characterized by fluorescence microscopy and cellular viability tests. Once the synchronized culture was obtained and characterized, preliminary tests of infection and viral production were realized.
The results suggest the usefulness of synchronizing cultures previous to infection; in order to obtain an optimized performance in producing occluded and budded viral forms.

BT-P21.

CLONING AND EXPRESSION OF FUSION PROTEINS BETWEEN LECTIN *Helix pomatia* AGGLUTININ (HPA) AND DSRED

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Cancer cells have different surface glycoproteins and glycolipids compared with normal cells, modifying cell adhesion properties. Lectins, sugar-binding proteins of non immunologic origin, could be used to detect changes in cell's surface. The *Helix pomatia* agglutinin (HPA-lectin) binding is associated with metastasic competence in several types of cancer. HPA binds to molecules involved in cell adhesion, migration or in antiapoptotic pathways. Most studies have detected HPA binding cell using antibodies, others have employed chemical modified HPAs. However, at present, HPA is isolated and purified from its natural source, the *H. pomatia*. Our objective was to obtain a recombinant form of HPA to incorporate it into a tumor cell specific delivery system. First, we cloned the HPA ORF into bacterial and baculoviral systems, and analyzed its expression. Furthermore, we make two fusion between HPA and DsRED, locating HPA sequences at the C- or N-terminal of the peptide, on pDsRED2-C1 y pDsRED2-N1 plasmids, respectively. These plasmids were used to study the specific binding ability of HPA to tumor cells. Plasmids were transfected into 293T cell line, and protein expression was followed by fluorescence microscopy. Then protein extracts obtained from HPA-DsRED producing cells, were used to verify the association of HPA-DsRED to tumor derived (HT29, F3II, MC7) cell lines.

BT-P22.

Z ALONE COULD DIRECT VLP`S FORMATION IN SF9 INSECT CELLS THROUGH BUDDING

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Junín virus, the etiological agent of Argentine hemorrhagic fever, belongs to the Arenaviridae family. This family has 23 recognized species with an ambisense-bipartite ssRNA genome. The enveloped virions include non-equimolar amounts of each genomic RNA, named L (ca. 7200 nt; L and Z proteins) and S (ca. 3500 nt; N and GPC proteins). The Z protein is a structural component of the virion that interacts with several proteins in infected cells, interfering with the normal protein expression pattern.
We analyzed Z amino acid sequences and divided it into three domains. The C-terminal region contains a domain involved in budding process. The core region comprises 8 amino acids corresponding to the ring finger structure. The N-terminal region, contains a conserved G at position 2, that would be myristoilated. This mediates protein localization in the plasma membrane, essential for viral particle formation. We expressed and purified Z protein in bacterial, insect and mammalian cells, from Candid#1 strain, and obtained a polyclonal serum anti-Z fused to thiorredoxin, used for immuno detection techniques. Insect cells-expressed His-tag-Z protein shows the lost of the tag after its expression a result agreeing with myristoilation. We verified the VLP´s formation, in insect and mammalian cells as consequence of the Z expression alone, by differential ultracentrifugation and western blotting.

BT-P23.

OBTAINING AND CHARACTERIZATION OF CASEIN HYDROLYSATES USING PROTEASES FROM *Bromelia hieronymi*

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Bioactive peptides are short amino acid sequences belonging to a protein that can be liberated employing an appropriate proteolytic enzyme. The aim of this work was the preparation of casein hydrolysates under different conditions and subsequent characterization and analysis of eventual biological activity in the peptides produced. Casein suspension (1.25 %) was obtained by solubilization in 0.1 M Tris HCl buffer (pH 8.5) at boiling temperature and further filtration. The proteolytic agent employed was a crude extract obtained by homogenizing in phosphate buffer frozen unripe fruits of *Bromelia hieronymi* Mez (Bromeliaceae), a species taxonomically close to pineapple growing in the NW of Argentina. The hydrolysis was stopped at 10 and 30 min and 1, 2, 3, 4, 5 and 6 h by adittion of 5% trichloroacetic acid or submitting each hydrolysate at 100ºC for 5 min. The hydrolysis degree was evaluated using the TNBS method employing L-leucine as standard. The molecular mass profile of the TCA soluble peptides was analyzed by tricine SDS-PAGE, showing a drastically reduction of higher peptides at short hydrolysis times: peptides higher than 14 kDa disappeared after 30 min hydrolysis and then the proteolytic profile remains unchanged. Results of hydrolysis degree assays showed acceptable correspondence with the electrophoretic patterns obtained.

BT-P24.

***ST*ASP-PSI DOMAIN IS THE KEY IN THE *ST*APS SPERMICIDAL ACTIVITY**

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We have isolated two potato aspartic proteases (*St*APs) with cytotoxic effect towards plant and human pathogens and on bovine and human sperm. *St*APs exert its cytotoxic activity by selective plasma membrane permeabilization. Cytotoxic activity of *St*APs is related with the presence in these proteins of a domain named *Plant Specific Insert* (PSI), which has high structural homology with proteins able to interact with different phospholipids (SAPLIPs). The aim of this work was to analyse the capacity of this domain, *St*Asp-PSIr, to interact with the plasma membrane and to exert spermicidal activity over bovine and human sperm, both cryopreserved and fresh. *St*Asp-PSIr reduced, in a dose-dependent manner (0.6-12,5 µM), bovine and human sperm total motility (100% at concentrations of 12,5 and 2,4µM respectively) and increased sperm membrane permeability (100 % at concentrations of 12,5 and 7,5µM respectively). These results suggest that *St*Asp-PSIr is the key domain into the *St*APs to exert spermicidal activity. However, contrary with the results reported for *St*APs, *St*Asp-PSIr was unable to bind to spermatozoa surface, independently of the specie and conservation method. Therefore, correct folding in the structure of mature protein or another *St*APs domain is necessary for binding to spermatozoa surface.
**Equal contribution*

BT-P25.
CHARACTERIZATION OF PROTEASES FROM LATEX OF *Vasconcellea quercifolia* USING PROTEOMICS TOOLS

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Vasconcellea quercifolia (Caricaceae) latex contains several cysteine endopeptidases with very high proteolytic activity, which are the active compounds used by the plant as a defence mechanism against herbivorous insects.
The crude extract presented several basic proteins with proteolytic activity and also other acid fractions without activity. The specific enzymatic activity (BAPNA) was 2.35 times higher in *V. quercifolia* latex than in *Carica papaya* latex. This higher activity is correlated with a higher concentration of enzymes in the latex of *Vasconcellea* fruits, but in addition, also could result from the presence of different cysteine proteinases.
The crude extract was purified (FPLC) by cation exchange chromatography (SP-Sepharose HR, Tris-HCl 50mM, pH 7.5 and 1mM sodium tetrathionate , using a linear gradient of 0.15-0.40M NaCl). From SDS-PAGE and blotting of the selected fractions, the N-terminal amino acid sequences of polypeptides were determined using the automated chemical Edman degradation. The Peptide Mass Fingerprinting (PMF) of the fractions was achieved by using a trypsin solution (0.4 µg/µl) for 12 h at 37ºC and analyzed by MALDI-TOF TOF/MS. The PMF results allowed the peptides characterization and comparison with other proteases. Both analyses confirmed the presence of several different cysteine proteinases in the latex of *V. Quercifolia.*

BT-P26.
ISOLATION AND CHARACTERIZATION OF PROTEASE ISOINHIBITORS FROM *Maclura pomifera* SEEDS

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Precise spatial and temporal regulation of proteolytic activity is essential to human physiology. Naturally occurring protease inhibitors control proteolysis within an organism, as well as inactivate proteases of competing or predatory species. Exploration of natural inhibitors and synthesis of peptidomimetic molecules has provided many promising compounds performing successfully in animal studies.
A purification protocol, involving buffer extraction, acetone precipitation, gel filtration chromatography on Sephadex G-50, ion exchange chromatography on Resource Q and RP-HPLC on a C18 column were employed to isolate three protease inhibitor isoforms (MpTI-1, MpTI-2 and MpTI-3) from *Maclura pomifera* seeds. These inhibitors strongly inhibited trypsin, but did not inhibited papain, cathepsin B or carboxipeptidase A. The new inhibitors were characterized by MALDI TOF-MS and SDS-PAGE and IEF, showing a molecular mass of about 6.5 kDa with similar pI values. N-terminal amino acid sequence (determined using the automated chemical Edman degradation) of the purified isoforms showed a high degree of homology among them. Notwithstanding, no homology could be found when compared with trypsin inhibitors known up to date.
CML and WDO, CONICET fellow; LMIL, CONICET Researcher Career.

BT-P27.
DENGUE PROTEIN PRODUCTION IN PLANT USING *Agrobacterium*-MEDIATED AND BIOLISTIC TRANSFORMATION

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Production of recombinant proteins in plant systems has emerged as a new alternative platform. Plant cells can be maintained in simple and economic media, without risk of contamination with bacterial toxins, virus or prions. Moreover, the plant cells can glycosilate and do the post-transcriptional arrangements need for complex glycoproteins. The envelope protein (E) is the major structural component and the most immunogenic of dengue virus proteins and it is involved in the induction of a protective immunity. E protein needs to be directed to the secretory pathway through the N-terminus signal peptide (SP), to be N-glycosilated. The presence of an ER retention signal in the C-terminus of some proteins increases their stability and the yield of recombinant protein in plant systems. The aim of this work was to produce E protein in a plant system to serve as a diagnostic reagent in the rapid detection of dengue virus. A gene encoding dengue type 2 E protein was successfully cloned in a binary vector and expressed in *N. tabacum* plant using Biolistic and *Agrobacterium*-mediated expression systems. The result indicates that expression systems used can produce the dengue virus antigen in plant. We are currently evaluating the integrity and the expressions level of this protein. The effect of different genetic sequences on the expression levels of the E protein will be assessed.

BT-P28.
INFLUENCE OF SHEAR STRESS ON ANTHRAQUINONES PRODUCTION BY *Rubia tinctorum* SUSPENSION CULTURES

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Elicitation of plant cell cultures is an effective strategy to enhance the production of secondary metabolites. Shear stress has been used as a mechanical elicitor to induce secondary metabolite accumulation in many plant species. *Rubia tinctorum* suspension cultures produce anthraquinones (AQs), secondary metabolites, which are important in the pharmaceutical and food industries. In this work the effect of different levels of shear stress on AQs production in *R. tinctorum* suspension cultures was studied. To induce shear stress 100 mL unbaffled and baffled shake flasks on an orbital shaker at 360 rpm were used. Controls were carried out in 100 mL unbaffled flasks agitated at 100 rpm. Cultures were grown at 25 °C with a 16 h photoperiod using cool white fluorescent tubes. Biomass concentration, cell death and AQs production were evaluated. Cell death was 16% and 17% after 24 h of shear stress in unbaffled and baffled flasks respectively. Regrowth assays showed that both controls and 24 h stressed suspension cultures reached similar biomass concentration after 7 days of incubation. AQs production increased 22% and 57% after 24 h of shear stress in unbaffled and baffled flasks respectively. These results show that shear stress elicits AQs production in *R. tinctorum* cell suspension cultures and could be a potential strategy for industrial production of these secondary metabolites.

BT-P29.

SCREENING OF ONE-BEAD-ONE-PEPTIDE COMBINATORIAL LIBRARY USING RED FLUORESCENT DYES

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Combinatorial peptide libraries using "one-bead-one-compound" (OBOC) method involves the synthesis of millions of peptides on beads so that each bead displays only one peptide entity. With the OBOC method, ligands with pharmacological and analytical uses and protein capture agents have been described. To screen OBOC libraries, beads are first mixed with a target molecule and those that interact with the target molecule will be isolated for compound structure determination. Herein we describe an OBOC peptide library screening using streptavidin (SA) as probe protein, labeled with a red fluorescent dye and using the COPAS BIO-BEAD flow sorting equipment to separate fluorescent from non-fluorescent beads. Red dyes investigated were ATTO 590 and Texas Red. After incubating the library with the SA-dye conjugate, positive beads due to peptide-SA interaction and false positive beads due to peptide-fluorescent dye interaction were isolated. Using control peptide-beads we realized that false positive had a bright homogeneous fluorescence while positive beads had a heterogeneous fluorescence exhibiting a characteristic halo appearance. Thus, positive from false positive beads could be manually isolated. The beads were analyzed by MALDI-TOF MS. Sequences obtained from positive beads had the His-Pro-Gln motif. Peptides from false positive beads were rich in Leu/Ileu, His, Phe and Tyr.

BT-P30.

OPTIMIZATION OF MALDI-TOF MS FOR THE ANALYSIS OF PEPTIDE BEADS FROM ONE BEAD-ONE PEPTIDE LIBRARIES

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One bead-one peptide combinatorial libraries, an important tool for new ligand identification, involves the synthesis of millions of peptides on beads so that each bead displays only one peptide entity. The library is screened against specific targets. Positive beads are isolated and analyzed. We reported a rapid and inexpensive method based on MALDI-TOF MS analysis. Contaminants, matrix clusters and metal ion adducts interfere with peptide ionization and mass spectrum interpretation. In this work we analyzed different strategies to improve MALDI spectra. We replaced the guanidine used for bead washing before MALDI analysis with a mixture of acetonitrile (MeCN), acetic acid (AcOH) and H_2O. Removal of guanidine as contaminant improved matrix crystallization. We optimized peptide-bead cleavage and peptide elution: beads were placed into microtubes in a drying chamber together with a flask containing NH_4OH. Cleaved peptides were eluted from the beads with 20 µl of AcOH- MeCN-H_2O and 0,5 µl of sample was loaded onto the sample plate. Elution of peptides in microtubes instead of placing the bead in the sample plate increased sample aliquots in case spectrum had to be repeated. Among the matrices analyzed, a-cyano-4-hydroxycinnamic acid induced higher peptide ionization. To minimize clusters and adducts, serine or $NH_4H_2PO_4$ were added to the sample yielding higher signal-to-noise ratio.

BT-P31.

MAGNETIC SILICATE BEADS FOR THE IMMOBILIZATION OF BIOMOLECULES USING SOL-GEL AQUEOUS ROUTE

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The use of biomolecules in clinical applications is limited due to their high cost, instability and low availability. This leads to an intense research in the field of biomolecules immobilization. Immobilized species are reusable, more stable and less costly. Our aim was to develop a versatile biomolecule immobilization system for its recovery after a biological assay and its reutilization. For this purpose urease was immobilized onto magnetic silicate beads by the sol-gel aqueous route.

A $FeCl_3$ and $FeCl_2$ aqueous mixture was coprecipitated with NaOH. The ferrofluid thus generated was mixed with a sodium silicate solution, and used as the aqueous phase of an inverse microemulsion. Magnetic beads were generated by an aqueous route sol-gel process. The magnetic beads obtained were functionalized with APTES to provide NH_2 groups and treated with glutaraldehyde, a bifunctional reagent that attaches to both APTES and urease free NH_2 groups. These beads are recoverable by means of a magnet after an assay. Glutaraldehyde concentration was crucial for the immobilized enzyme activity and stability. The immobilized enzyme maintains its activity during at least 30 consecutive reaction-recovery cycles. The reutilization of this system after 30 days storage at 4ºC showed no significative activity loss, demonstrating its stability.

BT-P32.

THE USE OF THE EFFICIENCY FUNCTION FOR COMPARING CATALYTIC COMPETENCE

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The use of k_{cat}/K_M to compare the catalytic competence of enzyme variants is inappropriate, since the ratio between the reaction velocities depends on [S] even when both variants have equal k_{cat}/K_M if they have different K_M values (Eisenthal, R. *et al.* (2007) *Trends Biotechnol* 25, 247).

In chemical catalysis, the efficiency function (*Ef*) is defined (Albery, W.J., Knowles, J.R. (1976) *Biochemistry* 15, 5631) as the ratio between the rate of the catalyzed reaction and the maximum theoretical rate, which is limited by diffusion of substrates to the active site. We derived a formalism by applying the steady-state approximation, and proposed its use for comparing the competence of enzyme-catalyzed reactions (Ceccarelli, E.A. *et al.* (2008) *Trends Biotechnol* 25, 119). *Ef* permits: i) a straightforward interpretation of changes in catalytic competence in terms of "efficiency" for the biotechnological practice; and ii) the evaluation of how far various engineered enzymes have approached the "catalytic perfection", viz. they are beyond diffusion control.

Ef provides a direct, easy and useful tool to make predictions on the conditions under which an enzyme variant could be efficiently employed. If physiological [S] are known, it can also be used to predict if expression of a mutated enzyme in a transgenic organism will improve the efficiency of conversion of a desired substrate.

BT-P33.

EFFECT OF DIFFERENT BIOSTIMULATION AGENTS ON HYDROCARBON DEGRADATION IN CONTAMINATED ANTARCTIC SOILS

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Bioremediation is considered an interesting tool to recover hydrocarbon contaminated soils. Biostimulation is the best strategy for the remediation of chronically contaminated soils and thus, it is important to evaluate several nutrients sources to select the most efficient one. Also, biostimulation combined with a surfactant could improve the removal of hydrocarbons from soil. A field assay under Antarctic environmental conditions was performed to evaluate the effect of different nutrients sources and their combination with a surfactant (Brij®700). Inorganic salts (NSS) and fish meal (FM) were evaluated alone and in combination with Brij®700 (NSSB and FMB). A commercial bioremediation product (CP) and a control without treatment (CC) were also included. The bacterial counts were evaluated in agar plates. Concentration of total hydrocarbons (TPH) was determined by the EPA 418.1 method. At the end of the assay, TPH showed to be significantly lower in all systems (CC: 6590.2 ppm; NSS: 4473.9 ppm; NSSB: 5271.2 ppm; FM: 4871.3 ppm; FMB: 6301.4 ppm; CP: 2649.3 ppm) compared with the initial value (9989.5 ppm). FM, FMB and NSSB showed no significant differences with CC. Results showed that inorganic salts and the commercial mix of enzymes, nutrients and surfactants (CP) exhibited the best efficiency. The use of inorganic salts could represent an adequate option on a cost evaluation basis.

CB-P01.

CYTOPLASMIC c-FOS ACTIVATES THE TRANSLATION IN DIFFERENTIATING PC12 CELLS AND *IN VITRO* SYSTEMS

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We have found that c-Fos, in addition to its activity as a member of the AP-1 family of transcription factors, associates to the ER and activates the synthesis of phospholipids in events that require membrane biogenesis. However, membrane biogenesis not only requires lipids, it also requires other components such as proteins. Consequently, we examined if c-Fos also activates protein synthesis by an AP-1 independent mechanism. Cultured PC12 cells induced to differentiate by feeding with NGF showed an increased in protein synthesis; this activation was abolished upon blocking of c-Fos expression; culturing cells with a peptide that specifically blocks the nuclear import of AP-1-c-Fos or with a specific inhibitor of p38 MAPKs that phosphorylate c-Fos transactivation domain shows that AP-1 is required to trigger neuronal differentiation whereas cytoplasmic c-Fos is necessary for differentiation to continue. Neuronal differentiation positively correlates with protein synthesis activation. *In vitro* translation shows activated protein biosynthesis in the ER that is dependent on the concentration of c-Fos and on the c-Fos/ER association. Similar results were found with a synthetic peptide containing the 22 basic aa of the BD of c-Fos. This is the first evidence that c-Fos can activate, in addition to the metabolism of lipids, protein metabolism.

CB-P02.

INHIBITORY EFFECTS OF DIETARY AND PHARMACOLOGICAL MELATONIN ON THE GROWTH OF A MURINE BREAST TUMOR

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Both physiological and pharmacological levels of melatonin (ME) exhibit substantial anticancer activity. However, its implicated mechanisms are still anclear. We investigated effects of dietary and pharmacological ME on the growth of a transplantable murine breast tumor (M3) and their correlation with LOX and COX metabolites, apoptosis, necrosis of tumor cells (TC), leukocytic tumor infiltration and tumor weight (TW). Three groups were fed on different diets. 1) Control group (C) basic diet plus 6% of Corn oil (rich in PUFAs n-6 and n-9); 2) PM group fed with C diet and pharmacological ME in water (3mg/l) and 3) Walnut group (W) fed with C diet replacing 50% of protein by Walnut flour (ME -3.5 +/- 1.0 ng/g- and PUFAs n-6 and n-9). We analysed eicosanoids (ng/1010NC/ml) derived from COX:12-HHT and for LOX:12-HETE and 13-HODE, by HPLC. Apoptosis was assessed by flow cytometry using Annexin V-FITC. Leucocyte infiltration (LI) in tumor tissues was evaluated by inmunohistochemical analysis with anti-CD3 mAb. Tumor weight was determined during necropsy. 12-HHT, 12-HETE and 13-HODE (p=0.05) were lowest for PM group. Apoptosis and necrosis were lowest for C while necrosis was higher for PM than W and inversely in apoptosis. LI was highest for PM (p=0.02) and lowest for C. TW was lowest for PM (p=0.05). Melatonin inhibited tumor growth in correlation with COX and LOX, cell death and LI.

CB-P03.

á–SYNUCLEIN OLIGOMERIZATION IN HeLa CELLS: ROLE OF MITOCHONDRIA ON AGGREGATION

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-Synuclein (AS), a small natively unfolded protein related to Parkinson´s disease, associates to form oligomers and insoluble fibrils. AS nitration by $ONOO^-$ has been found to play a role in aggregation: i) it induces AS oligomerization, ii) facilitates AS nucleation step, iii) displaces AS from lipid vesicles, and iv) protects the protein from proteosomal degradation. AS was found in the mitochondria of different cells, and there it regulated NO production. Some authors have found that oligomeric AS bound to mitochondria, but not the monomer. Whether mitochondria are capable of accelerating AS oligomerization, or AS binds mitochondria as a pre-formed oligomer remains to be elucidated. We observed that over-expressed AS entered the mitochondria of HeLa, whereas the endogenous protein was distributed in membrane and nuclei, and found colocalization of the aggregated form with the organelle. We employed a recombinant AS with a 12 amino acids tag with a binding site for the fluorogenic dye, FlASH, to map the protein to the mitochondria by flow cytometry on the isolated organelle. *In vitro* AS aggregation assays showed that mitochondria induced AS fibrillation. These findings altogether suggest a role for mitochondria on AS postranslational modifications which lead to the formation of potentially cytotoxic intermediates.

CB-P04.

A NOVEL MOTIF AT THE C-TERMINUS OF PALMITOYLTRANSFERASES IS ESSENTIAL FOR SWF1 FUNCTION *IN VIVO*

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S-acylation (also known as palmitoylation) is the addition of a lipid molecule to cysteine residues of a protein through a thioester bond. This modification is mediated by proteins referred to as Palmitoyltransferases (PATs), characterised by the presence of a conserved DHHC-Cysteine-Rich Domain. Swf1 is a yeast member of the DHHC family, and it is responsible for the S-acylation of several SNARES and other membrane proteins. Here we describe a novel 16 amino acid motif, present at the cytosolic C-terminus of PATs, that is required for Swf1 function in vivo. Within this motif, we have identified a single residue in Swf1, Tyr-323, as essential for function, and this is correlated with lack of palmitoylation of Tlg1, a SNARE that is a substrate of Swf1. Substitution of Tyr-323 with Alanine is the first phenotype affecting mutation uncovered that does not lie within the DHHC domain, for this or any other PAT. In silico analyses indicate that the motif is conserved in 70% of all PATs, and suggest that the motif may have once been present in all PATs. We named this motif “Palmitoyltransferase Conserved C-Terminus” (PaCCT). Additionally, while searching for a funtion for the PaCCT domain, we obtained preliminary evidence indicating that the Cytosolic C-terminus of Swf1 interacts with palmitoyl-CoA, the lipid donor in S-acylaction, albeit independently of the PaCCT domain.

CB-P05.

GENETIC EXPRESSION BY REAL TIME PCR OF *bglA, bgl, CspA, gyrB* AND 16S DNA GENES FROM *Shewanella sp.* G5

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Shewanella sp. G5 is a psychrotolerant Gram negative bacteria ß-glucosidases (ßGs) producers. This marine bacterium was isolate from the intestinal content of *Munida subrrugosa* in the Beagle Channel, Tierra del Fuego (Argentina); and grows between 4 and 37ºC using cellobiose as carbon source. ßGs are a heterogeneous group of enzymes with a broad substrate specificity range over different ß-glucosides. The genes (*bglA* and *bgl*) that encoding of two ßGs were characterized molecularly in previous studies. The primers design, RNA extraction, synthesis of cDNA and real time PCR assays using SYBRGreen were performed. The parameters assays to each condition were: temperature (10 and 30ºC), carbon source (cellobiose and glucose) and culture media. *bglA, bgl, CspA, gyrB* and DNAr 16S genes were quantified and expressed as relative genetic expression from of each assays using 2^{-Ct} method. Positive results were obtained for all genes during the optimization of the real time PCR. The amplification was verified by standard and dissociation curves analysis (reporter of Ct). Normalization assays using 2^{-Ct} method were carried out with the housekeeping genes (*gyrB*) also. The best values of relative genetic expression index for *bglA* and *bgl* were 27,67 and 30,17 respectively; and were obtained at the following assayed conditions: Brunner medium, 30 ºC and in presence of cellobiose.

CB-P06.

PROLIFERATIVE ABILITY IN *Phytomas Jma*; CORRELATION WITH THE ENDOGENOUS CONCENTRATION OF POLYAMINES

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Polyamines are fundamental for cell survival and proliferation; putrescine and spermidine being the most abundant in trypanosomatids. Putrescine can be synthesized from ornithine by ornithine decarboxylase (ODC), while spermidine derivates from putrescine via spermidine synthase. ODC activity was detected in phytomonads extracts and this activity was inhibited by DFMO. In vitro incubation with radioactive ornithine followed by electrophoretic analysis showed a stoichiometric equivalence between the amount of putrescine synthesis and the release of CO2, confirming the presence of ODC gene. In order to analyze the role of polyamines in *Phytomonas Jma*, they were cultivated in semisynthetic medium (SDM) in the presence or the absence of DFMO, showing in both cases a continuous growth. When levels of endogenous polyamines were measured by HPLC, the presence of DFMO in the medium caused an almost complete disappearance of putrescine while the concentration of spermidine was partially reduced . These results suggested that putrescine is not essential, because its absence did not affect the growth. On the other hand, when parasites were grown in the presence of both DFMO and CHA (spermidine synthase inhibitor) the growth was remarkably reduced wich correlated with very low levels of both putrescine and spermidine. This confirms that spermidine is essencial for Phytomonas Jma proliferation.

CB-P07.

CAD CELLS FORM NEURITES IN THE ABSENCE OF MAP1B, MAP2, TAU, STOP AND DOUBLECORTIN

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CAD cells proliferate in serum-containing medium and emit neurites in the absence of serum. Its re-addition induces neurites retraction. These cells lack several MAPs (microtubule associated proteins) and their microtubules (MTs) are very dynamic. Here we show that Tiam1 and Dishevelled1 (other MAPs) are present in CAD. Morphological, chemical and functional parameters of these cells are similar to those of normal neurons. Despite of lacking MAPs, neurites elongation rate is similar to that of axons in hippocampus primary cultures, challenging widely accepted concepts about the requirement of MTs stabilization by MAPs for neurite elongation. We also studied if MTs integrity is involved in neurite development and its retraction by serum re-addition. Nocodazol inhibited development and induced shortening of neurites. When MTs were stabilized with Taxol, subsequent treatment with Nocodazol produced no retraction, but serum re-addition did it. CAD cells lack acetylated tubulin (related to MT stabilization) but Trichostatin A induces its increment. This treatment did not prevent retraction by serum re-addition. Another way to stabilize MTs is depleting cells of ATP with sodium azide, however this drug did not prevent serum retraction either. So, even in the absence of many MAPs, CAD processes can be formed normally and their retraction is not impaired by stabilizing Mts.

CB-P08.

GROWTH HORMONE RECEPTOR AND GROWTH HORMONE BINDING PROTEIN IN PEJERREY *Odontesthes bonariensis*

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Growth hormone (GH) is a pluripotent regulator involved in metabolism and growth in vertebrates. The biological effects of GH depend on the target tissue, the number of receptors in the target cell, and the blood free GH concentration that depends on the presence of a growth hormone-binding protein (GHBP). GHBP was described in tetrapods and is a soluble version of the growth hormone receptor (GHR) that arise from alternative splicing of the mRNA or from the proteolytic clivage of the receptor. In fishes, two genes encoding for GHR (*ghr1* and *ghr2*) were described. We are particularly interested in the endocrinology of growth in Pejerrey. The aim of the present work is to characterize *ghr1* and *ghr2* genes, their expression patterns, and to determine the existence of a GHBP in pejerrey. We isolated and sequenced two fragments of 1240 and 1500bp cDNAs corresponding to GHR1 and GHR2 transcrips respectively, that showed 83% and 79% identity with GHR1 y GHR2 transcripts of *E. coioides* (Perciformes) respectively. Both transcripts were detected in 13 tested tissues, by RT-PCR analysis. In order to determine the presence of a GHBP in pejerrey, we performed an affinity chromatography using pjGH as a ligand. We isolated a single polypeptide of 68 kDa from serum that could correspond to the GHBP. Further tandem mass espectrometry analysis should be done to elucidate the protein identity.

CB-P09.
THE INSULIN RECEPTOR ON NORMAL AND DIABETIC MAMMARY RAT TUMORIGENESIS

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Several studies report that insulin hormone and its receptor (IR) may be associated to an increased risk for a number of malignancies. We demonstrated that N-Nitroso-N-methylurea (NMU) induced mammary adenocarcinomas in normal rats and more differentiated lesions in diabetic ones. The aim of the present study was to investigate the expression and localization of IR in mammary tissue during the carcinogenic process as well in mammary tumors, in both diabetic and non diabetic rats. Carcinomas were induced in animals by NMU injection to 50, 80 and 110 days old rats. Diabetes was induced by streptozotocin administration at 36 h of animals life. Breast tissue specimens were processed at 60, 90 and 120 days, as well as samples from developed tumors. Immunohistochemical analysis and Western blot were performed. Receptor expression was markedly increased in NMU-mammary glands (+++) vs normal ones (-/+). In diabetic rats IR expression was similar to normal glands (+), whilst in diabetic NMU-injected animals the expression was scarce (+/++). All tumors developed in non-diabetic rats presented high undifferentiated grade, cribriform pattern and marked IR expression (++++); tumors developed in diabetic animals showed differentiated pattern, low malignant grade and slight IR expression (+/++). Results suggest a correlation between IR expression and degree of differentiation on mammary tumors.

CB-P10.
CNBP IS REQUIRED FOR CRANIAL NEURAL CREST CELL DEVELOPMENT

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Cellular Nucleic acid-Binding Protein (CNBP) is a small protein that displays nucleic acid chaperone activity. Its gene general organization and amino acid sequence are highly conserved among vertebrates. Recently we reported that CNBP is required for forebrain formation by controlling the neural crest cell (NCC) proliferation/survival balance. In this study we used the zebrafish model to further understand the molecular role of CNBP during NCC development. We knocked down the CNBP embryonic expression by using both splicing and translation blocking morpholino oligonucleotides (MO). CNBP depletion resulted in forebrain truncation and developmental delay, but in normal development of the trunk. To observe a similar effect and phenotype with both MOs splicing MO was used at a concentration 3-fold lower than translation MO. It may be possible that dominant negatives have been translated from differentially processed transcripts. CNBP-depleted embryos showed a reduction in the expression of cranial neural crest markers, such as dlx, msx, and sox9. Instead, in these embryos the expression of trunk neural crest markers, such us sox10 and dct, was not affected. Hence, CNBP is required for craniofacial structures development but it seems not to be involved in melanoblast development. These data confirm that CNBP plays an essential and conserved role during vertebrate head development.

CB-P11.
ING1b TUMOR SUPPRESSOR PROTECTS GENOME INTEGRITY FROM OXIDATIVE STRESS IN MEFS

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Inhibitor of Growth 1b (ING1b), is a member of the ING protein family involved in various biological functions ranging from senescence, cell cycle arrest, apoptosis to DNA repair. ING1 mutations and altered expression levels are found in multiple human cancers. We investigated the role of ING1b in the cellular response triggered by oxidative stress and IR. We first examined the level of gamma-H2AX in wild type or ING1b gene trap MEFs (GG MEFs) by western blot, after H2O2 treatment. Expression peak of this damage marker, and its remanent level along time are greater in GG MEFs. Similar results were obtained after IR treatment. It is reported that gamma-H2AX level and size of foci are greater in relaxed chromatin after DNA damage. ING1b can target HDAC complexes to particular domains of chromatin. When MEFs were treated with the inhibitor of HDACs, Trichostatine A, before IR, there was an increase in DNA damage for both cellular types as indicated by the phosphorylated H2AX level. It seems that the absence of ING1b has an effect on chromatin, similar to that of TSA, confirming the notion of ING1b functioning as a bridge between HDACs complexes and nucleosomes. Finally, in UDS assay, we observed that ING1b mutated in its PHD finger domain could not repair peroxide damaged DNA as the wild type protein does. These results confer ING1b a protective role to cells under oxidative stress.

CB-P12.
OXIDATIVE STRESS INDUCES ACTIVATION OF THE TRANSCRIPTION FACTOR CREB IN NEURONAL CELLS

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Cyclic AMP response element binding (CREB) protein, a transcription factor, mediates responses to a number of physiological and pathological signals such as neurotransmitters, mitogens, and stress factors. Phosphorylation of CREB at Ser133, a well-characterized modification site, seems to be necessary but not sufficient to trigger its activation, suggesting a requirement for additional independent mechanisms including other phosphorylation sites and pathways. The aim of this work is to study the role of CREB in the DNA damage response in neuronal cells. In particular, the modification of CREB at phosphorylation sites in addition to Ser133 will be explored. Metabolic labeling of cells with ^{32}P-ATP followed by treatment with H_2O_2 showed that CREB becomes phosphorylated starting at 30 minutes post-treatment. However, unlike treatment with cAMP, addition of H_2O_2 did not induce phosphorylation of CREB at Ser133, as detected by Western blot. H_2O_2 also activated CREB transcriptional activity 3.5-fold in reporter assays using p4xCRE-CAT. This effect was partially blocked by inhibitors of Ras (PD152440) and MEK (PD98059) indicating that the Ras/ERK pathway is involved in the activation of CREB by H_2O_2. Taken together, these results suggest that H_2O_2 activates CREB by phosphorylation at sites other than the canonical Ser133 through a pathway that requires Ras and MEK activity.

CB-P13.

N-TERMINAL c-FOS TYROSINE PHOSPHORYLATION REGULATES c-FOS/ER ASSOCIATION AND c-FOS-DEPENDENT PHOSPHORHYLATION

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We have found that c-Fos, a well known transcription factor, associates to the endoplasmic reticulum (ER) and activates phospholipid synthesis. In T98G cells, c-Fos/ER association and consequently phospholipid synthesis activation is regulated by the phosphorylated state of c-Fos tyrosine residues. The small amount of c-Fos present in quiescent T98G cells is tyrosine-phosphorylated, is not associated to the ER and does not activate phospholipid synthesis. Furthermore, impairing tyr-dephosphorylation abrogates phospholipid synthesis activation and reduces proliferation rates to those of quiescent cells. c-Fos contains 4 phosphorylatable tyrosine residues at positions 10, 30, 106 and 337. Of these, only tyr 10 and 30 are relevant for this regulatory phenomenon. (Oncogene, 2007 26:3551-8).
Methods: Initially, an in silico search was performed using the Protein Human Data Base to find putative kinases or phosphatases acting on c-Fos. c-Src and EGFR were evidenced as putative kinases and TC-PTP phosphatase, respectively. To examine if c-Src phosphorylates tyr residues, recombinant c-Fos and c-Fos with tyr 10 and 30 substituted by phenylalanine were purified and incubated *in vitro* with recombinant c-Src. To determine if TC-PTP 45 dephosphorylates c-Fos, recombinant phosphatase was purified and incubated with phosphorylated-c-Fos (P-c-Fos).

CB-P14.

GALECTIN-3 IS TRANSCRIPTIONALLY DOWN-REGULATED DURING B CELL DIFFERENTIATION INTO PLASMA CELLS

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Galectin-3 (Gal-3) is an ubiquitous beta-galactoside-binding lectin implicated in cell growth, differentiation and apoptosis that plays an important role in inflammatory diseases. Here we report that B cells, with different functional behaviour, such as peritoneal B1 cells and conventional splenic B cells express similar levels of Gal-3 and that expression is 6.5-fold higher than T cells. Previously, we reported that Gal-3 expression increase during B cell differentiation to memory B cell whilst is almost null when B cells differentiate into antibody-secreting plasma cells (PC). To disect posible mechanisms involved in Gal-3 downregulation, conventional purified splenic B cells were stimulated with LPS, which induces B cell differentiation to PC via TLR4. After 72h of culture, LPS induced a strong decrease in Gal-3 expression determined at mRNA level by real time RT-PCR and at protein level by western blot. Immunofluoresce assays showed that PC shown a similar pattern of nuclear and citoplasmatic Gal-3 localization than resting B cell, although at a lower intensity. Blockade of RAS/MEK/ERK1/2 signalling with the PD98059 pERK inhibitor partially prevented Gal-3 dowregulation in LPS-stimulated B cells and reduced Ig release. Our results suggest that during the differentiation of conventional B cells to PC Gal-3 is transcriptionally down-regulated through the RAS/MEK/ERK1/2 pathway.

CB-P15.

DETYROSINATION OF TUBULIN AND Na+, K+-ATPASE ACTIVITY IN HYPERTENSIVE PATIENTS ERYTHROCYTES

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The acetylated tubulin associated to the plasma membrane is able to inhibit the Na+, K+-ATPase activity in different cells types including human erythrocytes. Hypertensive patients accumulates Na+ in the erythrocytes because in these cells the Na+, K+-ATPase is inhibited. In order to determine if tubulin is involved in the enzyme inhibition in these patients, we studied the erythrocytes membrane tubulin of hypertensive patients (MTHP). Recently we demonstrated that the MTHP is increased and provokes a decrease in the Na+, K+-ATPase activity. In this work we show that in hypertensive patients: a) the detyrosinated tubulin is increased in a higher proportion than the other tubulin isotype, b) the increase in the detyrosination of tubulin correlates with the formation of more stable microtubules in erythrocytes of hypertensive patients, c) the low levels of tubulin tyrosination is due to lower tubulin tyrosine ligase activity and this is correlated with a decrease in the amount of the enzyme in the red blood cells from patients with hypertension d) in vitro experiments showed that low levels of tubulin tyrosination affects Na+, K+-ATPase activity. These results show that tubulin of hypertensive patients has at least one difference in the composition of isoespecies tubulin that is involved in the regulation of the Na+, K+-ATPase activity.

CB-P16.

TUBULIN IN THE REGULATION OF GLUCOSE REDUCTASE AND NA+, K+-ATPASE IN RATS WITH INDUCED DIABETES

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Part of cytosolic tubulin is associated to plasma membrane and inhibit the Na+, K+-ATPase. We demonstrated that the ATPase inhibition by tubulin *in vitro* is reverted by glutamate and reversibly inhibited by glucose. In erythrocytes of diabetics patients (DP), the Na+, K+-ATPase is inhibited. To determinate if tubulin is involved in the inhibition of the enzyme in these patients, we studied the plasma membrane tubulin of DP's erythrocytes. Recently we demonstrated that erythrocytes of DP have increased more than two fold the content of membrane tubulin. Similar results were found when COS or CAD cells were incubated with glucose or sorbitol. This study demonstrate that the membrane tubulin increases significantly in brain of streptozotocin induced diabetic rats or rats treated with an intraperitoneal injection of sorbitol. This increase is correlated with a decrease in Na+, K+-ATPase activity. The origin of the sorbitol in DP is due to the reduction of glucose by glucose reductase, therefore we decided to study whether the tubulin had any influence on the activity of this enzyme. Cytosolic extracts from rat brain were incubated with a tubulin preparation of the same tissue. The results show that tubulin is able to inhibit glucose reductase *in vitro*. These results show that tubulin could be involved in regulating glucose reductase and Na+, K+-ATPase activities of DP.

CB-P17.
DEPLETION OF BOTH ER GTS CAUSES SEVERE DEFECTS IN *C. Elegans* DEVELOPMENT
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Quality control mechanisms are in place to ensure that newly synthesized proteins reach their properly folded conformation. N-glycans contribute to folding efficiency in the ER by a series of oligosaccharide processing and lectin binding reactions. The UDP-glycoprotein glucosyltransferse (GT) functioning as a conformational sensor is the key element in this mechanism. The human genome encodes two GT homologues, HUGT1 and HUGT2, which share 55 % identity, but only HUGT1 is active. *C. elegans* genome encodes two ER GTs (F48E3.3 and F26H9.8). We examined the consequences of depleting F26H9.8 and F48E3.3 proteins by RNA interference and the results showed that the expression of neither F26H9.8 nor F48E3.3 is essential for viability. However, RNAi against F26H9.8 and F48E3.3 causes morphological and developmental phenotypes and shortened their lifespan. The brood size was decreased and most of the interfered animals showed slow growth, and the development to progressive larval stages was retarded. We observed an impairment of the body movement and about 80% of the F483.3 interfered worms showed either a protruding vulva or had expulsed the intestine, suggesting that the depletion of these proteins causes progressive deterioration of muscle tissue. F48E3.3 RNAi elicits expression of the ER stress marker in SJ4400, suggesting the involvement of F483.3 in ER quality control.

CB-P18.
APOPTOSIS INDUCTION THROUGHOUT ACTIVATION OF ANG II AT_2 RECEPTOR
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Angiotensin II (Ang II) receptor expression is highly modulated during development, suggesting a role of these receptors in growth and organogenesis. AT_2 receptors are abundantly expressed in fetal tissues, where they probably contribute to development. However, the detailed mechanism is unclear. Here we report the induction of apoptosis via AT_2 receptor signaling, as a primary mechanism of tissue differentiation. To study the interaction between AT_1 and AT_2 receptors we design a co-transfection experiment to express both receptors in Hela cells. Receptors do not colocalize even after stimulation. In cells transfected with AT_2 receptors and stimulated with Ang II [10^{-7}] for different times, apoptosis appeared after 30 min stimulation. Cells were analyzed by immunofluorescence staining and confocal microscopy. We identified apoptotic cells by actin cytoskeleton depolymerization and chromatin condensation. Co-transfection with RhoA suggest a potential participation of this GTPase protein in the process. After quantification of apoptotic cells, the effect was statistically significant at 30, 60 to 240 min of stimulation. The present results showed no physical interaction between Ang II AT_1 and AT_2 receptors. On the other hand, AT_2 receptor stimulation induces apoptosis, evaluated at different levels in a time-dependent manner.

CB-P19.
THE NUCLEAR ENVELOPE DIFFUSION BARRIER IS REQUIRED FOR MOTHER-DAUGHTER ASYMMETRIC GENE EXPRESSION
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One of the mechanisms of cellular differentiation is asymmetric division, a process in which one cell divides to produce two different daughter cells due, for example, to asymmetric segregation of mRNAs or proteins that determine cell fate. We study one case of asymmetric gene expression in the yeast S.cerevisiae. Yeast cells form buds that separate to become daughters. Daughters and mothers are genetically identical but constitute distinct cell types. Early in the G1 phase of the cell cycle, at least eight genes are induced only in the daughter cell. Expression is controlled by the transcription factor Ace2, which enters both nuclei at the end of mitosis, but is soon re-exported from the mother nucleus. The source of asymmetry remains unknown. Recently, Scheprova et al (2008) described a lateral diffusion barrier between the mother and the growing bud nuclear envelopes that limits the translocation into the bud of all external nuclear membrane proteins, including the nuclear pores. Here we show, using quantitative time-lapse fluorescence microscopy, that cells delta-bud6, which are defective in this barrier, fail to establish mother- daughter asymmetric gene expression correctly. These results indicate that Ace2 is a better cargo of the mother export machinery and suggest that other cargoes might also be differentially exported, revealing a new source of cellular differentiation.

CB-P20.
GLYCOGEN PHOSPHORYLASE IS INVOLVED IN THE REGULATION OF THE YEAST STATIONARY-PHASE SURVIVAL
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We have previously showed that among the deletions of glucose metabolism enzymes in *S. cerevisiae*, the lack of glycogen phosphorylase (*GPH1*) lead to a strain with shortened stationary-phase survival (chronological lifespan) and hallmarks of oxidative stress: ROS increase, diminution of *SOD* expression and catalase activity. Snf1 pathway is key in regulating longevity in yeast. Upon glucose exhaustion, Snf1 is activated and phosphorylates the gene suppressor Mig1 derepressing enzymes for alternative metabolisms and genes related to yeast viability (stress response, Anaphase Promoting Complex, etc.). To evaluate the possible links between *GPH1* and the Snf1 path, we compared the survival of gph1 and snf4 (gene disruption of the Snf1 activator subunity). snf4 and gph1 showed similar lifespan decreases. Both lead to accumulation of ROS during the stationary-phase survival (146 and 188 AU/10^6 cel., *vs.* 45 in WT at day 7. $p < .05$). Induction of periplasmic invertase during stationary phase was assessed as a reporter of derepession, these values being 3-fold lower in both deletants than in WT after 7 d. This phenotype did not worse in the gph1 snf4 strain. Recent proteomic studies in *S. cerevisiae* show that Gph1 and Snf4 integrate a complex. We add evidence suggesting that Gph1 participates in a Snf1-mediated survival pathway in which Gph1 works upstream, enabling the activation by Snf4.

CB-P21.

POLY(ADP-RIBOSYL)ATION IN CELL CYCLE AND DNA DAMAGE SIGNALING IN *Trypanosoma cruzi*

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Poly(ADP-ribosyl)ation of different proteins is important for chromatin remodeling, an essential process that occurs in physiological and pathological conditions. The nuclear enzyme poly(ADP-ribose)polymerase (PARP) is able to transfer ADP-ribose from NAD^+ to target proteins, forming polymers (PAR) in response to DNA single strand breaks. In *Trypanosoma cruzi* only one PARP (TcPARP) has been identified. We have shown that TcPARP is present in all developmental stages of this parasite and, upon recognition of sbDNA, it activates, triggering an automodification reaction. Our aim is to study the role of TcPARP in chromatin dynamic structural changes throughout the cell cycle as well as in damage signaling. In synchronized cells, PARP content seems to be constant during cell cycle but PAR levels are high at G1 phase, then decrease to rise again in the late S and G2 phases. Regarding its role in DNA damage signaling, exposure of epimastigotes to UV radiation or H_2O_2, triggers the formation of PAR in a time exposition- or concentration-dependent manner. Post-injury survival assays have shown that the inhibition of TcPARP can allow the cells to avoid death when subjected to long UV irradiation times. We have demonstrated that, in vitro, TcPARP can ADP-ribosylate histones and AUK1 from this parasite, proteins that have been associated with both, cell cycle and DNA damage signaling.

CB-P22.

ROLE OF THE SNAIL FAMILY OF TRANSCRIPTION FACTORS IN HUMAN DISC LARGE TRANSCRIPTIONAL REGULATION

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A number of studies have demonstrated the involvement of human Disc large (DLG1) oncosuppressor in the control of both cell polarity and maintenance of tissue architecture. However, the mechanisms controlling DLG1 transcription are poorly understood. This is relevant since DLG1 is lost in many tumours during the later stages of malignant progression. We cloned and functionally characterized the DLG1 promoter region. We found, within the DLG1 promoter sequences, E-boxes consensus binding sites for the Snail transcription factors. Snail proteins are involved in the epithelial–mesenchymal transition, in the repression of epithelial markers, and are up-regulated in a number of tumours. Snai1 and Snai2 factors repress the transcriptional activity of the DLG1 promoter and down-regulate DLG1 endogenous levels. Ectopically expressed and endogenous Snail proteins bind to the native DLG1 promoter as ascertained by Chromatin immunoprecipitation assays. These interactions depend on the integrity of the E-boxes present in the DLG1 promoter sequences. These data suggest a role for Snail transcription factors in the control of DLG1 expression and provide a basis for understanding the transcriptional regulation of DLG1. Analysis of the correlation between DLG1 and Snail protein expression in tumour biopsies, by immunohistochemistry, are currently being performed.

CB-P23.

DOXORUBICIN EXACERBATES MEMBRANE MODIFICATIONS IN erbB4 KNOCKOUT MURINE MODEL

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Neuregulin (NRG) signaling through tyrosine kinase receptors erbB2 and erbB4 is critical for the maintenance of adult heart function. Combined therapy for breast tumors with antibodies blocking NRG signaling and anthracycline derivatives, may lead to a severe cardiomyopathy. We investigated the molecular consequences of an exacerbated cardiotoxicity in the ventricular muscle specific erbB4 KO mouse by the injection of doxorubicin (5 mg/kg, 3 times within a week). In this setting, there are significant changes in the expression of growth factors and of signals involved in stress and inflammatory process. One early event is a remarkable dilation of T-tubule membrane system. We therefore investigated molecules synergistically affected at the membrane and T-tubule structure. A group of membrane proteins involved in cytoskeletal pathways (ZO-1, desmin, erbB2) was found reduced in 50% relative to unchanged connexin-43 at the gap junctions and/or delocalized from Z-line and intercalated discs compared to Wt and KO mouse. A broader protein analysis is performed by employing a proteomic approach, initiated by a bidimensional protein separation (2D-PAGE) from membrane extracts. The identity of modified membrane components may reveal relevant underlying mechanisms of the aggravated myopathy.

CB-P24.

AKAP350 KNOCK DOWN INDUCES A DECREASE IN NPM EXPRESSION IN LEUKEMIA CELLS

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AKAP350 is a Golgi and centrosomal scaffolding protein associated with the modulation of microtubule cytoskeleton. Among the proteins scaffolded by AKAP350 to the centrosomes, Ran GTPase and CDK2 modulate the recruitment and release of nucleophosmin (NPM), a key protein in centrosomal duplication. Interestingly, 60% of normal karyotype acute myeloid leukemia (AML) present an isoform of NPM with changes in its centrosomal targeting domain (NPM_{c+}), representing the AML NPM_{c+} a primary event of AML. Our aim was to study if AKAP350 modulates NPM expression, and to analyze if this has a differential impact on AML cells expressing NPM_{wt} (THP1) and NPM_{c+} (OCI).We isolated centrosomes, and found that NPM centrosomal location did not differ between THP1 and OCI cells. We generated cells with decreased AKAP350 expression (A350-) by RNA interference. A350- cells exhibits a decrease in NPM levels in both cell lines (OCI -26%*; THP1 -34%*). In OCI, but not in THP1 cells, this decrease was associated with lost of cell viability (-9%*) and increase in the number of polynucleated cell-events (control: 0,95±0,58%; A350-: 3± 0,33%*). Our results indicate that AKAP350 down-expression induces a decrease in NPM levels that is specifically associated with a disorder in cell division in NPM_{c+} cells. These observations suggest alterations in centrosomal biology in NPM_{c+} AKAP350- cells. *P<0,05.

CB-P25.
DEHYDROLEUCODINE ARRESTS HeLa CELLS IN G2 PHASE
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Dehydroleucodine (DhL) is a sesquiterpene lactone isolated from the medicinal herb *Artemisia douglasiana*. The cell cycle is a collection of ordered processes that results in the duplication of the cells. The sequential phases G0/G1, S, G2 and M represent the major cell cycle states. Previous results from our laboratory showed that DhL delays the proliferation of HeLa cells. Our goal was to analyze in HeLa cells the cell cycle phase in which DhL arrests HeLa cells using flow cytometry and also to determinate the expression of cyclin B by western blot. For this propose $50x10^4$ cells in quiescence were stimulated with 10 % fetal bovine serum (FBS) with and without 4 µM DhL, 24 h The analysis of cell cycle phases indicated that at 24 h of stimulation 4,6% ± 1,0 (% ± SEM) and 11,1% ± 2,2 of the cells were found in G2 phase in the control and treated respectively. The statistical analysis indicated significant difference between the control and treated cells (p=0.05). The determination of cyclin B showed a reduction of the levels in treated cells. These results indicate that DhL induces accumulation of HeLa cells in G2 phase and inhibits the expression of cyclin B.

CB-P26.
NOVEL INVOLVEMENT OF NFkB SUBUNIT p65 IN THE LPS-STIMULATED NIS GENE EXPRESSION
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Bacterial lipopolysaccharide (LPS), an endotoxin from Gram-negative bacteria, exerts a variety of biological responses. The Na^+/I^- Symporter (NIS)-mediated iodide uptake is the main rate-limiting step in thyroid hormonogenesis. We have reported the ability of LPS to stimulate TSH-induced NIS expression. The aim of this work was to analyze the molecular mechanism involved in the LPS action in the thyroid cell line FRTL-5. LPS increased NIS mRNA and protein expression. TSH-induced transcription of the transfected rat NIS promoter was activated by LPS. Testing internal deletions of the promoter, we defined the NIS upstream enhancer (NUE) as responsible for the LPS stimulatory effect. NUE contains two Pax8 binding sites. Site-directed mutagenesis showed a critical role of Pax8 C site in LPS action. Bioinformatics analysis showed a novel conserved site for NFkB in NUE. Functional blockage of NFkB signaling antagonized LPS effect. Mutagenesis of NFkB site abrogates LPS stimulation. Co-expression of Pax8 and p65 in HeLa or Cos-7 cells synergistically activates NIS promoter transcription. A physical interaction between Pax8 and p65 was evidenced. Furthermore, ChIP experiments confirmed that NIS is a novel target gene for p65 transactivation in response to LPS. Our results thus reveal a new mechanism of p65 in regulating thyroid cell differentiation by a functional interaction with Pax8.

CB-P27.
MODIFICATIONS OF GLUCOCORTICOID STATUS PROMOTED BY PORPHYRINOGENIC DRUGS
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Hepatic enzyme phosphoenolpyruvate carboxykinase (PEPCK) is regulated by glucocorticoids (GC). Treatment with porphyrinogenic drugs, such as 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) and 2-allyl-2-isopropylacetamide (AIA) produces a gluconeogenic blockade at rat hepatic PEPCK level. Female Wistar rats were fasted during 8 h previously to the administration of DDC (50 mg / kg bw) and AIA, (100, 250 or 500 mg / kg bw) and sacrificed 16 h later. We studied the corticosterone (B) plasma level and its adrenal synthesis as function of drug dose, evaluating its relationship with PEPCK activity. Results show that treatment elicited a loss in the plasmatic levels of B being these decreases more marked in rats treated with the higher doses compared to controls: (4.1 ± 0.4 vs 10.2 ± 1.0) µg /100 mL) while the PEPCK showed a loss of about 40%. A disruption in the synthesis of B in the adrenals of treated animals was also observed. In porphyrin pathway, the results confirm an increase in liver ALA-S and a diminution in ferrochelatase activity. Response of GC in relation to PEPCK allows us speculate that the decline in activity of this enzyme could be the result of hormone alteration. Response of GC in relation to PEPCK allows us to speculate that the decline in activity of this enzyme could be the result of hormone disruption in this toxic acute porphyria model.

CB-P28.
SWELLING AND NAD(P)H DEPLETION INDUCED BY DIFFERENT BACTERIOCINS ON RAT HEART MITOCHONDRIA
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Mitochondria are considered to play a central role in apoptotic cell death. The opening of the "mitochondrial permeability transitions pore" (MPT) is known to occur under conditions of oxidative stress and matrix calcium overload. Next, mitochondria swell and release cytochrome c from the intermembrane space which is a critical early event in mitochondrial mediated apoptosis. In the present work we studied the effect of different bacteriocins: microcin E492, microcin V and enterocin CRL35 on mitochondrial swelling and oxidation of NAD(P)H. The swelling was studied following the absorbance at 540 nm and NAD(P)H oxidation by measuring its intrinsic fluorescence at 450 nm. These experiments were performed with succinate "activated" mitochondria in the presence or absence of either rotenone (the complex I inhibitor), cyclosporine A (a MPT inhibitor) or ascorbic acid as an antioxidant agent. We observed that all bacteriocins assayed, induced mitochondrial swelling and the oxidation of NAD(P)H, in a concentration dependent way. Effects that were inhibited by acid ascorbic and cyclosporine A. Our findings allow us to conclude that these peptides work through ROS generation.

CB-P29.

STUDY OF HUMAN PREGNANCY-SPECIFIC GLYCOPROTEIN GENE EXPRESSION DURING TROPHOBLAST DIFFERENTIATION

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Human pregnancy-specific glycoproteins (PSG) gene family is composed by 11 members with above 90% sequence similarity. Their expression is an early biochemical marker of trophoblast differentiation and reduced levels of PSG proteins are associated with poor pregnancy outcome. In this report we demonstrate a differential expression pattern for individual PSG genes by qPCR in two differentiation models, choriocarcinoma JEG3 cells and primary trophoblasts. Transient transfection assays of PSG genes constructs revealed that the promoter region governs differentiation-dependent activation in isolated primary trophoblasts. Meanwhile, the 5' proximal regulatory region is required to mediate transcriptional activation in JEG3 cells induced to differentiate. Epigenetic regulation of PSG genes was evaluated by treating JEG3 cells with histone deacetylases inhibitors, trichostatin A and sodium butyrate. Both reagents increase endogen PSG transcripts and protein production in a dose-dependent manner, as determined by qPCR and immunofluorescence. We conclude that PSG gene family members are transcriptionaly activated during trophoblast syncytialization reaching different expression levels among them. This activation involves regulatory elements and changes in trans-acting factor levels, as well as modifications in chromatin structure.
Supported by CONICET, FONCyT and SECyT-UNC.

CB-P30.

EXPRESSION AND LOCALIZATION OF KRÜPPEL-LIKE TRANSCRIPTION FACTOR 6 IN DIFFERENTIATING TROPHOBLAST

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Kruppel-like factor-6 (KLF6) is involved in differentiation, cell cycle control and proliferation in several cells. Klf6-/- knockout mice die by embryonic day 12.5 and are characterized by reduced hematopoietic differentiation in yolk sacs and impaired placental development. KLF6 is expressed in human term placenta, however little is known about its expression during trophoblast differentiation and its role in transcription of placenta specific genes. qRT-PCR and western blot revealed an increase in KLF6 expression which preceded morphological differentiation of cytotrophoblasts and Jeg3 cells. KLF6 alone or in cooperation with Sp1, activated promoter constructs of Pregnancy-specific glycoproteins genes, which are biochemical markers of trophoblast differentiation. Overexpressed KLF6 had a clear nuclear signal, while endogenous KLF6 was localized to the nucleus but mainly to the cell cytoplasm suggesting association with cytoskeletal filaments. No substantial change in subcellular distribution profile was observed in trophoblast cells induced to differentiate or cultured in hyperglycemia conditions. In contrast, KLF6 pattern was clearly modified in Jeg3 cells undergoing mitosis. These data are in line with a putative role of KLF6 in differentiation, proliferation and transcriptional regulation of placenta specific genes in trophoblast.
Supported by CONICET, FONCyT and SECyT-UNC.

CB-P31.

INSIGHTS ON GR ACTIVITY MODULATION THROUGH THE BINDING OF RIGID STEROID ANALOGS

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In previous works, we used molecular dynamics simulations to investigate the molecular basis of action of the selective antiglucocorticoid 21-Hydroxy-6,19-epoxyprogesterone, 21OH-6,19OP and the partial glucocorticoid receptor (GR) agonist 21-Hemisuccinate-6,19-epoxyprogesterone, 21HS-6,19OP. The simulations of GR Ligand Binding Domain (GR-LBD) suggest that the GR-LBD-21OH-6,19OP complex is not able to homodimerize. On the other hand, the presence of the bulky hemisuccinate group induced a significant displacement of the average position of Helix 12 in the GR-LBD-21HS-6,19OP complex, possibly altering cofactors binding ability. In order to confirm or rule out the simulation predictions we performed experiments on living cells. First, we used a GFP-GR chimera to show that the rigid steroid analogs were able to induce GR nuclear translocation. Second, we performed transactivation assays in the presence or absence of the co-activator TIF2. Results suggest that the partial agonist 21HS-6,19OP recruits TIF2 to GR while the transactivation antagonist 21OH-6,19OP does not. Third, we performed transrepression assays to confirm the ability of both steroid analogs to activate GR heterodimerization. Finally, we are currently studying the aggregation state of GR upon steroid binding through N&B analysis, a technique that will allow us to investigate GR dimerization state *in vivo*.

CB-P32.

BIOCHEMICAL MECHANISMS UNDERLYING PESTICIDE EFFECTS IN TOAD EMBRYO DEVELOPMENT

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The organophosphorus azinphosmethyl (AZ) is widely used for pest control. The evaluation of pesticide impact should also include studies on developmental alterations. We use amphibian embryonic development as a model in order to study the underlying mechanisms of AZ leading to teratogenesis. Toad (*C. arenarum*) embryos were exposed to 0.5-9 mg/L AZ from fertilization to operculum completeness (OC). Oxidative stress evidences are gradually observed during exposure. GSH content (measured by acid-soluble thiols) declines significantly (62%) at OC. GSH-dependent-(S-transferase (GST), peroxidase and reductase) enzyme activities are early induced by low AZ concentrations (23%, 39% y 66%), but inactivated by high oxidative stress at OC and higher concentrations. AZ also increases ornithine decarboxylase (ODC) activity (18X at OC) and affects polyamine (PA) levels: putrescine increases (60%) while spermidine and spermine decrease (56% and not detectable, by HPLC), probably through polyamine oxidase metabolism. PA catabolism contributes to the oxidative stress induced by AZ. GSH depletion is related to the degree of Protein Kinase C activation at OC (55% over basal histone phosphorylation). PKC may participate in the control of GST expression through Nrf2-ARE pathway, and ODC upregulation. Oxidative stress and PA depletion are underlying the observed alterations in embryo development.

CB-P33.

ALTERATIONS IN IRON AND NITRIC OXIDE PLASMATIC CONTENT IN ENDOTOXEMIA SEEMS AS INDEPENDENT EVENTS

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The aim of this work was to identify the possible link between the increase in nitric oxide (NO) and the plasma Fe content in endotoxemia. Female Sprague-Dawley rats were injected ip with saline solution (C) or LPS (4 mg/kg) and measurements were done 6 h after injections. Blood NO level, detected by EPR as the complex NO-Hb, was significantly higher in LPS as compared to C animals (22±2 and 0.8±0.5 AU respectively, $p<0.05$). Plasma Fe content (Fer-Color, Wiener lab) was 42% lower in LPS than in C, while labile iron pool (LIP), measured by the fluorescent probe calcein, was not significantly different between C and LPS (0.9±0.1 and 1.2±0.4 µM respectively). Protein carbonyl content and the ratio ascorbyl radical content/ascorbate content were increased by 28% and 73% in LPS treated animals as compared to C. No changes were observed in the level of thiols in plasma. Aminoguanidine (AG, a NO synthase inhibitor, 100 mg/kg, ip) inhibited by 68% the NO increase observed after LPS, but did not affect the decrease in plasma Fe. LIP was 1.4±0.2 and 1.5±0.3 µM after AG and AG+LPS treatments, respectively. These results suggest that Fe decrease would be independent of NO increase in circulation during endotoxemia and the increases observed in the oxidative stress markers in plasma after LPS administration seems not to be associated to LIP content.

Supported by UBACyT, ANPCyT and CONICET

CB-P34.

IRON, NITRIC OXIDE, AND OXIDATIVE METABOLISM IN *Mya arenaria*

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The main objective of this work was to analyze Fe role and oxidative and nitrosative metabolism in *Mya arenaria*. *M. arenaria* samples were collected in the German Wadden Sea. The ratio Ascorbyl radical ($A^{\cdot}$)/ascorbate content (AH^-) is understood as an index of oxidative stress. $A^{\cdot}$ content, assessed by quantification of electronic paramagnetic resonance (EPR) signals, was 0.04±0.01 pmol/mg FW, and the AH^- content was 478±12 pmol/mg FW. The ratio $A^{\cdot}/AH^-$ was (8±1) 10^{-5} AU, suggesting a minimum oxidative stress even under physiological conditions, presumably depending on basal metabolic functions. Total Fe content in *M. arenaria* was 1.9±0.7, 0.7±0.1 and 0.17±0.01 nmol/mg FW, in digestive glands (DG), mantle and gills, respectively. Labile Fe pool (LIP), assessed by EPR in DG, was 146±10 pmol/mg FW, and by employing calcein was 118±9 pmol/mg FW. The lipid radical content in the DG, assessed by EPR, was 27±1 pmol/mg FW, and the thiobarbituric reactive substances content amounted to 57±8 pmol/mg FW. The content of nitric oxide (NO) in DG was 99±3 pmol/mg FW. The generation rate of NO by nitric oxide synthase-like activity (assayed as NO production by EPR) was 3.16±0.06 pmol/mg FW DG/min. Further studies are required to analyze the effect of environmental stress conditions. The data presented here document the presence of highly reactive species in *M. Arenaria*.

CB-P35.

WM35 MELANOMA CELLS EXPRESS HISTAMINE H4 RECEPTOR. BIOLOGICAL RESPONSES ASSOCIATED TO THIS RECEPTOR

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Malignant melanoma is a rapidly spreading skin tumour with a very high invasive capacity and an incidence that continues increasing globally. Melanoma cells but not normal melanocytes contain large amounts of histamine that has been found to accelerate malignant growth. Additionally the three subtypes of histamine receptors, H1, H2 and H3 have been described in diverse human melanoma cells.
The aim of this work was to investigate the presence of H4 histamine receptor (H4R) in WM-35 cells (human primary melanoma cell line) and its associated biological processes. The expression of H4R was analyzed by RT-PCR and immunocytochemistry. To characterize the biological responses we evaluated cell proliferation by the clonogenic assay and cell counting, and also migration by the wound healing assay. Results indicated that WM-35 cells express H4R at the mRNA and protein level. By using specific histamine agonists and antagonists, we determined that histamine decreased proliferation in part through the stimulation of the H4R (70%).
In addition, the activation of the H4R produced a 2-fold decrease in migration capability. To our knowledge this is the first report that describes the presence of the H4R in melanoma cells. We conclude that the H4R is involved in the regulation of cell proliferation and migration, which are key processes in tumor progression.

CB-P36.

1á,25(OH)$_2$D$_3$ AND ANALOG INHIBIT THE GROWTH OF TRANSFORMED (KAPOSI SARCOMA) ENDOTHELIAL CELLS

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The Kaposi sarcoma-associated herpesvirus G protein-coupled receptor (KSHV-GPCR) is a key molecule in the pathogenesis of Kaposi sarcoma. In this work we have studied the effects of 1á,25(OH)$_2$D$_3$ (1,25D) and its TX527 analog on the proliferation of endothelial cells (EC) and transformed by viral GPCR (vGPCR). Proliferation assays showed that 1,25D and TX527 similarly decreased EC-vGPCR and EC cell number. VDR protein levels were induced by 1,25D or TX527 in time and dose-dependent fashion. In addition, basal VDR levels were increased in EC-vGPCR. The antiproliferative effects were also accompanied by a reduction in cyclin D1 levels and an accumulation of p27 monitored by WB analysis in EC but not in endothelial vGPCR. Induced VDR protein levels were blocked by stable transfection of shRNA against VDR in EC-vGPCR and the antiproliferative effect was decreased, demonstrating, that at least in part, the genomic pathway through VDR is involved in hormone and TX527 mechanism of action. In vivo experiments have shown the ability of 1,25D and TX527 to decrease EC-vGPCR tumor progression when the tumor cells were implanted in female nude mice. Although 1,25D was more successful than TX527 to inhibit tumor growth in vivo; TX527 was less calcemic and makes it more suitable for its potential use as therapeutic strategy for Kaposi sarcoma treatment.

CB-P37.

REGULATION OF BAP2 TRANSPORT ACTIVITY INTO THE YEAST *Saccharomyces cerevisiae*

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Amino acid transport in the yeast *Saccharomyces cerevisiae* is mediated by permeases with broad and restricted specificity. In previous communications, we described the isolation of mutants with altered amino acid transport and also with glycosylation reactions deficiency. Mutants were isolated by their resistant to Sodium Vanadate salt and their growth is inhibited by the antibiotics hygromycine and gentamicine. Six out of twenty mutants present a partial resistance to the toxic analog of L-leucine: trofluoroleucine (TFL). In this work, we developed western blot assays of such mutants, with an antibody (WBP1), raised against a subunit of yeast oligosaccharyltransferase (OST1) which indicates changes into the glycosylation levels in the endoplasmic reticulum. Complementation of these mutants with a genomic library enabled us the isolation of transformants with increased resistant to hygromycine. We also transformed four of the isolated mutants with a plasmid containing the BAP2 gene with a HA tail. The BAP2 permease constitutes a transporter for branched chain amino acids and L-threonine. The expression of the transporter in these mutants was studied by western blot analysis. Results indicate a correlation between the glycosilation deficiency and BAP2 activity for L-leucine transport.

CB-P38.

BIOCHEMISTRY STUDENTS' MISCONCEPTIONS RELATED TO SYSTEMIC INTEGRATION OF CARBOHYDRATE METABOLISM

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Carbohydrate metabolism is a central subject inside Biochemistry. An integral comprehension of this theme should include biochemical processes considering the organism as a whole.Our objective was aimed to explore the level of systemic comprehension achieved by students of Biochemistry in both Biology and Chemistry careers of the Faculty of Exact and Natural Sciences at the University of Buenos Aires. We use three different methodologies in order to validate the results: a) a questionnaire that confirmed the variables to investigate (n=171 students), b) concurrent and recurrent interviews (n=123 students), framed inside the model of Information Processing System, c) an open answer problem (n=123 students) to measure entropy as a parameter of the information dispersion. This last approach fits into the Mathematical Communication Theory and considers entropy as the pool of possible alternatives of answers for a specific situation.Results obtained by these methodologies suggest a new way to analyse the misconceptions of the biochemical processes and enabled us to clarify students' misunderstanding when the reasoning is not systemic, which is impossible to detect with the traditional evaluation tools. We also showed that the low entropy found in many answers do not demonstrate that students have much knowledge or use it in a right way, which questions the conclusions of previous works.

CB-P39.

ISOLATION AND CHARACTERIZATION OF MUTANTS SUPPRESSING AMINO ACID PERMEASE DEFECTS IN SHR3 STRAINS

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Ljungdahl *et al*. (1992) identified Shr3p as an endoplasmic reticulum protein that serves as a molecular chaperone by ensuring that nascent amino acid permease proteins fold correctly before being transported to the plasma membrane.
Ethylmethanesulfonate (EMS) was used to isolate suppressor mutants csu,(chaperone suppressor, mutants) from the two strains, RSY1383 and RSY1394, that carry disruptions in the SHR3 gene. EMS-treated cells were plated on minimal proline medium and colonies of proline-positive mutants we isolated as putative suppressor mutants. These strains were tested for suppression of two mutant traits: [1] Growth on proline as sole nitrogen source and [2] loss of resistance to canavanine. Although all six csu mutants of each shr3 disruption strain retained their ability to utilize proline as nitrogen source, only strains1383/csu6, 1394/csu4 and 1394/csu6 showed loss of resistance to canavanine. Therefore, there are at least two types of suppressor stains: [1] strains that show suppression of more than one aspect of shr3 phenotype and [2] strains that show selective suppression of one or more mutant trait. Because the csu mutants were produce by EMS treatment, it is unlikely that they are allelic to the multicopy suppressors isolated by Kota J, *et al*. (2007); Ljungdahl PO, *et al*. (1992); Cell 71(3):463-78; Kota J, *et al*. (2007); J Cell Biol 176(5):617-28.

CB-P40.

RETINOIC ACID AND *Xenopus laevis* MESODERM PATTERNING

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During *Xenopus* gastrulation different regions can be recognized in the mesodermal layer: head mesoderm, chordamesoderm, paraxial mesoderm and lateral plate mesoderm. Head mesoderm produces cephalic structures while paraxial mesoderm delivers somites. Although both mesoderm region give rise to the same tissue types (muscles and bones) they show distinct developmental programs. In order to elucidate the molecular setup of the head mesoderm and its relation to paraxial mesoderm we analyzed the expression of different molecular markers along the head-trunk patterning. We found head mesoderm is molecularly different from paraxial mesoderm. Only head mesoderm expresses *Tbx1*, *Pitx2* and *Nkx2-5*, while paraxial mesoderm molecular setup is represented by other markers as *pMesogenin1*, *Tbx6* and *PCNS*. Given that Retinoic Acid (RA) is involved in the patterning of the somitic mesoderm and considering that head mesoderm does not form somitic structures it is possible that head mesoderm is RA free mesoderm. To prove this hypothesis we analyzed the expression pattern of *RALDH2* (RA synthesis enzyme) and *Cyp26* (RA hydroxylase). *Cyp26* is expressed in head mesoderm and its overexpression produce alterations in the head-trunk mesoderm patterning. In avian embryos *Cyp26* is also only expressed in head mesoderm. These findings led us to suggest that the head-trunk molecular pattern is evolutionary conserved

CB-P41.

CELL-ADHESION GLYCOPROTEIN VITRONECTIN DURING *Xenopus laevis* EMBRYOGENESIS

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Vitronectin (VN) is a cell-adhesive glycoprotein present in blood and extracellular matrix of all vertebrates. Interestingly, we reported the presence of VN in yolk platelets during amphibian oogenesis. We demonstrated that maternal VN does not participate in the morphogenetic movements during amphibian gastrulation. We cloned *Xenopus* VN cDNA in order to determinate the zygotic expression. The temporal expression of VN was analyzed by RT-PCR, and the transcripts were detected from stage 28 onward. The spatial expression was determined by whole mount *in situ* hybridization. At tadpole stages, VN is expressed in heart, liver and foregut. The immunohistochemistry staining revealed that VN was located in heart, liver, foregut and pronephros at stages 43 and 47 of *Xenopus* embryos. In adult tissues, VN transcripts were observed in liver and kidney. In coincidence with our results, VN was found in the developing chick heart and was involved in the migration of endocardial cells. Also, VN and Sonic Hedgehog (Shh) morphogen act synergistically in the induction of avian motoneurons in embryonic neural tube. However, we have not found VN and Shh colocalization in amphibian embryos. The temporal and spatial VN expression in amphibians let us to suggest that this extracellular matrix glycoprotein could participate during organogenesis, however further work is required to precise its role.

CB-P42.

Wnt-6 AND Wnt11-R REGULATE *PARAXIS* EXPRESSION IN *Xenopus laevis* PARAXIAL MESODERM

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In all vertebrates the Paraxial Mesoderm (PM) segmentation involves the formation of metameric units called somites through a Mesenchymal-Epithelial Transition (MET). However this morphogenetic process is different in the amphibian *Xenopus laevis* because it does not form an epithelial somite but rather undergoes a complex cell arrangement. The molecular mechanisms that control these cell behaviours underlying somite formation remain elusive. *Paraxis* is a bHLH transcription factor expressed in vertebrate PM and later is localized in the somite. In mouse and chick embryos their expression is regulated by Wnt signalling pathway and is implicated in the somite MET. In order to establish if Wnt signalling pathway regulates *paraxis* expression and is involved in the rearrangement of *Xenopus* PM, we carried out experiments using an inhibitor of the Wnt signaling pathway, the Valproic Acid (AV). *In vivo* experiments show that AV produces a decrease of the *paraxis* expression in treated embryos. Also we carry out experiments of gain function by Wnt-6 and Wnt11-R mRNA microinjection. We find that the overexpression of these molecules produces an increment of the *paraxis* expression, suggesting that this gene could be regulated by Wnt-6 and Wnt11-R signal molecules.

CB-P43.

DIFFERENTIAL RESPONSE TO RETINOIDS IN A TUMOUR MAMMARY CELL LINE. EVIDENCE OF STEM/PROGENITOR CELLS

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Retinoids (Rds) regulate cell proliferation, diferentiation, apoptosis and affect oncogenesis through binding to RAR/RXR receptors. LM38-LP, a murine mammary tumor cell line composed by luminal (LEP) and myoepithelial (MEP) cells, expresses all RAR/RXR. We studied the mechanisms involved in Rtds effects in LEP/MEP interactions. We confirmed system functionality by using a RARE-luciferase gene reporter. Rds affected proliferation, survival and apoptosis in a differential way between cell lineages in LM38-LP. We found a decrease in pAkt and pErk expression (confocal/WB) and a higher number of senescent cells (SA-ß Gal activity), mainly in MEP. Rds modulated myoepithelial marker SMA and ALDH activity (cytometry), suggesting the existence of both stem and progenitor cells with various differentiation states and sensitivity to Rds. Previously we found that Rds inhibit proteases secretion (uPA and MMP-9 activity) and RARa antagonist Ro415253 seemed to act as a Rtd. Now we confirmed that activated RARs transrepressed these promoters by using an AP1-luc gene reporter. Surprisingly we detected that the antagonist induced the same decrease in AP1-luc activity as Rtds did, suggesting Ro415253 could work as agonist on AP1 sites. These results describe mechanisms involved in differential responses of LEP and MEP cells to Rds and suggest the existence of stem and progenitor populations in LM38-LP.

CB-P44.

APOPTOSIS INDUCED BY CALCITRIOL AND DL-BUTIONINE-S,R-SULFOXIMINE IN BREAST CANCER CELLS

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Breast cancer is one of the most frequent neoplasias. Calcitriol diminishes cancer risk and constitutes an alternative therapy to the usual with antiestrogens. The aim of the present work was to study the molecular basis of the anticancer effect of calcitriol in combination with DL-buthionine-S,R-sulfoximine (BSO), a glutathione (GSH) depleting drug, in order to potentiate the antiproliferative effect of the secosteroid on breast cancer cells (MCF-7). Cultured cells were treated for 96 hs with calcitriol (100 nM), BSO (20 µM), calcitriol + BSO or vehicle. Cell proliferation was evaluated by crystal violet staining while GSH content and the activity of the antioxidant enzymatic system, superoxide dismutase and catalase, by spectrophometry. Either calcitriol or BSO alone significantly reduced cell proliferation, being maximal with the combined treatment. Calcitriol induced reactive oxygen species (ROS) formation, which was potentiated by BSO. DNA fragmentation and citochrome c release were induced by the steroid and the combination. Total levels of GSH were decreased. The antioxidant enzymes were not altered by any treatment. In conclusion, BSO potentiated growth inhibition of calcitriol on breast cancer cells by apoptotic mechanisms involving ROS production and mitochondrial disruption.

CB-P45.
A STABLE INDUCIBLE CELL LINE: ROLE OF ACYL-COA SYNTHETASE IN BREAST CANCER CELLS

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Previously we demonstrated that the levels of an acyl-CoA synthetase (ACS4) correlate with aggressive cellular phenotype measured by cellular proliferation, migration and invasion. Taking MCF-7 and MDA-MB-231 cell lines as models of non-aggressive and aggressive human breast cancer, we showed that the aggressiveness of the cells changes by knocking down the expression of ACS4 by transient transfection of siRNA in MDA-MB-231 cells or by ACS4 overexpression in MCF-7 cells. Here we confirm these results generating a stable inducible cell line of MCF7, where overexpression of ACS4 can be switched off by tetracycline. MCF-7 cells were transfected with a regulator plasmid (pTet-Off) containing the tetracycline controlled transactivator and neomycin resistance. The selected clones were transfected with a responsive plasmid (pTRE) containing ACS4 cDNA and puromicin resistance. In the resulting stable cell line, ACS4 levels were 14-fold higher than MCF-7 non transfected or tetracycline treated cells. ACS4 levels correlated with the aggressiveness of the stable cell line measured by cellular proliferation and migration. ACS4 is part of the mechanism of COX-2 induction, an enzyme that plays a critical role in cancer progression. The combined use of inhibitors of both enzymes could be a potential approach of breast cancer treatment reducing the non desirable effect of COX-2 inhibitors.

CB-P46.
DIFFERENT CELLS SUBPOPULATIONS IN THE RGC-5 CELL LINE: MORPHOLOGY AND CALCIUM RESPONSES TO ATP

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RGC-5 cells are a new cell line derived from postnatal rat retinal ganglion cells (Krishnamoorthy R. et al., 2001, Mol. Brain Res). Although the RGC-5 cells are derived from a single clone; we investigate whether these cells constitute a morphologically homogeneous population. A least tree morphologically different populations of non differentiated RGC-5 in culture were identified by flow cytometry and immunocytochemistry. RGC-5 cells show differential calcium responses to ATP stimulation, some of them, exhibit a high amplitude positive response; other cells display a low amplitude positive response, while a third population shows a null response. These differences could be a direct consequence of heterogeneous expression of different ATP receptors in these cells. Surprisingly, RGC-5 cells do not respond to glutamate stimulation (100 and 200 ì M). Staurosporine administration (25ng/mL) induces morphological differentiation that correlates with a change in the flow cytometry pattern at 24 h post staurosporine administration. Interestingly, after 48 h of staurosporine treatment, cells died by apoptosis. The results strongly support the idea that this cell line is not a homogeneous population and this observation may result an important feature to be considered for future studies and for analyzing biochemical responses in these cells.

CB-P47.
SOLUBLE LYSOSOMAL HYDROLASES TRANSPORT IN THE PRIMITIVE EUKARYOTE *Giardia lamblia*

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Unlike yeast and mammalian cells, the lysosomal sorting pathway is not well defined in *Giardia*. For instance, in more evolved cells, soluble hydrolases are sorted from the trans-Golgi network to the endosomal/lysosomal system through a receptor-adaptin mediated process. We found that delivery of the soluble acid phosphatase (AcPh) to the lysosome-like peripheral vacuoles (PVs), involved an adaptin-clathrin dependent pathway. These strongly suggest that *Giardia* possesses a hydrolase receptor for PV delivery. Thus, we performed pull-down assays using a His-tagged AcPh to search for the hydrolase receptor. One of the associated proteins obtained contains a YQII lysosomal-sorting motif necessary for Adaptor Protein 1 (gAP1) binding. By fusion protein expression in transgenic trophozoites together with IFA and confocal microscopy, we found that this putative *Giardia* Hydrolase Receptor (gHR) colocalizes with AcPh around the nuclei and in the PVs. “In vivo” interactions of gHR with AcPh or gAP1 were confirmed by yeast two-hybrid and verified by IPP assays. In addition, ongoing experiments with PV-residents Cathepsin B, revealed that gHR might be a common hydrolase receptor. These studies will help to determine the minimal machinery necessary for lysosomal trafficking developed throughout eukaryotic evolution or whether it is a consequence of *Giardia* parasitic life style.

CB-P48.
ARGININE DEIMINASE SUMOYLATION: A NOVEL POST-TRANSLATIONAL MODIFICATION IN *Giardia lamblia*

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The protozoan parasite *Giardia lamblia* utilizes arginine deiminase (gADI) to produce energy from L-arginine under anaerobic conditions, a pathway restricted to prokaryotic organisms. Recently, analyzing the protein expression of gADI we found several bands including a major 85 kDa band instead of the predicted 64 kDa, that indicates a possible post-translational modification. Phosphorylation was predicted in several positions as well as a high probability of modification by the 11kDa SUMO-1 protein (small ubiquitin-related modifier protein). Immunoblotting experiments using anti-SUMO mAb detected an 85 kDa protein that corresponds to the gADI higher band. The ability of anti-SUMO mAb to immunoprecipitate the 85 kDa band of gADI confirmed the gADI-SUMO interaction. A predicted function of sumoylation of proteins is the cytoplasm/nuclei translocation, and we found that during differentiation of the parasite mainly the 85 kDa form of gADI translocate to the nucleus, inducing the downregulation of the encystation process. Further research into the possibility that gADI function could be regulated by sumoylation may provide insight into the biology of this important intestinal parasite and also contribute to the understanding of the evolution of protein modification in eukaryotic cells.

CB-P49.

EFFECT OF CALCIUM ON THE INTRACELLULAR LOCALIZATION OF *Trypanosoma cruzi* CALRETICULIN

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Calreticulin (CRT) is an abundant protein of the endoplasmic reticulum (ER) that works as a lectin-chaperone. In addition, CRT is one of the main intracellular calcium buffers. This activity depends on a highly acidic C-terminal domain that binds ~20 calcium ions with low affinity. Although CRT displays a hydrophobic export peptide and an ER retention-retrieval sequence, numerous reports have found it in the cytosol and nucleus. This anomalous localization can be accounted for by two mechanisms: (i) a suboptimal signal peptide or, (ii) the retrotranslocation of CRT from the ER lumen. It has been found the cytosolic localization of CRT depends on its C-terminal domain. Here we show that ER calcium depletion triggers a three fold increase of cytosolic CRT. This change was also observed in parasites incubated with cicloheximide, suggesting that CRT appears in the cytosol after being retrotranslocated from the ER. Moreover, a truncated form of CRT lacking the C-terminal domain remained in the ER regardless of its calcium content. This observation suggests a novel mechanism to regulate intracellular protein sorting by changes in the ER calcium concentration.

CB-P50.

ENDOPLASMIC RETICULUM CALCIUM REGULATES THE RETROTRANSLOCATION OF LUMENAL PROTEINS

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Calreticulin (CRT) is an abundant protein resident of endoplasmic reticulum (ER) that works as a lectin-chaperone and is one of the main intracellular calcium buffers. Although CRT displays an export peptide and an ER retrieval signal, several reports have found it in the citosol. Two theories account for this anomalous localization: (i) a suboptimal signal peptide that results in a poor initial translocation into the ER or, (ii) the retrotranslocation of the protein from the ER lumen. Previous works show that cytoplasmic localization of CRT depends on its C-terminal region. This 70 residues-long domain is rich in negative residues, binds ~20 calcium ions with low affinity and is responsible for the calcium buffering activity of CRT. By using CD and FRET experiments we found that calcium induces a more ordered and compact conformation of the C-terminal domain. In addition, ER calcium level affects the intracellular distribution of CRT. Digitonin permeabilization assays in HEK293 and COS 7 cells show that inhibition of the ER calcium pump results in a three fold increased of citosolic CRT. A similar result was observed in the presence of cicloheximide, showing that the increment of citosolic CRT results from its retrotranslocation from the ER. We speculate that this process could be exploited by the cell to integrate diverse signal transduction pathways.

CB-P51.

POLIQ HUNTINGTIN AFFECTS CCV ENDOCYTOSIS BY ALTERING DISTRIBUTION OF AP-2 IN STRIATAL NEURONS

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Clathrin coated vesicle-mediated endocytosis (CCVE) is known to be a mechanism by which neurons control diverse events. In brain, CCVs are required for recycling of membrane proteins after synapsis. Among several coat proteins, AP2 appears to contribute to the selectivity in the CCVE. In turn, huntingtin (htt) is a cytoplasmic protein that interacts with HIP1 involved in vesicle trafficking. Polyglutamine expansion (poliQ) in htt is responsible for the neuronal toxicity associated with Huntington´s disease (HD), that involves dysfunction and death of neurons, particularly the medium spiny neurons of the striatum. We proposed to evaluate if the expression of poliQhtt alters the levels and/or distribution of coat proteins. By Western blot, we evaluated the distribution of AP2, dynamin and HIP1 between cytosol and membranes from different brain areas in transgenic mice expressing poliQhtt (HD94) and in striatal cell lines established from wild-type and HdhQ111 kock-in embryos (Q111). We observed a decrease in AP2 bound to membranes in the striatum of HD94 mice and Q111 cells. No changes were observed in expression and distribution of the other coat proteins. Likewise, the redistribution of AP2 to cytosol affected the endocytosis of transferrin in Q111 cells. These results suggest that poliQhtt affects the normal distribution of AP2 and it may contribute to the pathogenesis of HD

CB-P52.

MIGRATION CHARACTERISTICS OF PERITUBULAR MYOID CELLS FROM SHR RATS

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Peritubular myoid cells (PMC) form a layer that surrounds the seminiferous tubules (ST) into the testis and these cells have characteristics of smooth muscle cells. Hypertension affects vascular smooth muscle cells (VSMC) proliferation and migration. In our laboratory we have seen that PMC from testis of SHR rat (hypertensive rat) are more proliferative than PMC from testis of WKY rat (control rat). We analyzed whether PMC migration is affected by hypertension. PMC from WKY and SHR rats were isolated and cultured in DMEM/F12 with 10% fetal bovine serum (FBS). Migration assays were performed by the scratch wound motility assay. PMC were seeded in 24-well plates and grew to confluence. After 24 h serum deprivation, PMC were incubated in 0.1% FBS with or without Angiotensin II (AngII) or Endothelin-1 (Et-1). Wound closure rates were analyzed by direct microscopic visualization at 0 and 24 h. The assay showed that the migration of CMP WKY was 96.01% ± 4.4 (% ± SEM) and 141.03% ± 10.34 with AngII and Et-1 respectively. While migration of PMC SHR was 140.5% ± 9.67 and 111.7% ± 5.69 with AngII and Et-1 respectively. These results indicate that PMC SHR respond to AngII in different way than PMC WKY. Also, PMC SHR has similar behavior to VMSC SHR showing that PMC, a kind of not vascular smooth cells, are affected by hypertension.

CB-P53.
HIGH-FRUCTOSE INTAKE INDUCE VASCULAR REMODELING IN APOLIPOPROTEIN E DEFICIENT MICE

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Fructose fed (FF) mice provides a model of dietary-induced insulin resistance, which has been used to assess the pathophysiological mechanisms of the cardiovascular changes associated to the metabolic syndrome. We hypothesized that chronic administration of fructose (10% in drinking water) to a murine model of atherosclerosis, apolipoproteína E deficient mice (apoE-/-), which develops hypercholesterolemia, would alter their metabolic profiles and result in arterial wall remodeling. Male ApoE-/- mice and their control C57BL/6 wild type were used (6-8 in each group). After 6 weeks fructose administration metabolic and morphometric variables were measured. Similar high cholesterol level was measured on both FF ApoE-/- and ApoE-/- group, instead, fasting glucose was significantly elevated only in FFApoE-/- group. The media cross-sectional area of vascular wall in the FF ApoE-/- group compared with ApoE-/- was significantly increased (67606,6 μm^2 ± 8908,3 vs 40317,0 μm^2 ± 7126,2 p=0,016) with a trend toward higher media/lumen ratio (1,3 ±0,61 vs. 0,40 ± 0,04). Tissue remodeling by matrix metalloproteinases (MMPs) is postulated to be involved in the pathogenesis of atherosclerosis. We found an increase of MMP-9 expression in FFApoE-/- arterial wall. These data support the hypothesis that hyperglycemia in the setting of elevated cholesterol accelerates the response to vascular injury.

CB-P54.
EFFECTS OF YACON (*Smallanthus sonchifolius* Poepp&Ende) ROOTS IN DIABETIC RATS

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Diabetes mellitus is a disease characterized by abnormalities in carbohydrates and lipids metabolism. In addition to synthetic drugs, several medicinal plants are actually used for their treatment, without proper scientific validation. *Smallanthus sonchifolius* [Poepp. & Ende] H. Robinson (yacon) is a plant originating from South America. Its roots contain low polymerization degree oligosaccharides (FOS) as the main storage saccharides. Several studies have demonstrated that fructans may affect the carbohydrates and lipids metabolism with healthy effects. In this study we examined the effects of 2 month oral administration of yacon roots in streptozotocin-induced diabetic rats. The treatment induced progressive weight loss associated with an important reduction of intraabdominal fat. Yacon supplement had no hypoglycemic effects, but total cholesterol, HDL, LDL and triacylglycerol plasma levels showed positive modifications attributable to yacon administration. Not significantly variation in serum insulin levels was observed, however, by immunocytochemical studies (anti- insulin, anti- proliferate anti- H_3 antibodies) and morphometric studies revealed an increase in pancreatic isolates. These results could be attributed to the high content of FOS in yacon roots. Further studies are necessary to evaluate the mechanisms involved.

CB-P55.
DOWN REGULATION OF GAP JUNCTIONS AND CADHERIN/â-CATENIN COMPLEXES IN DIABETIC SMALL INTESTINE

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Diabetes mellitus is associated with long-term damage of the gastrointestinal tract.To better understand the impact of hyperglycemia on intestinal intercellular communications, we analyzed the expression of gap junctions (GJ) and cadherin/ß-catenin complex in an experimental model of diabetes in rodents. High glucose down-regulated junctions in the mucosa and muscle layers of the intestine at early stages of the disease. Reduced expression of Cx43 and Cx45 proteins and aberrant cytoplasmic localization was determined in the mucosa layer and in the myenteric plexus cells. Results indicated that diabetes produced a Cx43 dephosphorilation. This could lead to an intracytoplasmic localization of this protein with a consequent loss of functional form. In the muscle layer the decreased in gap junction intercellular communication between smooth muscle cells exposed to high glucose was associated with a loss of Cx43 from the plasma membrane, as demonstrated by immunohistochemistry. Functional GJ requires appropriate cell adhesion mediated by the cadherin/ß-catenin complex. Diabetic injury produced a significantly reduction in the cadherin/ß-catenin complex contributing to abnormal intestinal cell-cell interactions. We propose that altered junctional adhesion molecules play a significant role at early stages of diabetic intestinal pathogenesis.

CB-P56.
CADMIUM INTOXICATED WATER MODIFIES ANTIOXIDANT ENZYME EXPRESSION AND ACTIVITY IN THE RAT HEART

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Cadmium-induced oxidative damage has been widely demonstrated by the increase of lipid peroxidation, and inhibition of enzymes required to prevent such oxidative damage. Objective: The aim of this study was to assess the effect of Cd intoxication on the antioxidant enzymes expression and activity, in rat heart. Experimental model: Male Wistar rats were separated into four groups: 1(Controls, tap water), 2, 3 and 4(tap water with 15 ppm Cd ion, during 15, 30 and 60 days, respectively). Food and water were administered ad libitum. Afterwards, animals were decapitated, under light ether anesthesia. Samples were kept at –20ºC, until processed. Methods: Antioxidant enzymes (GPx, CAT and SOD), and NOx expression levels were measured using RT-PCR. GPx and CAT activities were also assessed. Cd2+ levels in heart and serum were measured using atomic absorption spectrometry. Results: Cd2+ levels were elevated in heart, in groups 2, 3 and 4 ($p<0,001$). In serum, group 3 showed the highest levels ($p<0,05$). GPx and NOx expression increased in group 3 ($p<0,05$). SOD expression was higher in groups 2 and 3 ($p<0,05$). CAT expression levels weren't modified. GPx activity diminished in groups 2, 3 and 4, while CAT activity increased in groups 3 and 4. Conclusion: Cd2+ accumulates in heart, in a short exposure time. It causes an imbalance in the antioxidant enzyme system modifying expression and activity.

CB-P57.
ROLE OF GLUCOSIDASE II SUBUNIT IN THE QUALITY CONTROL OF GLYCOPROTEIN FOLDING

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Glucosidase II (GII) is a key player of the glycoprotein folding quality control in the lumen of the endoplasmic reticulum (ER). By this mechanism, misfolded glycoproteins are retained in the ER until proper folding is achieved or the protein is degraded. GII catalyzes the removal of the two innermost Glc residues of the $Glc_3Man_9GlcNAc_2$ oligosaccharides transferred to proteins during N-glycosylation. GII is a soluble protein composed of subunits GIIá and GIIß. GIIá holds the catalytic activity site and it lacks any known ER retrieval sequence. This last sequence is present in GIIß, whose function is controversial. Microsomal fraction prepared from fission yeast *Schizosaccharomyces pombe* mutant cells GIIá$^-$/GIIß$^-$ expressing GIIá with the ER retrieval signal VDEL hydrolyzes the substrate analogue p-NPG. Western blot analysis revealed that GIIá-VDEL is retained in the ER. This demonstrates that in *S. pombe* GIIß is responsible for GIIá retention in the ER but not for its folding or maturation. *In vivo* oligosaccharide labeling in GIIá$^-$/GIIß$^-$ mutants expressing GIIá-VDEL and *in vitro* activity assays of microsomes from these cells using physiological substrates demonstrate that cells lacking GIIß are totally and partially impaired of removing Glc from $Glc_1Man_9GlcNAc$ and $Glc_2Man_9GlcNAc$, respectively. This suggests that GIIß is responsible for oligosaccharide structure recognition by GII.

CB-P58.
ULTRA-STRUCTURAL ANALYSIS OF THE GOLGI COMPLEX IN RESPONSE TO RAB1b ACTIVITY

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Membrane transport from the Endoplasmic Reticulum (ER) to the Golgi complex requires the activity of the Rab1b GTPase. It is know that Rab1b downstream effectors as well as upstream modulators are required for maintenance of Golgi structure and biogenesis; however, direct impact of Rab1b activity on these functions has not been analyzed. In this work we study, at the ultra-structural level, the ER-Golgi interface in the context of Rab1b inhibition and activation. Our results showed that Rab1b silenced cells exhibited partial Golgi fragmentation with an important increase in the number of COPII-round shape profiles. On the other hand, expression of the Rab1b active mutant (Rab1bQ67L) induced central Golgi fragmentation and the Golgi ribbon was replaced by a huge number of COPI-like-round shape profiles located in the juxta-nuclear area. Moreover, over-expression of Rab1bwt induced enlargement of the Golgi complex without modifying its normal phenotype. Our results reveal that the Golgi complex can adapt its structure in direct response of Rab1 activity. Additionally, the fact that an increase in Rab1b levels leads to Golgi enlargement suggests that Rab1b can modulate Golgi biogenesis

CB-P59.
ANALYSIS OF RAB1b CHANGES IN A SECRETORY CELL LINE

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The isoforms of the GTPase Rab1 (Rab1a and b) are ubiquitous and essential for ER to Golgi transport but different tissues express dissimilar mRNA levels of Rab1a and Rab1b. Interestingly, levels of Rab1b mRNA are increased in secretory tissues such as the thyroid, prostate and epithelial lung cells. Our previous results shown that changes in Rab1b levels acted as a molecular switch to control the expression of a variety of genes by regulating both their promoter activity and protein levels. We postulate that activation of secretion could induce an increase in Rab1b levels that trigger signaling circuits essential for the synthesis of molecules necessary to coordinate the flux of membranes between trafficking organelles. To test this hypothesis, we used a thyroid secretory cell line to analyze protein changes after the addition of thyroid-stimulating hormone (TSH). We show that levels of some proteins involved in membrane transport, such us Rab1b, GM130, Pra1 and KDELR were increased upon the addition of TSH. Significantly, expression of the dominant negative Rab1b mutant inhibited the TSH-induced increase of GM130 and Pra1 suggesting that TSH treatment induce a global increase of Golgi proteins in a Rab1b-dependent mechanism. Our results indicate that Rab1b changes could play a role in the cellular response required for the adaptation to a physiological secretory stimulus.

CB-P60.
GLYCOSYLTRANSFERASE COMPLEXES IMPROVE GLYCOLIPID SINTHESIS

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The activities of the endogenous galactosyltransferase 1 (GalT1) and sialyltransferase 1 (SialT1) of CHO-K1 cells were increased 1.4 and 2.3 fold, respectively, in cells (ST18 cells) stably expressing sialytransferase 2 (SialT2). The activation was not due to protein stabilization or transcriptional activation of GalT1 and SialT1 genes. The N-terminal domain of SialT2, which participates in the formation of a complex with GalT1 and SialT1, was not able to activate them, indicating that the C-terminal catalytic domain was necessary for the activation (SAIB 2007). In this presentation we analyze the products of SialT1 activity in wt and ST18 cells. While in wt cells GM3 was the main product, in ST18, which showed higher SialT1 activity, the products were GM3, GD3 and GT3. This indicates that the product of SialT1 activity is being efficiently used by SialT2 to produce GD3, and that the presence of SialT2 stimulates the conversion of LacCer into GM3 by SialT1. Also, they suggest that, *in vitro*, the two enzymes are still part of a complex existing *in vivo*. Results of co-inmunoprecipitation experiments in homogenates of cells co-expressing SialT1-Flag and SialT2-HA indicate an interaction between both enzymes. These results indicate a topological organization of glycosyltransferases that improves glycolipid synthesis by a functional docking of participating enzymes.

CB-P61.

THE LOCALIZATION OF GANGLIOSIDE GLYCOSYLTRANSFERASES IS AFFECTED IN CELLS THAT DO NOT EXPRESS COG2

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Golgi resident glycosyltransferases are maintained in an asymmetric steady-state distribution within Golgi cisternae. This is a dynamic process with some Golgi glycosyltransferases cycling between their main site of residence and distal compartments or by constantly cycling between the Golgi and the ER. These proteins use COPI vesicles for retrograde transport.
The conserved oligomeric Golgi (COG) complex is a soluble hetero-octamer associated with the cytoplasmic surface of the Golgi complex. It has been characterized in yeast and mammalian cells as a Golgi-associated tethering complex necessary for Golgi retrograde trafficking of multiple Golgi processing proteins. We have found an interaction between the COG complex and the N-terminal domain of some glycolipid glycosyltransferases in which the subunit Cog2 plays an important role. These enzymes, that in CHO-K1 cells have a characteristic Golgi localization, in mutant cells lacking the Cog2 (ldlC) subunit, show a more reticular pattern and sometimes they concentrate in small round structures that could correspond to COG complex-dependent vesicles. In ldlC cells the metabolic labeling of gangliosides exhibit an altered pattern compared to CHO-K1 cells. These results suggest that COG complex is involved in the localization, transport and/or function of glycolipid glycosyltransferases.

CB-P62.

PARTICIPATION OF GQ ALPHA SUBUNITS IN PKD1-DEPENDENT INTRACELLULAR TRAFFICKING REGULATION

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We have previously described the role of heterotrimeric G proteins ß subunits in regulation of vesicle fission at the Trans-Golgi Network (TGN), where they activate a pathway that consist of Phospholipase Cß3 (PLCß3), PKC and PKD1. Since Gq a subunits are also able to activate PLCßs, we have decided to study the involvement of these proteins in traffic regulation.
Our results showed that constitutive active mutants of Gaq (QL mutants) were able to induce vesicle fission at the Golgi level, leading to a complete fragmentation of this organelle, while other GaQL mutants did not have any effect in fission. This vesiculation was accompanied with the activation of PKD1 by phosphorylation in its activation loop.
Specific PLC and PKC inhibitors, and an inactive PKD1 mutant (PKD kinase dead) were able to block the effect of GaqQL in membrane fission, suggesting that these subunits participate in the same or in a similar pathway as the one initiated by bg subunits. On the other hand, a non-palmytoylated mutant of GaqQL could not induce Golgi fragmentation, demonstrating that its localization in membranes is necessary to be completely effective. Finally, *Pasteurella multocida* toxin, a Gaq activator, produced an augment in transport due to an increase in vesicle fission, confirming the participation of these G protein subunits on the regulation of TGN to cell surface trafficking.

CB-P63.

VINCULIN IS DELIVERED TO A RECYCLING COMPARTMENT AFTER BRADYKININ TREATMENT OF COLLECTING DUCT CELLS

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Focal adhesions (FA) are structures of cell attachment to the extracellular matrix. Previously, we showed that bradykinin (BK) induces a restructuring of FA by dissipation of vinculin-stained FA, concomitant with the appearance of vesicles containing vinculin and PIP2 in the citosol. Now, we performed vinculin pixel intensity profile analysis from cell edge towards cell center to explore the cellular distribution of these vesicles on cultured rat renal papillary collecting duct cells treated with BK. After 1 min of BK treatment, vesicles accumulated around the nuclei, and at 5 and 10 min were diffusely distributed in the citosol. Pre-treatmente of cells with a selective BK-B2 antagonist, Hoe 140, avoid the dissipation of vinculin-stained FA. We observed that vinculin-containing vesicles did not colocalize with caveolin-1 nor with the fluid phase-endocytosis marker, dextran (Overlap coefficients= $R<0.7$). By contrast, vinculin colocalized with the marker of recycling endocytic pathway, transferrin receptor (TfR) after BK stimulation ($R>0.7$). These observations suggest that BK produces the internalization of vinculin by its delivering to a recycling compartment, located in the perinuclear area. From here, vesicles containing vinculin migrate to the cell membrane to allow FA restructuration at 10 min after BK stimulation, when the physiological condition is restored.

CB-P64.

IS ARGINYLATED CALRETICULIN IMPLICATED IN CELLADHESION?

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The post-translational arginylation of proteins is the covalent union of arginine to an acidic amino acid at the N-terminal position and is mediated by arginyl-tRNA protein transferase (Ate 1). We demonstrated the posttranslational arginylation of calreticulin (CRT) *in vitro* and in living cells. The arginylated form of CRT (R-CRT) is present in the citosol, in contrast to the non arginylated CRT, resident of endoplasmatic reticulum. It has been shown that CRT affects cell adhesion by regulating vinculin expression. This may be mediated by direct interaction between CRT and the KxGFFKR of integrins, that imply a cytoplasmic localization of CRT. By immunocytochemistry, using an antibody against R-CRT we observed an enhanced colocalization of R-CRT and vinculin in cells subjected to focal adhesion disassembly and reassembly, similarly to that observed between RCRT and integrins. Further studies performed in cells lacking Ate1 (ate1 -/-) show a differential attachment to laminin and polylisine when compared to cells ate1 +/+. After transfection with CRT the ate1 -/- cells did not improve their adhesiveness to substrata as ate1 +/+ cells. Thus, this evidence supports the idea that R-CRT is implicated in cell adhesion and posttranslational arginylation of CRT seems to regulate its cytosolic function.
Supported by SECyT-UNC, CONICET, ANPCyT-PICT.

CB-P65.

DYNAMIC AND SELECTIVE ASSOCIATION OF ARGINYLATED CALRETICULIN TO STRESS GRANULES IN CELLS

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Post-translational arginylation of proteins is the covalent union of arginine to an acidic amino acid at the N-terminal position. We characterized calreticulin (CRT) as substrate of this modification. This modified protein (R-CRT) showed a sub-cellular localization in the cytosol different from that known for CRT in the endoplasmic reticulum. We determined that the decrease in cytosolic calcium concentration increases the production of R-CRT, witch is associated with stress granules (SGs). Furthermore, we found R-CRT associated to SGs in cells exposed to oxidative stress and heat shock, similar to the calcium stress condition. However, in cells treated with UV, R-CRT does not aggregate with SGs. In correlation with these observations, cytosolic calcium decreases in both oxidative stress and heat shock conditions, whereas after the UV, calcium levels are unchanged. After withdrawal of the stressor, SGs rapidly disappear but the R-CRT remains aggregated after 2 h. Thus, there are different rates of disassociation between SGs and R-CRT aggregates after the environmental stress has ended. These changes correlate with the restoration of calcium levels, which in itself depend on the magnitude of the stress.
In conclusion, intracellular calcium changes promote the selective association of R-CRT to SGs under particular stress conditions.
Supported by SECyT-UNC, CONICET, ANPCyT-PICT.

CB-P66.

MICROTUBULE-DEPENDENT POSITIONING OF PROTEIN TYROSINE PHOSPHATASE PTP1B IN CELL-MATRIX ADHESIONS

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PTP1B is an ubiquitous ER-bound tyrosine phosphatase implicated in a variety of cellular processes; thus, it requires a precise subcellular targeting. PTP1B has the potential for substrate dephosphorylation throughout the branching network occupied by the ER. Here, we provide new insights on the mechanism through which PTP1B is targeted to cell-matrix adhesion sites. We developed a microscopy-based assay for quantifying coincidence events between microtubules (MTs), PTP1B and cell-matrix adhesions in B16F1 melanoma cells and fibroblasts. We found that GFP-PTP1B bearing the substrate-trapping mutation D181A (DA) localized at MT plus ends in cell-matrix adhesions visualized by interference reflection microscopy. ER/MT dissociation prevented PTP1B-DA but not MT targeting to the adhesion sites. Similar results were observed using the protein CLIP-170 as a marker of growing MT plus ends. PTP1B-DA at MT plus ends localized mostly at the distal tips of foci containing paxillin, and to a less degree, at foci containing zyxin, markers of nascent and mature adhesions, respectively. TIRFM confirmed that coincidence events occurred near the ventral cell surface. Our results suggest that PTP1B is positioned to the distal tip of newly forming cell matrix adhesion sites through the dynamic activity of MTs. PTP1B may contribute to the maturation of cell-matrix foci.
Supported by ANPCyT.

CB-P67.

PROTEIN TYROSINE PHOSPHATASE 1B (PTP1B) REGULATES CELL MIGRATION THROUGH A FAK/SRC DEPENDENT PATHWAY

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To identify molecular pathways regulated by PTP1B in cell-matrix adhesion complexes we searched for conditions that prevented the accumulation of the substrate trapping mutant PTP1B-D181A in these complexes. We started using cell lines in which different mediators of integrin signaling were knocked out or mistargeted. Cells knock out for c-Src and the adaptor protein paxillin allows accumulation of the PTP1B-DA while cells triple knockout for c-Src, c-Fyn and c-Yes do not. Reconstitution with wild type c-Src or c-Fyn, and mutants of the regulatory tyrosines 416 and 527 rescued the PTP1B-DA accumulation. FAK-null cells lost the accumulation of PTP1B-DA, and this is rescued by re-expression of FAK. FAK displacement from cell adhesions and/or block of autophosphorylation of FAK-Y397 (the Src-recruiting motif) eliminated the accumulation of the PTP1B-DA. PTP1B-DA tightly co-localized with c-Src and c-Fyn in adhesion sites. Imaging of living cells show dynamic projections of ER-bound GFP-wild type PTP1B over nascent paxillin-rich foci, which often is followed by their grow in size. PTP1B null cells display fast cycles of lamellar extension/retractions, and frequent changes in the direction of migration compared to the WT cells. We postulate that PTP1B regulates the FAK/Src signaling pathway, promoting the maturation of adhesions and the directional cell migration.
Supported by ANPCyT.

CB-P68.

GANGLIOSIDES ARE APICALLY SORTED IN MDCK CELLS AND INTERNALIZED BY CLATHRIN-INDEPENDENT ENDOCYTOSIS

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Gangliosides are glycosphingolipids mainly present at the plasma membrane (PM). After synthesis in the lumen of the Golgi apparatus, gangliosides leave the organelle via transport vesicles destined to PM. In this study, we analyzed the synthesis and membrane distribution of GD3 and GM1 gangliosides endogenously synthesized both in polarized and non-polarized Madin-Darby canine kidney (MDCK) cell lines stably expressing ganglioside glycosyltransferases. By using biochemical techniques and confocal microscopy we demonstrated that GD3 and GM1, after being synthesized at the Golgi apparatus, are sorted and accumulated mainly at the PM of non-polarized MDCK cells. Interestingly, both complex gangliosides were found enriched mainly at the apical domain when these cell lines were induced to polarize. We demonstrated that after arrival to PM, GD3 and GM1 are endocytosed by using a clathrin-independent pathway. Then, internalized GD3 is accumulated in endosomal compartments and sorted back to the PM. On the other hand, endocytosed GM1, in association with cholera toxin, is transported to endosomal compartments en route to the Golgi apparatus. The genetically modified MDCK cell lines should provide an excellent model system for studying synthesis, trafficking and polarity of glycolipids in epithelial cells as well as the biological significance of the polarized distribution of these lipids.

CB-P69.

ACYLATION OF GAP-43 IS REGULATED BY ELECTROSTATIC INTERACTIONS BETWEEN ITS POLYBASIC DOMAIN AND PI4P

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The growth-associated protein-43 (GAP-43) is a dually acylated protein at cysteines 3 and 4 and contains a polybasic domain (aa 32-55) which is able to bind anionic lipids. The aim of this study was to investigate if the polybasic domain modulates the acylation state of GAP-43. Using biochemical assays and live cell fluorescence microscopy, it was demonstrated that after synthesized in the cytosol, GAP-43 is acylated at the trans-Golgi network (TGN), which is necessary for its stable association with membranes. By comparing N-terminal region (aa 1-13, N13GAP-43) and full-length GAP-43 (GAP-43full), it was found that basic residues of GAP-43 could be modulating both its adsorption to TGN and palmitoyl-acyl transferase accessibility. We speculated that the polybasic domain from GAP-43 could be electrostatically interacting with PIP4, an anionic phospholipid highly concentrated at the TGN through an ADP-ribosylation factor 1 (Arf1)-mediated process. In this sense, we observed that Arf1 inhibition did not affect acylation and TGN and plasma membrane association of N13GAP-43 but severely affected subcellular distribution of GAP-43full and its adsorption to TGN, thereby increasing the cytosolic pool of this protein. We conclude that Arf1 activity, through its effector PI4P, is required for TGN adsorption of GAP-43full via its polybasic domain.

CB-P70.

THE GTPASE RAB14 IS RECRUITED TO *Chlamydia trachomatis* INCLUSION

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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 an inclusion that is trafficked to the peri-Golgi region. This inclusion receives material from the biosynthetic via by fusing with vesicles released from the TGN. The specific host and bacterial factors that mediate this intracellular trafficking to the *chlamydia* vacuole remain undefined. Rab GTPases are the molecules in charge of controlling intracellular transport. Since Rab14 is a key regulator of vesicular transport between the TGN and early endosomes, and is critical for the maintenance of *Mycobacterium tuberculosis* phagosome maturation arrest; we postulate that Rab14 is involved in *chlamydia* inclusion development. We examine by confocal microscopy the intracellular localization and function of this Rab and its inactive mutants: Rab14 S25N (Rab14 GDP-bound form) and Rab14 ÄGCGC (Rab14 not associated to membranes) fused to EGFP in Hela cells infected with *C. trachomatis* biovar LGV. We observed a marked recruitment of EGFP- Rab14 to the membrane of *chlamydia* inclusions. These data suggest that *Chlamydia trachomatis* selectively interferes with critical components of the trafficking machinery of the host cell in order to generate an intracellular niche favorable for its surviving and development.

CB-P71.

PHAGOCYTOSIS OF *Coxiella burnetii* REQUIERES PIP5K AND ARF6

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Coxiella burnetii (*Cb*) is an intracellular pathogen that causes Q Fever desease. This pathogen entries into the host cell by phagocytosis. Phagocytosis is a process that involves actin dynamic regulated by Rho and Arf GTPases. During phagocytosis Arf6 (class III Arf) actives PIP5K which generates PI(4,5)P2 that interacts with actin cytoskeleton effectors. We analyzed if Arfs, in paticular Arf6, and PIP5K are involved in the *Cb* internalization by the host cell.
We tested the effect of brefeldin A (BFA), a class I and II Arfs inhibitor, upon the internalization process. HeLa cells were treated with BFA, then infected and processed for indirect immunofluorescence and analyzed by confocal microscopy. We observed that BFA did not affect *Cb* internalization. We decided to check if Arf6, a BFA-insensitive Arf, could be involved in that process. Before infection, Hela cells were transfected with pEGFP-Arf6 wild type (WT) or -Arf6T27N (inactive mutant). We observed that Arf6T27N diminished the number of internalized *Cb* compared with WT. To study if PIP5K is recruited to the plasma membrane during *Cb* uptake, HeLa cells were tranfected with pcDNA3Myc-PIP5K and processed as above. We observed that PIP5K was recruited at the bacterial entry sites. These results sugget that Arf6 and PIP5K are involved in the host cell interaction of *Coxiella burnetii*.

CB-P72.

RHOA SIGNALLING PATHWAY IN INVOLVED IN *Coxiella burnetii* PHAGOCYTOSIS

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Phagocytosis is an important defense process against pathogens. This process is triggered by the interaction of pathogen ligands with host cell specific receptors such as CR3. The actin cytoskeleton is necessary for phagocytosis and Rho GTPases are well known regulators of actin dynamic. In particular, RhoA is mainly involved in CR3-dependent phagocytosis. On the other hand, it is known that Src and ROCK kinases are part of the Rho signalling pathway. *Coxiella burnetii* (Cb) is an obligated intracellular pathogen that causes Q fever in humans. Cb phase II is an avirulent form that is phagocytosed through aVß3 and CR3 receptors. Since Cb interacts with CR3, and RhoA is activated during CR3-mediated phagocytosis, we decide to analyze if Cb internalization induces RhoA activation in the host cell. Rho activation was measured using a RBD domain of Rhotekin, fused to GST,that recognizes Rho-GTP form and a pull-down assay. We observed that heat-killed Cb induced RhoA activation during HeLa cell infection. To assess the participation of Src and ROCK in Cb internalization, HeLa cells were incubated with PP2 or Y-27632, specific inhibitors of Src and ROCK, respectively, before infection. We observed that Cb internalization decreased significantly in the presence of both inhibitors. Al together, these results suggest that the RhoA, Src and ROCK are involving in heat-killed Cb phagocytosis.

CB-P73.

THE INTERNALIZATION OF *Coxiella burnetii* BY THE HOST CELL IS REGULATED BY CORTACTIN

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Cortactin is a protein involved in the actin cytoskeleton organization regulating ruffles and lamellipodia formation during cell migration. In those processes cortactin is recruited to membrane in a PI3K-Rac1 dependent manner. Cortactin has different domains to interact with actin, and actin efectors such as Arp2/3 and N-WASP. The interaction is regulated by Tyr and Ser phosphorylation catalyzed by Src and Erk kinases, respectively.
It has been shown that cortactin plays role in host cell-pathogen interaction. Adhesion, invasion or actin-based motility of pathogens such as *E.coli*, *Chlamydia*, *Shigella* and *Listeria* are regulated by cortactin. *Coxiella burnetii* (Cb) is an obligated intracellular pathogen that after phagocytosed form a parasitophorous vacuole in the host cell. We previously observed that cortactin regulates the Cb phagocytosis. To study if PI3K and Src proteins are involved in the internalization of Cb, HeLa cells were treated with wortmanin (Wn) or PP2, inhibitors of PI3K and Src, respectively, before infection. The adhered and intenalized bacteria were estimated by indirect immunofluorescence and confocal microscopy. We observed that Wn did not affect adhesion but inhibited internalization, while PP2 stimulated adhesion and internalization. These results suggest that PI3K and Src are involved in the interaction of Cb with host cell likely through cortactin.

CB-P74.

THE SECRETED ALPHA-HEMOLYSIN OF *S. Aureus* ACTIVATES THE AUTOPHAGIC RESPONSE IN THE HOST CELL

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S. aureus induces a caspase-independent cell death, with the participation of autophagy. This involves the sequestration of cytosolic components, organelles and microorganisms in a vacuole, the autophagosome, which finally fuses with the lysosome. Our purpose was to identify the factor(s) and the signalling pathway that participates in the activation of the autophagic response caused by *S. aureus*.α-Hemolysin is a pore forming toxin secreted by *S. aureus*.CHO cells over-expressing GFP-LC3 (an autophagosome marker) were incubated with the toxin. This caused a marked activation of autophagy in a concentration-dependent manner. In order to address if the toxin is the only secreted factor responsible for the activation of autophagy, CHO GFP-LC3 cells were infected with the following *S. aureus* strains: wt, a mutant deficient for α-Hemolysin (Hla-) and the Hla (-) mutant expressing an α-Hemolysin plasmid. *S. aureus* wt as well as the mutant expressing the plasmid stimulated autophagy upon infection. In contrast, the Hla(-) mutant was unable of activating this pathway. In addition, we demonstrated that autophagy activation was calcium dependent, since this was hampered by the intracellular calcium chelator BAPTA-AM. Interestingly, the toxin effect was not prevented by the classical autophagy inhibitors 3-MA and Wortmannin, suggesting that the action of α-Hemolysin is independent of PI3K.

CB-P75.

EFFECT OF OCHRATOXIN A ON CYTOKINES PRODUCTION BY PBMC: MODULATION BY PROBIOTIC *Lactobacilli* STRAINS

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Ochratoxin A (OTA) is a toxin produced by some fungus species and it is commonly found in alimentary products. This toxin is pro-apoptotic and carcinogenic and changes cytokines profile of some cells. Previous studies in our laboratory showed the immunomodulatory capacity of *Lactobacillus acidophilus CRL 1014* and *L. reuteri* CRL 1098. The aim of this work was to investigate the capacity of these strains to modulate the changes in cytokines induced by OTA in human peripheral blood mononuclear cells (PBMC). $2x10^6$ PBMC were treated with OTA (2-100 µg/ml) at different incubation times at 37ºC and 5% CO_2. A pro-inflammatory cytokine (TNF-á) and a regulatory cytokine (IL-10) produced by PBMC were measured by ELISA kit. The lowest concentration of OTA (2 µg/ml) increased TNF-á production (4 times), while higher concentrations (5 to 100 µg/ml) produced an inhibitory effect (43-93%) at 4h incubation. IL-10 was inhibited at all OTA concentrations tested. PBMC exposed to 7.5 µg/ml OTA were co-cultured with *L. reuteri* and *L. acidophilus* for 4h. Results showed that *L. reuteri* increased TNF-á inhibition produced by OTA; in contrast, *L. acidophilus* reverted the toxin effect. Neither strains modified the inhibition of IL-10 production by OTA. These results are encouraged, indicating that certain lactobacilli strains may protect the host against the adverse health effects of OTA.

CB-P76.

DISRUPTION OF LIPID RAFTS MODIFIES THE IMMUNOMODULATORY EFFECT OF *Lactobacillus acidophilus* IN PBMC

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Among the multiple health beneficial effects proposed to probiotics, is outlined their capacity to interact with the immune cells. However, up to date, the mechanisms of this effect remain unclear. We investigated whether disruption of lipid rafts could influence the effect of probiotic *L. acidophilus* CRL 1014 on cytokines production by human peripheral blood mononuclear cells (PBMC). To disrupt lipid rafts, PBMC were treated with 10 mM methyl-ß-cyclodextrin. Control (untreated) and disrupted-lipid rafts (d-lr) cells were co-incubated with: alive, heat-killed and UV-irradiated *L. acidophilus* and with bacterial cultures supernatant, at 37ºC and different incubation times. TNF-á and IL-10 were detected in co-cultures supernatant by ELISA. The results show that alive and UV-irradiated *L. acidophilus* stimulated TNF-á production (3 times) of control cells at 4h incubation. Similar effect was observed when PBMC were incubated with bacteria culture supernatant. This stimulatory effect was increased in d-lr cells with respect to control cells. In contrast, heat-killed bacteria produced no effect on TNF-á release by control and d-lr cells. Similar, but less pronounced effect, was observed in IL-10 release at 4 h incubation in both control and d-lr PBMC, and was significantly increased at 8 h incubation. Studies are currently in progress to further define the rafts role in this response.

CB-P77.
ROLE OF CLUSTERED PROTOCADHERINS IN CELL ADHESION AND CELL SIGNALING
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Clustered protocadherins (Pcdh) are a group of neuronal surface glycoproteins that are expressed in specific regions of the central nervous system (CNS) of vertebrates (brain cortex, olfactory bulb, spinal chord, hippocampus), and are enriched at synapses. They belong to the Cadherin superfamily, involved in Ca2+ dependent cell adhesion by dimerization, but their function remains unknown. They are arranged in three clusters. Single neurons express Pcdh isoforms monoallelically and stochastically, with the exception of the "c2 type" isoform.
Our goal is to understand the role of clustered Pcdh in the CNS. We want to establish whether clustered Pcdh are involved in homo or heterophilic interactions and whether these trigger cell adhesion and/or signaling processes. Our model consists of transfection of a mouse neuronal cell line with plasmids expressing one isoform at a time, and then challenging them with a different isoform. As clustered Pcdh do not establish strong adhesion contacts, we constructed chimeric plasmids that express a given Pcdh isoform fused to the cytoplasmic domain of E-cadherin. Correct expression on the membrane of a transfected mouse neuronal cell line is monitored in fusions to green fluorescent protein. Formation of cellular aggregates of cells expressing the same or different Pcdh isoforms allows us to test for homo vs heterophilic interactions.

CB-P78.
ß-AMYLOID-MEDIATED INDUCTION AND ACTIVATION OF p19INK4d INVOLVES CALCIUM-DEPENDENT SIGNALING
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ß-amyloid, a key player in neurodegenerative diseases and the main component of senile plaques, acts by causing oxidative stress: lipid and protein oxidation and DNA damage. In response to genotoxic stress, p19INK4d, a member of the INK4 family of cell cycle inhibitors, becomes transcriptionally induced and phosphorylated. p19 improves DNA repair and reduces apoptosis. However, the mechanism leading to p19 induction and activation in response to ß-amyloid remains unknown. Our working hypothesis is that ß-amyloid, by increasing intracellular Ca2+ levels, activates calpains, which convert CDK5 regulatory subunit p35 to its active form, p25. We show that p19 is phosphorylated by CDKs. We used SH-SY5Y cells differentiated with retinoic acid as a neuronal model. By Northern blot and metabolic labeling with 32P, ß-amyloid treatment of both differentiated and undifferentiated cells induced the expression and phosphorylation of p19 in a dose-dependent manner, as well as the expression of p35. Similar results were obtained with A23187, a Ca2+ ionophore. Antisense downregulation of CDKs prevented the ß-amyloid induced phosphorylation of p19. Our results show that Ca2+-dependent signaling becomes activated upon ß-amyloid treatment and appears to be involved in the induction and activation of p19INK4d.

CB-P79.
EFFECT OF ANDROGENS AND MELATONIN ON GLUCOCORTICOID RECEPTOR REGULATION IN TOAD TESTES
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Bufo arenarum is an anuran amphibian characterized by having the highest concentration of plasma glucocorticoids (GC) during the breeding season. The sensitivity to GC is regulated by the number and affinity of the glucocorticoid receptor (GR). A cytosolic GR has been described in Leydig and Sertoli cells. Seasonal studies of GR apparent number of binding sites have shown significant differences during the year. Melatonin and androgens have been reported to regulate GR expression in other biological systems, so the aim of the present work is to analyse the effect of these hormones in the amount of GR in *B. arenarum* testicular cells. The number of binding sites and GR protein levels (Western Blot analysis) were measured in testes homogenate after *in vitro* incubation of testicular fragments in the following conditions: testosterone (0,1 µM), dihydrotestosterone (0,1 µM), casodex or hydroxyflutamide (testosterone receptor antagonists) (10 µM), cyanoketone (a 3ß-hydroxysteroid dehydrogenase inhibitor) (10 µM), melatonin (0,2 and 0,9 nM). Neither androgens nor androgens antagonists modified the number of binding sites. In addition, the inhibition of steroidogenesis does not affect the number of receptors. On the other side, 0,2 nM melatonin decreases the number of binding sites and GR protein level suggesting that this circadian responding hormone might regulate the GR in the toad testes.

CB-P80.
BIDDER'S ORGAN AND AROMATASE ACTIVITY IN THE MALE TOAD *Bufo arenarum*
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The Bidder's organ (BO) is a rudimentary ovary located at the anterior end of the testis in male true toads (Bufonidae), its development being arrested by the presence of functional testes. Since *Bufo arenarum* testes lack aromatase activity (ARO), it has been suggested that BO could be the source of circulating estradiol (E2). The main goal of this work is to determine the expression of ARO and other steroidogenic enzymes in BO as well as the correlation between glucocorticoids (GC), androgens and the development of BO. The expression of the cytochrome P450 SCC and 3ß-hydroxysteroid dehydrogenase/isomerase was detected by immunocytochemistry and substrate conversion, respectively. Moreover, ARO activity was detected by the conversion of testosterone (T) into E2 and by Western blot analysis. These results indicate BO has the capacity to synthesize estrogens from cholesterol. In addition, specific activity of ARO, expressed as nmole per mg protein, increased with organ development. The presence and weight of BO positively correlates with T concentration but no correlation between those parameters and GC was found. Finally, the presence of ARO activity in BO encouraged us to study this enzyme in other peripheric organs. Results indicate that ARO activity is also present in liver but not in fat bodies, suggesting that the liver could contribute to the overall production of E2.

CB-P81.

ROLE OF THE Na^+/K^+ ATPASE IN THE SPERMIATION OF *Bufo arenarum*

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In the toad *Bufo arenarum*, spermiation occurs either as a consequence of mating or *in vitro*, as a response to LH/hCG. It has been proposed that a decrease in Na^+/K^+ATPase activity is associated to spermiation in response to hCG. The main objective of this work is to analyze, in *B. arenarum*, the participation of the Na^+/K^+ATPase in hCG-induced spermiation and androgen synthesis as well as to study the role of PKA and PKC on hCG action. Testicular fragments were incubated in buffer (KRGH) with and without hCG and with different concentrations of ouabain, H89 (PKA inhibitor) and PMA (PKC activator). Spermiation was quantified as the number of spermatozoa released in incubation media by using a Neubauer's chamber. Testosterone synthesis was determined by RIA. Ouabaine, Na^+/K^+ATPase inhibitor, blocked hCG-induced spermiation without affecting steroidogenesis. This result confirms previous results of our laboratory showing that both processes are induced by different ways. In addition, H89 blocked induced- spermiation but not steroidogenesis, suggesting that PKA activation could play a fundamental role in the induction of spermiation by hCG. PMA also blocked hCG-induced spermiation but stimulated steroidogenesis mimicking hCG. In conclusion, these preliminary results suggest that activation of PKC is not involved in hCG-spermiation but positively regulates steroidogenesis.

CB-P82.

AROMATASE ACTIVITY IN BIDDER'S ORGAN OF *Bufo arenarum* LARVAE

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Bufonids tadpoles are good models for the study of sex differentiation due to the presence of a structure called Bidder's organ (BO). BO differentiates in both sexes as a rudimentary ovary in the cephalic portion of the genital ridge even before the differentiation of gonads. Until today, in anuran it is unclear the mechanism conducing to differentiation of the genital ridge into ovary and testis. In general, steroids, such as estrogens (E) and androgens, have major functions in sexual differentiation. Adult male of *Bufo arenarum* conserves the BO, which contains previtellogenic oocytes. Preliminary results of our laboratory show that BO expresses steroidogenic enzymes. Particularly, in the follicular cells of BO it has been detected the syntheses of E from testosterone. The main objective of this work is to establish the stage during the larval life of *B. arenarum* in which BO starts the synthesis of E that could affect the gonadal differentiation. Aromatase (ARO) and 5α-reductase activities in the BO-gonad-kidney complex of individuals in different stages were measured. The presence of ARO was also detected by immunohystochemistry. Results indicate that ARO is expressed in BO as early as in stage 38 (Gosner), suggesting that the appearance of this enzyme precedes the differentiation of gonads. As well, a low expression of 5α-reductase and 17-hydroxysteroid dehydrogenase was detected.

CB-P83.

INFLUENCE OF CALTRIN PROTEIN ON THE SPERM INTRACELLULAR Ca^{2+} AND MEMBRANE K^+ CHANNELS

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Ca^{2+} signaling plays a central role in the sperm physiology during mammalian fertilization. Sperm cytosolic [Ca^{2+}] can be raised by extracellular Ca^{2+} influx or Ca^{2+} release from intracellular stores, and it induces acrosomal exocytosis (AE) in the head and hyperactivation in the tail. On ejaculation, epididymal sperm bind caltrin (calcium transport inhibitor) a protein that inhibits extracellular Ca^{2+} uptake and the onset of Ca-dependent processes. Thus, ejaculated sperm are unable to carry out them without help of physiological inducers. To explore the action of caltrin we examined its effect on the intracellular Ca^{2+} in bovine epididymal sperm subjected to capacitation before being exposed to caltrin, and the activity of K^+ channels in somatic cells. Sperm loaded with Calcium Green were incubated for 2 h and then exposed to caltrin for 30 min, while K^+ currents were measured by patch clamp in HEK cells. Sperm images analyzed with the Image J program showed intense fluorescence in the head as a result of Ca^{2+} influx but, when the cells were exposed to caltrin, it was localized just in the midpiece. Data suggest that caltrin cancels Ca^{2+} influx into the sperm head which probably initiate AE, without affecting the channels in the midpiece where Ca^{2+} is stored in the mitochondria. K^+ currents were reduced by caltrin suggesting an inhibitor effect on Ca^{2+}-activated K^+ channels.

CB-P84.

PRESENCE AND ROLE OF ß1 INTEGRIN IN THE GAMETES OF THE AMPHIBIAN *Bufo arenarum*

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We previously found that the ß1 integrin expresses in the plasma membrane of *B. arenarum* oocytes and has a role in the process of fertilization (this was determined by *in vitro* fertilization assays in the presence of the peptide RGDS). Our aims now were to: i) begin the molecular characterization of *B. arenarum* ß1 integrin, ii) analyze the presence of ß1 integrin on the male gamete (sperm) and its role during fertilization.

We carried out ovary total RNA isolation, retrotranscription and performed PCR using degenerated primers. The amplified DNA was cloned and sequenced. The obtained 235 bp sequence had strong homology to ß1 integrins (563-639 amino acid sequence). The presence of ß1 integrin in sperm was assayed by Western Blot and immunofluorescence using the 8C8 antibody (Hybridoma Bank). These studies revealed that the protein is present predominately in sperm heads. Preliminary *in vitro* fertilization assays using sperm pre-treated with the RGDS peptide (a known integrin interacting motif) suggest that sperm ß1 integrin would not be necessary for the fertilization process to occur (in fertilization percentage, mean + SEM: Control = 56.1 + 2.8; RGDS pre-treated sperm = 59.4 + 6.5, n=3). Our results suggest that although ß1 integrin is present in both *B. arenarum* gametes: sperm heads and oolemma, only plays a role in the oocyte during fertilization.

CB-P85.

HAVE HYPERCHOLESTEROLEMIC AND MEDITERRANEAN DIETS CONSEQUENCES ON SPERM PHYSIOLOGY?

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Hypercholesterolemia is a causal factor of atherosclerosis and coronary heart diseases. Diet alterations might lead to plasma membrane modifications that have impact over cellular physiology. Our current model includes the spermatozoa as target cell. The present study was carried out to investigate the effects of hypercholesterolemic (HCd) and Mediterranean diet (olive oil supplemented, Md) on rabbit sperms (Sp). The HCd treatment (16% bovine fat/total intake) caused significant ($p < 0.05$) increase in total cholesterol levels in serum (121.8 ± 5.3 vs. 20.4 ± 2.7 mg/dl; control). Concerning Sp cell, we found changes in subcellular lipid distribution in Sp heads, reduction in Sp motility (53.6 ± 5,9 % vs. 72.4 ± 3.4 %, control; $p < 0.05$) and the percentage of acrosome-reacted Sp (AR) showed a 20% decrease ($p < 0.05$) compared to control conditions. Olive oil supplemented diet (14%) significantly ($p < 0.05$) improves both, membrane integrity and AR. Experimental diets did not cause significant changes over parameters as animal weight (control: 3.9 ± 0.5; HCd: 3.7 ± 0.3; Md: 3.8 ± 0.5 kg), libido and semen physical characteristics. In conclusion, saturated fat consumption promotes an increase in serum cholesterol, changes in cellular lipids distribution and Sp specific functions, motility, and AR. Md might be associated to an improvement in Sp quality.

CB-P86.

RAT SPERM ROSETTES: IDENTIFICATION OF EPIDIDYMAL PROTEINS INVOLVED IN SPERM ROSETTES ASSEMBLING

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In many mammals' species, sperm associate during their epididymal trip. This association could be related to sperm maturation and protection during epididymal storage. In the rat these association were called Rosettes and appeared in the distal cauda. The aim of this work was isolate proteins from caudal epididymal fluid (EF) with activity to re associate sperm *in vitro* trying to mimic the *in vivo* phenomenon (*in vitro* sperm re association test: i.v. Rosette test). Mature and motile sperm (1.106 cells/ml) was incubated with EF fractions (1 mg/ml) and the number of Rosettes obtained *in vitro* was recorded (expressed as Rosettes number/60 microscopic fields, 400 X). Using gel filtration –proteins separated by size range (Sephacryl S200) - and affinity chromatographies –Glucose/mannose affinity proteins fractionation (Concanavalin A sepharose) - EF was fractionated in eight fractions.
All fractions were tested by i.v. Rosettes test and the most active fraction (with MW from 40 to 80 KDa) and Concanavalin A affinity were analysed by MALDI-TOF-MS. Two proteins with serine protease inhibitor activity (serpins) were identified: á1-Antitrypsin and a new protein with a á1-Antitrypsin like domain. These results and previous indicate that Rosettes are assembled during the epididymal trip by several factors: sperm motility inhibition, linking proteins, proteases and their inhibitors.

CB-P87.

SUBCELLULAR LOCATION AND CHARACTERIZATION OF SPERM THIOL PROTEINS

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During epididymal trip the sperm proteins support a thiol oxidation. This redox changes from thiol to disulfides bonds are associating with the acquisition of sperm motility, capacitation, acrosome reaction and fertilization. Ours objectives were isolate different sperm subcellular fractions with proteins rich in thiol groups. Using different detergent and sucrose gradient, we fractionated this cell such as head, acrosomal proteins, principal and terminal piece, outer dense fibers and fibrous sheath. Then we certificated the success of this procedure by electron microscopy. On the other hand we used monobromobimane, a fluorescent reactive that labels with protein`s thiols groups. We localized, by fluorescence microscopy, witch cellular fraction reacted. Others aliquots, were prepared for SDS-PAGE. About 27 kDa band display a great fluorescence as well as the tail of the sperm. With this positive band we raised polyclonal antibody immunizing rabbits. We saw an immune positive reaction with sperms tail. These results suggest that we successfully isolated protein/s rich in thiols groups and we are in condition to initiates the molecular characterization of this protein/s.

CB-P88.

A NOVEL EPAC-RAB3 SIGNALING PATHWAY IS ESSENTIAL FOR SPERM EXOCYTOSIS

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The single secretory granule present in sperm undergoes regulated exocytosis (the acrosome reaction, AR) near the time it encounters the egg's zona pellucida at fertilization or following pharmacological stimulation *in vitro*. Our laboratory has identified a number of proteins required for sperm exocytosis; our current goal is to identify new components of the fusion machinery and reveal the sequence in which they act. To this end, we promote or perturb specific interactions between exogenous (e.g., neurotoxins, antibodies, recombinant proteins, second messengers, etc) and endogenous elements of the fusion machinery at different stages of the AR, and analyze the results by means of functional (exocytosis) and biochemical (pull down, subcellular fractionation, partition, Western blot, etc) assays. We show here that the AR is a bifurcated pathway consisting of two limbs, one driven by Rab3A, PTP1B, NSF, and Munc18-1, which arrives at the SNARE-mediated docking of the acrosome to the plasma membrane. The other is governed by Epac, Rap, and a PLC, and elicits an efflux of calcium from the acrosome. Rab3A is activated and targeted to membranes upon treatment with AR inducers. Subsequently, PTP1B dephosphorylates NSF, allowing it to disassemble unproductive SNARE complexes. Munc18-1 binds monomeric syntaxin and helps to nucleate SNAREs in fusion-competent complexes

CB-P89.

PHOSPHORYLATION/DEPHOSPHORYLATION OF SYNAPTOTAGMIN 6 DURING ACROSOMAL EXOCYTOSIS

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The acrosomal exocytosis (AE) is a calcium regulated exocitosis essential for fertilization. We have demonstrated that synaptotagmin 6 (Syt 6) is phosphorylated in resting sperm, and that its C2B domain presents a polybasic region which is a target for PKC. As we predicted that threonine 419 (T419) has more probability to be phosphorylated by this kinase, we created a phosphomimetic mutant (T419E). Using a FRET assay we demonstrated that T419E lost the ability to bind liposomes in a Ca^{2+}-dependent way and its inhibitory effect in an AE assay. These results suggest that the PKC mediated regulation of C2Bwt activity depends on T419 phosphorylation. However, T419 is not the only target for PKC; the T419E mutant was still able to incorporate ^{32}P in an *in vitro* phosphorylation assay. We previously demonstrated that Syt 6 is dephosphorylated after sperm stimulation. Calcineurin (CaN), a calcium/calmodulin-activated protein phosphatase that is present in sperm and is required for AE, could be responsible of Syt 6 dephosphorylation. In an *in vitro* phosphorylation/dephosphorylation assay with ^{32}P, using a constitutively active form of CaN, we showed that the Syt 6 C2B domain was dephosphorylated by CaN. We conclude that Syt 6 activity is regulated by PKC phosphorylation at T419 and needs to be dephosphorylated by CaN to participate in AE.

CB-P90.

c-FOS, A NOVEL TARGET TO CONTROL TUMOR DEVELOPMENT IN CENTRAL AND PERIPHERAL NERVOUS SYSTEM

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c-Fos is a member of the family of AP-1 transcription factors that heterodimerizes with c-Jun and regulates the expression of genes involved in mitosis and differentiation. We found that it also associates to the endoplasmic reticulum (ER) and activates the synthesis of phospholipids for membrane biogenesis involved in cell growth of brain human tumors.

Herein, this phenomena was examined in central and peripheral tumors (neurofibromas) occurring in human patients and NPcis mice, an animal model for the Neurofibromatosis Type I (NF1) disease. High levels of c-Fos were observed co-localizing with ER markers both in brain tumors and neurofibromas. These levels correspond with high rates of cell proliferation. Similar results were observed in stable cell lines generated from both neoplasias. c-Fos expression blockage on NPcis mice brains lowered phospholipid synthesis and proliferation levels selectively in treated areas respect to non-treated ones. Regarding peripheral tumors, this treatment decreased tumor burden significantly. NPcis mice on a fos-/- background, are not capable of developing PNS tumors, corroborating the importance of c-Fos on such malignancies. These results point to the importance of cytoplasmic c-Fos in supporting normal and exacerbated growth of cells derived from the nervous system and may be a therapeutical target for controlling tumor growth in Nf1.

EN-P01.
EFFECT OF SUBVERSIVE SUBSTRATES ON *Entamoeba histolytica* THIOREDOXIN REDUCTASE ACTIVITY
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E. histolytica, usually lives and multiplies within the human gut under reduced oxygen pressure. During tissue invasion, it is exposed to increased amounts of reactive oxygen species, which are highly toxic for the parasite. The metabolic pathways used by this organism to cope with such environmental changes and redox homeostasis are a matter of our work. Recently, we characterized in *E. histolytica* a functional thioredoxin system, which is composed by three thioredoxins and a thioredoxin reductase (TRXR). The system serves as a reduction equivalents donor for different disulfide proteins. Our data in vitro show a central role of TRXR in the functionality of the system, being the enzyme a potential key molecular target for the development of new drugs to be used in the treatment of amoebiasis. In this work, we studied the effect of methylene blue (MB), essential oils from *Bystropogon mollis* ("peperina") and *Thymus vulgaris* ("tomillo") on the TRXR activity. Results agree with the view that these compounds are subversive substrates of the enzyme, as they stimulate its oxidase activity with a concomitant inhibition of the disulfide reductase activity. This work strongly supports the potentiality of both essential oils and MB for designing new anti-parasite agents to treat emergent cases of amoebiasis resistant to conventional drugs.
Granted by UNL, CAI+D 2006; ANPCyT, PICTO 05 13129.

EN-P02.
THREE ENZYMES INVOLVED IN GLUCOSE METABOLISM IN *Streptomyces coelicolor* A3(2)
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Streptomyces are filamentous soil bacteria producing a wide range of secondary metabolites including more than half of the known microbial antibiotics. These metabolites are synthesized in dedicated biosynthetic routes linked to primary metabolism in ways not completely understood. Looking for a deeper comprehension of central metabolic pathways in *Streptomyces*, we characterized three enzymes involved in glucose utilization in *S. coelicolor* A3(2). The genes *glgC*, *glgA* and *gtaB*, respectively coding for ADP-glucose pyrophosphorylase (EC 2.7.7.27; ADPGlcPPase), glycogen synthase (EC 2.4.1.21) and UDPGlcPPase (EC 2.7.7.9) were cloned, expressed in *Escherichia coli*, and the proteins purified to electrophoretical homogeneity. The enzymes were characterized in their kinetic properties. Allosteric effectors were found specifically for ADPGlcPPase. Results contribute to a better understanding of enzymes involved in central metabolic pathways and allow to hypothesize about regulation of glucose partitioning in *Streptomyces* spp. Obtaining knock out mutants of these genes will further help to elucidate their physiological role.

EN-P03.
THE ROLE OF C-TERMINUS IN THE CATALYTIC MECHANISM OF A BACTERIAL FERREDOXIN-NADP+ REDUCTASE
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Ferredoxin-NADP$^+$ reductases (FPRs) are flavoenzymes that catalyze reversible reactions between obligatory one-electron carriers and two-electron donors/acceptors. Structural analysis of reductases from different sources revealed that these flavoproteins are made up of two domains that bind the FAD and NADP(H) molecules, and helped to identify structural features that account for the difference in catalytic competence. Enzymes found in photosynthetic organisms are characterized by an extended FAD conformation and an invariant tyrosine residue at the C-terminus, responsible for high catalytic efficiency. Instead, the enzyme from eubacteria bind FAD in a bent conformation and the amino acid facing the flavin may be aromatic or aliphatic.
In the *Rhodobacter capsulatus* reductase, the binding of NADP(H) leads the displacement of the C-terminal tail of the protein to accommodate the nicotinamide ring for hydride transfer. We evaluate the role of the terminal residues A267 to I272 on the coenzyme binding and the catalytic activity. FAD was stably bound to all studied mutants after uv-visible spectroscopy. A six fold decrease of activity resulted from the A266Y mutation, but a still higher fall of catalytic rates corresponded to the A266Y and A266 mutants. Our results evidence the contribution of the C-terminus structure to the catalytic mechanism in bacterial ferredoxin-NADP$^+$ reductases.

EN-P04.
A MOLECULAR DYNAMICS STUDY OF GLYCOGENIN AUTOGLUCOSYLATION MECHANISM
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Glycogenin (GN) is a glucosyltransferase involved in the de novo biosynthesis of glycogen. Two different reaction mechanisms have been proposed for retaining glycosyltransferases such as GN: a double displacement (SN2) with a short-lived glycosyl-enzyme intermediate, and a single front-side displacement (SNi). The molecular nature of GN autoglucosylation is discussed since its crystallographic structure was described, because the distances measured between the amino acids involved in both mechanisms are long enough to preclude them. So, in order to understand at a molecular level the autoglucosylation of GN, the dynamic behaviour of the protein should be considered. Therefore, we performed a molecular dynamics study to analyze GN dimer movement. From the obtained trajectories, we calculated the distances between the amino acids involved in both mechanisms, considering an intra-monomeric reaction or an inter-monomeric one. Our preliminary results suggest that the dynamic behaviour of the protein favours SNi reaction mechanism. In agreement with in vitro experimental results, we also found that dimeric GN is more efficient to catalyze its autoglucosylation than the monomer.

EN-P05.
THE INTRAMOLECULAR AUTOGLUCOSYLATION OF MONOMERIC AND DIMERIC GLYCOGENIN
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It was described that glycogenin exists in solution and crystallizes as dimer at high protein concentrations. These results, together with the enzyme concentration independent specific rate of glucosylation, lead to propose an inter-subunit, intradimeric reaction mechanism for autoglucosylation. We analyzed the size of glycogenin by gel filtration and the specific rate of autoglucosylation at low protein concentrations and describe that the enzyme exist as monomer at low concentrations, showing a glucosylation kinetics also consistent with an intramolecular reaction mechanism. Accordingly, the dimeric glycogenin subunit might glucosylate as the monomer do, by a similar intra-molecular instead of intermolecular, intersubunit mechanism. The specific rate of autoglucosylation of the dimeric enzyme is up to two times the rate of the monomeric form. Referring to which form, monomer or dimer, would actually initiate the autoglucosylation *in vivo*, it is conceivable that just after synthesized, a molecule of glycogenin initiates its autoglucosylation without the need to reach the concentration required for dimerization. The monomer to dimer conversion resulting in the increase of the autoglucosylation efficiency, would allow glycogenin to modulate the *de novo* biosynthesis of proteoglycogen at the initial step of the glucose polymerization.

EN-P06.
GLYCOGEN BIOSYNTHESIS: GLYCOGENIN-BOUND MALTOSACCHARIDE INACCESSIBILITY TO FURTHER AUTOGLUCOSYLATION
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The intramolecular autoglucosylation of glycogenin (Gn) initiates the *de novo* biosynthesis of proteoglycogen, with formation of the Gn-bound maltosaccharide primer required by glycogen synthase for subsequent glucose polymerization. The tyrosine-bound maltosaccharide, produced by incubation of Gn with UDP-glucose, reaches a maximum polymerization degree of 13 glucose units at cessation of the reaction. No exhaustion of the substrate donor occurred at the autoglucosylation end and the full autoglucosylated enzyme continued catalytically active for transglucosylation of the alternative substrate dodecyl-ß-maltoside. Even though the autoglucosylation ceases once Gn acquired the mature maltosaccharide moiety, Gn species ranging rM 47 to 200 kDa, isolated from purified proteoglycogen after controlled amylolytic digestion, as well as proteoglycogen, were able to autoglucosylate. It was described that a-1,4-linked glucoses adopts a left-handed helical structure in maltoheptaose. Such a helical structure adopted by the mature Gn-bound maltosaccharide might leave the last incorporated glucose spatially far from the glucosylation site, a restriction which might be overcome by branched-bound a-1,4-glucans. Our work provides the first evidence to date for Gn intramolecular autoglucosylation ending by inaccessibility of the built maltosaccharide to the glucosylating site of the enzyme.

EN-P07.
SEX, INGESTION AND TEMPERATURE CONDITION THE EXPRESSION OF GPDH IN *T. Infestans* FLIGHT MUSCLES
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Triatoma infestans (*T. infestans*), Chagas' disease vector, acquires wings and the ability to fly after the last molt from fifth instar nymph to adult. The ability to fly is important for insect dispersion. Glycerol-3-phosphate dehydrogenase (GPDH) isozymes are differentially expressed during flight muscles development. The aim of this work is to begin GPDH transcripts expression studies during flight muscles development in both sexes and with different ingestion and room temperatures of 22 and 28°C.GPDH transcripts patterns were studied by RT-PCR, and the PCR-bands were semiquantified with a graphical method. The pattern of expression differs between sexes. The expression of GPDH-1 begins earlier in females and GPDH-2 is more abundantly expressed than in those from males. The expression pattern showed changes with increase of ingestion quantity. Before the last molt, GPDH-1 is 2 fold higher and GPDH-2 is 25 fold higher. In young adults, GPDH-1 decreased in 20% and GPDH-2 increased in 40%.In 30 days old adults, the GPDH-2 was 20% higher and GPDH-1 had no significant modification. During flight muscles development the expression pattern was different at room temperatures of 22 and 28°C. At 22°C the pattern showed delayed changes. These results are consistent with the expression and the metabolic role described for GPDH isozymes in thoracic muscles of flying insects.

EN-P08.
HETEROLOGOUS EXPRESSION AND CHARACTERIZATION OF *P. Tricornutum*'S PHOSPHOGLYCERATE KINASE-1
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Diatoms are key constituents of aquatic phytoplankton communities. Their contribution to marine primary productivity was estimated between 30-40%. Because of its small genome and the possibility of being routinely transformed, *Phaeodactylum tricornutum* has emerged as a model species for dissecting diatom biology. Our interest is to characterize enzymes involved in carbon metabolism in the diatom. Phosphoglycerate kinase-1 (E.C. 2.7.2.3; PGKase-1), which is a key step in the glycolytic pathway, generates ATP by conversion of 1,3-bis-phospho-glycerate into 3-phospho-glycerate, in a reversible reaction that needs Mg^{2+} as essential cation. Expression of the gene encoding *P. tricornutum* PGKase-1 in *Escherichia coli* rendered a soluble, active and recombinant enzyme. After purification, kinetic, regulatory and structural properties of the recombinant enzyme were characterized; and the properties were compared with those reported for PGKases from other sources. The specific activity of the purified diatom enzyme was 10.7 U/mg; and it exhibited optima values of temperature (of 30 ºC) and pH (of 8.0). Structural characterization was carried out through native PAGE and homology modeling, results agreeing with PGKase-1 being a monomeric enzyme with two possible conformations: open, in absence of substrates; and closed, when the enzyme is forming a ternary complex with its two substrates.

EN-P09.

CHARACTERIZATION OF *Entamoeba histolytica* UDPGLC-PYROPHOSPHORYLASE. STUDIES ON REDOX REGULATION

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Amoebiasis is an intestinal infection caused by the human pathogen *Entamoeba histolytica*. The parasitic disease is the third leading cause of death in the world (100,000 deaths annually). *E. histolytica* displays anchored-cell surface glycoconjugates involved in host-parasite interactions. The pathways for biosynthesis of glycoconjugates have not yet been fully elucidated. In parasitic organisms the production of structural oligo- and polysaccharides occurs via UDPGlc; with UDPGlc pyrophosphorylase (EC 2.7.7.9; UDPGlcPPase) catalyzing its synthesis. We report the molecular cloning of the gene coding for UDPGlcPPase from genomic DNA of *E. histolytica*. We used BL21(DE3)/pRSETB for expression and convenient purification of the recombinant His-tag protein product. The purified enzyme exhibited typical hyperbolic saturation kinetics for substrates. The enzyme activity was affected by redox modification of thiol groups. Different oxidants, including diamide, hydrogen peroxide and sodium nitroprusside inactivated the enzyme. The process was conveniently reversed by reducing agents, mainly DTT and thioredoxins from *E. histolitica* and *Trypanosoma brucei*. Results suggest the occurrence of a physiological redox mechanism for modulation of UDPGlcPPase activity, which would be critical to regulate carbohydrates metabolism in protozoa.
Granted by CAI+D 2006, PAV 137 and PICTO'06 15-36129.

EN-P10.

SIMULTANEOUS ALLOSTERIC ACTIVATION OF ADP-GLUCOSE PYROPHOSPHORYLASE: A SIMPLIFIED KINETIC MODEL

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Agrobacterium tumefaciens ADPGlc PPase (EC 2.7.7.27) is activated by two metabolites: pyruvate and fructose 6-phosphate (F6P). Both compounds mainly increase V_{max}, although at different levels for each. In this work, we studied the simultaneous effect of pyruvate and F6P, and found a decrease in $A_{0.5}$ corresponding to one activator in the presence of the second one, which may correlate with a mutual positive cooperative binding. Nevertheless, the effect on the catalytic step is not mutually positive in cooperativity, since at saturating concentrations of both activators, pyruvate leads activation even when it exerts a lower effect than F6P. We postulate a simple mechanism in which both activators bind to the free enzyme, to the complexes enzyme-susbtrate and enzyme-second activator. Using the MATLAB program, experimental data were fitted to the velocity equation and kinetic parameters were evaluated. Calculated substrate and activators apparent dissociation constants from enzyme complexes with both activators bound, were lower than those from enzyme complexes with only one activator. Also, data envisage that at saturating levels of both activators the predominant activated conformational state corresponds to that produced by pyruvate. These results preclude the assumption of a competitive behaviour and suggest that the binding site is not exactly the same for both activators.

EN-P11.

A WAY TO SEARCH OUT THE *Pseudomonas aeruginosa* ACETYLCHOLINESTERASE GENE

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P. aeruginosa acetycholinesterase (AchE) is induced by choline and involved in the corneal infection. Therefore, we focused our attention on the gene responsible for AchE synthesis. Although the catalytic properties of AchE are similar to those described in eukaryotic organisms, we could not find proteins with homology to AchEs of different organisms. Without success, we tried to obtain a transpositional mutant. After protein purification and analysis by MALDI-TOF, PA4496 was our first probable candidate, but later it was discarded. Our attempts were focused on PAGE gels under native, denatured and renatured conditions. The SDS denatured AchE could be easily renatured by dialysis with Triton X-100. AchE activity was identified with the Karnovsky-Roots specific staining and the isolated gel band analyzed by MALDI-TOF. Two main proteins belonging to PA5378 and PA1342 genes were identified. Both genes were cloned, the proteins expressed in *E. coli* and in *P. aeruginosa* and even both gene were deleted. None of these experiments allowed us to identify the *achE* gen. PA1342 and PA5378 shared the characteristic of belonging to periplasmic components of ABC transporter family. PA5378 was noted as a probable choline-betaine binding protein. At present, we have a strain deleted in PA1342 protein which has similar MW and pI to AchE and will probably help us to find the *achE* gene.

EN-P12.

MITOCHONDRIAL ATP SYNTHESIS IS NOT THE REVERSAL OF ATP HYDROLYSIS-DRIVEN H^+ TRANSLOCATION

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Sulfate is a partial inhibitor at low and a non-essential activator at high [ATP] of the ATPase activity of F_1. Therefore, a catalytically-competent ternary F_1•ATP•sulfate complex can be formed. In addition, the ANS fluorescence enhancement driven by ATP hydrolysis in submitochondrial particles is also stimulated by sulfate, clearly showing that the ATP hydrolysis in its presence is coupled to H^+ translocation. However, sulfate is a strong linear inhibitor of the mitochondrial ATP synthesis. The inhibition was competitive (K_i = 0.46 mM) with respect to Pi and mixed (K_i = 0.60 and K'_i = 5.6 mM) towards ADP. Since it is likely that sulfate exerts its effects by binding at the Pi binding subdomain of the catalytic site, we suggest that the catalytic site involved in the H^+ translocation driven by ATP hydrolysis has a more open conformation than the half-closed one ($ß_{HC}$), which is an intermediate in ATP synthesis. Accordingly, ATP hydrolysis is not necessarily the exact reversal of ATP synthesis.

EN-P13.

INTRACELLULAR COPROPORPHYRINOGEN OXIDASE LOCATION IN HEXACHLOROBENZENE TREATED RATS

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Hexachlorobenzene (HCB) chronic exposure of rats produces an experimental model for human porphyria cutanea tarda. After 8 weeks of treatment, rats showed higher porphyrin excreta and 50% inhibition of liver uroporphyrinogen decarboxylase activity. In feces isocoproporphyrin from abnormal pentaporphyrinogen decarboxylation by liver coproporphyrinogen oxidase (CPO), became the main porphyrin. Trypsin-treated mitochondria showed the outer and inner membrane (IM) permeability barrier highly conserved against enzyme release and trypsin accessibility, even after HCB intoxication. In digitonin-treated HCB mitochondria, CPO was free in the mitochondrial intermembrane space (IMS), whereas in normal (N) mitochondria, 30 to 50% remained anchored to the IM depending on the pH. HCB led to an uncoupled mitochondria. Albumin restored oxidative-phosphorylation, indicating no irreversible IM damage. N and HCB mitochondria oscillatory studies exhibited similar damping factor values, showing that HCB had no significant effect on membrane fluidity and elasticity. Mitochondrial uncoupling could explain the free-state of the enzyme within the IMS. The free state of the enzyme makes it more flexible and would allow pentaporphyrinogen, whose levels are increased, to compete with coproporphyrinogen and being transformed into dehydroisocoproporphyrinogen, liver forerunner of fecal isocoproporphyrin.

LI-P01.
CHOLESTEROL HOMEOSTASIS DEPENDS ON MEMBRANE LIPID COMPOSITION

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Cholesterol homeostasis is crucial for cell viability, but specially, its removal from peripheral cells is critical in order to prevent its accumulation on the artery wall. High density lipoproteins (HDL) and their major apolipoprotein apoA-I, play a key role in the Reverse Cholesterol Transport, which results in the removal of cholesterol (Chol) excess, and its transport towards the liver. It is well-known that this process is dependent on the activation of ABCA1 transporter. Here we suggest that membrane composition and/or organization is also strongly involved in cholesterol homeostasis. In order to test this hypothesis, we constructed a cell line overexpressing Stearoyl CoA desaturase, (SCD-cells, SAIB 2007), which results in the enrichment of monounsaturated fatty acids in the plasma membrane, without altering ABCA1 levels. Now we characterize membrane composition and analyze lipid removal, caveolin-1 expression and cell viability mediated by apoA-I. Our results show that plasma membrane is slightly enriched in Chol but has a lower phospholipids/sphingomyelin ratio in SCD-cells, which show less-efficient cholesterol removal mediated by apoA-I. Instead, they export more caveolin to the medium and are more resistant to Chol toxicity. We discuss our results in terms of a dynamic equilibrium between cell viability and Chol homeostasis.

LI-P02.
STEROLS: EFFECTS ON THE DYNAMICAL AND FUNCTIONAL PROPERTIES OF PLASMA MEMBRANES IN INSECT CELLS

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As insect cells lack the capability to synthesize sterols, the lipid profile of plasma membrane in cultured insect cells is dependent on the culture medium composition. Cultures of the lepidopteran cell line UFL-AG-286 adapted to grow in a cholesterol-free culture medium are refractory to baculovirus infection. Moreover, infection kinetics appeared to be influenced by the type of sterol used to feed them. The aim of this work is to correlate the dynamical properties of the plasma membrane of UFL-AG-286 cells, with the incorporation of different sterols, and their response to baculovirus infection. Cells were treated with methyl-beta-cyclodextrin that reduced 80% of the cellular sterol content. Then, aliquots of treated cells were fed with either cholesterol or sitosterol emulsion, while other aliquot was not fed with sterols. The membrane fluidity in each group of cells was estimated through EPR measurements with spin labels, while the susceptibility to viral infection was determined following the kinetics of AgMNPV virus adsorption. The results indicate that fluidity was increased in sterol depleted cells, but similar values were found in the aliquots fed with sterol and in untreated cells. The cells fed with sitosterol showed a quick virus adsorption at short times, while those fed with cholesterol had a slower initial kinetics but resulted more efficient in the infection.

LI-P03.
STRUCTURAL CHARACTERIZATION OF VERY HIGH DENSITY LIPOPROTEIN FROM THE SPIDER *Polybetes pythagoricus*

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We have previously isolated, purified and characterized an hemolymphatic VHDL from *Polybetes pythagoricus*. This lipoprotein contains hemocyanin (Hc) as the major apoprotein, and as a novelty, it transports most of the circulating lipids. In the present work, for the first time in arachnids, we studied size, shape and structure of *P. pythagoricus* VHDL using electronic microscopy, MALDI-TOF-MS, circular dichroism, partial proteolysis, N-terminal sequencing, and lipid and copper gel specific staining.
Results showed that VHDL has spheroidal morphology with an estimated size of 11.2 ± 0.025 nm. As seen by SDS-PAGGE, it contains one main band corresponding to monomers of Hc (70 kDa), and two minor subunits corresponding to non-respiratory proteins of 105 and 120 kDa. These last two proteins are not linked by disulfide bonds, they don´t include lipids or copper in their structure, and they would be more exposed to the aqueous medium in native conditions. Using circular dichroism we observed that it contains 20% á-helix, 29% ß-sheet, 22.7% turns and 29.7% random. By MALDI-TOF MS, 15 polypeptides present in the monomer were found to be homologous to subunit 3 of the spider *Cupiennius salei* hemocyanin; and 27 polypeptides from the 105 kDa subunit showed homology to a protein from the insect Anopheles gambiae.

LI-P04.
***Ulemoides dermestoides* IN ALTERNATIVE MEDICINE: LIPIDIC COMPOUND INHIBITED CELL PROLIFERATION**

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The tenebrionid beetle *Ulomoides dermestoides* (Fairmaire, 1893), a stored grain product pest, known in Argentina as gorgojo, is reared by people for ingestion alive as alternative medicine in the treatment of different illnesses, specially cancer. The aim of this study was to improve the knowledge of the defense compounds released by stressed insects and determine their potential cell inhibitory activity. The analysis was performed with solid-phase microextraction (SPME) coupled to CGC-MS. In inhibitory assays we used an extract with the same composition. The effects on cellular proliferation and viability were analized on A549 cell line using MTT test and trypan blue cell counting. The major components of the extract are 1,4-benzoquinones (BQ) and 1-pentadecene (n-C15:1), in a 1,8 ratio. The IC50 value calculated with the MTT test was 0.678 eq/ml (1 equivalent = the amount of components extracted per beetle with CH_2Cl_2 during 20min). The same concentration produced 40% inhibition of cell proliferation on A549 cells. We also tested the synthetic compounds individually and combined. The inhibition of A549 cells proliferation with BQ was similar to that obtained with the insect extract. C15:1 did not shown any inhibitory effect. We conclude that the BQ present in the insect extract is responsible for inhibit cell proliferation and metabolic activity.

LI-P05.
FATTY ACIDS DESATURASE IN *Bradyrhysobium* PEANUT NODULANT
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Desaturases (Des) of fatty acids (FA) have been described in eukaryotic as well as prokaryotic organisms. These introduce double bounds in fatty acids of the membrane phospholipids; their activity is regulated so that the fluidity of the bilayer stays constant, mainly when some changes on the environment happen. In this work we identified a possible desaturase gene in a nodulant peanut rizobio strain and verified the presence "*in vivo*", of a desaturation system of stearic acid (18:0).
DNA from Bradyrhizobium TAL 1000 and Ensinfer meliloti (control strain) were extracted and submitted to PCR amplification, employing primers of gene des sequence from *E. meliloti*. The PCR product was sequenced. The enzymatic activity was measured in B. TAL1000, using 18:0 [1-^{14}C] as substrate after 24 hs of growth at 28ºC. Lipids were extracted and fatty acid methyl esters were obtained and separated by $AgNO_3$-TLC.
A fragment of 1 Kb, having a high identity with predicted des gene of *E. meliloti* and related bacteria, was amplified by PCR. When Des activity was measured, 35% of the radioactivity from stearic acid was recovered in monounsaturated FA fraction. The above results are indicative that B. TAL1000 would own a desaturation system to generate monounsaturated fatty acids, which would be complementary of the anaerobic system of unsaturated FA formation.

LI-P06.
SALINE AND OSMOTIC STRESS EFFECT ON PHOSPHOLIPID METABOLISM IN COLEOPTILE AND ROOTS OF BARLEY
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The plants developed a variety of physiological and biochemical responses to adapt to adverse environmental conditions. Saline stress is certainly one of the most serious abiotic factors that limit the normal development of the plants and display diverse signaling processes. Our aim was to study physiological and biochemical changes at the level of signaling lipids and proline accumulation during early germination under saline and osmotic stress. A decrease of growth in seedlings, in dark conditions with different NaCl and mannitol concentrations was observed. We also analyzed lipid kinase activities through incorporation of phosphate from [d-^{32}P]ATP to their endogenous substrates. Phospholipids (PLs) identified were PIP2, PIP, DGPP, LPA and PA. In coleoptile and roots controls, PIP was more abundant whereas in aleurone were DGPP and PA. These observations suggest that the lipid metabolism is modified during the transition on dormition state to germination. Saline stress produced an increase of root PA, however in coleoptile no change was observed. Osmotic stress produced an increase in PA and DGPP only in roots. Both stresses increased proline levels only in roots. Although plants respond physiologically to stress as a whole, the metabolic responses differ from an organ to other and according to each type of stress analyzed.

LI-P07.
STRUCTURAL CHARACTERIZATION OF ACIDIC GLYCOLIPIDS IN *Trypanosoma cruzi*
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Although acidic glycosphingolipids are ubiquitous in eukaryotic cells, very little is known about their role in parasites. Among acidic glycosphingolipids, gangliosides (containing sialic acid) and sulfoglycolipids which contain sulfate monoester groups are usually found as membrane components distributed on the cell surface. They are active participants in adhesion processes in many cellular systems and appear to be involved in the regulation of cell proliferation, differentiation and other developmental cellular events. In parasites, lipid metabolism has been attracting a lot of attention with respect to basic biology and applications for chemoterapeutic purposes, but although a lot has been reported about the relation of *Trypanosoma cruzi* glycosphingolipids with chagasic autoimmunity, structures and the biological role of acidic glycosphingolipids of *T.cruzi* has not been characterized so far. In this work we show by extraction, purification, TLC and MALDI-TOF mass spectrometry analysis, the structure of two acidic glycosphingolipids, a sulfatide and a ganglioside, named STc1 and GTc, respectively, from epimastigote forms of *T. cruzi*. Interestingly, parasite treatment with an inhibitor of the glycolipidic pathway caused an arrest on parasite development associated to variations in the acidic glycolipidic biosynthetic levels.

LI-P08.
CHEMICAL VALIDATION OF *Trypanosoma brucei* FATTY ACID DESATURASES AS DRUG TARGETS
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Trypanosomatids are causative agents of several diseases in developing countries. The drugs used to treat the parasites have important disadvantages such as severe side effects, strain resistance, and variable efficacy. So, the development of more secure and efficient drugs is needed. Thiastearic acids (TS) showed toxic effect on *C. fasciculata* and *Leishmania* spp. while Isoxyl was used previously to treat tuberculosis. Both compounds seem to inhibit unsaturated fatty acid (UFA) synthesis. In this work, we propose that UFA synthesis is essential for parasites, and inhibitors of this pathway could affect trypanosomes growth. Then, we treated cultures of *Trypanosoma brucei*, bloodstream (BSF) and procyclic (PCF) forms with 4 positional isomers of TS and Isoxyl. In PCF, 9-TS (TS with sulfur at position 9) showed poor growth inhibition while 10-, 12-, 13-TS and Isoxyl showed a concentration dependant growth inhibition. 10-TS effect was reverted by oleate, indicating that its target was the Ä9 desaturase. 12- and 13-TS were synthesized as putative inhibitors of Ä12 desaturase, which was confirmed by analyzing the fatty acid profile of treated and untreated cells.
BSF showed a slightly different behavior, but parasite growth was inhibited when drug concentration reached a minimum inhibitory concentration with all compounds, validating both desaturases as promissory drug targets

LI-P09.
BIOPHYSICAL CHARACTERISTICS OF TESTICULAR SPHINGOMYELINS WITH VERY LONG CHAIN POLYUNSATURATED FATTY

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Very long-chain (C24 to C36) polyunsaturated fatty acids (VLCPUFA) are important constituents of sphingomyelin (SM) of mammalian spermatogenic cells and spermatozoa. The most abundant VLCPUFA in rat testicular SM are 28:4n-6 and 30:5n-6, followed by 32:5n-6. In order to study some of the biophysical properties of these molecular species, liposomes were prepared from three rat testis SM subfractions differing in the fatty acids bound to sphingosine: I) C16:0-C18:0, II) C22:0-24:0, and III) C28-C32 VLCPUFA. Their thermal behavior was measured by determining the generalized polarization (GP) of the fluorescent probe Laurdan as a function of temperature. Despite the difference of about 6 carbon atoms in their acyl chains, subfractions I and II showed a similar transition temperature (Tt), around 45ºC. In contrast, SM fraction III (on average 7 carbon longer than fraction II) showed a significantly lower Tt, about 25ºC. Thus, the degree of unsaturation in the amide-bound fatty acid of SM overrides the effect of chain length, causing a low GP value in the liposomes at physiological temperatures. Opposite to the typical ability of most naturally occurring species of SM to form highly-ordered, closely-packed lipid domains, VLCPUFA-rich SMs may form liquid-disordered lipid domains in liposomes and perhaps also in bilayers of the specific germ cells and gametes in which they occur.

LI-P10.
LIPIDS WITH VERY LONG CHAIN FATTY ACIDS (VLCPUFA) IN GERM CELLS AND RESIDUAL BODIES

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In seminiferous tubules, Sertoli cells and spermatogenic cells at different stages of differentiation coexist. In this work pachytene spermatocytes (PS) and round spermatids (RS) were isolated to study how ceramides (Cer) and sphingomyelin (SM) with VLCPUFA are formed in the testis. Although the concentration of phospholipids including SM was similar in both cell types, there were many differences in their polyenoic fatty acids (FA). In glycerophopholipids, the 20:4n-6/22:5n-6 ratio was significantly higher in PS than in RS. In SM (and Cer), 28:4n-6, 30:5n-6, 32:5n-6 were the main FA in PS, whereas 28:4n-6 predominated in RS. By contrast, in the latter, an important proportion of SM (and Cer) FA were recovered as a-hydroxylated versions of VLCPUFA (2-OH 28:4 to 32:5). The residual bodies (RB) contain materials –including lipids– that are discarded from late spermatids (elongated forms) as they differentiate to spermatozoa, to be phagocytized and "recycled" in Sertoli cells. The RB were very rich in glycerophopholipids and triglycerides with 22:5n-6. The SM of RB contained virtually no VLCPUFA but was extremely rich in 2-OH VLCPUFA. The results point to fatty acid desaturase, elongase and hydroxylase activities that are highly specific for long-chain and very long-chain polyenoic fatty acids encoded by genes that are expressed sequentially as cell-differentiation proceeds.

LI-P11.
RAPID AND PERMANENT LIPID CHANGES AFTER DEATH OF TESTICULAR CELLS INDUCED BY EXPOSURE TO CADMIUM

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Because it selectively damages testicular capillary endothelial cells, exposure to cadmium results in hemorrhagic ischemia and hemato-testicular barrier disruption. Tight junctions between Sertoli cells are disassembled, followed by sloughing of dead Sertoli and germ cells to seminiferous tubule lumen. Here we studied the early and late effects of $CdCl_2$ on rat testicular lipids. The early effects were similar to those of ischemia induced by testicular artery ligation. Two days after a single $CdCl_2$ dose, visible hemorrhage and inflammatory edema was accompanied by a massive hydrolysis of germ cell 22:5-rich glycerophospholipids (GPL) (to free fatty acids and diacylglycerols), and of sphingomyelins (SM) containing normal and hydroxylated polyenoic fatty acids (to the corresponding ceramides, Cer). Thirty and 45 days later, the testicular weight was reduced to less than one third and most of the original 22:5-GPL and their products had been removed from the testis. Cholesterol esters and triglycerides mirrored the GPL changes, suggesting they were formed as GPL fatty acids were eliminated, probably by phagocytes. By contrast, the Cer initially produced remained high and unchanged in the tissue. Whether this is due to physical seclusion of Cer in a space inaccessible to phagocytes or to a Cd-related inhibition of ceramidase remains to be established.

LI-P12.
DIMETHOATE-INDUCED LIPID PEROXIDATION IN LEYDIG CELL IS REVERTED BY *IN VITRO* TOCOPHEROL ADDITION

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Dimethoate (D) is involved in deleterious effects on testicular physiology. We studied the impact of a sub-chronic administration of D to Wistar rats (15 mg/Kg body weight ip, 5 weeks) on the neutral (N) and polar (P) lipid composition, antioxidant status and peroxidability of microsomal (Mi) and mitochondrial (Mt) membranes isolated from Leydig cells. N and P fatty acyl chains from Mi membranes were enriched in PUFAs compared with Mt fraction. D treatment did not modify their composition; however, it produced a significant decrease in the tocopherol (Toc) content. The loss of Toc correlated with both an increased TBARS production, and a high sensitivity to peroxidation (PX). The time-curse of the in vitro t-BHP-induced PX was measured as TBARS generation under standard incubation conditions. At zero time, membranes isolated from D-treated rats showed an increase of TBARS. The lag of induction almost disappeared in cells isolated form intoxicated animals and the rate of propagation and the maximal production of TBARS were significantly increased. These alterations were almost reverted by in vitro Toc addition. The protective effect was dose-dependent and followed a sigmoidal behavior. Toc may be effective in the protection of Mi and Mt membranes of D-intoxicated cells and in the preservation of PUFAs intimately involved in androgenic function and spermatogenesis.

LI-P13.

INTOXICATION IN PROFESSIONAL SPRAYERS: OXIDATIVE STRESS BIOMARKERS AND OTHER BIOCHEMICAL PARAMETE RS

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We study the impact of involuntary exposure to residual pesticides in a population of 54 professional sprayers (S) (32 males, 22 females; 25 ± 5 year old) exposed to pesticides and fungicides during approx. 5 years by determining various oxidative stress biomarkers (OSB) and other parameters in blood. Results were compared with a similar group of volunteers (C) selected from urban areas. S showed hematological, renal, and hepatic biomarkers within the normal range of values established for the general population, including acetylcholinesterase activity and fructosamine levels. In spite of that, the entire biochemical test was significantly altered compared to C. Plasma tocopherol content, and the total reducing ability of plasma (FRAP assay) were significantly decreased, while plasma protein carbonyls, thiobarbituric acid-reactive substances, total glutathione, ceruloplasmine, total copper, and the sum of nitrates and nitrites were increased. This investigation demonstrated that screening laboratory tests are completely insensitive for demonstrating sub-clinical intoxication and/or occupational risk. It also proved that OSB should be included in this kind of evaluation procedures to better design new strategies of prevention. Chronical exposure to residual pesticides and to other agrochemicals is intimately linked to an increased incidence of degenerative illnesses in humans.

LI-P14.

COPPER OVERLOAD IN HUMAN A-549 CELLS: EFFECTS ON LIPID COMPOSITION AND ANTIOXIDANT DEFENSE SYSTEM

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We studied *in vitro* Cu^{++} overload in human alveolar malignant cells A-540 to test the response of the antioxidant defense system and the possible impact on the fatty acyl composition of total cellular lipids. Exposition to Cu^{++} (0-180 µM /24 h) increased thiobarbituric acid-reactive substances, nitrates+nitrites, protein carbonyls, total glutathione, peroxidase and glutathione-transferase activities; and decreased alpha-tocopherol content, total reducing ability (FRAP assay), and glutathione reductase and catalase activities. Superoxide-dismutase was incremented at low Cu^{++} overload and inhibited at higher concentrations. The rate of cellular growth was strongly depressed in a concentration-dependent manner. Changes were associated to an increased milli- and microcalpaine activation, and with a caspase-3 increment at 40 (but not at 80) µM Cu^{++}. Fatty acyl composition of total cellular lipids exhibited progressive changes which depend on the Cu^{++} concentration; mainly a relative increase of saturated fatty acids (especially 16:0 and 18:0) and a substantial loss of PUFAs. Concomitantly, unsaturation index decrease progressively in a [Cu^{++}]-dependent fashion. Modifications induced by Cu may be important in the understanding of the damages associated to involuntary Cu exposure, and also in the etiology of human illnesses such as neurodegenerative disorders.

LI-P15.

PEROXIDATION OF RETINAL LIPOSOMES ANALYZED BY CONJUGATED DIENES, TRIENES, TBARS AND PHOTOEMISSION

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The retina is highly susceptible to oxidative damage due to their high content of polyunsaturated fatty acids (PUFAs), mainly docosahexaenoic acid (22:6 n3). The lipid peroxidation (LP) process is thought to be involved in various physiological and pathological events. Many model membranes can be used to learn more about issues that cannot be studied in biological membranes. Multilamellar (ML) and unilamellar liposomes (UL) prepared with lipids isolated from bovine retina were subjected to LP, under air atmosphere at 22°C, with Fe^{2+} or Fe^{3+} in different aqueous media. Conjugated dienes (CD) and conjugated trienes (CT), determined by absorption at 234 and 270 nm respectively, thiobarbituric acid-reactive substances (TBARS) and photoemission were measured as a function of time. Our results demonstrated that both liposome types were susceptible to Fe^{2+}-initiated lipid peroxidation and they were poorly peroxidized by Fe^{3+}. On the other hand we noticed that lipid peroxidation of retinal liposomes is affected not only by the type of initiator but also by the aqueous media. Our model constitutes an appropriate system to study formation of lipid peroxidation intermediaries and products in an aqueous environment and is useful to assay the effect of different antioxidants.

LI-P16.

ENDONUCLEAR LIPIDS HAVE A HIGH PROPORTION OF NEUTRAL LIPIDS

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Nuclear lipids are mostly found in the double nuclear membrane, and in a minor proportion they are associated to chromatin and to the nuclear matrix (Mx). Composition and physico-chemical properties of these lipid pools are very important as they are the sources of nuclear signal transduction system of PI, PC and SM as well FA involved in genetic regulation. The aim of this work was to analyze nuclear neutral lipids composition (NL), their location and nuclear fluidity (GP: Generalized polarization). Thus, whole nuclei and nuclear double membrane–depleted nuclei (Mx) were isolated from rat liver cells. Purity of nuclear fractions was determined by electronic microscopy and marker proteins. Nuclei lipids mainly contain 84% and 16% of PL an NL, respectively, as follows: PC>PE>TAG>PI>Cho>SM/PS>>CE. Endonuclear lipids (Mx) are mainly constituted of 57% PL and 43% NL, as follows: TAG=PC>PE=Cho>SM/PI/PS/CE. Thus, the mayor lipid pool in nuclei is PC, while TAG and PC are mainly found inside nuclei. Mx were analized by Laurdan GP (espectrofluorometric and 2 Foton microscopy), presenting less fluidity than nuclei. In conclusion, endonuclear lipids are enriched in NL (TAG, Cho, CE). Despite the minor amount of PL, these are enriched in SM and PS. TAG and PC were found to be the major lipids inside the nuclei, and they may be alternative sources of FA with different properties and regulation.

LI-P17.
STUDIES ON PROTEIN PALMITOYLATION IN *Toxoplasma gondii*
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Palmitoylation is a post-translational modification that provides proteins with enough hydrophobicity to interact with membranes. In *Toxoplasma gondii*, little is known of this important modification. Metabolic labeling of *T. gondii* tachyzoites with [^{3}H]-palmitic acid revealed several radioactive proteins that are modified with this fatty acid, indicating that protein palmitoylation occurs in this parasite. Furthermore, incubation of *T. gondii* tachyzoites with a palmitoylation inhibitor 2-Br-palmitate blocked the invasion process. In addition, we identified TgHSP20, a small heat shock protein, to be palmitoylated "*in vivo*" in *T. gondii*. TgHSP20 is a stripe-arranged chaperone localized to the inner membrane complex (IMC) of the parasite. TgHSP20 association to the IMC is quite striking since the protein does not contain any hydrophobic region or transmembrane domains. Compared alignment of HSP20 aminoacidic sequences from different apicomplexa parasites revealed a set of cysteines that are conserved among them. Interestingly, these conserved cysteine- residues are predicted to be palmitoylated by "in silico" analysis. Taken together, the data obtained shows that protein palmitoylation is an operating system in *T. gondii* that may be important for the parasite invasion process. Besides, we have started to identify proteins that are palmitoylated in this parasite.

LI-P18.
PRESENCE OF PHOSPHATIDYLCHOLINE DERIVED SIGNALLING IN SYNAPTOSOMAL LIPID RAFTS
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We have previously determined the coexistence of phosphatidylcholine specific phospholipase C (PC-PLC) and phospholipase D (PLD) pathways in synaptic endings obtained from rat cerebral cortex . These enzyme pathways generate the lipid messenger, diacylglycerol, (DAG) by catalyzing phosphatidylcholine (PC) hydrolysis. The objective of the present work was to characterize lipid rafts obtained from purified synaptosomes (Syn). We also investigated the presence of the above mentioned lipid messengers pathways in this fraction. The detergents resistant membranes (DRM) were obtained from purified synaptic endings from adult and aged rats according to the procedure of Brown and Rose (1992). DRM lipid composition was determined by TLC and protein components were evaluated by Western blot. DRM membranes showed an increase in sphingomyelin and cholesterol levels with respect to the values found in purified synaptosomes. The enrichment in ceramide, caveolin and c-src was also observed in DRM with respect to entire synaptosomes. DRMs membranes from adults and aged rats were able to generate DAG from PC when they were incubated in the presence of exogenous PC. Our results showed that lipid messenger pathways that generate bioactive lipids from PC are present in DRM fraction obtained from synaptic endings.

LI-P19.
LIPIDS FROM VIRULENT AND ATTENUATED *B. bovis* INDUCE DIFFERENTIAL TLR2–MEDIATED MACROPHAGE ACTIVATION
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Babesia bovis, an intraerythrocytic apicomplexan protozoa of cattle, causes an acute infection with parasite persistence and its resolution depends on products of activated macrophages. In this sense, it has been demonstrated that the lipid fraction of *B. bovis* (mexican strain)-infected erythrocytes stimulates the production of nitric oxide (Brown WC, 2000). Herein, we report that the lipid fractions from *B. bovis* merozoites of R1A (attenuated) and S2P (pathogenic) strains induced higher TNFá and KC release in peritoneal macrophages (Mo) from wild type mice (WT) compared to unstimulated cells (ELISA). In contrast, this effect was inhibited in Mo from knock out toll like receptor 2 mice (KOTLR2). Moreover, the induction of lipid body formation (Osmium staining) in murine Mo by R1A and S2P lipids was significantly inhibited in TLR2-defective Mo when compared with WT cells. Interestingly, in both cases R1A lipids induced a significantly higher response than S2P. R1A lipids also induced the expression of Ciclooxigenase 2 (COX-2) in WT cells whereas this effect was not observed in KOTLR2 cells (Immunoblot). No COX-2 induction was detected in WT and KOTLR2 cells stimulated with S2P lipids. These results suggest that activation of Mo by *B. bovis* lipid fractions is mediated by TLR2 and the decreased inflammatory response observed with S2P lipids could be an immune modulation mechanism.

LI-P20.
ROLE OF PHOSPHATIDATE KINASE AND PHOSPHATIDATE PHOSPHOHYDROLASE IN ABA SIGNAL
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Abscisic acid (ABA) exerts an important role in seed germination since it inhibits the response to gibberellin (GA) in aleurone. Phosphatidic acid (PA) is an intermediary in ABA signal however diacylglycerol pyrophosphate (DGPP) role in germination processes is not clearly established. Here, we showed that PA produced by phospholipase D (PLD) during antagonist ABA effect in GA signal was rapidly phosphorylated by phosphatidate kinase (PAK) to DGPP. This fact is critical for aleurone since dioleoyl-DGPP exogenously added was able to inhibit the á-amylase secretion. Inhibition of PLD activity by 1-butanol during ABA treatment resulted in a normal secretory activity. This effect was overcome by addition of dioleoyl-DGPP. It also showed that ABA decreased the activity of Mg^{2+}-independent and NEM-insensitive form of phosphatidate phosphohydrolase (PAP2). Using *Arabidopsis thaliana* Lpp protein sequences as queries we identified two putative molecular homologues, which we termed HvLpp1 and HvLpp2, encoding putative Lpps. The presence of well conserved structural Lpp domains in these sequences and the detection of protein by immunoblots suggest that both proteins are functional enzymes. Results shown are consistent with the role of DGPP as a regulator of the antagonistic effect of ABA in GA signal.

LI-P21.
ROLE OF THE PHOSPHATIDIC ACID IN RESPONSE TO OSMOTIC STRESS IN *Trypanosoma cruzi*
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The aim of this work was to determine the relationship between phospholipid metabolism and parasite differentiation in response to osmotic stress. We found that the addition of NaCl or Mannitol to parasite culture medium increased the intermediate forms between epimatigotes and trypomastigotes, similarly to the differentiation process that occurs in the small intestine and the rectum of the insect vector. We also found that osmotic stress evoked an increase in lipid kinases analyzed. By using [-^{32}P]-ATP and endogenous substrates, the diacylglycerol kinases (DAG-K) was the main enzyme modulated by osmotic stress since its product phosphatidic acid (PA) increased 2.5 fold respect to the control. PA was also phosphorylated to diacylglycerol pyrophosphate (DGPP) by phosphatidate kinase. In another type of experiments with parasites in exponential phase of growth, the stimulus with NaCl 0.5 M for 5 min produced also an increase in DAG-K. It is shown that the generated PA can be desphosphorylated by the activity of Mg2+ independent and NEM-insensitive form of phosphatidate phosphohydrolase (PAP2) and that the osmotic stress increased the PAP2 activity. By using *Arabidopsis thaliana* Lpp protein sequences as queries it was also identified putative molecular homologues in the parasite. These results are in agreement with the role of PA level during the morphological changes of parasite evoked by osmotic stress.

LI-P22.
OSMOTIC ACTIVATION OF PHOSPHOLIPID BIOSYNTHETIC PATHWAYS INVOLVES DIFFERENT REGULATORY MECHANISMS
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Renal medullary collecting ducts are immersed in the highest interstitial osmolality of the body. To function they have developed various protective mechanisms. In rat renal medulla, we have shown that membrane phospholipid (PL) turnover acts as a defensive mechanism against osmolality. With the purpose of determining the molecular mechanisms that govern membrane renewal in renal cells, in the present work we studied how hyperosmotic medium modulates PL synthesis and content, and the role of PLC, PKC and ERK1/2, showed to be activated by osmotic stress. MDCK cell cultures were grown during 24 h in hyperosmotic medium made by Urea, NaCl or both (NaU) addition to isotonic medium (Iso: 298, HOs: 370 to 700 mOsm/Kg H2O). PL biosynthetic activity was evaluated by measuring ^{14}C-glycerol incorporation to PL. NaU700 caused more than 100 % increase in radiolabelled PL. The highest biosynthetic activity was associated to phosphatidylethanolamine (PE), followed by phosphatidylinositol (PI) and phosphatidylcholine (PC). Hyperosmolality increased PL content per cell and changed PL membrane profile. U73122, PLC inhibitor, decreased all ^{14}C-PL by 30% and PKC inhibition only affected ^{14}C-PI. ERK1/2 inhibitor U0126 selectively dropped ^{14}C-PE by 70 %. Our data show that the PL synthesis that leads to membrane renewal and osmotic cytoprotection involves exclusive regulatory mechanisms for each PL.

LI-P23.
CHANGES IN KEY ENZYMES OF HEPATIC LIPID METABOLISM INDUCED BY COMMERCIAL OILS-SUPPLEMENTED DIETS
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Previous studies from our lab demonstrated that commercial oils used as dietary lipid source (70 g/Kg diet for 60 days) can induce oxidative stress in liver, brain, kidney, and testis of Wistar rats fed soybean (S), coconut (C), olive (O) or grape-seed (G) oil-supplemented diets. In liver, these changes increased free radical production in G and S dietary groups, compared with C, and O rats. We investigated the impact of these diets on the main hepatic enzymes involved in fatty acid (FA) metabolism. Long-chain FA synthetase was decreased in S and G groups compared with O and C. Ä9 Microsomal FA desaturase activities for palmitic and stearic acids were decreased in S and G groups, while Ä6 (linoleic and linolenic FA as substrates), and the Ä5 (eicosatrienoate), decreased in the order O>S>G>C. Phospholipase A2 activity was incremented in the order G>S>O>C. Rate of ß-oxidation (mitochondrial fraction) was depressed in G and S group compared with O and C. FA-CoA synthetase from S and G was incremented compared with that of O and C groups. Nitrite+nitrate concentration in liver homogenates parallel the concentration of ONOO.- which directly correlate with [Ca]. This finding may be the causal factor that explains the observed changes. Thus, dietary lipid may have a direct influence on lipid metabolism through the modifications induced in some of the most important enzyme activities.

LI-P24.
ALTERATION IN THE LEVELS OF CCTb2 EXPRESSION AFFECTS NEURONAL DIFFERENTIATION
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The morphological hallmark of neuronal differentiation is neurite sprouting and elongation. This process increases the demand for membrane components. Phophatidylcholine (PC), the predominant membrane phospholipid, is synthesized in neurons by the Kennedy pathway. The enzyme CTP:phosphocholine cytidylyltransferase (CCT) regulates this pathway. Two CCT isoform have been described; CCTa is ubiquitously expressed in all tissues whereas CCTb is the most abundant isoform in brain and gonads. Little is known about CCTb transcriptional regulation and their physiological role.
We used Neuro2a cells as an in vitro differentiation model. In our study we attend to vary the PC levels in these cells by modifying the CCTb2 expression levels; we use specific siRNA (siCCTb2) to knockdown its expression and a plasmid to overexpress this isoform. Subsequently, we analyzed how neuritogenesis is affected by measuring cell differentiation parameters such as neurite number, length and branching. We observed that down regulation of CCTb2 changes the differentiation parameters respect to the control cells. However, overexpression of CCTb2 in Neuro2a cells has no effect on the differentiation process. Because CCTa overexpression induces differentiation, our results could suggest that there is a crosstalk mechanism between the CCT isoforms in Neuro2a cells physiology.

LI-P25.

CULTURES OF FIBROBLASTS DISPLAY DAILY VARIATIONS IN THE BIOSYNTHESIS OF PHOSPHATIDYLCHOLINE

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The circadian system that controls temporally physiology and behavior is composed of central pacemakers located in the brain and a network of peripheral oscillators distributed throughout the body. We have reported that the biosynthesis of phospholipids oscillates daily in synchronized fibroblast cultures under an intrinsic clock control involving the expression of the clock protein mPer1. However, it is unknown how the synthesis of phosphatidylcholine (PC), the most abundant eukaryotic cell lipid, is regulated along the day in cell cultures. The main pathway of PC synthesis in all mammalian nucleated cells is the CDP: choline pathway -Kennedy pathway- with CTP: phosphocholine cytidylyltranferase (CCT) as the key enzyme. Here, we show that the synthesis of PC in synchronized cultures of NIH 3T3 cells exhibits a daily variation with higher levels at 6.5 h post serum shock. Similar changes were found in total CCT activity presenting higher levels at 6.5 h and 35 h post stimulation ($p<0.00965$ by ANOVA). We found detectable transcript levels of CCTá1, CCTß2 y CCTß3 but not of CCTá2 by RT-PCR in the cultures. Moreover, a daily variation in CCTß mRNA and protein levels was found. The results clearly indicate that the biosynthesis of PC display a daily variation accompanied by similar changes in CCT enzyme activity, and mRNA and protein levels for the isoform ß2.

MI-P01.

HBV VARIANTS AND NATURAL RESISTANCE TO NUCLEOS(T)IDE ANALOGUES

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Naturally occurring HBV resistance mutations to a given anti-HBV drug are defined as those variants exhibiting any aminoacids replacement associated to a/n (reportedly known) antiviral resistance, despite the absence of such an antiviral treatment.
Objective: To study in chronically HBV-infected patients in Argentina the development of natural antiviral resistance to at least one of the current nucleos(t)ide analogues (NAs) approved for chronic hepatitis B treatment.
Matherials and Methods: DNA was extracted from serum of 13 Argentinean patients with chronic hepatits B. Five of them had ever undergone antiviral therapy with NAs and 8 only received LMV. HBV S gene was amplified by PCR, and amplicons were sequenced.
Results: we detected 13 cases of chronically HBV-infected patients natural antiviral resistance to at least one of the current NAs approved for chronic hepatitis B. Five of them are also carriers of S-escape mutants with primary resistance to ADV.
Conclusion: With the circulation of HBV variants displaying natural resistance to current NAs, their detection before an antiviral therapy regimen is set up should be a strategic way to optimize the treatment for chronic hepatitis B. Finally, circulation and transmission of S-escape variants with primary resistance to antiviral drugs should be of public health concern as they may represent a potential risk for the community.

MI-P02.

INFLUENCE OF THE HCV NS5A PROTEIN IN TRANSCRIPTION OF GENES INVOLVED IN LIVER DETOXIFICATION

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The HCV NS5A protein interacts with cellular factors, affecting signalling pathways modulation, apoptosis, cell cycle and gene transcription. ABC family's proteins are ATP-dependent transmembrane pumps. MDR1/3, MRP1/2 and BCRP are involved in cellular detoxification processes. According to previous reports, the interaction between NS5A and HNF1 promotes MRP2 transcription, which would have been implicated in the resistance to antiviral-tumoral drugs.
Objective: To study the effect of the NS5A expression in Huh7 and HeLa cells over the different mRNAs that encode ABC family's proteins. The relative expression was measured by RT-Real time PCR in Huh7 and HeLa cells transiently expressing NS5A protein. A down-regulation in the expression of both *mrp1/2* genes was observed in Huh7 cells expressing NS5A. Such effects were not observed in HeLa cells. Up-regulation of *mrp1/2* genes was registered when a HBV protein was expressed in Huh7. The expression of *mdr1/3* (Huh7) and *bcrp* (HeLa) decreased in NS5A transfected cells. The difference in *mrp1/2* relative expression between several cell lines might implicate that NS5A protein would exert its effect across factors present in hepatoma cells only. Down-regulation of genes implicated in liver detoxification by NS5A protein would induce the accumulation of toxic biliar compounds, producing cholestasis and liver injury.

MI-P03.

UNRAVELING THE ROLE OF AURORA KINASES IN *Trypanosoma cruzi*

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Cell division and differentiation are key processes for protozoan pathogens to colonize and cause disease. *Trypanosoma cruzi*, the etiological agent of American Trypanosomiasis, possesses a complex life cycle that required strict checkpoints. Currently the regulation of cell division in *T. cruzi* is poorly understood. Aurora kinases (AUKs) are a family of conserved S/T kinases that are essential regulators of many events during cell division. Previously, we reported the identification of three AUK orthologs (TcAUK1, 2 and 3) in *T. cruzi* and expressed them in *E. coli*. In the present work, to investigate the AUK1 role in *T. cruzi*, we used pTREX and pTEX-GFP vectors to overexpress this enzyme either as a native protein or fused to the green fluorescent protein (GFP). As an alternative strategy to determinate the subcellular localization of this protein, we are using polyclonal antibodies against TcAUK1 in immunofluorescense assays. Considering that we failed to measure kinase activity of recombinant TcAUKs when we used commercial histones mix as substrate and the fact that *T. cruzi* histones show high divergence compared to other eukaryote ones, we decided to clone and express histones H3, H3 variant and H4 from *T. cruzi* in bacterial systems to use them as substrates in kinase activity assays. These results are a new step toward the clarification of TcAUKs role in the parasite life cycle.

MI-P04.

FUNCTIONAL STUDY OF ELONGATOR PROTEINS IN *Trypanosoma cruzi*

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N-terminal tails of nucleosomal histones are subjected to post translational modifications (PTM) that regulate several cell processes such as chromatin structure, transcription, DNA damage repair and replication. Lysine acetylation of this tails is a dynamic PTM that involves histone acetyltransferases (HATs) and histone deacetylases (HDAC). Growing evidence correlate histone acetylation with gene activation, and histone deacetylation with gene repression. We have previously reported the PCR-amplification of a *Trypanosoma cruzi* sequence with Elp3 identity named TcElp3. This sequence was not able to complement an Elp3 deficient yeast strain and the recombinant protein expressed in bacteria did not show enzymatic activity. In this work we analyze the overexpression of TcElp3 in epimastigotes of CL Brener and Tulahuen 2 strains using pTREX vector. Immunofluorescence microscopy showed that TcElp3 has a perinuclear localization that is enhanced in overexpressing parasites. We also studied the effect of histone deacetylases inhibitors trichostatin A (TSA) and sodium butyrate on epimastigotes grown *in vitro*. When parasites were treated with 50 nM TSA strong proliferation inhibition was observed. In addition a sub G1 peak and cell cycle alterations were observed in flow cytometry DNA histogram. We are currently repeating these experiments with overexpressing parasites.

MI-P05.
SUBSTRATE SPECIFICITY AND POSSIBLE FUNCTIONS OF THE METACASPASES OF *Trypanosoma cruzi*
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Metacaspases (MCAs) are distant caspase relatives present in plants, fungi and protozoa, but absent in animals. We have reported the existence of two MCA genes (TcMCA3 and TcMCA5) in *T. cruzi*. We have now determined the catalytic properties of both enzymes and got some evidence on their possible role in the parasite. Substrate specificity and autoprocessing were studied using full length, active site mutants and truncated versions of purified recombinant TcMCA3 and TcMCA5 produced in *E. coli*. Both full length enzymes showed substrate specificity for Arg at P1, thus differing from the Asp-P1 specificity of caspases, but in good agreement with results reported for *T. brucei* and *Leishmania major*. At variance with executioner caspases, TcMCA3 and 5 are enzymatically active without proteolytic processing. The presence of the putative prodomain plus an intact His-Cys catalytic dyad was essential for activity, at variance with the C-terminal extension of TcMCA5. Since overexpression of TcMCA3 in epimastigotes was apparently lethal, we chose the pTcINDEX inducible system.
We found that TcMCA3 overproduction leads to an arrest of growth, most likely interfering with cell cycle progression. Thus, our results, as well as those of other research groups, suggest that metacaspases might differ from classical caspases not only in their biochemical properties, but also in function.

MI-P06.
GALACTOSYLCERAMIDE SYNTHASE OF *Trypanosoma cruzi*: PURIFICATION AND CHARACTERIZATION
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Ceramide UDP-GalT [E.C.: 2.4.1.45] is a key enzyme in the biosynthesis of cerebrosides catalyzing the transfer of Galactose from UDP-Galactose to Ceramide yielding Galactosylceramide (GalCer). This glycosphingolipid serves as a precursor for a few simple glycolipids, sulfatide, galabiosylceramide, and the ganglioside sialo-Galceramide, however Galactosylceramide is the major constituent of myelin where the Ceramide UDP-GalT activity changes during the myelination process. It is also highly enriched in many epithelial cells where it is thought to play an important role in lipid and protein sorting. Although the main pathways for glycosphingolipid biosynthesis are relatively well understood in mammalian cells, little is known about them in parasites. In the present work we describe the purification and partial characterization of the Ceramide UDP-GalT obtained from epimastigote forms of *Trypanosoma cruzi*. The enzyme activity was determined in total lysates obtained from Tul-2 and CL-Brenner strains as well as semipurified membrane fractions. Fractionation on ConA-Sepharose column yielded a protein band with an apparent MW range of 62-68 kDa whose activity was shown using UDP-Gal and NBD-Ceramide as sustrates. On going studies of Maldi mass spectrometry analysis to determine the sequence of the purified protein and biochemical characterization will be described.

MI-P07.
PURIFICATION OF AN ACTIVE GLUCOSYLCERAMIDE SYNTHASE IN *Trypanosoma cruzi*
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The genome determination of three human-infective protozoa, including *Trypanosoma cruzi*, the agent of Chagas disease, has prompted the research to the identification and validation of novel drug targets. Glycosphingolipids are important regulators of many different cellular processes such as signaling control, cell cycle progression and differentiation. Thus, attention has been focus on them as potential drug targets to treat a wide variety of diseases. Glucosylceramides are membrane lipids that contribute to the physical properties and physiological functions of membranes, they serve as basic precursors for over 300 species of glycosphingolipids and its synthesis and degradation seem to contribute to the control of the level of ceramide, which is regarded as a second messenger involved in many biological processes. Previously, we have shown the presence of an active Glucosylceramide synthase (GCS) in *T.cruzi* which is the key enzyme in this biosynthetic pathway. In the current work we present a method for the isolation of GCS from epimastigotes forms of *T. cruzi*. Purification of GCS was achieved via a 5 step protocol involving a Green-dye agarose affinity column. This procedure led to a 70 kDa protein whose specific activity was proven using NBD-Ceramide and UDP-Glucose as susbstrates. On going studies are being carried out to make the biochemical characterization of the enzyme.

MI-P08.
***Trypanosoma cruzi* CYTOCHROME P450 REDUCTASES: STRUCTURAL AND BIOLOGICAL CHARACTERIZATION**
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Three different *Trypanosoma cruzi* genes codifying for cytochrome P450 reductases named TcCPR-A, TcCPR-B and TcCPR-C have been identified in our laboratory. The aminoacidic sequences present the characteristic binding domains to FMN, FAD and NADPH, with 11% identity, differing mainly in their amino-end. The recombinant enzymes expressed in bacteria demonstrated characteristic NADPH dependent cytochrome c reductase and dealkylkase activity. To produce high amounts of soluble recombinant proteins we co-expressed them in combination with chaperones. The soluble proteins were used to perform biochemical and structural characterization and to produce polyclonal sera against the TcCPRs. The Ni-NTA agarose purified proteins exhibited typical flavoprotein visible absorbance spectra. Subcellular localization was analyzed by Western blot and confocal microscopy using the specific antiserum. The expression vectors pTREX and pTEX-GFP, were used to generate the following constructions: pTREX-TcCPR-A, pTREX-TcCPR-B, pTREX-TcCPR-C and pTEX-TcCPR-C-GFP. Epimastigotes of the strain CL Brenner were transfected with these constructions and overexpression assessed by Southern, Northern and Western blot. TcCPR-B overexpression confers augmented resistance to the trypanocidal Nifurtimox and Benznidazole. We are currently establishing the redox state and survival in parasites challenged with these drugs.

MI-P09.
PRO-INFLAMMATORY PROPERTIES OF A NATURAL COMPOUND WITH ANTIVIRAL ACTIVITY

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The limonoid 1-cinnamoyl-3,11-dihydroxymeliacarpin (CDM), isolated from purified leaf extracts of *Melia azedarach* L., exhibits anti-HSV-1 and anti-HSV-2 activities in Vero cells and increases the antiherpetic activity of IFN- and IFN- . CDM inhibits HSV-1-induced NF- B translocation in an infected macrophage cell line and increases TNF- production. Also, in the presence of CDM, murine peritoneal macrophages stimulated with HSV-2 increment TNF- secretion.
Taking into account that during herpes infection IFN- has a spectrum of immunoregulatory effects besides the production of an antiviral state, we investigate the effect of CDM on NF- B activation and cytokine secretion in peritoneal macrophages induced with IFN- . The induction with both HSV-2 (used to mimic a herpetic infection) and IFN- produced a synergistic effect in TNF- á production, which is maintained until 24 h in the presence of CDM. IFN- and CDM synergized TNF- secretion as well. In addition, CDM induced persistent NF- B translocation in macrophages as determined by an immunofluorescence staining, showing a similar pattern of fluorescence to that observed with IFN- as stimulus. Our data suggest that CDM not only increases the antiherpetic activity of IFN- but also synergizes and sustains TNF- expression, a prolonged pro-inflammatory response necessary to obtain a complete clearance of infecting microbes.

MI-P10.
MOLECULAR SYSTEMATICS OF *OITHONA NANA* (COPEPODA, CYCLOPOIDA) OF THE BUENOS AIRES PROVINCE

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Oithona nana is one of the dominant copepod species in coastal waters of the Argentine Sea. In order to confirm its taxonomic identity, molecular analysis was performed, comparing the 28S sequences of specimens from the type locality (Gulf of Naples) and the Buenos Aires province ("EPEA Station", 38º 28'S, 57º 41'W, and "El Rincon" area). The 5´ end of the large subunit (28S) is appropiate to distinguish between closely related taxonomic groups such as genera and species. *O. nana* females were obtained and preserved in 95% ethanol. Each specimen was squashed in a PCR tube and a DNA fragment corresponding to 28S ribosomal was PCR amplified using universal primers P63 and R635. The product was cloned and sequenced. A phylogenetic tree was constructed using the package MEGA. Three groups corresponding to different cyclopoid families were well resolved (high bootstrap values). Within the Oithonidae family, *O. nana* sequences from Buenos Aires province were closely related to that from the type locality (less than 5% distance) showing a strong agreement between the morphological and molecular taxonomy of *O. nana* from the Argentine Sea. The obtention of the 28S "reference sequence" of *O. nana* constitutes an important advancement towards its specific confirmation, and contributes to strengthen the public databases.
Partially suported by CONICET PIP 5009, PICT 15227/03, UNMDP 15/E269.

MI-P11.
GENE DISCOVERY THROUGH ORESTES SEQUENCING IN *Triatoma infestans*

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Triatoma infestans is one of the major vectors of Chagas disease in Argentina. The main objective of this project is to provide new data for exploring alternative strategies towards the development of novel and effective ways to prevent the disease transmission. Expressed Sequence Tags (ESTs) sequencing is a cost-effective and useful advance in gene discovery of human disease vectors, which might result in the identification of new targets for the aforementioned aim.
Here, we used the ORESTES (Open Reading frame ESTs) strategy to generate ESTs from mRNA isolated from stage IV nymphs. This strategy preferentially generates ESTs of the central, and thus most informative portion of the transcript, and frequently identifies less abundant mRNAs. We generated four mini-libraries with the use of arbitrary primers and a low-stringency RT-PCR protocol. From these libraries we obtained 156 sequences that were compared against the NCBI nr database using the BLAST suit of programs. 10% matched *T. infestans* or other triatomine sequences, 25% were homologous to other organisms, and 26% did not hit any sequence in the database and thus might represent *T. infestans*-specific sequences. Up to now, we have identify 30 putative new genes for *T. infestans*, which are represented by those ESTs with positive matches against non *T. infestans* transcripts.

MI-P12.
EST SEQUENCING FROM *Triatoma infestans* cDNA LIBRARIES

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Triatoma infestans is one of the main vectors of Chagas disease in Argentina and South America. In order to contribute to the generation of its transcriptome, we are constructing cDNA libraries from IVth instar nymphs to obtain Expressed Sequence Tags (ESTs). In a first attempt, we constructed a standard cDNA library. The obtained sequences were compared against the nr nucleotide collection of the NCBI database using the BLAST tool. So far, we have sequenced and analyzed 131 ESTs. We obtained 37% of sequences with non-significant similarity to any known sequence, and 12% with significant similarity to mRNAs of other organisms. These two subsets of ESTs are interesting for gene discovery and will be further analyzed. We also obtained 22% of sequences with significant similarity to rRNAs of *T. infestans* or other triatomine species, and 27% of sequences with identity with Triatoma virus, a RNA virus widely distributed in *T. infestans* populations. The clustering of these last two subsets of ESTs revealed that most of them were redundant. Finally, 2% of the sequences had identity with mRNAs of *T.infestans* already annotated. With the aim of increasing the proportion of ESTs from low abundance transcripts, we are currently generating normalized cDNA libraries using the Clontech PCR-Select cDNA Subtraction kit. The comparative analysis of the libraries will be shown in the poster.

MI-P13.
PUTATIVE ROLE OF TCSR62 FROM *T. Cruzi* IN THE METABOLISM OF mRNAs IN THE CYTOPLASM
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TcSR62 is a RNA binding protein (RBP) from *Trypanosoma cruzi* belonging to the SR-related family. SR and SR related proteins have multiple roles in mRNA metabolism, particularly as regulators of pre-mRNA processing. More recently, it has been shown that several members of these families have additional roles in transport, quality control, stabilization, and translation regulation of mRNAs. TcSR62 is present in all stages of the parasite and localized mainly in the nucleus in a speckled pattern and in the cytoplasm, where it displays a homogeneous distribution. Previous results from our laboratory suggest that the protein is implicated in pre-mRNA processing. Here, we present evidences suggesting that in addition to its nuclear function(s),TcSR62 also plays a role in the metabolism of mRNA in the cytoplasm. In order to start analysing its putative role in the cytoplasm, we performed immunoprecipitations of mRNAprotein complexes from cytoplasmic extracts with a specific antiserum against TcSR62. After isolating the mRNA fraction, a cDNA library was constructed using a modification of the PCR-Select Subtraction Kit from Clontech. Several clones were sequenced and analyzed to identify the encoded transcripts. Up to now, we have identified more than 100 different transcripts with no apparent relationship among them. These results suggest that TcSR62 may be a relatively nonspecific RBP.

MI-P14.
EVALUATION OF RIBOSOMAL REGIONS SEQUENCING AS TOOL FOR WINE YEAST IDENTIFICATION
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Yeast identification is important in wine industry to resolve microbiological problems and improve fermentation management. Different molecular techniques have been proposed to identify yeast species. The aim of this work was to evaluate if direct sequencing of two ribosomal regions allow a rapid and accuracy identification of yeasts related to wine industry. Fifty yeasts were evaluated; reference and native strains were included. ITS1 and ITS4 primers were used to amplify a region of the rRNA gene 5,8S and ITS1-ITS2, and NL1 and NL4 primers to amplify the domain D1/D2 of the 26S rRNA gene. Sequencing was done with an automatic sequencer. Sequences were aligned and compared in Genebank database using the BLAST tool. 5.8S-ITS RFLP was done to confirm the results. 5.8S-ITS sequencing allowed identify 84% of strains, 9 strains remained misidentified. 5.8S-ITS RFPL helped to resolve species assignation, but some strains remained doubtful. 26S sequencing allowed 90% of correct identification. Combining both techniques, complete identification was reached. Results showed that 5.8S-ITS RFPL and 26S sequencing could be suggested to unequivocal identification of yeast species closely related or not. Moreover, yeast database of RFLP is available and facilitate the species assignation, and the most commonly utilized D1/D2 region is also very accessible and it can be determined in one experiment.

MI-P15.
COMPARATIVE DIVERSITY OF NARG GENE IN THE SUQUIA RIVER SEDIMENTS
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We report the molecular characterization of narG gene in sediments isolated from two distant sites of the Suquía River of Córdoba Province, where different nitrate concentrations were observed. This analysis was performed using narG RFLP, cloning and sequencing. A total of 195 narG clones of two libraries were screened. Among 51 RFLP patterns identified, only one was found in both sediments. Rarefaction curves of the amino acid sequences predicted a greater diversity from the narG genes obtained in the sediment where the higher freshwater nitrate levels were found. Ninety different sequences were obtained and the identities between the deduced amino-acid sequences and those from cultivated or environmental denitrifier bacteria deposited in the databases ranges from 57-100%. Phylogenetic analysis grouped the 195 *narG* clones in seven clusters related to the -proteobacteria (33%), -proteobacteria (30%), -proteobacteria (3%), -proteobacteria (2%) and *Thermus thermophilus* (10%) *narG* genes. The rest of the clones were less than 68% similar to narG sequences available in databases suggesting that they are indigenous of the Suquía River. The results indicate that the nitrate reducing communities present in the Suquía River sediments are phylogenetically diverse and divergent from previously descripted sequences.
Supported by CONICET, FONCyT and SECyT-UNC.

MI-P16.
PLANT GROWTH PROMOTING *Rhizobacteria* IMPROVE GROWTH AND ESSENTIAL OIL YIELD IN SWEET MARJORAM
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Effects of root colonization by plant growth promoting rhizobacteria (PGPR) on biomass, and qualitative and quantitative composition of essential oils, were determined in the aromatic crop *Origanum majorana* L. (sweet marjoram). PGPR strains evaluated were *Pseudomonas fluorescens*, *Bacillus subtilis*, *Sinorhizobium meliloti*, and *Bradyrhizobium* sp. Only *P. fluorescens* and *Bradyrhizobium* sp. showed significant increases in shoot length, shoot weight, number of leaf, number of node, and root dry weight, in comparison to control plants or plants treated with other PGPR. Essential oil yield was also significantly increased relative to non-inoculated plants, without alteration of oil composition. *P. fluorescens* has clear commercial potential for economic cultivation of *O. Majorana*.

MI-P17.
ROLE OF *LPSB* IN *Sinorhizobium meliloti* BIOFILM FORMATION
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Biofilms are populations or communities adhered to an interface and surrounded by an extracellular matrix. *S. meliloti*, a symbiotic Gram negative bacteria, is able to form biofilms *in vitro*. In this bacteria, *lpsB* codes for a glucosyltransferase that is involved in LPS (lipopolysaccharide) core biosynthesis, mutation of this gene results in an altered LPS composition. In order to study the impact of *lpsB* in *S. meliloti* biofilm formation, we performed confocal laser scanning microscopy and cristal violet adhesion experiments in two *lpsB* mutant strains. Rm6963 (Rm2011 *lpsB*::Tn5) showed a reduced adhesion to polypropylene and polystyrene, as compared to Rm2011 (wild type strain). In contrast, such adhesion-deficient phenotype was not observed in Rm8530 *lpsB* as compared to Rm8530 (Rm1021 derivative, expR$^+$, galactoglucan producer). Cell-cell interactions and mature biofilm structures were altered in both mutants: a partial inhibition of lateral interactions and characteristic arrays of bacteria with polar interactions were observed. The mature biofilm structure was slightly altered in Rm6963, as compared to Rm2011. In contrast, a stronger alteration was observed in Rm8530 *lpsB*, as compared to Rm8530. In conclusion, *lpsB* has a role in determining cell-cell interactions and mature biofilms structures, but adhesion to hydrophobic surfaces behaviors of *lpsB* mutants are strain-dependent.

MI-P18.
GENETIC RELATIONSHIP AMONG NORTH-WEST ARGENTINEAN STRAINS OF XAC CAUSING CBC
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Forty-two strains of *Xanthomonas axonopodis pv. citri* (Xac), the causal agent of bacterial canker of citrus, isolated from Citrus limon in north-western Argentina, were characterized phenotypic, biochemistry, and molecularly. The colonies of most isolates were mucoid, convex and yellow on nutrient agar plates as other xanthomonads, except for str 44, which produced rough structures. Simultaneously, with the confirmation of pathogenicity, the isolates were identified to species level by analysis of hydrolysis of gelatine and carbon source utilization. Molecular analysis were also performed using a previously described primer designed to be universal for all citrus canker strains and a new set of primers, that we developed, for specific identification of CBC type A strains. This primer is based on a sequence within xps gene of Xac str 306, which encodes for the general secretion pathway protein D. All strains characterized were identified as Xac. Pathogenicity tests revealed that only str 44 was unable to produce canker on lemon leaves. To evaluate the diversity of *Xanthomonas* strains causing CBC in NOA and to make a deeper characterization of str44 we performed DNA fingerprinting analysis using rep-PCR. This result was used to build a dendogram that shows the close relationships among *Xanthomonas* strains. Further analysis must be done to understand the less pathogenicity of str 44.

MI-P19.
CARACTERIZATION OF BACTERIOCINS PRODUCED BY PGPR *Pseudomonas*
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The objective of the present study was to investigate the production of bacteriocin in native *Pseudomonas* spp. isolated from wheat root.
Materials and methods: bacteriocin production was evaluated according to Parret et al. (2003). The supernatant of *Pseudomonas* was precipitated with ammonium sulphate and dialysed. Aliquots of crude bacteriocin were tested for their susceptibility to proteolytic enzymes and resistance to heat. Samples of bacteriocin were applied to a column of biogel P-100 and eluted with phosphate buffer. Fractions were collected, evaluated for antimicrobial activity an analyzed by gel SDS-PAGE.
Results: *Pseudomonas* sp. SF4C synthesized a bacteriocin of probably high-molecular weight because it diffused near the colony. It is resistant to proteinase K and sensible at 75°C. The bacteriocin produced by *Pseudomonas* sp 39a diffused further from the colony than SF4c bacteriocin. It is resistant to proteinase K too, but antimicrobial activity was lost after incubation at 42°C. Clarified culture supernatant from SF4C was precipitated at 60% saturation of ammonium sulfate (crude extract). Fractions with antibacterial activity were eluted early from the biogel P100 column. These fractions were subjected to SDS-polyacrylamide gel and two bands were detected. Other studies are being carried out to determine which band is the responsible of antimicrobial activity

MI-P20.
BIOCORROSION STUDIES IN NUCLEAR INDUSTRY ALLOYS INDUCED BY A STRAIN OF *Bacillus cereus*
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Biocorrosion is caused by electrochemical and biological processes that take place on the metal surfaces by the metabolism of organisms attached to them. Events attributed to microbiological corrosion in alloys used in the nuclear industry are reported in the present study. To obtain the microorganisms, samples of water were taken from oligotrophic environments and their bacterial populations were isolated and characterized. This study shows the action of a bacterium characterized as *Bacillus cereus* (named *B. cereus* RE10) on several kinds of materials: carbon steel, stainless steels AISI 304 and AISI 316, high purity aluminum and aluminum alloy AA6061. After 20 days of immersion in highly diluted culture, ball-like deposits were observed in high purity aluminum and AA6061 but not on carbon steel and stainless steels alloys. The *B. cereus* RE10 showed a clear tendency to adhere on the AA 6061 surface. Only in AA 6061 local attack in the form of pits with irregular borders was observed under these deposits, being this kind of attack compatible with microbiological corrosion. Inside the pits, magnesium and iron were found by Energy Dispersive X-ray Microanalysis. Taking into account that there was not attack in samples of aluminum, it is hypothesized that the presence of Mg_2Si, ( )Al-Fe-Si Fe and Al_3Fe inclusions in AA6061 could promote the localized attack induced by *B. cereus* Re10.

MI-P21.

A POLYPHASIC STUDY OF THE MICROBIAL COMMUNITY STRUCTURE IN SOILS FROM NORTHWESTERN ARGENTINA

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In this study we compared the capacity of three microbial community profiling methods to describe differences in the soil microbial community associated with deforestation and agriculture. We used biochemical (phospholipid fatty acid analysis, PLFA), physiological (community level physiological profiling, CLPP, using a BD oxygen biosensor system), and molecular (16S rDNA polymerase chain reaction-denaturing gradient gel electrophoresis, PCR-DGGE) fingerprinting methods. We analyzed samples from pristine soils, as well as adjacent deforested and agricultural soils with different land-use histories (40 or 100 yrs sugarcane and 20 yrs soybean monocultures).
All fingerprinting methods distinctly characterized the microbial community structure of each soil and detected changes in the soil microbial population related to land use. PLFA analysis revealed that total microbial biomass was much more abundant in pristine forest soils than in those under agricultural use or that had been recently deforested, where the microbial biomass was severely reduced. Analysis of CLPP and DGGE profiles indicated that microbial communities from soils under agricultural management were functionally and genetically different from those characterizing pristine soils. The structure of microbial communities was different between the soils analyzed and these differences were detected by all the approaches used.

MI-P22.

CHARACTERIZATION OF PRISTINE AND AGRICULTURAL SOILS BY CATABOLIC PROFILING OF MICROBIAL COMMUNITIES

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The advance of the agriculture frontier in Northwestern Argentina affects soils, especially those that were subjected to deforestation followed by monoculture agriculture. In this work we analyzed the community level physiological profiling (CLPP) of microbial communities to compare soils from the low-altitude pristine forests of the Yungas eco-region with soils under 40 and 100 years of sugarcane monoculture. Two techniques were applied, a BD oxygen biosensor system (BDOxy) and a variant of the Biolog system. The BDOxy method allows the direct measurement of microbial respiration using a fluorescent dye, while the cheaper but less sensitive Biolog system estimates metabolic activity indirectly through color change of a redox dye. The multivariate dataset was summarized using Analysis of Principal Components. Both methods showed differences in the catabolic profiles for the three soils tested, with the agricultural soils sharing a higher similarity between them. Using microcosm experiments we found that the microbial community of the pristine soil was more resilient to environmental stresses, specifically changes in temperature. Although it can be argued that the BDOxy and the Biolog methods estimate the catabolic activities of different community fractions, our results suggest that both can be used as indicator of soil characteristics and community dynamics.

MI-P23.

EXPRESSION PROFILES OF CATALASE GENES IN THE PHYTOPATHOGEN *Xanthomonas axonopodis* PV *Citri*

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Xanthomonas axonopodis pv. *citri* (Xac) is a Gram-negative obligate aerobic bacterium that infects citrus plants. Pathogenic bacteria are usually exposed to H_2O_2 produced either by normal aerobic metabolism or as a part of the plant defense response against microbial invasion. In order to survive and colonize plant tissues Xac must overcome H_2O_2 toxicity, and catalases are enzymes employed for its detoxification. We determined Xac catalase activity in different growth phases and observed that it was higher in stationary-phase compared to early-exponential phase. This was consistent with the capacity of cultures to resist H_2O_2. Semi-quantitative RT-PCR analysis showed that three catalase genes are expressed in Xac during growth in rich media. The genes *srpA* and *katG* are expressed throughout all phases of growth whereas *katE* transcripts are only detected as cells enter the stationary phase. We also analyzed the expression profiles of catalase genes in Xac cultures grown in standard media and in XVM2, suspected to mimic the environment of plant intercellular spaces. We found that mRNA levels of *katE* are significantly higher in XVM2, which could account for the higher catalase activity previously observed in this media. Our results suggest that KatE may be the major catalase induced in Xac in starvation conditions and in the environment found during plant-pathogen interactions.

MI-P24.

CONTROL OF LEMON GREEN MOLD CAUSED BY AN IMAZALIL-RESISTANT *Penicillium digitatum* ISOLATE

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P. digitatum is the causal agent of citrus green mold. To avoid this pathogen, lemons have been treated with fungicides (mainly imazalil), leading to the proliferation of resistant strains and the increase of toxic residues in the fruit. International markets have implemented strict limits on such residues and thus, it is urgent to find alternative methods for decay control. We standardized, *in vitro*, an oxidative sequential treatment (OST) to kill *P. digitatum* conidia. It consists in 2-min incubations of conidia at 20ºC: first with NaClO and then with H_2O_2 and $CuSO_4$. Here, we analyze if the OST and some variations of it are effective on an imazalil resistant isolate. The effect of the treatment was checked *in vitro* by viability on plate and by infectivity on lemon fruits. The OST was effective against imazalil-resistant isolate showing a total loss of viability and infectivity. Moreover, the decay control was evaluated by *in situ* applications of OST, in lemons inoculated (24 h before) with non-treated conidia. The decay was significantly delayed in the fruits subjected to *in situ* treatment in comparison with those treated with water. Additionally, the *in vitro* protocol was improved by incubations at 42ºC. Our OST may have a potential application for postharvest disease control, and the procedure at 42ºC would be suitable since this temperature activates natural defenses of lemons.

MI-P25.

ROLE OF RAPA1 IN CELLULAR INTERACTIONS DURING BIOFILM FORMATION BY *Rhizobium leguminosarum*

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It is widely accepted that many bacteria grow in the form of biofilms. Either spontaneously or by environmental pressure, bacteria associate to produce complex structures, usually embedded in exopolymers. *Rhizobium leguminosarum* was shown to form biofilms, and these biofilms are dependent on the presence of the type I protein secretion system PrsDE. Exopolysaccharides (EPS) play an important role in biofilm formation and several EPS-modifying enzymes are exported via PrsDE. Besides, a family of proteins, named *Rhizobium* adhering proteins (Raps) are also secreted by this system. Using confocal laser-scanning microscopy (CLSM) we sought to determine the role of RapA1 in biofilm formation. RapA1 was expressed in higher levels than wild type (4 to 5-fold) in 3 different strains of *R. leguminosarum*, and CLSM was used to follow biofilm formation in minimal medium (Y). Surprisingly, we observed that RapA1-overproducing strains have altered cell-to-cell interactions resulting in "less ordered" biofilms. In addition, cells of RapA1 over-producing strains did not aggregate or settle from cultures when they were left standing. We also observed that in Y-Congo red plates, the colony morphology was altered when RapA1 was increased. We hypothesize that a specific amount of RapA1 on the cell surface is required for the cells to interact in the correct manner and form an "ordered" mature biofilm.

MI-P26.

***Xanthomonas campestris* SUPPRESSES STOMATAL DEFENSE**

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Pathogen-induced stomatal closure is part of the plant innate immune response. In order to penetrate leaves through stomata, pathogens must avoid the stomatal response of the host. In this work we describe a factor secreted by the bacterial phytopathogen *Xanthomonas campestris pv campestris* (*Xcc*) that is capable of interfering with stomatal closure induced by bacteria or by abscisic acid (ABA). We found that living *Xcc*, as well as ethyl acetate extracts from *Xcc* culture supernatants, are capable of reverting stomatal closure induced by bacteria, lypopolisaccharide (lps) or ABA. *Xcc* ethyl acetate extracts also complemented the infectivity of *Pseudomonas syringae pv tomato* (*Pst*) mutants deficient in the production of the coronatine (cor) toxin, which is required to overcome stomatal defense. By contrast, the *rpfF* and *rpfC* mutant strains of *Xcc*, that are unable to respectively synthesize or perceive a diffusible molecule involved in bacterial cell to cell signaling, were incapable of reverting stomatal closure, indicating that suppression of stomatal response by *Xcc* requires an intact *rpf*/DSF system. In addition, we found that guard cell-specific MAP kinase 3 (MPK3) Arabidopsis antisense mutants were unresponsive to bacteria or lps in promotion of stomatal closure, and also more sensitive to Pst cor deficient mutants, showing that MPK3 is required for stomatal immune response.

MI-P27.

STRUCTURAL CHARACTERIZATION OF THE LIPOPOLYSACCHARIDE FROM *Xanthomonas axonopodis* PV. CITRI

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Plants have basal perception systems for characteristic patterns from different classes of microorganisms, named pathogen-associated molecular patterns (PAMPs), which are recognizable by the innate immune system of plants. In plant pathogenic bacteria, lipopolysaccharides (LPSs) are important virulence factors and are increasingly recognized as major PAMPs for plants. Citrus canker, one of the most devastating citrus diseases in the world is caused by *Xanthomonas axonopodis* pv. citri (Xac). In this context, the chemical structure characterization of LPSs obtained from the wild type and mutants is important to understand the role of Xac LPS in this disease. Lipid A was obtained from purified wild type Xac LPS by acid hydrolysis. Analysis by TLC showed a heterogeneous complex pattern. Studies by UV-MALDI-TOF mass spectrometry revealed the presence of two main cluster ions, using nor-harmane as matrix in the positive and negative ion mode. One of them, with ions ranged from m/z 1350 to 1450 corresponding to pentacylated species that carried two glucosamine and two phosphate groups with acyl chains of different lengths. The other one, ranged from m/z 1500 to 1610 attributed to the presence of an additional residue of 2-aminoethylphosphate and one 2-aminoethyl phosphate groups. Interestingly, the same analysis performed on lipids A from mutants evidenced structural differences.

MI-P28.

IDENTIFICATION OF POTENTIAL CELL WALL ANTIGENS FROM *M. Aavium* AND *M. avium* SUBSP. *Paratuberculosis*

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Mycobacterium avium subsp. paratuberculosis (MAP) is the causal agent of bovine paratuberculosis or Johnes disease, a significant economical disease in cattle. The Mycobaterial cell wall is a subcellular fraction known to play a major role in pathogenesis. Antigenic proteins in this fraction are involved in virulence and cell-pathogen recognition. Identification of specie-specific proteins that discriminate bovines with MAP and bovines exposed to Mycobacteria are one of the most important objectives in bovine paratuberculosis research. The aim of this work is to recognize potential antigens and compare cell wall antigens from *M. avium subsp paratuberculosis* and *M. avium*. In order to complete our purpose we extract, separate and resolve by 2D electrophoresis proteins from MAP and MA cell wall. The proteins obtained were transferred and incubated with bovine sera from not infected animals and with paratuberculosis infected animals, procedure developed with and without *M. phlei* sera absorption. The reactive spots were selected based on reactivity, pI and mass. Proteins were identified by N-terminal and/or MS. In this work we identify cell wall antigens from *Mycobacterium avium subsp. paratuberculosis* and *Mycobacterium avium* in bovine paratuberculosis.

MI-P29.

CHARACTERIZATION OF A KNOCKOUT STRAIN OF *Mycobacterium bovis* IN P27 GENE

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Introduction: P27 lipoprotein was previously described as a virulence factor in *Mycobacterium tuberculosis*. This protein is encoded by the P27 (*lprG*) gene and forms an operon with Rv1410 that encodes for an efflux pump, P55. Objective: The aim of this study was to determine the role of P27 during the infection of *Mycobacterium bovis.*

Method: a mutant of *Mycobacterium bovis* not producing P27 was obtained by two-step mutagenesis using the counterselectable *sacB* marker in the shuttle plasmid p2nil. Complemented strains carrying a copy of each genes or the entire operon in a replicative plasmid were also obtained. An intrathacheal model of mice infection was used to assess the *in vivo* replication of strains.

Results: Western blot experiments using anti-P27 polyclonal sera showed that the P27 protein was present both in the parental and in complemented strains, in which either the entire P27-P55 operon or P27 was reintroduced, but absent in the mutant strain. All of the strains showed similar growth kinetics and characteristics in culture broth. Finally, the replication of the P27 mutant was impaired in mice.

Conclusion: These results indicate that P27 is also relevant for the replication of *M. Bovis.*

MI-P30.

STUDY OF THE IMMUNOLOGICAL PROFILE TOWARDS *Mycobacterium bovis* ANTIGENS IN NATURALLY INFECTED CATTLE

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Introduction: A number of studies have determined the contribution of TH1 and TH2 responses to protective immunity and pathology of *Mycobacterium bovis* infection. However, much of that information is derived from experimentally infecting cattle with *M. bovis* and few data from naturally infected animals is available.

Objectives and Methods: The aim of this study was to characterize the immunological profile towards *M. bovis* antigens of naturally infected cattle by measurement of cytokine mRNA expression in peripheral blood mononuclear cells (PBMCs) and to determine which lymphocyte subsets are involved in recall proliferation of PBMCs from *M. bovis* infected cattle in response to *M. bovis* antigens.

Results and Conclusions: Consistent with data from experimentally *M. bovis* infected cattle, naturally infected animal displayed a Th1 cytokine profile in response to PPDB stimulation. Production of INF-g mRNA by PBMCs after PPDB stimulation statistically distinguishes between infected and healthy herds, suggesting this molecule as an *M. bovis*-infection marker. As it happens in experimental infected cows, CD4 and gamma-deltaTCR cells from a naturally *M. bovis*-infected herd are the predominant T cell responding in recall responses to PPDB.

MI-P31.

EFFECTS OF VANCOMYCIN (VAN) ON VIRULENCE FACTORS OF *Staphylococcus aureus*

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The glycopeptide VAN is a bacteriocin produced by *Streptomyces orientalis* that is used for serious infections caused by methicillin-resistant *S. aureus*. VAN blocks the action of transpeptidase during the synthesis of peptidoglycan. Subinhibitory concentrations (SubMIC) of some antibiotics can affect the expression of bacterial virulence factors. We hypothesized that subMIC of VAN may have effects on virulence factors of *S. aureus*. The VAN MIC of the Newman strain was 7 µg/ml. After subMIC exposure, a reduction of polysaccharide capsular production was determined by immunoprecipitation assays. Proteins profiles of Newman strain and its isogenic regulatory mutants cultured with or without VAN were analyzed by SDS-PAGE. We observed that several cell wall proteins regulated by *sae* were inhibited by VAN. In contrast, VAN induced the increase of several cell wall protein regulated by *mgrA*. Moreover, a particular surface protein of Newman strain showed to be increased after growth with VAN. The pattern of haemolysins

expression of Newman and its regulatory mutants was not modified by VAN. Our results suggest that subMIC of VAN reduce the capsule production and alter the expression of surface and cell wall proteins patterns of Newman strain.

MI-P32.

CHARACTERIZATION OF ANTISEPTIC EFFLUX SYSTEMS IN COAGULASE-NEGATIVE *Staphylococci clinical isolates*

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In the hospital environment, handwash is a very valuable tool to control nosocomial infections and several antiseptic agents are used in soaps. A molecular analysis of efflux systems codified by the *qac* genes was performed in 35 coagulase-negative staphylococci human clinical isolates of species different from *S. epidermidis*. The Minimum Inhibitory Concentration (MIC) in the presence/absence of m-chlorophenylhidrazone (CCCP) was performed according to the CLSI guidelines using *S.aureus* ATCC 29213 and an *E.coli* MG1655 mutant (AcrAB/TolC overexpression). The presence of *smr, qacA/B, qacG, qacH* and *qacJ* genes was determined by PCR and the expression of them was analyzed by RT-PCR. Of the species studied, *S. haemolyticus* showed efflux to ethidium bromide, SDS, sodium deoxycholate and benzalkonium chloride. The *qacA/B* gene was found in 16 isolates, *qacG* in 11, *qacH* in 10, *qacJ* in 5 whereas *smr* was found in all of them. RT-PCR showed only the expression of *smr* in several of the *S. haemolyticus* isolates. In conclusion, the resistance to antiseptics includes a myriad of *qac* genes distributed homogenously in each isolate. The notable issue that the *qacG, qacH* and *qacJ* genes were found in isolates from human origin, demonstrating the broad spreading of antiseptic resistance, a fact that emphasizes the importance of the rational use of antiseptics.

MI-P33.

HYPERMUTABILITY AND ADAPTIVE PHENOTYPES IN CYSTIC FIBROSIS ISOLATES OF *Pseudomonas aeruginosa*

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During Cystic Fibrosis (CF) pulmonary chronic infection, *P. aeruginosa* diversifies into phenotypes with particular traits -such as mucoids, avirulent, hypermutators and multiresitant antibiotic variants- which favor *P. aeruginosa* long-term persistence and prevent its eradication. The acquisition of these phenotypes mostly involves mutations in target genes which would be catalyzed by hypermutators. Indeed, the link between antimicrobial resistance and hypermutators was suggested. Also, we established a link between hypermutability and emergence of mucoid and avirulent variants *in vitro*. In order to study the role of hypermutability in *P. aeruginosa* phenotypic diversification *in vivo*, we analyzed the association between hypermutator phenotype and mucoid, avirulent and multi drug resistant variants (*mucA*, *lasR*, *mexZ* mutants respectively) in 40 *P. aeruginosa* isolates from 27 CF patients. We observed that 20 isolates (50%) were hypermutators and 14 patients (55.5%) had hypermutators. Regarding *mucA*, *lasR* and *mexZ*, two isolates (5%) showed unaltered sequences in the three genes and a non mutator phenotype. Also, 75% of the isolates showed mutations in *mexZ* of which 40% were hypermutators. In addition, 65% of isolates showed mutations in *mucA*, of which 46% were hypermutators. Finally, a lower percentage of isolates (37%) harboured mutations in *lasR*, however, 66% of them were hypermutators.

MI-P34.

ANIMAL MODEL FOR STUDYING THE EARLY STAGES OF REACTIVE ARTHRITIS AFTER *Salmonella enteritis*

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Reactive arthritis (ReA) is a sterile arthritis triggered by a distal mucosal infection. Studies on ReA pathogenesis are difficult because of the limited tests that can be performed in humans. We describe a model useful for the study of the early stages of *Salmonella* ReA. BALB/c mice were orally infected with 10^3 CFU of a virulent strain of *S. enteritidis*. After 21 days mice showed gut inflammation and synovitis in the knee joints. Bacteria were not recovered from joint or draining lymph node cultures at any time studied, ruling out a septic arthritis. Antibodies against *Salmonella* were neither detected in plasma nor in joints. Flow cytometry revealed a significant increase of CD4+ lymphocytes in the popliteal lymph nodes of mice receiving a single oral dose of *S. enteritidis* infection. We investigated the local response to a second exposure to the antigen challenging infected mice in the footpad with heat-killed *Salmonella*. Results showed a higher number of CD4+ lymphocytes in the popliteal lymph nodes, compatible with a secondary response to *Salmonella*. Using an *invG* mutant we found that the proinflammatory signalling mediated by *Salmonella* type 3 secretion system-1 (TTSS-1) in the gut is required for the induction of joint sequelae. This highly reproducible and easy to perform model provides great potential for investigating both host and bacterial contributions to ReA.

MI-P35.

***STAPHYLOCOCCUS AUREUS* AUXOTROPHIC MUTANT AS NEW TOOL OF BACTERIAL INTERFERENCE IN NASAL COLONIZATION**

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S. aureus nasal carriage is a risk factor for infection in humans, particularly in the hospital environment. The active colonization with a strain of *S. aureus*, which possess minimal pathogenic properties, is able to prevent colonization by more virulent strains. In this work a murine model of *S. aureus* nasal colonization was developed. Mice were inoculated by intranasal (ina) via with a suspention of the auxotrophic mutant of *S. aureus* (NA412, obtained in our lab previously) during three consecutive days. At fourth day, mice were challenged by ina via with one of the representative clinical strains of *S. aureus* in Argentina [pediatric (HDE288) and cordobes (HU-71) clones]. Bacterial interference was evaluated by colony counting from nasal homogenates at 24 h post-challenge. Viable counts of the clinical strains in the groups of NA412 mice colonized (named G1) decreased significantly when compared with those groups of animals without pre-colonization (named G2) (HU-71 G1: 120 CFU/ml and G2: 9415 CFU/ml, $p<0.0003$, HDE288 G1: 45 CFU/ml and G2: 1270 CFU/ml, $p=0.02$, Mann-Whitney test). Therefore, previous NA412 nasal colonization significantly reduces the colony number of the most prevalent clones of *S. aureus*. Undoubtedly, NA412 mutant interferes in colonization and establishment from other pathogenic strains of *S. aureus* able to produce serious infections.

MI-P36.

***Yersinia* LIPOPOLYSACCHARIDE INDUCES INCREASED PRO-INFLAMMATORY RESPONSE IN TNFRP55-/- MACROPHAGES**

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Yersinia enterocolitica causes reactive arthritis and macrophages mediate synovitis. We studied the impact of TNFRp55 deficiency in macrophages stimulated with *Yersinia* antigens. Peritoneal macrophages from wild-type and TNFRp55-/- C57BL/6 mice were stimulated with *Y. enterocolitica* O:3 outer membrane (OM) or lipopolysaccharide (LPS). Supernatant nitric oxide (NO) by Griess reaction, and TNF- and IL-6 by ELISA were measured. Toll-like receptor (TLR)-2 and TLR4 mRNA expression was evaluated by RT-PCR, and iNOS protein expression by western blot. Higher NO levels were secreted by TNFRp55-/- macrophages stimulated with 5 or 10 g OM ($p<0.03$ or $p<0.006$, respectively compared with wilt-type). This was in line with iNOS protein and TLR-4 mRNA expression. TLR-4-/- macrophages did not respond to OM stimulation. In addition, higher NO levels were secreted by of TNFRp55-/- macrophages stimulated with 100 or 1000 ng LPS ($p<0.011$ or $p<0.005$, respectively). Similarly, increased IL-6 levels were found after 10, 100 or 1000 ng LPS stimulation ($p<0.0004$, $p<0.05$ and $p<0.007$, respectively). Polymixine B treatment decreased significantly NO and IL-6 secretions ($p<0.0027$ and $p<0.0008$). These results suggested that TNFRp55-/- deficiency increases macrophage pro-inflammatory activity, and that *Yersinia* LPS is the main OM component that induces this activation.

MI-P37.
ACTIVITY OF MCCJ25 AND ITS ANALOGOUS MCCJ25-T9P ON *Salmonella newport* INSIDE OF MACROPHAGE
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The microcin J25 (MccJ25) is an antibiotic of 21 amino acid residues, active against *Escherichia coli*, *Salmonella* and *Yersinia* strains. Convincing evidence shown that RNA polymerase (RNAP) and respiratory chain membrane are the targets for MccJ25 action. We study the ability of this antibiotic to internalize inside of macrophages reaching an intracellular concentration required to inhibit the *S. newport* cells replication. The monolayer of infected macrophages was treated with 1 and 5 µM MccJ25 and a recount of CFU/ml at different times was carried out. The viable recount with 1µM of MccJ25 was of 6.1, 5.55 and 4.95 units $\log_{10}$ and with 5 µM of MccJ25 it was of 5.71, 5.37 and 4.94 units $\log_{10}$, at 6, 18 and 24 hours post-infection, respectively. In both cases we observed a decrease of two $\log_{10}$ units, approximately, compared wiht the untreated macrophage as a control. The same difference in viable recount was obtained when the experiment was performed with 1 and 5 µM of the MccJ25 variant, MccJ25 T9P. Any effect of the MccJ25 on the *Salmonella* invasion ability was observed when the macrophages were pretreated with the antibiotic. No morphological or viability changes was observed when the macrophages were incubated with MccJ25 or MccJ25 T9P. Our results could be useful for potential therapeutic application of this antibiotic in the control of *Salmonella* human infection.

MI-P38.
FITNESS AND FTSZ PLACEMENT RESTORATION BY INCORPORATION OF ALTERED PBP GENES IN *Pneumococcus*
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-Lactam (L) resistance in *Streptococcus pneumoniae* (or pneumococcus) is caused by mutations in penicillin-binding proteins (PBPs), mainly PBP1a, PBP2x, and PBP2b, which are enzymes involved in cell wall synthesis and cell division cycle. Previously, we found that *pbp2b* mutants, obtained by transforming *pbp2b* PCR products from L-resistant isolates into CP1015 strain, showed decrease fitness, and morphological alterations. By using a fluorescent derivative of vancomycin (Fl-Van), we observed abnormal simultaneous parallel septal and equatorial staining. We also found by inmunofluorescence microscopy a FtsZ delocalization indicating cell division defects. Because no alterations were observed in the original L- resistant strains, we constructed double and triple mutants to analyze if *pbp* genes association may compensate those alterations. The double *pbp2b/2x* or *pbp2b/1a* mutants resulted only in a partially restored morphology. Double pbp mutants neither showed a complete FtsZ placement correction nor fitness restoration, whereas the triple *pbp2b/2x/1a* mutant was similar in morphology, fitness and Fl-Van staining to Cp1015, and it also showed a correct FtsZ localization. Our results suggest that acquisition by horizontal transfer of *pbp2x/pbp1a* mutations have compensatory effects on fitness and cell division process, in addition to development of L resistance.

MI-P39.
A GENOMIC AND FUNCTIONAL APPRAISAL OF TWIN-ARGININE TRANSLOCATION SYSTEM IN INTRACELLULAR BACTERIA
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The TAT system is a novel protein transport complex that target folded proteins across the inner membrane of Gram negative bacteria. Tat membrane components, *tatA, B,* and *C* have been identified in most of the bacterial sequenced genomes. However the extent to which this secretory pathway is utilized is not well characterized. Comparative genomic analysis throughout alphaproteobacteria confirmed the presence of *tatA, B,* and *C* in almost all studied genomes. Synteny analyses allow us to split the class in two groups with either operon or scattered organization. qRT-PCR assays using *A. marginale* and *B. abortus* RNA were performed to confirmed expression and determine the stoichiometric ratio of *tatABC* mRNA so as to characterize transcriptional regulation mechanism. The ability of *tatABC* genes to compensate for the absence of the cognate *E. coli* one was attained. Furthermore, a genome wide identification of putative tat substrates among alphaproteobacteria was performed using TatFIND1.4. Since some organisms appear to make extensive use of this pathway, others seem to secrete very few products through it. GO terms and clustering analysis led to the classification of the organism into groups regarding to the properties and number of predicted substrates. Whether or not this classification could be in accordance to adaptive mechanism for very different ecological niches will be discussed.

MI-P40.
***MGTA* EXPRESSION IS INDUCED BY ROB AND MEDIATES *Salmonella* CYCLOHEXANE RESISTANCE**
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mgtA codes for a P-type ATPase which is known as one of the three Mg^{2+} transporters in *Salmonella enterica*, It has been previously demonstrated that *mgtA* expression is regulated at the transcriptional level by the extracellular Mg^{2+}-responsive PhoP/PhoQ two-component system and post-transcriptionally by an intracellular Mg^{2+}-sensitive riboswitch. In our laboratory, while searching for factors that would modulate the expression of *Salmonella mgtA*, independently of the PhoP/PhoQ system, we identified Rob. Rob is a member of the a subfamiliy of AraC/XylS-type transcriptional regulators implicated in bacterial multidrug, heavy metal, superoxide and organic solvent resistance phenotypes. We demonstrate that, in *Salmonella*, Rob expression (but not its paralog proteins SoxS or MarA) is able to induce *mgtA* transcription in a PhoP-independent fashion by binding to a conserved rob/mar/sox motif localized downstream the PhoP-box, and overlapping the PhoP-dependent transcriptional start site. We found that Rob-induced *mgtA* expression confers *Salmonella* a low-level cyclohexane resistance. Because *mgtA* intactness is required for Rob-induced cyclohexane resistance provided the AcrAB multidrug efflux pump can be expressed, we postulate that MgtA is involved in the AcrAB-mediated cyclohexane detoxification mechanism promoted by Rob expression, disclosing a novel role for MgtA in *Salmonella*.

MI-P41.

CHARACTERIZATION OF A TRANSCRIPTIONAL REGULATOR OF MYCOLIC ACID BIOSYNTHESIS IN *Mycobacterium*

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The most relevant lipids present in *Mycobacterium* cell envelope are mycolic acids. These unusual fatty acids are essential for survival and virulence of *M. tuberculosis*. However, there is no information about the regulation of the synthesis of these lipids, which involves two different fatty acid synthase (FAS) systems. Our working hypothesis is that these two systems should be strictly co-regulated in order to maintain lipid homeostasis in this organism. We have characterized a transcriptional regulator that controls the expression of genes involved in FAS systems. This protein, whose structure appears to be unique within mycobacteria, is highly conserved in actinomycetes but lacks a close homolog in eukaryotes and in the host gut flora. We have identified a conserved 21 bp motif present in the promoter regions of these genes and demonstrated binding of purified protein to 300 bp DNA probes containing the motif. DNase I footprinting analysis verified that the protein under study protects the region containing the conserved sequence. Using *M. smegmatis* as a working model we found that this interaction modulates the expression of a number of FAS-related genes. This regulator, predicted to be a growth-essential gene in *M. tuberculosis*, is an attractive target for the development of new and specific antimycobacterial agents.

MI-P42.

THE ESSENTIAL CARBOXYLTRANSFERASE ACCD6 AS A TARGET FOR NOVEL ANTI-MYCOBACTERIAL AGENTS

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The hallmark of mycobacteria is their lipid-rich cell wall. Much work has been done solving the structures of these unique lipids and their biosynthetic pathways. However, almost no information is available regarding the biosynthesis of the precursors for these complex molecules. Our working hypothesis is that the alphacarboxy acyl-CoAs utilized in the biosynthesis of the membrane and cell-wall fatty acids are the product of the Acyl-CoA Carboxylase complexes (ACCase) present in *Mycobacterium tuberculosis*. The ACCase 6 complex, formed by the carboxyltransferase AccD6 and the biotinylated protein AccA3, has been proposed to be the essential ACCase that provides the malonyl-CoA for the elongation steps of both fatty acid and mycolic acid biosynthesis, although this has not been demonstrated experimentally so far. We generated a conditional mutant in the *accD6* gene of *M. smegmatis*. Our analysis of this mutant demonstrated that AccD6 is the essential carboxyltransferase component of the ACCase 6 enzyme complex, and that this complex is implicated in the biosynthesis of malonyl-CoA for both fatty and mycolic acids. Our results shed light on the biological roles of the key ACCases in the biosynthesis of cell wall fatty acids, as well as providing a new target for tuberculosis drug-development.

MI-P43.

***Bordetella bronchiseptica* DEEP ROUGH LPS INDUCES IL-10 BONE MARROW DENDRITIC CELL RESPONSE**

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Though it is well known that *B. bronchiseptica* LPS induces a TLR4-dependent innate cytokine production, the role of the different LPS portions in such induced immune response has not been yet elucidated. To get a better insight on this aspect we examined cytokine production on dendritic and macrophage like cells following exposure to different purified LPS structures. To this end purified BMDC were exposed for 24 h to different *B. bronchiseptica* LPS structures (10 ng ml-1). It was observed that both the smooth (wild type, named LPSBb9.73) and the deep rough (derived from *waaC* mutant, named LPSBbLP39.) LPS induced similar levels of IL-1 , TNF- and IL-6. Only in presence of the LPSBbLP39, IL-12p40 and IL-10 were secreted in high level and the concentration of these cytokines induced correlated with the amount of LPS employed. In parallel, murine J774 macrophages were incubated with LPSBb9.73 or LPSBbLP39. While no significantly differences in IL-1 or IL-12p40 concentrations were observed between both LPS, TNF- and IL-6 production were enhanced following stimulation with LPSBb9.73 (TNF- : 2.4±0.5 ng/ml LPSBb9.73 vs. 0.1±0.03 ng/ml LPSBbLP39, IL-6: 5.2±0.2 ng/ml LPSBb9.73 vs. 2.6±0.2 ng/ml LPSBbLP39). The data showed that a LPS without the branched oligosaccharide might interact with TLR4 inducing a stronger anti-inflammatory response suggesting a regulatory role for this structure.

MI-P44.

***Brucella abortus* PPX IS REQUIRED FOR WILD-TYPE VIRULENCE IN BALB/C MICE**

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Inorganic polyphosphates (polyP) are linear polymers of orthophosphate residues linked by high-energy phosphoanhydride bonds. The enzyme primarily responsible for polyP synthesis in *Escherichia coli* is polyP kinase (PPK), which uses the gamma phosphate of ATP to make the polymer. PolyP can also be hydrolyzed to Pi by exopolyphosphatase (PPX). *In silico* analyses revealed the presence of a gene coding for a putative PPX in the *B. abortus* genome. The *ppx* gene was cloned and the *Brucella abortus ppx* null mutant was obtained by deletion and insertion of Km cassette. The mutant grew as well as the wild-type in Brucella Broth complex medium but its survival was reduced during late stationary-phase of growth. Cell infection assays in J774 murine macrophage-like cell line suggested that *ppx* mutant is less resistant in the initial phase of infection to killing by macrophages than the wild-type strain. However, once this phase is bypassed, the multiplication rate is similar to the one observed with the virulent strain. BALB/c mice infection assays showed that the mutant display reduced virulence at all times tested, thus indicating that *ppx* is required for efficient infection process in mice.

MI-P45.

MICE IMMUNIZATION WITH LIVE *Lactococci* EXPRESSING ROTAVIRUS VP8 ANTIGEN

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In the last decade, several studies have used lactic acid bacteria as antigen delivery vehicles to develop safe, live vaccine. Recombinant *Lactococcus lactis* strains expressing heterelogous antigens have been successfully used to elicit an immune response against bacterial or viral antigens. In this context, we use *L. lactis* for the expression of rotavirus antigens in order to develop an useful tool for immunization against this virus. For this purpose, we constructed two recombinant *L. lactis* strains targeting VP8 antigen to the cytoplasm (LL-VP8cyt) or to the cell wall (LL-VP8cwa). The expression capacity of such strains was analyzed by Western blot. Then, their ability to induce an immune response in mice was compared. Groups of eight Balb/c female mice were immunized intragastrically with the induced *L. lactis* strains. Control mice received identical quantities of wild type *L. lactis* (LL). After the last boosting, mice were sacrificed and levels of specific serum IgG and intestinal IgA against VP8 were measured by ELISA and Western blot. In this way, it was determined that the group of immunized mice with LL-VP8cyt produced a level of IgA significantly higher than the control. On the other hand, the group that received the LL-VP8cwa strain showed both increased levels of intestinal IgA and serum IgG. These strains are thus good candidates for the immunization against rotavirus.

MI-P46.

UNEXPECTED USES OF AN OLD DRUG: ANTI-MYCOBACTERIAL ACTIVITY OF AZOLE ANTI-FUNGAL COMPOUNDS

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Econazole is a potent anti-fungal drug inhibiting CYP51, a Cytochrome P450 enzyme involved in the synthesis of the fungal cell wall. Mycobacterial genomes contain several genes encoding CYPs, but there is no information on their physiological role. It has recently been shown that econazole is active on mycobacteria but its mechanism of action or target affected are unknown. Considering the potential utilization of azole compounds as novel anti-tubercular drugs, we undertook the study of their mechanism of action by isolation of econazole- resistant *M. smegmatis* mutants. Four mutants isolated at very low frequency were characterized by colony morphology, pattern of drug susceptibility, growth in liquid and solid medium, biofilm formation, sliding motility and cell envelope composition. Our results show that their sensitivity to a panel of antibiotics was the same than the one obtained for the parental strain. Three of the mutants show differences in Congo Red accumulation, biofilm maduration and sliding motility suggesting cuali or quantitative differences in cell envelope composition. Two of the mutants display altered growth in liquid medium with larger duplication times and cross-resistance to another azole drug, clotrimazole. These mutants will allow the study of the mechanism(s) of action of azole drugs and the ensuing mechanisms of resistance staged by mycobacteria.

MI-P47.

DIVERSITY OF QUORUM SENSING SIGNAL SYNTHASE GENES IN *Acinetobacter* CLINICAL ISOLATES

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Opportunistic pathogens of the genus *Acinetobacter* represent a serious threat in nosocomial environments due to its wide distribution and multi-resistant profile. In this study, four representative isolates of the genus, characterized by DNA-DNA hybridization as *A. calcoaceticus, A. baumannii, A. genosp 3 and A. genosp 13TU*, all from nosocomial sources, were compared with respect to virulence- related phenotypic and genotypic traits. In particular, haemolysin, lipase and protease activity, biofilm formation, QS signal detection by TLC and putative genes involved in the QS signal generation were analysed. For gene search, full length and inner primers of conserved regions from the *abaI* gene (*A.baumannii* synthase) and of the *hdtS* gene (*P. fluorescens* synthase) were used as templates in PCR amplifications. All isolations showed 1, 2, or 3 QS signals of similar Rf´s, 0.2, 0.4 or 0.5, differing only on their relative intensity. In spite of the similarity in QS signals, the genetic pattern was quite diverse. While *abaI* was present in *A.baumannii* and *hdtS* was detected in *A. genosp3*, both as sole genes, *A.calcoaceticus* contained the genes for both synthases while *A. genosp13* contained none. Our results indicate that *Acinetobacter* shares a common QS phenotype to communicate cell density within the genus but the signal generator genes can radically differ among species.

MI-P48.

ROLE OF ALKALINE/NETURAL INVERTASES IN *Anabaena* STRAINS IN RESPONSE TO A SALT STRESS

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In *Anabaena* (*Nostoc*) sp. PCC 7120, we had identified and biochemically characterized two alkaline/neutral invertase genes (*invA* and *invB*) coding for proteins of about 53-55 kDa. The aim of this work was to investigate if those isozymes play different roles in the *Anabaena* filaments. Expression analysis, localization of InvA and InvB in N2-fixing filament cells, evidenced with transcriptional fusions of an optimized version of the green fluorescent protein (GFP) gene (gfp-mut2) to putative promoters of *invA* and *invB*, and phenotipic characterization of *Anabaena* mutant strains where *inv* genes were disrupted, supported the importance of alkaline/neutral invertases, and particularly *invB*, in diazotrophic growth. On the other hand, a key function of alkaline/neutral invertases in salt tolerance was demonstrated by expression analysis at the transcriptional, traslational and activity level. The impair in alkaline/neutral invertase activity affect salt tolerance. Taken together our results, we conclude that a high sucrose turnover seems to be important to cope salt stress.

Supported by ANPCyT (PICT Nº 2004), UNMdP, PIP 6105 and FIBA.

MI-P49.

CYANOBACTERIAL SUCROSE METABOLISM AND THE SYNTHESIS OF SUCROGLUCANS AFTER A SALT TREATMENT

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Whereas many plant species synthesize sucrosyl oligosaccharides to cope with abiotic stresses, some filamentous nitrogen-fixing cyanobacteria (photosynthetic oxygen-evolving prokaryotes) accumulate water-soluble polymers with the general name of sucroglucans as a response to environmental stress. The structure of the members was recently elucidated, consisting in a series of non-reducing sucrose derivatives, where glucose is linked through its hemiacetalic hydroxyl to the 2 position of the Glc moiety of Suc. We hypothesized that sucroglucans may be synthesized by a series of glucosyl transfers from UDP-Glc, first to Suc, and then to each higher homolog. We investigated the accumulation of sucroglucans in *Anabaena* (*Nostoc*) sp. PCC7120 derivative mutants that have either increased sucrose content or that are sensitive to salt. We analysed the expression of genes related to sucrose metabolism in the wild-type and mutant strains. We conclude that salt sensitivity is not cope by the only presence of a high sucrose content, when it is accompanied by the abolishment of sucroglucans accumulation. This is in agreement with preliminary results that indicate that sucrose level would not be involved in the polymer synthesis. Genetic work is in progress to characterize the enzymes involved in sucroglucan metabolic pathway.

Supported by ANPCyT (PICT Nº 2005), UNMdP, PIP 6105 and FIBA.

MI-P50.

THE CARO PROTEIN OF *Acinetobacter baumannii* EXISTS AS A FAMILY OF MULTIPLE ALLELES

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We have recenty showed that the outer membrane protein CarO from the pathogen *Acinetobacter baumannii* functions as a channel for L-ornithine, arginine and the last-generation antibiotics carbapenems. In this work we studied the polymorphism of *carO* within a wide collection of *A. baumannii* clinical strains obtained from Argentine and USA during a 20-year period. Our study identified the presence of 4 different *carO* variants (I-IV) among the population. Sequence analysis revealed that an active diversification has occurred within this gene. Also, sequence diversity within each group of *carO* variants was minimal, suggesting that after selection a clonal spread has taken place. Genetic variation in *carO* is strikingly concentrated in three regions which notably superimpose with the surface exposed loops. Besides, the CarO variants exhibited differential inmunogenicity patterns as determined by the use of specific antibodies, indicating the existence of specific epitopes between the variants. They did not show statistically significant result, whereas programs designed to detect recombination indicated the presence of breakpoints boundering the variable sequences. These results pointed out the differential phylogenetic origins of the variable regions relative to the conserved ones, and suggested that horizontal gene transfer may account for the polymorphism observed in *carO*.

MI-P51.

PRODUCTIVE FOLDING OF METALLO- -LACTAMASES IN THE PERIPLASM OF GRAM-NEGATIVE BACTERIA

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Periplasmic proteins are synthesized as precursor proteins in the cytoplasm and acquire their native structure in their final destination. While cytoplasmic chaperones have been well characterized, much less is known about periplasmic folding assistants. We studied here by genetic methods the role of proposed periplasmic chaperones in the biogenesis of the metallo -lactamase (M L) GOB. We observed that the production of the GOB precursor in *Escherichia coli* confers -lactam resistance, but that deletion mutants in putative periplasmic chaperones such as DsbA, DsbC, DsbG, PpiA, PpiD, SurA, FkpA or DegP did not affect resistance levels indicating that these proteins do not participate individually in GOB biogenesis. *Salmonella enterica* insertion mutants were generated by MudJ transposition, and clones with diminished -lactam antibiotic resistance were isolated. Sequence analysis of a particular mutant called RDC, showed disruption of *phsB*, a gene part of the *phsABC* operon that encodes periplasmic thiosulfate reductase responsible for H_2S production during anaerobic respiration. Biochemical assays indicated that this mutant still generate H_2S, suggesting that PhsB is not essential for this function. Based on these observations, we propose that this redox protein is implicated in the productive folding of metalloenzymes such as M Ls in the bacterial periplasm.

MI-P52.

MEMBRANE-PERMEABILIZING PEPTIDE ACTS SYNERGISTICALLY WITH MCCJ25 AGAINST *Salmonella typhimurium*

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The microcin J25 (MccJ25) is an antimicrobial peptide that inhibits the RNA polymerase activity and the membrane respiratory chain. MccJ25 is active on some *Salmonella* serovars, while *Salmonella typhimurium* is completely resistant because the inability of its FhuA protein to mediate penetration of the antibiotic. In the present study we test the adjuvant effect of the membrane-permeabilizing peptide (MPP) KFFKFFKFFK when it is administrated together with MccJ25. We demonstrated that under the above condition *Salmonella typhimurium* 14028s became sensitive to the MccJ25 antibiotic. The *in vivo* transcription and oxygen consumption assays showed that the mix MccJ25-MPP was active on both antibiotic targets in *Salmonella typhimurium*. We also study the antimicrobial activity of MccJ25MPP against *Salmonella typhimurium* inside of macrophage. We determined that the bacterial replication ability was hardly inhibited by the presence of MccJ25-MPP compared with untreated control. Additionally, the bacterial invasion ability was not affected by the treatment. Our results demonstrated that MPP perturbs the membrane to allow the MccJ25 penetration in an FhuA-independent way. According with our findings, the mix MccJ25-MPP would be considerate as a therapeutic agent against pathogenic *Salmonella* strains.

MI-P53.
THE ANTIBIOTIC MICROCIN J25 IS A REDOX PEPTIDE
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The microcin J25 (MccJ25) is a lasso-peptide antibiotic of 21 L-amino acid residues (G^{1}-G-A-G-H^{5}-V-P-E-Y-F^{10}-V-G-I-G-T^{15}-P-I-S-F-Y^{20}-G). Two cellular targets for this antibiotic, the RNA polymerase and the membrane respiratory chain via the superoxide production, were previously reported by our group. Also, we showed that Tyr9 is the key amino acid in the membrane respiratory chain target of the antibiotic. Now, we studied the electronic flow from NADH to MTT through phenazine methosulfate (PMS) by spectroscopy techniques, 2D-FTIR and oxygen consumption, in the presence of MccJ25. MccJ25 was capable to inhibit the tetrazolium salt (MTT) reduction in a concentration dependent way. We observed an increment of both i) Tyr radical band located at 1,478 cm^{-1} in the IR spectrum and ii) the oxygen consumption. In the absence of MccJ25 the electrons flow directly to MTT since no oxygen consumption was observed. The last fact suggested that microcin is able to transfer electrons to oxygen molecule. FTIR spectroscopy was also used in order to detect the peptide´s Tyr reduction in the presence of potassium ferro/ferricyanide. The wavenumber related with the Tyr-OH specie shifted from 1,514.6 to 1,512.2 cm^{-1}. These data led us to propose MccJ25 as a redox antibiotic peptide.

MI-P54.
ANTIBIOTIC ACTIVITIES OF A *Pseudomonas aeruginosa* SUPERNATANT
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An antibiotic producing strain was isolated from a human sample. Genus and speciation determination were carried out by 16S rDNA and *gyrB* sequencing and subsequent analysis of the sequences using BLAST. The strain was identified as *Pseudomonas aeruginosa*. The supernatant showed activity against different human and plant pathogens. The antimicrobial substance diffused through a dialysis membrane with a 12kDa cut off. The antibiotic activity in the supernatant was resistant to all proteases tested. The activity partially decreased after exposing the culture supernatant to 121ºC for 30 minutes. When the supernatant was adsorbed on a C8 cartridge two different fractions, with antibiotic activity, were eluted at methanol/water (40: 60) and methanol/water (80: 20). The two fractions show dissimilar action spectra. The 40% fraction was susceptible to high temperature whereas the 80% fraction was totality resistant. These last results indicated that the supernatant of this strain contains at lest two different antibiotics. An *E. coli* strain mutant in *tolC* gene was hiper-susceptible to the two fractions suggesting a possible role of TolC protein in the exportation of these antibiotics. Identification and characterization of this compound produced by *Pseudomonas aeruginosa* may represent a novel antibiotic discovery.

MI-P55.
IN-VITRO PROINFLAMMATORY RESPONSE MEDIATED BY *Shigella sonnei* VIRULENCE PLASMID SPONTANEUS MUTANT
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The pathogenesis of *Shigella* is focus in the capacity to invade intestinal epithelial cells, replicate and escape from the infected enterocytes triggering a proinflammatory immune response sense by Nod1 intracellular receptors. For this purpose *Shigella* have a virulence plasmid of~220 kb (pINV) that encodes essential products, some of them are included in *ipaBCDA* operon. In previous works we have shown the absence of amplification and expression of the genes of *ipaBCDA* operon in *Shigella sonnei* clinical isolates by PCR and western blot. Moreover, these *ipaBCDA* negative mutants showed ability to invade Hep-2 and Caco-2. In this work the ability of *S. sonnei ipaBCDA*$^{-}$ to induce the expression of CCL20 and IL-8 as a measure of the proinflammatory response in Caco-2 cells was studied. Caco-2 cells containing *ccl20:luc* reporter construction were incubated with heat killed *S. sonnei* IpaBCDA^{-} and *S. flexneri* IpaBCDA^{+} clinical isolates, and assayed for luciferase activity. In a second approximation Real Time PCR was performed using specific primers for *ccl20*, *il-8* and *actin B* as housekeeping gene. Despite of the absence of virulence proteins, known as necessary for invasive phenotype and Nod1 proinflammatory pathway, we have found by both methods similar expression pattern of chemokines, CCL20 and IL-8, induced either by *S. sonnei* ipaBCDA^{-} or *S. Flexneri*.

MI-P56.
INFLUENCE OF SALICYLATE ON EXPRESSION OF *Staphylococcus aureus* EXTRACELLULAR ADHERENCE PROTEIN
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One of the most important adhesins in *Staphylococcus aureus* is the Extracellular Adherence Protein (Eap) which is found in 98% of clinical isolates. *eap* transcription is mainly regulated by *sae*. Several environmental signals affect virulence factor expression in *S. aureus*. In particular, salicylate (SAL) was shown to reduce staphylococcal virulence. The aim of this work was to study the effect of SAL on *eap* and *sae* expression and its consequences on internalization of *S. aureus* into MAC-T epithelial cells. We demonstrated that *eap* expression is enhanced after treatment with SAL through the *eap* promoter fused to the gfp_{uvr} reporter gene. We also showed by Real-time PCR that levels of *eap* and *sae* transcripts were increased with SAL. Moreover, we observed the increase of Eap protein after SAL treatment. By fluorescence microscopy, we observed that *eap* expression is required to invade MAC-T cells and that SAL enhances internalization. Indeed, the Newman strain pretreated with SAL ($1.5x10^{5}$ CFU/ml) showed a significantly higher ability to become internalized within epithelial cells when compared with the untreated strain ($7x10^{4}$ CFU/ml, $p<0.0001$, Mann-Whitney test). Altogether, the results demonstrate that SAL increases internalization of *S. aureus* due to enhanced Eap production caused mainly by a positive effect of SAL on *sae*.

MI-P57.
INTERACTIONS OF THE CHEW PROTEIN WITHIN THE CHEMOSENSING COMPLEX: A GENETIC SUPPRESSION STUDY
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Chemotactic behavior allows bacteria to find and colonize their specific habitats. In *E.coli*, swimming cells respond to chemical gradients by modulating the frequency of direction changes. The basic unit that "smells" the chemicals consists in a ternary complex composed by chemoreceptors, a histidine kinase called CheA and a receptor-kinase coupling protein called CheW. Although there is quite a lot of structural information for the individual proteins, a reliable model for the precise structure of the complex that might help explain how the kinase activity is modulated is still missing.
The coupling protein CheW is an absolute requirement for the receptors being able to activate the kinase, but its role is not yet completely understood. Here, a genetic suppression study was conducted in order to look for suppressor mutations in CheA or in the serine chemoreceptor Tsr that restore chemotactic function to cells that express a defective CheW mutant. Suppressor mutations were found in both partner proteins. However, most of them lack allele specificity, suggesting that they act mainly through phenotypic compensation rather than through actual conformational suppression. However, the obtained results allowed us to identify some positions that might represent actual contact residues and will be explored as such through *in vivo* crosslinking studies.

MI-P58.
ELECTRON DONORS FOR THE 5- ACYL-LIPID DESATURASE IN *Bacillus subtilis*
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Unsaturated fatty acids (UFAs) synthesis is carried out by desaturases. The reaction requires oxygen and utilizes NAD(P)H and flavoproteins as electron donors. The electron transport system of the 5-acyl lipid desaturase from *B. subtilis* has not been yet characterized. We have demonstrated that mutants in ferredoxin are not impaired in UFAs synthesis. The *in silico* analysis of the genome of *B. subtilis* reveals the existence of two flavodoxins YkuN and YkuP, and we propose that they could act as putative electron donors for the 5-desaturase. We constructed conditional mutants in the *ykuNOP* operon and the effect of the mutation on UFAs synthesis was evaluated after labeling the fatty acids with [14C]-palmitate followed by TLC. The results showed that absence of flavodoxins did not cause differences in the UFAs levels compared to the wild type strain. In order to verify our model that flavodoxins can act as electron donors for the 5-desaturase we constructed mutants in the *ykuNOP* operon and *fer* gene and evaluated the UFAs production. The results revealed that in *B. subtilis* the function of de 5-desaturase is impeded when both ferredoxin and flavodoxins are absent. However, UFAs production was observed when flavodoxin was present and in the absence of ferredoxin. These results indicate that both flavoproteins can act as electron donors for UFAs synthesis in *B. Subtilis*.

MI-P59.
PHYSIOLOGICAL ROLE AT LOW pH OF THE C4 METABOLIC PATHWAY IN *Enteroccocus faecalis*
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Lactic Acid Bacteria (LAB) are recognized as safe microorganisms that are capable to improve the quality of diary products. When the LAB *Lactococcus lactis* is employed as a starter strain for the production of fermented foods, high quantities of important aroma compounds such as diacetyl are generated by means of the C4 metabolic pathway. This route depends on the expression at low pH of the *als*, *aldB*, *aldC* and *butBA* genes encoding the enzymes involved in the conversion of pyruvate into aroma compounds. In the non-starter LAB *Enterococcus faecalis*, a bioinformatic search determined that only *als* and the *L. lactis aldB* orthologous gene *alsD* are present in its genome. Our studies showed that these genes compose a single operon, which is maximally transcribed during the mid-exponential phase of growth from a promoter located 34 nucleotides upstream from the ATG of *als* gene. The trancriptional analysis indicated that this operon is induced in the presence of pyruvate. The disruption of the *als* gene, produced a C4 deficient strain sensible to low medium pH and high concentrations of pyruvate. Moreover, we found that the putative transcriptional regulator LytR, transcribed divergently to the *als* operon, is not involved in its induction. Finally, we determined that LytR is associated to cell wall maintenance since a *lytR* deficient strain showed a higher rate of autolysis.

MI-P60.
THE METAL SELECTIVITY OF GolS IS ACHIEVED BY SPECIFIC AMINO ACIDS IN THE C-TERMINAL BINDING LOOP
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Salmonella depends on two MerR regulators to monitor the environmental concentration of monovalent metal ions: the copper-regulator CueR which governs copper homeostasis, and the *Salmonella*-specific GolS, responsible for gold resistance. CueR is able to detect Au or Cu ions with similar affinities. On the other hand, GolS is 52 times more responsive to Au(I) than to Cu(I), and this metal-discrimination relies on the C-terminal CX_2C loop of the sensor protein. Replacement of the C residues of the metal-binding loop or of a conserved S residue within the dimerization domain abrogated metal-dependent activity of the regulator, indicating that these residues are essential for the regulator's function. To dissect the role of the individual amino acid residues within the metal-binding region of GolS in metal-selectivity, a series of mutant proteins were constructed, in which each residue was replaced by the one present in CueR-like regulators. The replacement of an A residue in the position 113 of GolS for a P rendered a mutant regulator with similar affinities to Au(I) or Cu(I). Our results suggest that metal-discrimination in GolS relies on the spatial conformation that the metal-binding loop adopts rather than on the presence of particular residues within it, favouring the interaction of Au(I) with a larger ionic radius, and hindering the interaction of Cu(I) with a smaller ionic size.

MI-P61.

DEVELOPMENT OF A REPORTER GENE ASSAY FOR SCREENING OF *DESK* MUTANTS IN *Bacillus subtilis*

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The *B. subtilis* Des pathway is composed by the membrane delta5-acyl lipid desaturase and the two- component system DesK-DesR. DesK is a histidine kinase located in the membrane and DesR is a cytoplasmatic response regulator that binds specifically to the promoter region of the *des* gene. Induction of the Des pathway is brought about by the ability of DesK to assume different signaling states in response to changes in membrane fluidity. A random mutagenesis approach of the transmembrane segments of DesK was initiated to unveil the mechanistic details of membrane fluidity sensing. The aim of this work was to develop an easy screen system in *B. subtilis* for analysis of punctual mutations in DesK, based on sporulation as reporter phenotype. RapA is a phosphatase of the Spo0F response regulator, a key component of sporulation initiation cascade. Sporulation is impaired when RapA is expressed without its inhibitor (PhrA), so we built a *rapA-phrA* null mutant expressing ectopically *rapA* under the P*des* promoter (VRA2). As the Des pathway is negatively regulated by unsaturated fatty acids, *des* gene of VRA2 was disrupted, rendering VRA2des-. In sporulation medium this strain showed a Spo+ phenotype at 37º C and Spo- after a downshift from 37 to 25º C. These results support the concept of a useful screening system to be used in spore forming bacteria.

MI-P62.

RESPONSE OF *Pseudomonas aeruginosa* TO LETHAL AND SUBLETHAL UVA IRRADIATION: ROLE OF QUORUM SENSING

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Bacteria present in natural environments are frequently exposed to UVA irradiation, whose effects are mediated mainly by oxidative stress. Quorum sensing (QS) is a cell-cell mechanism employed by bacteria to regulate transcription in response to population size. In *Pseudomonas aeruginosa*, QS involves two signaling systems, *las* (QS I) and *rhl* (QSII), that function as activators of hundred of genes. In this work, we report the response of *P. aeruginosa* to lethal and sublethal UVA irradiation, and the participation of QS mechanism in this response. Wild type PAO1 strain and its QS I or QS II defective derivatives were irradiated at stationary phase, when most of QS controlled genes are induced. The QS II defective strain was more sensitive to lethal radiation than the strain defective in QS I and the wild type, measured as loss of cell viability. When a sublethal fluence of UVA was applied to PAO1 strain, a dose-dependent growth delay, not yet described in *Pseudomonas*, was observed. Under sublethal irradiation, the QS II defective strain exhibited an increased growth delay when compared with QS I mutant or the wild type, which presented a similar behavior. It is concluded that QS II is important in the response of stationary cells of *P. aeruginosa* to both lethal and sublethal fluences of UVA irradiation, probably by modulating the expression of genes involved in antioxidant processes.

MI-P63.

HETEROLOGOUS EXPRESSION OF THE HALOARCHAEAL ATP-DEPENDENT LON PROTEASE IN *E. Coli*

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The ATP-dependent Lon protease family can be divided into two subfamilies, LonA (bacterial and eukaryotic) and LonB (archaeal), based on the sources and the domain structures of the proteins. Although haloarchaea (optimum growth in 20% NaCl) encode Lon homologs, these enzymes have yet to be characterized and the biology of these enzymes in archaea is poorly understood. In order to determine the catalytic properties of the haloarchaeal LonB, the gene encoding the LonB protease from the haloalkaliphilic archaeon *N. magadii*, *NmlonB*, was cloned, sequenced and expressed in *E. coli* cells. The full-length *lon* gene with a His6 tag was subcloned into pET24b and amplified in *lon*-deficient *E. coli* Rosetta cells. A major polypeptide of the expected size (115 kDa) was detected in cell lysates after induction with IPTG. This polypeptide was subjected to trypsin digestion and MALDI-TOF/MS analysis and four peptides were detected which corresponded to *Nm*LonB protease. Archaeal LonB proteases are membrane-bound, therefore, *Nm*Lon was solubilized with Triton X-100 and purified by Ni^{2+}-TED affinity chromatography. Protein purification was monitored by SDS-PAGE/Coomassie Blue staining and Western blotting using Anti-His antibody. By using this protocol, *Nm*Lon was successfully purified with high yield (20 g protein/ml cell culture) and high purity from *E. coli*.
Supported by CONICET, ANPCyT and UNMdP.

MI-P64.

FUNCTIONAL CHARACTERIZATION OF *Bacillus sp.* ACYL-LIPID DESATURASE

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The biosynthesis of unsaturated fatty acids is carried out by desaturases, a family of enzymes that introduce a double bond at specific positions in fatty acids and play an important role in determining the fluidity of cell membranes. Knowledge about the determinants of their enzymatic specificity is still not understood. Our main goal is to understand the specific role of amino acid residues in substrate recognition and double bond-positioning activities of desaturases. *In silico* analysis on *Bacillus* genomes allowed us the identification of highly similar desaturases. We have characterized two desaturases from *B. licheniformis* and two from *B. cereus*. These enzymes were expressed in a *B. sutilis des* mutant and the fatty acids patterns were analised by GC-MS. *B. licheniformis* BLO2106 and *B. Cereus* BC2983 are 5-desaturases, 70-71 % identical to *B. subtilis* orthologous. *B. cereus* BC0400 is a 10-desaturase and BLO2692 did not present activity. Site-directed mutagenesis and domain swapping techniques will help us to elucidate which residues or regions in these enzymes that are important for the substrate specificity and/or region selectivity.

MI-P65.

EFFECT OF THE RCSB REGULATOR EXPRESSED FROM DIFFERENT PROMOTERS, IN *Salmonella typhimurium* VIR

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The Rcs phosphorelay system consist of the sensor RcsC, the response regulator RcsB, and the histidine-containing phosphotransfer protein RcsD, an intermediary in the phosphoryl transfer from RcsC to RcsB. Although the signal activating the system remains unidentified, the activation can occurs under certain growth conditions. Previously, we demonstrated that the RcsB overproduction promotes the Rcs system activation, in *rcsC* or *rcsD* mutants, but not in the double mutant *rcsD rcsC*, suggesting that RcsC or RcsD can independently transfer the phosphate group to RcsB. On the other hand, we found that *rcsB* is expressed under the control of two promoters, P_{rcsDB} and P_{rcsB}, and that the *rcsB* overexpression negatively regulates the Rcs system repressing the P_{rcsDB} activity. Here, we show that the levels of *rcsB* expression from each promoter are required at different growth phase and different levels to control specific physiological process. In this way, the *rcsB* expression from P_{rcsB} activity is required to maintain the repression of the RcsB-dependent motility phenotype. Also we observed a differential requirement of *rcsB* from P_{rcsDB} or P_{rcsB} activity during the phagocytosis and replication in macrophages. Taken together, our findings enhance the phosphorelay pathway and the biological significance of the Rcs system activation in *Salmonella* pathogenesis.

MI-P66.

RECOMBINATION OF THE CASSETTES AND PSEUDOCASSETTES EMBEDDED IN THE CLASS 2-INTEGRON IN2-8

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The *Tn*7::In2-8 transposon, described in *A. baumannii* multiresistant isolates, contains an unusual class 2 integron array. This integron has the particularity that downstream the stop codon of the *catB2* gene a sequence with 100% identity to the *attI2* was found. A non-elucidated mechanism might be involved in the acquisition of the *catB2* pseudocassette. In view of the uncommon features of this class 2 integron, the integrase-mediated site-specific recombination of the cassettes and pseudocassettes embedded in the In2-8 were studied. The cassettes and pseudocassettes were cloned in order to use them for in vivo recombination assays. *E. coli* cells harboring the *intI1* gene and the plasmid that served as substrate were cultured in the presence of IPTG, and plasmid DNA was isolated. Specific PCR primers were used to identify plasmid species where excision has occurred. We observed that the IntI1 excised the *attC-catB2(attI2)-dfrA1-attC* cassette as a module, although it was shown that IntI1 could also excised *attC-catB2(attI2)* and *(attI2)-dfrA1-attC*. We also found the excision of *aadA1, dfrA1, sat2, aadB* and orfX. We demonstrated that the IntI1 recognized all the cassettes and pseudocassettes embedded in the In2::8, showing that the *attI2* could be used as a substrate to mediate the specific recombiantion reaction.

MI-P67.

PRESENCE OF CLASS C GROUP II INTRONS AMONG *Shewanella* ISOLATES

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Bacterial group II introns are organized in several classes, from A to F, and distributed in several microorganisms. These elements have the ability to move to new regions in a genome through an RNA intermediate by a retrohoming event. Previously, we found that a class C group II intron from *Shewanella baltica* OS185 shared a common ancestor with the *S.ma*.I2 intron from the clinical isolate *Serratia marcescens*. The aim of this work was to identify the class C group II introns among *Shewanella* isolates and to determine their genetic contexts. We searched for *S.ma*.I2 homologues in 10 *Shewanella* clinical and 7 environmental isolates using the PCR technique with specific primers. All the isolates harboured a group II intron, while the *Shewanella algae* Sh10 isolate had 100% nucleotide identity with *S.ma*.I2. *In silico* analysis showed that over 17 complete and 7 partial genome projects 5 harboured a class C intron in their chromosomes. Alike *S.ma*.I2, these introns were located downstream of secondary structures indicating a similar mechanism of recombination. Also, a second class of introns, the cloroplast-like class, was also present in *Shewanella* coexisting with the class C introns. The *Shewanella* bacteria harbour different intron classes and are potentially involved in the horizontal transference of class C introns to clinical niches.

MI-P68.

ROLE OF CLPLP- AND SSRA-DEPENDENT PROTEOLYSIS IN THE ACIDIC STRESS-INDUCED LYSIS OF *S. pneumoniae*

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In *S. pneumoniae*, we demonstrated that acidic stress triggers a autolysin-induced lysis that is regulated by the ComE and CiaR response regulators, which play a favoring or protective role, respectively. We demonstrated that the ClpL ATPase, which is highly expressed during acid exposure, is required for the acidic stress-induced lysis (ASIL). ClpL carries out a chaperone function and is also involved in proteolysis in association with the serine protease ClpP. The SsrA-SmpB system mediates peptide tagging of nascent truncated proteins, which are targeted for proteolysis via the ClpP-chaperone complex. We constructed a *clpL* mutant that showed a complete inhibition of ASIL, indicating that this chaperone is required to fold proteins that promote ASIL. To study the proteolysis contribution to ASIL induction, we also obtained mutants of *clpP* and *smpB* genes. When the ASIL phenotype was analyzed, both *clpP* and *smpB* mutants did not autolyse at acidic pH. Considering these results, we hypothesized that acidic stress-unfolded proteins are *ssrA*-tagged and degraded by ClpLP complex, facilitating the ASIL process. However, we cannot exclude the possibility that putative inhibitors of ASIL could be degraded directly by ClpLP through the recognition of *ssrA*-like tags present in their C-terminal regions, as described in *E. Coli*.

MI-P69.
EFFECT OF PURINE METABOLISM ON POLYKETIDE PRODUCTION IN *Streptomyces coelicolor*
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Bacteria respond to nutritional stresses by producing an intracellular alarmone ppGpp which triggers the stringent response resulting in growth arrest and expression of resistance genes. Under amino acid limiting conditions, the RelA protein associated with ribosomes synthesizes ppGpp from ATP and GTP. In *Streptomyces* sp. several studies have noted a correlation between ppGpp synthesis and antibiotic production. Although it is clear the role of RelA for the synthesis of ppGpp, little is know about the effect of regulation of purine metabolism on synthesis of ppGpp. In aerobic condition, the committed step in the degradation of purine bases is the oxidation of hypoxanthine and xanthine to uric acid and then to allantoin, which is further metabolise by the allantoin pathway. In this work we have identified a cluster of genes *sco6243*, *sco6244*, *sco6247* and *sco6248* from *S. coelicolor* encoding putative proteins involved in allantoin metabolism that are next to *sco6246* gene encoding a protein homologue to AllR, the allantoin regulator of *E. coli*. Genetic studies with a *sco6246* mutant strain demonstrated that AllR encode a repressor of this pathway and the deregulations of the pathway strongly impair antibiotic production. These results could suggest that availability of precursors for synthesis of ppGpp is a check-point for the production of secondary metabolites in *Streptomyces*.

MI-P70.
TRANSCRIPTIONAL ACTIVATION OF THE FATTY ACID BIOSYNTHETIC GENES IN *Streptomyces coelicolor*
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Fatty acid biosynthesis is not only an essential process for most living cells, but it is also energetically expensive. Thus organisms have developed homeostatic mechanisms that maintain the concentration of lipids at particular levels. In *Streptomyces coelicolor* the essential FAS genes are clustered at a single location within its genome (*fab* operon). Bioinformatics analysis of the *fab* genes locus revealed the presence of a highly conserved open reading frame located upstream of *fabD*, named *fasA*, which has homology with transcriptional regulators. To investigate the functional role of FasA in the transcriptional regulation of the *fab* operon, we initially constructed a *fasA* disruption mutant (AM6). This strain showed an evident reduction in vegetative growth rate and a delay in the onset of sporulation. AM6 also presented diminished FAS activity, distorted composition of total fatty acids and reduced triacylgliceride content compared to the wild type strain. Further the analysis of a transcriptional fusion to *fabD* showed an 8-fold reduction in its expression in AM6 cells. To confirm that this effect was exclusively related to the absence of FasA, we complemented the AM6 mutant. In this genetic background the *fabD* transcription, as well as the growth rate, was restored to the wild type levels. All these results suggest that FasA is involved in transcriptional activation of *fab* operon.

MI-P71.
EXPRESSION AND BIOCHEMICAL CHARACTERIZATION OF GUMI, A BACTERIAL -MANNOSYLTRANSFERASE
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Xanthomonas campestris is a Gram negative bacterium that produces an exopolysaccharide known as xanthan gum. Xanthan is involved in a variety of biological functions, including pathogenesis, and is widely applied in food and non food industry. Although the genetics and biosynthetic process of xanthan are well documented, the enzymatic components remain very poorly characterized. We describe here the expression, purification and functional characterization of the *gumI* gene product, an essential protein for xanthan synthesis. Complementation studies and immunoblots showed correspondence between the proposed gene and the 39-kDa protein product, and that it is membrane-associated. The membrane association was independent of the remaining Gum proteins. The protein was overexpressed in *Escherichia coli*, solubilized and purified in active and stable form using Ni-chelating and size-exclusion columns. The purified protein catalyzed the transfer of a mannosyl residue from GDP-mannose to glucuronic acid- -1,2-mannose- -1,3-glucose- -1,4-glucose-P-P-polyisoprenyl with formation of a mannose- -1,4-glucuronic acid linkage. The enzyme shows no homology with any of the 91 of the currently known carbohydrate-active enzyme families. The procedure described here produced good yield of pure protein through two chromatographic steps, suitable for further structurefunction studies.

MI-P72.
CHARACTERIZATION OF THE *Enterococcus faecalis* OXALOACETATE DECARBOXYLASE MEMBRANE-BOUND COMPLEX
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Citrate metabolism in *E. faecalis*, as in other citrate-fermenting bacteria, involves the conversion of citrate to oxaloacetate and acetate by the enzyme citrate lyase. Then, oxaloacetate is decarboxylated to pyruvate by the enzyme oxaloacetate decarboxylase (OAD). This compound is subsequently degraded to different end products. Analysis of the gene cluster involved in the citrate degradation pathway in *E. faecalis* revealed the presence of all the enzymes required for this metabolism. Interestingly genes coding for two types of OADs were found, *citM* homologous to *L. lactis* soluble OAD and *oad* genes encoding a membrane-bound complex homologous to *Klebsiella pneumoniae* OAD. In this work, we report on the characterization of the OAD membrane complex. The expression of the biotinylated -subunit was detected by Western blot in wild type cell extracts (grown in the presence of citrate) but not in a cit-deficient strain. The expression was restored in a complementant strain. Moreover, the -subunit was localized in the citosolic as well as in the membrane fraction of cell extracts, suggesting a dynamic complex. In order to confirm the expression of all the subunits, OAD complex was purified, and the recovered subunits were identified by MALDI-TOF.

MI-P73.

REGULATION BY METALS OF PHOSPHATE-DEPENDENT *NADH* EXPRESSION IN *Escherichia coli*

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The *ndh* gene, which codified NADH dehydrogenase-2 in *E. coli*, is highly regulated at transcriptional level by diverse external stimuli. Our laboratory reported that *ndh* expression was maintained in stationary phase when cells were grown in the presence of at least 37 mM phosphate. Preliminary observations also indicated that *ndh* expression was decreased when cells were grown in the presence of copper. The aim of this work was to study the regulation of phosphate-dependent *ndh* expression during stationary phase by transition metals. The experiments were performed using chromosomal fusions of the complete promoter or its deletions with *lacZ*. A concentrations range of Cu^{2+} was added at different times of growth. -Galactosidase activity and viability were determined. In exponential phase cells, *ndh* expression was not regulated by copper. However, during stationary phase, the phosphate-dependent gene expression was totally repressed when cells were incubated for 1 h with 1 mM $CuSO_4$. This copper effect was also observed with *ndh* promoter deletions from -250 to -100. Viability was not modified in any of the conditions tested. To analyze a possible copper-specific *ndh* repression, others metals that regulate metal-responsive promoters were assayed. Further studies should be done to elucidate this metal-mediated regulation of *ndh* during stationary phase.

MI-P74.

LACTIC ACID PRODUCTION IN A *CCPA* MUTANT OF *Lactobacillus casei*

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Lactic acid has applications in the pharmaceutical, chemical, textile and foods industries. It has received great attention as a precursor of the biodegradable polylactic acid. Great amounts of lactate are produced by Lactic Acid Bacteria, like *Lactobacillus casei*. The catabolic repression can be an important limiting factor in the lactic acid synthesis. In an attempt to use industrial by-products like cheese whey, we evaluated the fermentative capacity of a mutant strain of the catabolite repression regulator CcpA (BL71). We found that this strain is unrepressed for the nitrogen and carbohydrate metabolism. The proteolytic and peptidase activity were analyzed at the transcription and protein level. Transcription of the *prtP* gene was evaluated by Real Time PCR. The *ccpA* mutant showed to be derepressed. Also milk-grown cultures of strain BL71 were quickly acidified, lowering the pH to one unit less than the wild type. The L-lactic acid production yield was also higher. Different substrates and growing conditions for BL71 were analyzed in order to increase lactate production in low cost medium, like cheese whey. Our results indicated that the *ccpA* mutant is a good candidate for future developments in biotechnology.

MI-P75.

CLONING AND EXPRESSION OF C-TERMINAL MOTIF OF S-LAYER OF *Lactobacillus acidophilus* ATCC 4356

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Lactobacillus acidophilus is one of the major species of the genus *Lactobacillus* found in human and animal intestines and believed to possess probiotic properties. In the outermost envelope of *Lb. acidophilus* ATCC 4356, an S-layer structure is found. It is composed of 45Kb protein monomers encoded by a *slpA* gene. The lytic activity of this S-layer was showed against *Salmonella newport* as a pathogen model. The activity was not detected against *Bacillus* and *Lactobacillus* cell walls. This gene was sequenced and showed 99% homology with other *slpA* from *Lb. acidophilus* strains and *in silico* analysis of the deduced protein showed a C-terminal motif which aligned with murein hydrolases of several *Lactobacillus* strains. The C-terminal motif was cloned in plasmid pHMC05 with an IPTG inducible pSpac promoter. The plasmid introduced in *E. coli*JM109 showed high instability and decreased growths in particular when IPTG was present.We assume that expression resulted in a lethal effect. Therefore we introduced it in *Bacillus subtilis* 168 and induced expression by adding IPTG. Activity of the C-terminal S-layer was evident in *Bacillus subtilis*, where it conserved the murein hydrolase activity. The new activity here described may contribute to the already reported probiotic properties of this bacterium.

MI-P76.

MODIFICATIONS IN D-ALANYLATION OF WALL TEICHOIC ACIDS DURING THE OSMOADAPTATION OF *Lb casei*

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The envelope of Gram-positive bacteria is essentially composed of a thick wall of peptydoglycan chains (PG) and teichoic acids (TA). TA are composed of long chains of olyglycerophosphates linked to either peptydoglycan (wall teichoic acids WTA) or anchored to the membrane through a glycolipid (lipoteichoic acids LTA). We previously showed that In *Lb casei* BL23 growing in hyperosmotic conditions, the PG structure was modified. We wanted to analyze if TA were also modified and in particular the role of the D-alanylation was investigated. For this purpose, walls from cultures grown in MRS and MRS containing 0.7 M NaCl media were prepared and WTA was purified. The content of phosphorus and D-Alanine were determined. We found a 2 fold increase in the D-alanylation when WTA was obtained from cultures grown in high salt. Furthermore, WTA subjected to PAGE allow to visualize the polymer chain length. Those obtained from the high salt condition showed a shift mobility suggesting longer chains and/or differences in charge than those extracted from the control condition. The *dlt* operon of *Lb casei* is responsible for D-alanine esterification of TA In order to evaluate if this operon was derepressed, its expression in different osmotic conditions was analyzed by Real Time PCR. The comparison of these data with those of the D-alanine content of WTA will be discussed.

MI-P77.

CLONING AND EXPRESSION OF A BILE SALTS HYDROLASE GENE FROM *Lactobacillus reuteri* CRL 1098

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Bile acids (BA) are biological detergents that play an essential role in fat digestion. They are synthesized from cholesterol in the liver and then released into the intestines, where may be extensively modified by the indigenous intestinal bacteria. Microbial *B*ile *S*alt *H*ydrolase (BSH) is an important enzyme that hydrolizes the amide bond of the glycine/taurine moiety of BA from the steroid core. A close relationship has been demonstrated between BSH and the ability of the sourdough isolate *Lactobacillus (L.) reuteri* CRL 1098 to remove cholesterol in vitro. In this work, the *hsb* gene from this strain was amplified by PCR techniques. The deduced sequence was shown to have 99% similarity with a choloylglycine hydrolase from *L. reuteri* F275. The *hsb* gene with its putative promotor region was cloned and expressed in *Escherichia coli* and DH10B and in *Lactococcus lactis* NZ9000. *L. lactis* NZ9000 (BSH+) cell free extracts (CFE) showed BSH activity towards all BA assayed (5 mM of GDCA, TDCA, GCA or TCA). For some BA, the BSH values were similar or even higher than those detected in the wild-type *L. reuteri* CRL 1098. In particular, higher values were observed towards GDCA in boths strains. These results confirmed the functionality of the hsb gene of *L. reuteri* CRL 1098 and will contribute to the knowledge of BA metabolism in probiotic microorganisms.

MI-P78.

CITO REGULATES THE CITRATE DEGRADATION GENE CLUSTER OF *Enterococcus faecalis*

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We demonstrated that CitO, a member of the GntR family of transcriptional regulators, is a novel positive regulator involved in the expression of the *cit* operons in *Enterococcus faecalis*. The transcriptional analysis of the *cit* clusters revealed two divergent operons, *citHO* and *oadHDBcitCDEFXoadAcitMG*, which were activated by specific addition of citrate to the medium. In this work, we showed by means of DNAseI footprinting and EMSA assays that CitO binds to the *cis*-acting sequences O1 and O2, present on the *citHO oadH* intergenic region. Its affinity for the binding sites is increased in the presence of citrate allowing in this way the induction of both *cit* promoters. To demonstrate that CitO specifically recognizes the intergenic region *in vivo*, we used a plasmid containing the O1 and O2 sites (pO1O2). Transformation with the empty vector resulted in normal transcriptional induction, while transformation with plasmid pO1O2 reduced the activity of the promoters. To define the role of CitO binding regions in the mechanism of transcription regulation, a set of DNA fragments covering different regions of the *cit* promoters were fused to a promoterless *lacZ* reporter gene. Altogether, the results presented indicate that the two CitO binding sites are very likely to be the fundamental *cis*-acting elements for the regulation of the *cit* promoters mediated by CitO *in vivo*.

MI-P79.

ACTIVITY OF MUTS OLIGOMERS ON *Pseudomonas aeruginosa* MISMATCH REPAIR

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MutS is an ABC ATPase that recognizes misspaired bases and activates downstream events in the postreplicative DNA Mismatch Repair (MMR). *P. aeruginosa* MutS is an 855 amino acid protein with different oligomeric species. At present, the identification of the *in vivo* functional oligomeric state of this protein is matter of extensive study. We have previously shown that native *P. aeruginosa* MutS forms only tetramers in solution and that the 57 C-terminal amino acids are indispensable for the formation of this oligomeric state. Furthermore, we found that R842 and K852 are key residues in MutS oligomerization since R842E mutation impairs tetramerization and K852A mutation alters the oligomerization equilibrium. In the present work, we investigate the *in vitro* activities and the *in vivo* performance of both, the dimeric MutSR842E and tetrameric MutSK852A. Both mutants showed ~2-fold reduction in ATPase activity and a lower affinity for heteroduplex DNA (Kd = 544nM and 598nM, respectively) compared with native MutS (Kd =376nM). Analysis of *P. aeruginosa* strains expressing these mutated versions of MutS showed that the tetramer is more efficient that the dimer to repair mismatches under highly mutagenic conditions induced by the base analogue 2-aminopurine. Our results indicate that the tetrameric species could be considered the active form of MutS in *P. aeruginosa* MMR.

MI-P80.

LIGHT SENSITIVITY OF TWITCHING MOTILITY IN *Acinetobacter baylyi* STRAIN ADP1

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Photoreceptors are traditionally associated with phototrophic organisms. Recently, however, photosensory proteins have also been found in chemotrophic bacteria, mostly with unknown function. A BLAST search revealed four putative Blue Light with FAD (BLUF)-domains in the sequenced genome of *Acinetobacter baylyi* (ADP1). This strain produces peritrichous thin pili implicated in adhesion, and polar thick pili correlated with twitching motility. Interestingly, both appendages are relevant for opportunistic infections. In order to determine whether twitching motility can be regulated by light in ADP1, 1 µl of an ON culture was placed in the center of soft agar plates, which were then sealed and incubated in the presence and absence of white light (intensity ~50µE). ADP1 showed a spreading area of 1,1± 0,41 cm2 in the light and 13,7±5,99 cm2 in the dark. This result leads to the conclusion that light inhibits twitching motility in ADP1. A *fimA* mutant of ADP1 (strain N100, kindly provided by Dr. B.Averhoff), lacking thin pili, showed an area of 0,6±0,27 cm2 in the light and 24,0±6,58 cm2 in the dark This experiment leads to the conclusion that the inhibition of the twitching motility does not depend on the thin (type I) pili. Furthermore, N100 showed an increment in the spreading zone with decreasing light intensity. It is clear that light suppresses twitching motility in *A. Baylyi.*

MI-P81.

PHOTO-SENSITIVITY OF DIVERSE *Micrococcus* SPECIES IS INDEPENDENT OF THEIR CAROTENOID CONTENT

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It is generally accepted that carotenoids protect bacteria from damage induced by sunlight. Nevertheless, except for photosynthetic organisms, this function has been demonstrated in a few bacterial species. Our objective was to evaluate the photo-protective effect exerted by carotenoids in a group of non-photosynthetic bacteria that are closely related but produce different carotenoids, in order to study an eventual influence of carotenoid structure on photo-protection. Several *Micrococcus* strains, exhibiting different colony colours, were isolated and identified. Their carotenoids were extracted and characterised by HPLC and absorption spectroscopy. Isolated bacteria were exposed to sunlight and survival curves were obtained. Unexpectedly, carotenoid depletion by diphenylamine treatment or mutations had negligible effect on sunlight response of these organisms. In contrast with results from early studies about carotenoid functions in *Micrococcus*, it seems that pigmentation is not involved in prevention of light-induced damage in the isolated organisms. Carotenoids should exert alternative functions in these organisms and bacterial strains isolated in this study could be a useful tool to explore these functions.

MI-P82.

INVOLVEMENT OF ALTERNATIVE FACTORS IN THE RESPONSE TO THE STRESS INDUCED BY UVA IN *S. typhimurium*

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Bacteria exposed to sub-lethal UVA radiation exhibit a transient growth delay (GD) as a consequence of the inactivation of certain tRNA. This GD is enlarged by the stringent response and several membrane alterations. A preventive action of a pre-exposure to very low fluences was also established. Since alternative factors play a key role for bacteria survival under stress condition, the involvement of RpoS and RpoE in the UVA effect and its prevention was investigated in *Salmonella enterica* serovar Typhimurium. The extent of GD was similar in the wild type strain and in an *rpoS* mutant, but it was increased in an *rpoE* mutant. Prevention by previous UVA exposure in *rpoS* mutant was less efficient than in the wild type strain, while *rpoE* had no effect on the photoprotection. The *relA spoT* mutant (unable to synthesise ppGpp) exhibited a short GD which was no modified by pre-irradiation treatment. The preventive action of pre-irradiation was impaired by the chloramphenicol addition. Results suggest that RpoE is required for the recovery probably at the level of the envelope or periplasmic damage. Prevention of GD requires RpoS functions and protein synthesis, and probably dependent on the stimulation of RpoS expression and/or function by ppGpp. This study suggests that both RpoE and RpoS participate in the UVA induced stress response and its prevention.

MI-P83.

CELLULAR Cu(II)-REDUCTION BY *E. coli* RESPIRATORY CHAIN IN COPPER EXPORTERS DEFICIENT STRAINS

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Copper is both an essential nutrient and a toxic element able to catalyze free radical formation. In *E. coli* there are two copper exporters: a P-type ATPase CopA and a multi-component transport system CusCFBA. All proteins of the copper homeostatisis use Cu(I), although the copper reductase activity is still unclear. Previous results of our laboratory have shown that electron flow through the *E. coli* respiratory chain promotes the reduction of cupric ions by NADH dehydrogenase-2 and quinones. Here we measured Cu(II)-reduction as the rate of Cu(I) appearance in supernatant of cellular suspensions exposed to sub-lethal Cu(II) concentrations using strains lacking several respiratory chain components and/or copper exporters. Without CopA or Cus system, the Cu(II)-reduction rate decreased around 50% respect to the wild-type strain. In the absence of NDH-2, this decrease was nearly 10%, but interestingly a double mutant lacking CopA and NDH-2 recovered the reduction level of the wild-type. In the absence of quinones, the Cu(II)-reduction rate decreased 60-80%. To understand the mentioned events, membranes NADH and D-Lactate:Cu(II)-oxidoreductase activities of different mutants were assayed. We proposed two copper reduction mechanisms in *E. coli*: one of them occurs in cytoplasm and is mediated by both NDH-2 and quinones and the other to the periplasmic side and is mediated only by quinones.

MI-P84.

STUDY OF THE REGULATION AND FUNCTION OF THE INNER MEMBRANE PROTEIN SBMA

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The SbmA protein transports MccB17, MccJ25, bleomycin and proline rich peptides into the *Escherichia coli*. Homologs of the *sbmA* gene are found in a variety of bacteria suggesting an important physiological role might exist for these genes, however it remain unknown. In this work we described the study of the *sbmA* regulation in *Escherichia coli* and *Salmonella typhimurium*. Using the chromosomal transcriptional *lacZ* fusion in the *sbmA* gene of these two gram-negative bacteria, we demonstrated that *sbmA* expression is regulated by the PhoP/PhoQ two-component system. Previously, we demonstrated that the double mutant *sbmA tolC* shows a Tc hypersensitivity phenotype, so we studied the *sbmA::lacZ* expression in a *tolC* mutant. We observed high level of -galactosidase activity in this genetic background, indicating a positive regulation by *tolC* mutation. We tested the expression of *sbmA::lacZ* in *E. coli tolC* mutant growing in minimal medium with low Mg^{2+} concentration to determine a hypothetical additive effect of two regulation mechanisms. Interestingly, we observed that there is a relationship between *tolC* mutant effect and the PhoP/PhoQ-dependent activation. Moreover we observed a deficient growth phenotype of *sbmA* mutant in medium containing low magnesium. The future understanding of *sbmA* regulation mechanism would let to hypothesize a physiological function for this membrane protein.

MI-P85.

A ROLE FOR THE TYPE II SECRETION SYSTEM IN BIOFILM FORMATION IN *Xanthomonas axonopodis pv* CITRI, XAC

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Biofilms are multicellular aggregates of bacteria, enclosed in a self-produced polymeric matrix attached to an abiotic or biotic surface. Within the biofilm, bacteria are protected from different harmful factors. Biofilm formation involves the attachment of free-leaving bacteria facilitating the attachment of others. The biofilm grows through cell division and recruitment of new cells. In the final stages the biofilm changes and water channels appear. The shape becomes mushroom like. Xac, the etiological agent of canker disease has two operons *xcs* and *xps* encoding for different type II secretion systems (SSTII). Mutations in different genes of these operons have shown to confer phenotypic alterations in plant symptoms and to affect the initial steps of biofilm formation. The analysis of the biofilm by confocal microscopy has also shown that *xcs* and *xps* mutants are impaired in the development of mature structures. The evidences of the involvement of SSTII in the ability of bacteria to adhere to a surface and biofilm formation and the high identity of these systems with the type IV pilus indicate that Xcs and Xps systems could be directly involved in the adhesion process. However, the analysis of secreted enzymes showed that the *xps* operon is responsible for degrading different kinds of substrates, such as starch and proteins, while the *xcs* operon is not.

MI-P86.

ILE[13] OF THE MICROCIN J25 MOLECULE IS INVOLVED IN INTERACTION WITH THE RECEPTOR FHUA

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E. coli RNA polymerase (RNAP) is the target of microcin J25 (MccJ25). MccJ25 enters cells after binding to specific membrane transporters: FhuA in the outer membrane and SbmA in the inner membrane. We studied a MccJ25 mutant carrying a substitution of Ile13 by Lys (I13K). The results showed that the I13K mutant inhibited RNAP *in vitro* . However, the mutant was defective in its ability to inhibit cellular growth. A possible explanation could be that the mutant is unable to reach the cytoplasm of the sensitive cells or that it has a higher affinity by YojI, a MccJ25 export pump. Following this hypothesis we tested the activity of the mutant against strains with a chromosomal deletion of *yojI* and hyperexpressing FhuA and SbmA. The mutant was almost as active as wild-type microcin only against an *E. coli* strain carrying a plasmid with *fhuA* cloned, suggesting that the lower activity of the mutant is due to a deficient transport into the cell. Moreover, in experiments in which the FhuA protein was bypassed, the mutant showed an activity similar to wild-type MccJ25. In addition, in a competition assay with colicin M, MccJ25 was able to reduce colicin uptake by FhuA, while the I13K mutant did not. Together, these results suggest that the Ile13 residue in the MccJ25 molecule is needed for uptake of the antibiotic by FhuA, but it is not required for MccJ25 inhibition of RNAP.

MI-P87.

IDENTIFICATION OF NEW *Lactobacilli* SPECIES ABLE TO PRODUCE COBALAMIN (VITAMIN B12)

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Cobalamin (CBL), a very complex vitamin made by some bacteria and archea, is essential to humans and animals. Recently we have discovered that a strain of *Lactobacillus reuteri* is able to produce this vitamin. Genomic analysis of some *Lb. reuteri* strains suggested that the *cob* genes involved in CBL biosynthesis were acquired by horizontal transfer. The aim of this work was to evaluate the distribution of *cob* genes and the ability to produce CBL in 16 *Lactobacillus* strains used in the development of a bio-fortified functional food. A biological assay showed that *Lb. coryniformis* CRL 1001, *Lb. curvatus* CRL 1000, *Lb. murinus* CRL1104, and *Lb. reuteri* CRL 1101, 1324 and 1327 complemented the CBL requirements of the auxotrophic strain *Escherichia coli* 113. Higher levels of CBL biosynthesis were observed in the presence of the CBL precursors, dimethylbenzimidazole (DMB) and uroporphyrinogen III. It was shown that some strains harbored the *cbiF* and *hemC* genes which show homology with the enzymes Precorrin-4 C11-methyltransferase and Porphobilinogen deaminase respectively, which are involved in the CBL biosynthetic pathway. Moreover the key gene c*obT* was amplified by PCR and cloned into the pBluescript vector to perform regulatory studies. These results allow us to conclude that the CBL production is a strain dependent property supporting the horizontal transference hypothesis.

MI-P88

PEPTIDIL-PROLYL ISOMERASES IN TRYPANOSOMATIDS

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The peptidyl-prolyl cis/trans isomerases (PPIases) catalyze the cis/trans isomerization of the peptide bonds preceding proline residues. PPIases include three subfamilies: the cyclophilins, FKBPs and the parvulins. Studies in multiple systems have suggested that parvulins play a critical role by controlling cell-cycle progression. In *T. cruzi* we have previously identified two parvulins that closely resembles that of the human proteins hPin1 and hPar14 respectively. Here, we describe a novel member of the parvulin family of PPIases named TcFHAPIN. This parvulin contains an N-terminal forkhead-associated motif and a C-terminal parvulin-like catalytic domain. A comparison of the relative specificity constants for various substrates shows a strong preference for a substrate with the basic arginine residue preceding proline. To our knowledge, TcFHAPIN is the first example of a FHA domain present in the same polypeptide chain with a PPIase catalytic domain. The presence of an FHA domain strongly indicates that the FHA-containing protein will interact with a protein partner in a process regulated by reversible protein phosphorylation. Given the significance of Pro isomerization and phosphorylation in diverse cellular functions, the characterization of a novel parvulins with nuclear localization will shed light on the cellular importance of these processes in trypanosomatids.

NS-P01.

PARTITION OF NICOTINIC ACETYLCHOLINE RECEPTOR IN MODEL MEMBRANES

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Nicotinic acetylcholine receptor (AChR) affinity-purified from *T. californica* membranes and a synthetic peptide corresponding to the M4 transmembrane region (ãM4) of the receptor were reconstituted into synthetic liposomes with lipid compositions resembling that of raft domains (PC:SM:Chol, 1:1:1). The preferential localization of AChR or ãM4 in such synthetic membranes was analyzed using detergent-resistant (DRM) or detergent-soluble domains (DSM) obtained by treatment with 1% Triton X-100 at 4°C followed by SDS-PAGE and Western blotting. The influence of the ganglioside GM1 on AChR was also analyzed with this techniques. The efficiency of the Förster resonance energy transfer (E) between the protein intrinsic fluorescence and dehydroergosterol (fluorescent cholesterol mimetic) served as a measure of the protein location in the membrane. Purified AChR displayed no preferential partition between raft and non-raft domains whereas the ãM4 peptide preferentially partitioned into ordered domains. The presence of GM1 increased the proportion of AChR in the DRM fractions, and antibody-mediated crosslinking of the ganglioside induced a significant redistribution of the AChR towards the DSM fraction. Thus, the preferential localization of the AChR protein in the membrane may not only be governed by its lipid-protein interface.

NS-P02.

INTRACELLULAR GSH MEDIATES THE DENITROSYLATION OF PROTEIN NITROSOTHIOLS IN THE RAT SPINAL CORD

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Protein S-nitrosothiols (PrSNOs) have been implicated in the pathophysiology of neuroinflammatory disorders characterized by extensive nitrosative stress. Using rat spinal cord slices we have previously established that S-nitrosoglutathione (GSNO) is a viable intercellular S-nitrosylating agent. Moreover, generation of PrSNOs with GSNO occurs exclusively via a S-transnitrosylation mechanism. Although the metabolical instability of PrSNOs is well known, there is little understanding of the factors involved in the cleavage of S-NO linkages in intact cells. To address this issue, we conducted chase experiments in spinal cord slices incubated with GSNO. The results show that removal of GSNO leads to a rapid decreasing of PrSNOs ($t_{1/2}$ ~ 2h), which is greatly accelerated when glutathione (GSH) levels are raised with the permeable analogue GSH ethyl ester, suggesting that GSH plays a key role in the denitrosylation process. Inhibition of both GSH-dependent enzymes and enzymes that could mediate denitrosylation do not alter the rate of PrSNO decomposition. The lack of protein glutathionylation during the chase suggest that most proteins are denitrosylated via rapid transnitrosylation with GSH. The differences in the denitrosylation rate of individual proteins would indicate the existence of additional structural factors in this process.

Supported by NIH grant NS 47448.

NS-P03.

MODULATION OF ACH RELEASE AT THE EFFERENT-IHC SYNAPSE BY THE GABAERGIC SYSTEM

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Before the onset of hearing, inner hair cells (IHC) of the mammalian cochlea are transiently innervated by medial olivocochlear (MOC) efferent fibers. This synapse is cholinergic, inhibitory and mediated by the á9á10 nicotinic receptor. Although ACh is the main transmitter released at this synapse, there is evidence showing that the GABA is present at MOC synaptic terminals and that GABAA receptors are expressed in outer hair cells (OHC). However, no GABAergic currents have been recorded so far in IHC or OHC. Moreover, the possibility that synaptically released GABA could be modulating the cholinergic input at MOC-synapses by acting on presynaptic GABAB receptors has not been investigated yet. In the present work, we therefore evaluated the effects of a GABAB antagonist (CGP55845) on the quantal content of transmitter release at the MOC-IHC synapse. Postsynaptic cholinergic currents, evoked by electrically stimulating the efferent fibers, were recorded in voltage-clamped (-90 mV) IHCs from acutely isolated mouse organs of Corti. The quantal content of evoked release was significantly increased by 0.3 ì M CGP55845 (60 ± 25 %). The lack of effect of CGP55845 on spontaneous synaptic current amplitude indicates that the site of action of this drug is presynaptic. Our results suggest that GABA might be exerting a negative feedback control on the release of ACh at this synapse.

NS-P04.

FIRST SNAIL EGG PROTEIN WITH A NEUROTOXIC EFFECT ON MICE

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While many invertebrates sequester toxic compounds to endow eggs with chemical defences, here we show, for the first time to our knowledge, the identification of a neurotoxin of proteinaceous nature localized inside an egg.

Egg extracts from the freshwater apple snail *Pomacea canaliculata* displayed a neurotoxic effect in mice upon intraperitoneal injection (i.p.) (LD50, 96 h 2.3 mg/kg). Egg protein and total lipids were analysed separately and the only fraction displaying a highly toxic effect (LD50, 96 h 0.25 mg/kg, i.p.) was further purified to homogeneity as an oligomeric glyco-lipoprotein of 400 kDa indistinguishable from the previously described perivitellin PV2. The neurotoxin was heat sensitive and there was evidence of circulating antibody response to sublethal i.p. doses on mice. Clinical signs, histopathological and immunocytochemical studies revealed damage mostly in mice spinal cord. Experiments showed chromatolysis and a decreased response to calbindin D-28K associated with a significant increase of TUNEL-positive cells (terminal deoxynucleotidyl transferase (TdT)–UTP–biotin nick end labelling) in the dorsal horn neurons.

These results suggest that calcium buffering and apoptosis may play a role in the neurological disorders induced by the toxin in mammalian central nervous system.

NS-P05.

COOPER, CERULOPLASMINE, AND OXIDATIVE STRESS BIOMARKERS LEVELS IN HUMAN NEURODEGENERATIVE DISORDERS

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Cu imbalance and oxidative stress (OS) are documented in brain tissue of Alzheimer patients (AD). However, their utility as prognostic parameters in peripheral blood is still poorly understood. We studied the levels of TBARS, nitrate + nitrite [NOx], protein carbonyls (PCs), total glutathione (GSH), tocopherol (Toc), antioxidant enzymes, Cu, Se, Zn, and ceruloplasmine (CRP) in plasma and erythrocytes from AD, Parkinson (PD) and vascular dementia patients (VD). Results were compared with first order relatives (R) and a control population. All OS markers were altered in AD, PD and VD compared with the corresponding control group. Superoxide dismutase, GSH-reductase, and catalase activities in erythrocytes, as well as TBARS, PCs, and [NOx] in plasma were increased; while GSH and Toc in AD, PD and VD were decreased. Cu level was increased in severe AD and PD patients, and in all stages of VD. R of AD and VD also exhibited increased [Cu] not associated to substantial and clear alterations in OS biomarkers. CRP was increased only in VD samples. We conclude that peripheral OS biomarkers should be poor discriminating indexes for the screening of the type and severity of neurodegenerative disorders; however, the CRP/Cu ratio may be useful in estimating the progression from a sub-clinical condition to a symptomatological illness, especially in R of VD.

NS-P06.

GSK3â AND ERK ARE ACTIVATED DOWNSTREAM OF PI3K IN SYNAPTIC ENDINGS DURING OXIDATIVE INJURY

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Iron induced oxidative injury is comparable to that of ß-amyloid peptide on the brain of Alzheimer's disease patients. Our purpose was to evaluate the state of PI3K pathway and its downstream effectors Akt and GSK3ß in cerebral cortex synaptosomes exposed to Fe^{2+} (50 µM) for different periods of time (5, 30 and 60 min). The increase in Akt phosphorylation in serine 473 and threonine 308 was temporally coincident with PI3K activation (5 min). GSK3ß, the downstream effector of Akt, was also phosphorylated after 5 and 30 min of iron exposure and this phosphorylation was inhibited by LY294002. Additionally, Erk activation was also observed after 5 and 30 min of insult exposure, and this activation was PI3K-dependent. Immunoprecipitations carried out with anti-cSrc demonstrated a strong association between activated Akt and this tyrosine kinase induced by oxidative stress. Our results demonstrate that oxidative stress triggers the activation of different synaptic signaling pathways that operate downstream PI3K/Akt.

PL-P01.

IS THE CELL WALL REINFORCEMENT A POTENTIAL TOOL FOR THE EARLY SCREENING OF RESISTANCE IN BREEDING PROGRAM?

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Horizontal resistance is a valuable tool for potato Late blight control, caused by the oomycete *Phytophthora infestans*. In Argentina several sources of horizontal resistance were identified. In particular, the wild specie *Solanum tarijense* (trj) has shown high levels of resistance. Previous results with trj clones moderately resistance to Late Blight, showed high levels of PR activities, such as, ß-1,3-glucanases, chitinases, peroxidases (POX) and polyphenol oxidases (PPO). The use of these chemical markers as a potential tool to screen for horizontal resistance was studied. The defense response in trj clones through the induction of lignin, callose, phenolic compounds, as well as the activities involved in the cell wall strengthening, POX and PPO, were analyzed.
Leaf tissue from resistant clones responded to *P. infestans* infection though the deposition of lignin, callose and phenolic compounds. All these changes were preceded by the activation of the enzymes involved in the synthesis and polymerization of these compounds. However, for the susceptible clones slight increases were detected. These results suggest that the analysis of the compounds related with the cell wall strengthening could be a potential tool for early screening of horizontal resistance in breeding program.
Supported by SECyT, INTA and Banco Santander Rio.

PL-P02.

EFFECTS OF PHOSPHITE TREATMENTS ON CELL WALL COMPONENTS OF POTATO TUBERS

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Phosphites (Phi) are alkali metal salts of phosphorous acid, with the ability to protect plants against different pathogens. Molecules involved in the protection given by phosphites are still unknown. Preliminary studies of our laboratory showed that the periderm thickness of potato tubers increased in tubers from treated plants (Phi). The aim of this work was to analyze modifications in cell wall proteins and pectins in tubers from treated plants with potassium phosphite (KPhi). The tubers from cvs. Shepody and Bannock Russet showed an increase in the resistance to *F. Solani*, *P. infestans* and *E. carotovora*. Differences in SDS-PAGE pattern of cell wall proteins were observed between tubers from treated plants respect to controls. On the other hand, pectin extracts, obtained by different methodologies, showed that they are modified by KPhi treatments and also after wounding or *F. solani* infection. The type of pectin and the accumulation were analyzed by immunoblot using JIM5 and JIM7 antibodies. The differences produced in proteins and pectins by KPhi, also showed variations depending on potato cultivar. Morpho-histological analysis will contribute to know if these changes in the cell wall architecture are part of the defense responses induced by KPhi.
Supported by UNMDP, CONICET, ANPCyT

PL-P03.

DOES PHOSPHITE TRIGGER THE DEFENSE RESPONSE IN *Fusarium* INFECTED TUBERS?

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Plants react against pathogens developing an array of responses that helps them to counteract this attack.
Previous results in our group showed that potassium phosphite (Kphi) applied to foliage protects tubers against *Fusarium solani*. In order to correlate the protection extent with the fungal invasion, the activities of two fungal enzymes were measured: FESP (Fusarium extracellular serine protease) and polygalacturonases (PG). Potato tubers (cv Bannock Russet) from treated plants and infected with *F. solani* showed reduced FESP and PG activities. These results were correlated with a low degree of infection. The activities of two enzymes related with the defense, peroxidases and polyphenoloxidases were quantified. Both activities decreased in Kphi treated plants compared to control ones.
By using antibodies against different PRPs, the accumulation of these proteins in potato tubers from treated plants and infected was analyzed. The chitinase accumulation increased while glucanase content clearly decreased.
Proteinase inhibitor and polygalacturonase inhibiting protein contents were increased by KPhi treatment both in infected and non infected tubers.
These results suggest that all these proteins are involved in the mechanism by which KPhi protects tubers against *F. solani* during the infection process.
Supported by UNMdP, CONICET, ANPCyT, McCain Argentina SA. and AgroEmcodi SA

PL-P04.

INNATE IMMUNE GENES ARE DIFFERENTIALY EXPRESSED IN TMV RESISTANT CP-MP TRANSGENIC PLANTS

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Viral diseases produced considerable economic losses worldwide. It is necessary to find new genetic strategies to obtain resistant crops. Transgenic co-expression of TMV movement protein (MP) and a mutated capside protein (CPm) in *Nicotiana tabacum* confers resistance to viral infections along with developmental defects, some of them similar to the symptoms produced by the infection. These plants also have increased levels of a set of microRNAs that produce similar phenotypes when overexpressed.
To determine differences in gene expression between transgenic and control plants, we used a Tomato Affymetrix microarray chip. After statistical data analysis we selected 26 genes, 8 upregulated and 18 downregulated in transgenic plants. Bioinformatics analysis showed that some of these genes seem to be regulatory members of innate immune response; some are inhibitors of SAR (downregulated in transgenic plants) and other enhancers (upregulated). Another upregulated group of genes belonged to the RNA processing machinery, and there were also putative transcriptional factors and carbon metabolism related genes. These candidates are being validated by qRT-PCR.
The complexity of immune response and its relation to development and growth are tightly regulated in plants. MicroRNAs could be involved in this regulation and consequently play a role in viral resistance.

PL-P05.
OXIDATIVE STRESS AND IMMUNOLOCALIZATION OF HEME OXYGENASE IN *Soybean* PLANTS
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We have previously demonstrated that the induction of heme oxygenase-1 (HO-1) (EC 1.14.99.3) plays a protective role for plant cells against oxidative stress. Here, we investigated the possible signal transduction pathways involved in HO-1 induction in soybean leaves and we determined for the first time the tissular localization of HO-1 in soybean nodules subjected to salt stress.
Treatment with 100 mM NaCl in leaves increased 30% TBARS content, whereas GSH decreased 50%, respect to controls. These effects were prevented by preincubation with NADPH oxidase inhibitor, guanylate cyclase inhibitor or $LaCl_3$ (calcium channel blocker).
Treatment with 100 mM NaCl were used for immunohistochemical studies which did not allow properly establish the enzyme localization in soybean nodules, but electron-microscope examination of immunogold-labeled sections of nodules showed that the inducible isoform of HO was only present on the peribacteroid membrane.
These results clearly demonstrated that the signal transduction pathways involved in oxidative stress, triggered by salt were similar to those implicated in HO-1 induction, and provide additional information suggesting that HO might play a key role in the antioxidative protection machinery of higher plants. Besides, these results undoubtedly demonstrated that this enzyme is localized in the plant tissue and no in the bacterial symbiont.

PL-P06.
DEVELOPMENTAL FEATURES AND STRESS-INDUCED RESPONSES IN KNOCKDOWN L-PROLINE DEHYDROGENASE PLANTS
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We have recently found that tissues triggering the Hypersensitive Response (HR) upon recognition of avirulent pathogen display early transcriptional of proline (Pro) degradation genes. Activation of ProDH (Pro dehydrogenase) and reduction of P5CDH (P5C dehydrogenase) are detected at the center, but not the periphery, of the HR lesion. Such responses are induced in wild type plants treated with exogenous salicylic acid (SA). Consistently, *sid2* and *npr1* mutant plants defective in the SA pathway, do not activate these changes in response to avirulent pathogen. Interestingly, ProDH knockdown plants (siRNAProDH) show reduced cell death during incompatible interactions suggesting the involvement of ProDH in HR generation. Putative contributions of this enzyme to HR factors are analyzed. We also evaluate developmental features of the siRNAProDH lines.

PL-P07.
TRANSCRIPTIONAL REGULATION OF PROLINE-CATABOLISM GENES UNDER STRESS CONDITIONS
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Proline (Pro) catabolism involves the consecutive activity of Pro dehydrogenase (ProDH) and P5C dehydrogenase (P5CDH), which generate Ä¹-pyrroline-5-carboxilate (P5C) and glutamate, respectively. Both enzymes have mitochondrial localization. ProDH gene expression is induced by exogenous Pro and salicylic acid (SA), as well as by the attack of avirulent pathogen. Some of these conditions also alter *P5CDH* gene expression. In addition, *P5CDH* transcript levels are regulated by *SRO5-P5CDH* natural siRNA, induced by oxidative stress and salinity. Uncoupled ProDH and P5CDH activities may generate accumulation of P5C, a proposed inducer of apoptosis in animals. P5C is a highly unstable metabolite which quantification in plant tissues has severe limitations. We evaluated if in tissues triggering cell death, *ProDH*, *P5CDH*, and *SRO5* transcript levels are compatible with P5C accumulation. Also we analyze transcriptional cross-regulation between *ProDH* and *P5CDH* by evaluating stress-induced gene expression changes in ProDH knockdown plants and in *p5cdh* T-DNA mutant lines.

PL-P08.
THE INVOLVEMENT OF HYDROGEN PEROXIDE IN POLYAMINE- INDUCED CELL DEATH IN *Nicotiana tabacum*
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Polyamines (PAs) are ubiquitous in living organisms and have been implicated in a wide range of processes, including plant growth and development. It is suggested that they are involved in cell proliferation and cell death. The aim of this work was to evaluate the effect of exogenously added PAs in cell death events in tobacco. Leaf discs from 70-day-old wild type SR1 tobacco plants were treated for 2, 16 and 24 h with 0.1mM or 1mM Put, Spm or Spd. All PAs concentrations tested increased superoxide as well as H_2O_2, in a dose dependent manner, evidenced by using nitrobluetetrazolium and diaminobencidine staining, respectively. Additionally, Spm was the most deleterious PA in relation to membrane damage, increasing Evans blue staining 10 times and electrolyte leakage 85% over the controls at 24 h of exposure. Previous addition of catalase (1500 U/ml) significantly reversed (50%) the increased in solute leakage in Spm-treated leaves, suggesting that H_2O_2 derived from Spm catabolism was the molecule involved in cell death. Chlorophyll content was not altered by any of the treatments whereas TBARS content, measured as an index of lipid peroxidation, was increased from 16h with 1 mM Put or Spm. These preliminary results suggest that H_2O_2 could be the main oxidant molecule involved in PAs toxicity, and this damaging effect could be partially prevented by catalase in the case of Spm.

PL-P09.

OVERPRODUCTION OF PUTRESCINE CHANGES THE RESPONSE TO AN OXIDATIVE CHALLENGE IN *Arabidopsis thaliana*

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Polyamines (PAs) have been involved in a wide range of physiological processes in plants, such as stress responses and are considered as potential free radical scavengers and antioxidants. *Arabidopsis thaliana* plants (AT) that accumulate high levels of Put (line 9.12) were challenged with paraquat (PQ), to study if the susceptibility to oxidative stress was altered in transgenic plants. Analysis were performed in leaves from 30d-old wt or 9.12 plants, incubated in a rotatory shaker for 2 or 21 h with 10 or 100 µM PQ. We evaluated superoxide and H_2O_2 formation using NBT and DAB staining, lipid peroxidation, chlorophyll and electrolyte leakage. The 9.12 line showed an enhanced Put content (58 %) measured in 30-d old entire plants. Formation of superoxide was decreased, while the content of H_2O_2 was increased at 21 hours, in wt or 9.12 lines treated with 10 or 100 µM PQ. The lower PQ concentration increased 6 times the leakage of solutes at 21 h, without detectable differences between wt or 9.12 lines, doubled TBARS levels and decreased chlorophyll to half of the controls, being these parameters slightly more affected in the transgenic cultivar. PQ at 100 µM severely altered the studied variables. These preliminary results suggest that leaves of transgenic AT plants with high Put content could present slightly higher susceptibility to the oxidative damage induced by PQ.

PL-P10.

***Arabidopsis* TIR1/AFB AUXIN RECEPTOR FAMILY IS INVOLVED IN THE ADAPTATIVE RESPONSE TO ABIOTIC STRESS**

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Recently, auxin regulation has been involved in plant-pathogen interactions. However, the role of auxin signaling pathway during abiotic stress remains unclear. The aim of this work was to evaluate auxin receptors (TIR1, AFB1, AFB2 and AFB3) participation in the adaptative response of *Arabidopsis thaliana* plants against abiotic stress conditions. Double mutant plants *tir1/afb1*, *tir1/afb2* and *tir1/afb3* were analysed under oxidative stress caused by methyl-viologen (MV), hydrogen peroxide (H_2O_2) and salt stress. Thus, *tir1/afb2* and *tir1/afb3* double mutant seedlings growing in 10 nM MV presented 20 % less inhibition of root growth compared to WT plants, while *tir1/afb1* showed similar response than WT plants. Quantification of cell death symptoms indicated that *tir1/afb2* and *tir1/afb3* mutant seedlings were also more resistant against oxidative treatments mediated by 20 mM H_2O_2. Superoxide ion (O_2^-) production in leaves, after 50 uM MV treatment, showed a reduce O_2^- production in *tir1/afb2* mutant compared to WT. Moreover, *tir1/afb2* double mutants exhibited reduce sensitivity against salt treatments during germination and root growth. These results allowed us to propose that *A. thaliana* plants repress auxin signaling as part of the response to abiotic stress as an adaptative strategy to modulate growth and stress tolerance.

Partially supported by CONICET; UNMDP; ANPCyT.

PL-P11.

EVIDENCES OF POSTTRANSCRIPTIONAL CONTROL DURING THE DEFENSE RESPONSE OF POTATO TUBERS

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One important disease of the potato (*Solanum tuberosum*) tubers is the dry rot caused by Fusarium species. In our lab, around 500 cDNAs were identified after the differential screening of a potato library from *F. eumartii* infected tubers. Here we report the expression analysis of 358 cDNAs from the collection using DNA macroarray technology. Changes in gene expression of potato tubers wounded or wounded and inoculated with *F. Eumartii* at 0 and 24 hs post-treatments were monitored. About 85% of the cDNAs were induced at least two-fold after the treatments. Approximately150 of these cDNAs were only induced by *F. eumartii* at 24 h post inoculation. Infected tubers specifically accumulated transcripts encoding amino acid metabolism enzymes which are involved in nitrogen recycling and mobilization. The mobilization of nitrogen might be a strategy used by the fungus to ensure successful colonization. In addition, *F. eumartii* induced differential abundance of transcripts for several proteins involved in RNA processing and export. These genes have not been related to the potato defense response and belong to different families of RNA-binding proteins. Three of these cDNAs showed conserved RNA helicase domains. These results contribute to the identification of new candidate genes that might regulate at the posttranscriptional level the potato tuber responses against *F. eumartii*.

Supported by UNMdP, ANPCyT and CONICET

PL-P12.

NITRATE REDUCTASE ACTIVITY AND RESPONSE TO FIXATION NITROGEN IN DIFFERENT PEANUT GENOTYPES

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Nitrogen (N) is the major limiting nutrient for the most plant species. Plants require N from soil or from atmosphere, by symbiotic N_2 fixation. Soil-derived N, generally in the form of nitrate, is reduced to ammonia by two-step process. The first step, reduction of nitrate to nitrite, is catalyzed by the plant nitrate reductase (NR), which is an inducible enzyme, depending on the availability of nitrate. The objectives of this work were to evaluate NR activity and the nitrogen fixation in peanut genotypes fertilized with KNO_3 or inoculated with *Bradyrhizobium* sp. SEMIA 6144 (microsymbiont peanut). The assay was conducted in greenhouse and the physiological and symbiotic parameters (shoot and nodule dry weights, number of nodules and nitrogen content), the activity of NR and nitrate content in roots, leaves and nodules at 40 days after planting were evaluated. TEGUA genotype showed higher values of N_2 fixed (44.64 mg/plant) and symbiotic effectiveness compared with other genotypes. In the contribution of N to plant, the TEGUA and ASEM-485 genotypes presented a better response to inoculation and the UTRE genotype presented a better response to nitrogen fertilization. The NR activity and nitrate content showed variations in the analyzed organs according to the genotype and nitrogen source used.

PL-P13.

INDUCED RESISTANCE IN THE BIOCONTROL OF *Sclerotium rolfsii* BY A PGPR STRAIN ON PEANUT

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Plant growth promoting rhizobacteria (PGPR) are colonizing bacteria with beneficial effects including plant growth promotion and disease control such as the induction of systemic resistance (ISR). In the present study, the enzymatic activities of phenylalanine ammonia-lyase (PAL), peroxidase (PO) and ß 1-3 glucanase were evaluated in roots and leaves from peanut plants inoculated with the PGPR bacteria SEHEP2 and with the fungal pathogen *Sclerotium rolfsii*. SEHEP2 is an epiphytic bacteria isolated from peanut leaves. In order to test its ability to induce ISR, peanut plants growing in Hoagland solid medium were inoculated with SEHEP2 strain in their leaves and the fungal pathogenic *S. rolfsii* in the culture medium. Twenty two days after planting, the enzyme activities were analyzed in four treatments: control; SEHEP2; *S. rolfsii*; SEHEP2 and *S. rolfsii*. The highest specific activity of PAL and ß 1-3 glucanase were founded in the leaves of plants treated with both SEHEP2 and *S. rolfsii*. In contrast, the enzyme PO showed a significant increase in plants coinoculated with SEHEP2 and *S. rolfsii* in relation to those inoculated only with *S. rolfsii*. We speculate that plant defense enzymes are stimulated in peanut plants by this PGPR bacterium with ISR property.

PL-P14.

HAWRKY6, A TRANSCRIPTION FACTOR FROM *Helianthus annuus*, IS POTENTIALLY INVOLVED IN DEFENSE PATHWAYS

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WRKY transcription factors constitute a family of more than 70 members in plants, divided in several subfamilies. A few members were characterized as participants of biotic and abiotic stresses responses. Here we describe a partial functional characterization of HAWRKY6, a member of group IIa of the WRKY encoding genes family. A genomic clone was isolated from a BAC library encoding a complete cDNA plus 900 bp upstream of the transcription initiation site. The coding region exhibiting a WRKY domain as well as a leucine zipper was subcloned under the control of the 35S CaMV promoter in order to transform Arabidopsis plants. The promoter region presenting 3 putative W-box binding sites within region comprised between -240 and -70; two sites involved in the response to jasmonate and ethylene and a c-AMP response element, was cloned directing the GUS reporter gene expression in order to transform plants and establish the expression pattern. In parallel, expression studies performed with qRT-PCR indicated that this gene is strongly induced by SA and ACC as well as by the infection caused by *Pseudomonas syringae* and repressed by MeJA. Moreover, ACC treatment reverted the MeJA repression effect. We postulate that HAWRKY6 may participate somehow in the SA/JA/ET pathways in response to pathogen infections. Experimental work is in progress in order to corroborate this hypothesis.

PL-P15.

EFFECTS OF CADMIUM ON CATALASE ACTIVITY AND EXPRESSION IN *Sunflower* SEEDLINGS

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Catalase (CAT) dismutates the reactive oxygen species H_2O_2 into water and dioxygen and in plants; it is located in peroxisomes and glioxysomes. In the present study, we investigated the effect of cadmium at 10 µM and 100 µM (a well-known oxidative stress inducer) on catalase in roots and cotyledons of developing sunflower seedlings. Although germination was unaltered after 48h of exposure to 100 µM Cd^{2+}, root length was significantly reduced. CAT activity was also significantly reduced, but this activity was completely restored (10 µM treatment) or even enhanced (100 µM treatment) 24h later. Although CAT protein abundance remained similar to control in roots and cotyledons of Cd-treated seedlings, cadmium produced CAT protein oxidation, indicating that the mechanism of CAT inactivation by Cd^{2+} involves oxidation of the protein structure. The transcripts of the four genes described for sunflower (*CATA1* to *CATA4*) increased after cadmium treatment; *CATA1* and *CATA2* were the most over expressed in cotyledon and root, respectively. The differential expression of catalase genes in sunflower seedlings under Cd stress might be related to the synthesis of CAT isoforms less sensitive to oxidation, which would prevent enzyme inactivation and H_2O_2 accumulation.

PL-P16.

A PROPOSED LINK BETWEEN NR-DEPENDENT NO PRODUCTION AND RESPONSES TO IRON DEPRIVATION IN *Arabidopsis*

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Iron is an esential element for plants. In *Arabidopsis thaliana,* iron uptake depends on the reduction of Fe^{3+} to Fe^{2+} and the internalization of Fe^{2+}. These actions are carried out by a Fe^{3+} reductase, (FRO), and an iron transporter (IRT), respectively. Expression of IRT1 and FRO2 are regulated by the transcription factor FIT1. When iron is not available for growth, reactive oxygen species (ROS) and nitric oxide (NO) levels increase, as well as FRO, IRT and FIT expression. To evaluate the role that NO might have in the activation of plant responses to iron deficiency in *A. thaliana*, plants grown in iron deficient conditions were treated with the NO scavenger methylene blue (MB) and the nitrate reductase (NR) inhibitor tungstate (Tg). NR is a NO producing enzyme in plants. Seedlings growing with low iron supply showed symptoms of chlorosis. Roots display an increase in root hair and lateral root density, higher NO and H_2O_2 levels and an increase in FIT1 and FRO2 mRNA levels, as well as higher iron reductase activity. MB treatment provoked a reduction in NO and H_2O_2 levels, FIT1 and FRO2 mRNA levels and in the reductase activity. Tg also decreased NO levels and iron reductase activity. Results obtained with MB and Tg suggest the levels of transcripts responsive to iron deficiency, H_2O_2 and iron reductase activity would be induced by an NR-dependent NO production.

PL-P17.

NO AND ROS REGULATION OF FIT1 AND FRO2 EXPRESSION UNDER IRON DEFICIENCY IN *A. thaliana* ROOTS

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Iron deficiency alters plant molecular physiology.Under these conditions, *Arabidopsis thaliana* have developed a strategy that implies rizosphere acidification,reduction of Fe^{3+} by the Fe^{3+}-reductase FRO2 and the uptake of Fe^{2+}. The bHLH transcription factor FIT1, whose expression is regulated by nitric oxide (NO) under iron deficiency in tomato,regulates FRO2 mRNA levels. Preliminary evidence showed that iron deficiency increases NO and ROS levels. Crosstalk between ROS and NO has been extensively studied in many patho-phisiological processes. We decided to study NO and ROS effects during iron deficiency in *A. thaliana* roots. Treatments with the H_2O_2 scavenger, and catalase produced an increase in NO and a decrease in FRO2 and FIT1 transcript levels under iron deficiency.The NADPHoxidase (NOX) is a superoxide producing enzyme. In order to elucidate the enzymatic source of ROS under iron deficiency we inhibited NOX with apocynin.This treatment did not show any differences in mRNA levels of FRO2 and FIT1. However, it was observed an increase in NO levels under these conditions. Results indicated that scavenging of H_2O_2 and inhibition of NOX did not prevent the accumulation of NO produced under iron deficiency. However, the presence of H_2O_2 would be necessary for the full FRO2 and FIT1 induction under iron deficiency suggesting it acts in concert or downstream of NO in plant response to iron deprivation.

PL-P18.

EXPRESSION OF GENES ASSOCIATED TO BIOTIC AND ABIOTIC STRESSES IN PEANUT AND MAIZE

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The aims of the present study were to analyse the expression of pathogenesis-related protein in peanut under biotic and abiotic stresses. On the other hand, we analysed the expression of the Na^+/H^+ antiporter ZmNHX in maize under abiotic stress conditions. The analysis of PR-10 gene expression was performed by Quantitative Real- Time PCR using total RNA obtained from leaves and roots of 15 days old peanut plants grown under salt stress conditions or infected with *F. solani* or *S. rolfsii*. In maize, the analysis of *ZmNHX* gene expression was carried out from plants growing in presence of 150 mM NaCl and inoculated with *Pseudomonas* sp. 3b at 24 h and 15 days post-treatment.
Results obtained in peanut revealed that both biotic and abiotic stresses induced the expression of PR-10. While no differences were observed between leaves and root level expression under biotic stress. The gene expression in roots of plants under abiotic stress was higher than in leaves.
Results obtained in maize indicated that only abiotic stress (at 15 d post-treatment) induced the *ZmNHX* gene expression. These results indicated that the inoculation of *Pseudomonas* sp. 3b in maize plants did not induce the *ZmNHX* gene expression under saline stress conditions. PR-10 was expressed in peanut plants under both biotic and abiotic stress conditions.

PL-P19.

PARTICIPATION OF *Xanthomonas axonopodis* pv. citri EFFECTOR PROTEINS IN CITRUS CANKER

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Xanthomonas axonopodis pv. *citri* (Xac) causes citrus canker, a serious disease that results in important losses. Xac is a Gram (-) bacterium and uses secretion systems for translocation of pathogenicity and avirulence proteins to the plant cells. There are many genes characterized like elicitors of the host plant response and/or of hypersensitive response (HR) in non host plants. In the sequenced genome of Xac several genes have been identified as putative effector proteins that exert their function in the plant cell, among these are *XAC3090*, *avrXacE1* (*XAC0286*) and *avrXacE2* (*XAC3224*). We isolated *XAC3090*, *avrXacE1* and *avrXacE2* of Xac and constructed mutants in these genes, using a suicide vector transferred by biparental mating. For disease symptoms assays, bacterial suspensions were infiltrated into leaves of host plant orange (Citrus sinensis) and in non-host plants tomato, tobacco and cotton, showing differences in virulence phenotype only in the interaction with the host plant. No differences were observed in bacterial growth curves in host plant, but when photosynthetic parameters were analyzed, we detected differences between mutant and wild type Xac. The results suggest that these effector proteins could have different roles in pathogenicity, but may not be indispensables in citrus canker.

PL-P20.

EFFECTS OF MUTATIONS IN THE *Xanthomonas axonopodis pv citri* LIPOPOLYSACCHARIDE ON CITRUS CANKER

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A broad range of pathogens is recognized by plants through so-called pathogen-associated molecular patterns (PAMPs), which are highly conserved fragments of pathogenic molecules. In plant pathogenic bacteria, lipopolysaccharide (LPS) is an important virulence factor and it is being recognized as a PAMP. Stomatal closure is part of a plant innate immune response. Citrus canker is produced by *Xanthomonas axonopodis* pv. citri (Xac). The aim of this study is to determine the effect of Xac mutant strains defective in genes (*wzt* and *rfb303*) involved in LPS biosynthesis on citrus canker. The structures of LPS from Xac-*wzt* and Xac-*rfb303* were determined and differences between LPS from Xac and mutants strains were observed. Incubation of orange leaves with LPS isolated of Xac wild-type (wt), Xac-*wzt* and Xac-*rfb303* induced stomatal closure after 2 hours. On the other hand, incubation of orange leaves with bacteria induced stomatal aperture after 3 hours. It is established that bacterial LPS can induce accumulations of transcripts of a number of defense-related genes like PR, PrxA, GST and MAPKs in plants. Expression changes in genes that are involved in basal defense were observed in orange leaves infected with Xac wt, Xac-*wzt* and Xac-*rfb303* at 6 and 24 hours post-infiltration. These results suggest that LPS of Xac acts as a PAMP in Xac-orange interaction.

PL-P21.

ANTIFUNGAL ACTIVITY OF RECOMBINANT SNAKIN-1 (SN1R) PROTEIN INVOLVES MEMBRANE PERMEABILIZATION

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Snakin1 (SN1) is a cysteine-rich peptide isolated from potato tubers with a MW of 6,922 kDa. Previous reports show that SN1 has antimicrobial activity on fungal and bacterial plant pathogens. In order to analyze in depth the antimicrobial mechanism of action, we have successfully subcloned the SN1 cDNA into pQE70 vector, expressed into M15 cells, induced with 1mM IPTG after 2 h. and purified in Ni-agarose column. Recombinant SN1 (*St*SN1r) was able to inhibit germination of *F. solani* spores and growth of hyphae at the same concentrations reported for SN1*wt in vitro*. When *F. solani* hyphae and spores were incubated with different concentrations of *St*SN1r, membrane permeabilization of these structures was observed, as shown by the uptake of the fluorescent dye SYTOX Green. These results show that, as reported for other plants antimicrobial cysteine-rich peptides, cytotoxic activity of *St*SN1 involves plasma membrane permeabilization.

PL-P22.

HISTOCHEMICAL LOCALIZATION OF CADMIUM IN RANGPUR LIME (*Citrus limonia L. osbeck*) ROOTS

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The first manifestation of Cd^{2+} toxicity in plants takes place at radicular level because root is the first organ interacting with metals. Rangpur lime is used as a lemon rootstock in many countries and little is known respect to its response to heavy metals. The aim of this work was to study the distribution and effect of different Cd^{2+} concentrations in roots of Rangpur lime seedlings at the level of the absorption zone. Thirty five days old seedlings were transferred to 0 (control), 5 and 10 µM $CdCl_2$ in hydroponic culture for 7 days . Excised roots were fixed and cut into semithin sections (2 cm upper the apex). For microscopic analyses, sections were clarified with NaClO and stained with specific dyes: 2-(8-quinolylazo)-4,5-diphenylimidazole (cadmium), toluidine blue (lignin, pectin), and Sudan IV (suberin). No changes were found in total root and vascular cylinder diameters or epidermis and exodermis thickness. Cd^{2+} was detected in both exodermis and vascular cylinder of roots treated with 5 and 10 µM $CdCl_2$. Lignin was detected in the xylem of both control and Cd^{2+} treated roots, but in the last one the colour intensity was lower. Pectin was only detected in the cortex of Cd-exposed roots, while suberin was detected in both exodermis and endodermis of control and 5 µM treated roots. Results indicated that Cd^{2+} affected mainly the cell wall structures but not the root morphology.

PL-P23.

FUNCTIONAL ANALYSIS OF A SA-INDUCIBLE LECTIN PROTEIN IN PATHOGENS DEFENSE RESPONSE IN *Arabidopsis*

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Salicylic acid (SA) is a crucial hormone for the establishment of defense responses to biotic stress. Genes activation mediated by this hormone is essential for this process. We previously identified a group of early SA-inducible genes by transcriptomic analysis in Arabidopsis. Within this group, *LLP*, coding for a lectin like protein, was selected and its activation by SA and by inoculation with *Pseudomonas syringae* pv tomato (Avr Rpm1) was validated. The product of *LLP* has not been associated to any biological function.
We are using functional genetic approaches to evaluate the function of LLP in microbe-induced defense response. We obtained homozygote transgenic lines over-expressing *LLP*, by using *Agrobacterium*-mediated transformation. We also isolated and characterized a homozygote and null T-DNA insertion line for *LLP* gene. By using these lines, we are currently evaluating the role of LLP in the defense response to *Pseudomonas syringae* pv tomato (virulent and avirulent races). Our preliminary results suggest that *LLP* is involved in the defense response against *Pseudomonas*.
Furthermore, analysis of the subcellular localization of LLP, by confocal microscopy of plants expressing the LLP-GFP fusion protein indicate that LLP-GFP is probably associated to the plasma membrane.
Financed by FONDECYT-CONICYT (1060494) and Millennium Nucleus for Plant Functional Genomics (P06-009-F).

PL-P24.

ANALYSIS OF *Arabidopsis thaliana* 2-Cys PEROXIREDOXIN B PROMOTER REGION

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The 2-Cys Peroxiredoxin B (2-Cys Prx B) is a chloroplastic protein that reduces a broad spectrum of peroxides like H_2O_2, lipid hydroperoxide and peroxinitrite. The 2-Cys Prx proteins are proposed to have chaperone activity as well.
To understand the role of this protein in the defence system of plants we studied the activity of 2-Cys Prx B promoter by inserting the putative promoter region in the pBI101 plasmid and transforming *Arabidopsis thaliana* (Ecotype Col-7), setting the ß-glucuronidase gene (GUS) under the control of the promoter.
We analyzed the expression of the reporter gene under several stress conditions, such as heat, cold, osmotic stress, salinity stress, oxidative stress and fungal elicitors. Results indicated that 2-Cys-Prx B promoter region is highly active in leaves under control conditions. In addition, expression levels change under some stresses.
These results suggest that 2-Cys Prx B could be involved not only in the antioxidant response but also in the response to other stresses in *Arabidopsis thaliana*.

PL-P25.
HOW TOMATO PLANTS ARE GETTING RID OF SSA
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GABA accumulates in immature tomato fruits and in response to a variety of abiotic stresses in other plants. It is probably due to activation of glutamate decarboxilase (GAD), one of the three enzymes of the "GABA shunt" together with GABA transaminase (GABAT) and succinic semialdehyde dehydrogenase (SSADH). Arabidopsis was the only plant specie where this pathway was studied. Under physiological conditions, GABA is catabolized via GABAT to SSA, a highly reactive molecule, and further to succinate via SSADH. The activity of SSADH could be restricted under stressful conditions, although SSA did not accumulate suggesting that an alternative pathway for SSA catabolism may be active such as SSA reductase (SSR). In this work we investigated the levels of SSA and transcripts of SSADH and SSR as well as their activities in different organs of tomato plants. We analyzed tomato fruits during ripening process, as a continuous transition. We observed a decrease in the activities of both enzymes and in SSA content. However, the transcript level of SSADH increased during the ripening process. The activity of these enzymes and the levels of SSA were also studied in other organs of the plant. In spite of SSA toxicity, we observed that SSA accumulated at a certain level under physiological conditions, suggesting complementary roles for SSADH and SSR in keeping low SSA levels in tomato organs.

PL-P26.
IDENTIFICATION OF A NEW *GDH* GENE IN *Solanum lycopersicum*
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Glutamate dehydrogenase (GDH) catalyses a reversible reaction for the reductive amination of a-ketoglutarate to glutamate. Although GDH is ubiquitous in plant tissues, its physiological role is a subject of controversy. GDH is a hexamer comprised of two subunit polypeptides a and ß, that differ slightly in mass and charge. The association of both subunits yields seven possible GDH isoenzymes. In several plant species these subunits are encoded by at least two distinct genes, however in tomato (*Solanum lycopersicum*) only one gene, coding for the ß subunit (*Slgdh*B) was isolated.
Looking for others functional gdh genes in tomato, we searched in the transcript and genomic sequences available at Sol Genomic Network database (www.sgn.cornell.edu). We found a complete cDNA of 1604 bp, with a 56.9% of identity to *Slgdh*B. This clone was highly similar to the genes encoding the a subunit of *N. plumbaginifolia* (87%) and *N. tabacum* (88%). We isolated the corresponding *Slgdh*A putative promoter and analyzed the predictive cis-acting elements. The expression of *gdh*B and *gdh*A was studied by real time PCR and western blot analysis using antibodies that recognizes both subunits or only the a subunit. We found that the expression of both *gdh* genes and the isoenzyme profile were diverse in the different tomato tissues. These data suggest that *gdh*B and *gdh*A are independently regulated in tomato plants.

PL-P27.
GAMMA CARBONIC ANHYDRASE 2 FROM *Arabidopsis thaliana* FORMS TRIMERS *IN VITRO*
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Mitochondrial electron chain Complex I of Arabidopsis includes five structurally related plant-specific subunits representing gamma-type carbonic anhydrases termed CA1, CA2, CA3, CAL1 and CAL2. These proteins share relatively high similarity to CAM, the only fully characterized and crystallized gamma CA from *Methanosarcina thermophila*. Crystallographic studies of CAM have shown a quaternary homotrimeric structure coordinating a Zinc ion in the catalytic site.
In silico modelling analysis shown that Arabidopsis CAs possess all important aminoacid needed for catalysis, and the possibility to form homotrimeric and heterotrimeric functional structures. *In vivo* studies have shown that The CAs protein could form homodimers with functional active sites. In contrast, CAL1 y CAL2 did not. In the present work, to make an attempt to test the results obtained by informatics and *in vivo* analyses, the gene encoding carbonic anhydrase 2 from *Arabidopsis thaliana* was over-expressed in *Escherichia coli. T*he heterologously produced enzyme was purified from inclusion bodies to apparent homogeneity, refolded, and subjected to FPLC and native gel analyses. These experiments prove that Arabidopsis carbonic anhydrase 2 forms trimers *in vitro*.

PL-P28.
PROTEOMIC ANALYSIS OF MUTANT CELL CULTURES FOR TWO MITOCHONDRIAL CARBONIC ANHYDRASES LIKE GENES
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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 higher homology with the highly characterized gamma-CAs from the archaea *M. thermophila*, and two CA-Like genes (*CAL1* and *CAL2)* show 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. By bioinformatic analysis, CALs proteins lack a number of functional aminoacids important to form an active site in a homotrimer, but they could form a mature heterotrimer together with two CA subunits. By proteomics analysis, we showed that *ca2* mutant cell culture exhibited a 80% reduction in Complex I levels, but this feature was not observed in *ca1* or *ca3* mutants. In this work, we analyzed the mitochondrial proteome of *cal1* mutant and *cal1cal2* mutant cell cultures. *cal2* cell cultures were unsuccesful. We observed that *cal1* and *cal1cal2* cell cultures presented normal amounts of all mitochondrial complexes. A detailed analysis showed that both mutants lacked the same two bands in silver stained 2D BN-SDS gels and only one band reacted with an antibody recognizing all CAs. These results suggest an epistatic relationship between CAL1 and CAL2.

PL-P29.

***Wheat* CYTOSOLIC GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASES ARE DIFFERENTIALLY REDOX REGULATED**

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In plants, glycolysis is a complex network where specific steps are critical for regulation. Thus, oxidation of Ga3P to 3P-glycerate in the cytosol of plant cells is achieved by two different pathways. One involves phosphorylating Ga3P dehydrogenase (Ga3PDHase, EC 1.2.1.12) and 3P-glycerate kinase, where triose-P derives in the synthesis of ATP and NADH. The other route, catalyzed by the non-phosphorylating Ga3P dehydrogenase (npGa3PDHase; EC 1.2.1.9), leads triose-P to the synthesis of NADPH. An ongoing and challenging problem is to elucidate the regulation of these alternative reactions of glycolysis, whereby extracellular stimuli coordinate the intermediary metabolism. We characterized redox modifications occurring on cytosolic Ga3PDHase and npGa3PDHase from wheat. Both enzymes were inactivated by different thiol oxidants as diamide, hydrogen peroxide, sodium nitroprusside, oxidized glutathione and S-nitrosoglutathione. The loss of activity was effectively reversed after incubation with various reducing agents, suggesting that the process could function physiologically. Interestingly, Ga3PDHase was markedly more sensitive to oxidants than npGa3PDHase. We conclude that under oxidative conditions, the oxidation of triose-P is preferably utilized for NADPH generation, being a key metabolic point to regulate carbon, energy and redox equivalents partitioning in plant cell cytosol.

PL-P30.

ANALYSIS OF NDPK EXPRESSION IN POTATO PLANTS

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In plants, response mechanisms to biotic and abiotic stresses are regulated by different pathways that depend on early gene activation. Numerous kinases are involved in stress responses. Nucleoside diphosphate kinases (NDPKs) were long considered as house-keeping enzymes with a primary metabolic function, however, recent studies indicate that these enzymes might have a regulatory role in signal transduction events in plant responses to wounding, heat shock and pathogen infection. Three NDPK isoforms have been described in plants: NDPK1 in cytosol, NDPK2 in chloroplast stroma and NDPK3 in the chloroplast lumen and in the mitochondrial intermembrane space. Using primers designed on ESTs present on potato microarrays we amplified a 555 nt PCR fragment 89% homologous to NDPK3 from *Spinacia oleracea*. This isoform was expressed in buds, swelling stolons, sprouts and leaves of potato plants but not in dormant tubers. The primers amplified a 1694 nt PCR fragment when genomic DNA was used as template. This fragment was used as probe in a Southern blot assay. Primers that span an intronic region were designed to study the localization of this StNDPK gene in the potato genome. Semiquantitative RT-PCR assays are being performed to study NDPK3 expression in potato plants exposed to cold, high salt, defense signals (chitosan, PGA), hormones (GA_3, ABA, AIA) and in PVY infected leaves.

PL-P31.

DIFFERENTIALLY EXPRESSED GENES IN POTATO SPROUTS IN LIGHT/DARK CONDITIONS DETECTED BY MICROARRAYS

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Tuber sprouting initiates after a period of dormancy under favorable conditions. In order to analyze genes expressed upon sprouting in different light conditions, tubers were grown for 10 days under continuous light (10L) or for 10 or 35 days under complete darkness (10D, 35D). Total RNA was obtained from sprouts, labeled with Cy3 or Cy5 and hybridized to potato microarrays produced by TIGR. A group of genes related to photosynthesis, carbon metabolism and light perception was enhanced (>5 fold induction) in 10L (light induced genes) while in 35 days etiolated sprouts different proteinase inhibitors (PIs): Kunitz-type PIs, protease inhibitor seed storage lipid transfer protein (LTP), Metallocarboxypeptidase inhibitor IIa (MCPI2) were drastically induced (13-fold). These results were confirmed by RT-PCR. PIs play a potent defensive role against predators and pathogens and interfere with physiological processes such as germination and maturation. PIs may regulate cell proteolysis by inhibition of endogenous proteases and thus control protein turnover and metabolism. Diverse functions have been proposed for PIs, ranging from regulators of endogenous proteinases to storage proteins. In chick pea, PIs could be involved in xylem vessel formation and a cell wall PI in the elongation process. We suggest that in potato, PIs might be involved in regulating the elongation of etiolated sprouts.

PL-P32.

CRYPTOCHROME AND HETEROTRIMERIC G PROTEIN REGULATE COMMON DEVELOPMENTAL RESPONSES IN *Arabidopsis*

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Plants sense and interpret the light environment and adjust their growth and development accordingly. In *Arabidopsis thaliana* blue light is perceived by two cryptochromes (cry1 and cry2). Arabidopsis has a single gene encoding for the alpha subunit of the heterotrimeric G protein (GPA1). Heterotrimeric G proteins have been largely implicated in blue signaling during Arabidopsis seedling development. Here we combine a biochemical, molecular and genetic approach to study signaling pathway connections between GPA1 and cry1. GTP binding activity in the *cry1* mutant was significantly reduced to *gpa1* mutant levels. GPA transcript or protein levels were normal in the *cry1* mutant and in our experimental conditions we did not find protein–protein interaction. *cry1* mutants failed in some typical gpa1 mediated responses in darkness and *cry1 gpa1* double mutants studies showed genetic epistasis. Microarray experiments showed cry1 and gpa1 coregulate the expression of several genes. Finally, adult *cry1 gpa1* double mutants showed altered leaf shape, trichome formation and rosette size. All these results suggest cry1 and gpa1 regulate common processes during Arabidopsis seedling development.

PL-P33.

EXTRACELLULAR ATP (eATP) SIGNALING IN PLANTS: ROLE OF PHOSPHOLIPASES, Ca^{2+} AND NO

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ATP is a vital molecule used by living organisms as a universal source of energy. In animals, extracellular ATP (eATP) is a regulatory signal involved in numerous physiological processes. In plants, eATP induces increases in citoplasmic Ca^{2+} concentration from both the extracellular and intracellular stores, however it is not known whether phospholipase C (PLC) derived inositol-3-phosphate (IP_3) is involved in this process. PLC has role in signaling generating, IP3 and diacylglycerol (DAG). In plants DAG is converted to phosphatidic acid (PA) via DAG kinase (DGK). Another source of PA is phospholipase D (PLD). We have previously shown that eATP induces an increase in nitric oxide (NO) production downstream of PA. The aim of the present work was to elucidate the enzymatic sources of PA production during eATP treatments and to analyze their roles in cytosolic Ca^{2+} increase and NO production. Time course experiments showed that PA is generated within minutes. Preliminary results showed that both PLD and PLC/DGK pathways are involved not only in generating PA but also required for NO production. Our results suggest that extracellular Ca^{2+} is involved in the response of eATP, upstream of NO production. Current studies are in progress in order to elucidate the role of Ca^{2+}, PA and NO in eATP-signaling physiological responses.

PL-P34.

PROTEIN-PROTEIN AND PROTEIN-DNA INTERACTION STUDIES *OF A. thaliana* CLASS I TCP TRANSCRIPTION FACTORS

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TCP proteins are plant-specific transcription factors involved in the regulation of cell growth and proliferation. These proteins contain the TCP domain, a basic helix-loop-helix (bHLH) structure required for DNA binding and dimerization, unrelated to the canonical bHLH found in eukaryotic proteins. Members of the TCP family can be grouped into two classes, I and II. The DNA sequences recognized by both classes share a core sequence but differ in flanking nucleotides. To characterize the DNA binding properties of class I proteins, we performed *in vitro* DNA binding assays with oligonucleotides containing variants of the previously determined consensus binding site and proteins TCP11, TCP20, TCP15 and TCP21 from *A. thaliana*. The results showed that, while subtle differences in nucleotide preferences were evident for the other proteins, TCP11 shows a clearly different DNA binding specificity. A more detailed picture of binding to DNA was obtained by missing nucleoside experiments with hydroxyl radical. These assays indicated the presence of different contacts in the interaction of TCP proteins with DNA. We also identified potential TCP11 interaction partners by yeast two-hybrid screening. *In vitro* and *in vivo* protein-protein interaction assays revealed that TCP11 interacts with TCP15 and TCP21. The DNA binding and dimerization specificity of class I TCP proteins is discussed.

PL-P35.

ANALYSIS OF THE COX5B-2 PROMOTER FROM *Arabidopsis thaliana*

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The promoter of the Arabidopsis nuclear gene *COX5b-2* (At1g80230), encoding subunit 5b of mitochondrial cytochrome c oxidase, was analyzed using plants stably transformed with different promoter fragments fused to the glucuronidase reporter gene. Histochemical staining indicated that the COX5b-2 promoter directs tissue-specific expression in meristems, cotyledon tips, anthers and pollen grains. The analysis of progressive upstream deletions of the COX5b-2 promoter suggested the presence of a negative regulatory element, possibly a G-box, between nucleotides -700 and -650 from the translation start site. Site-directed mutagenesis of two copies of the site II element (TGGGCC/T) present at -148 and -172 or the four copies of the Initiator element (YTCANTYY, Y = C or T) between nucleotides -112 and -85 produces a general decrease in GUS expression levels. In addition, mutagenesis of all copies of both elements completely abolishes expression of a reporter gene in all organs/tissues. Mutations in site II elements abolishes induction by sucrose. Proteins present in nuclear extracts were able to specifically bind to these elements in electrophoretic mobility shift assays. The results suggest that the combined action of site II and Initiator elements is essential for COX5b-2 expression and that site II elements have an additional role in the response of this gene to carbohydrates.

PL-P36.

CHARACTERIZATION OF ARABIDOPSIS GENES ENCODING CYTOCHROME C OXIDASE ASSEMBLY FACTORS

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The biogenesis of cytochrome c oxidase (COX) requires accesory proteins, including COX11, COX17, and SCO1, that have been implicated in copper metallation of two COX subunits. Another protein, COX19, is essential for COX biogenesis but its exact function is unknown. Based on structural similarities with COX17, it has also been implicated in metal transport. The Arabidopsis genome contains one *AtCOX11* gene and two genes for each *AtCOX17*, *AtCOX19*, and *AtSCO1*. It is noteworthy that *AtCOX11*, *AtCOX17* and *AtCOX19* genes contain an intron located near the translation start site and share certain motifs in their promoter proximal regions. Expression of *AtCOX17* and *AtCOX19* genes was localized preferentially in young roots, anthers and siliques and was induced by different treatments that produce oxidative stress in plants. Expression of these genes was completely dependent on the presence of the intron and site II motifs located around -200 from the translation start site. For *AtCOX11*, similar expression patterns were observed, but induction by stress and dependence on the intron and site II motifs was not evident. Our results indicate that, in addition to the structural similarities observed for COX17 and COX19 proteins, the expression mechanisms of these genes are highly conserved. This further supports the view that these copper chaperones have closely related functions in COX assembly.

PL-P37.
CHARACTERIZATION OF ATNFS1, A MITOCHONDRIAL CYSTEINE DESULPHURASE FROM *Arabidopsis*
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Iron-Sulfur (Fe-S) clusters are ubiquitous prosthetic groups required to sustain fundamental life processes. The assembly of Fe-S clusters requires the coordinated delivery of both iron and sulfide and involves at least ten proteins. The first step in biosynthesis is carried out by cysteine desulfurases (NifS like proteins) which catalyze the formation of elemental sulfur and alanine from cysteine. The Arabidopsis genome encodes two cysteine desulfurases AtNfs1 and AtNfs2. AtNfs2 has been characterized as a plastid protein whereas AtNfs1 has been localized to the mitochondria. In order to characterize this first step in Fe-S cluster biosynthesis in plant mitochondria, we expressed, purified and performed kinetic characterization of the recombinant protein AtNfs1m (lacking the mitochondrial transit peptide). We also examined possible protein interaction of AtNfs1 with the Arabidopsis frataxin homologue, AtFH (a potential donor of iron for the assembly of cluster Fe-S). The results confirmed that AtNfs1m displays cysteine desulfurase activity; moreover, it is possible to speculate that AtFH modulates the activity of AtNfs1.

PL-P38.
PARTICIPATION OF ATFH IN THE BIOGENESIS OF HEME-CONTAINING PROTEINS
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Frataxin, a nuclear-encoded mitochondrial protein, has been proposed to participate in Fe–S cluster assembly, mitochondrial energy metabolism, respiration, and iron homeostasis. However, its precise function remains elusive. Mutations in the frataxin gene cause a reduced expression of this gene and an inherited recesive ataxia (Friedreich's ataxia, FRDA). The analysis of *Arabidopsis thaliana* T-DNA insertional mutants deficient in AtFH expression suggest that AtFH is involved in Fe-S protein biogenesis in plant mitochondria. In the present work, we evaluate the effect of frataxin deficiency in heme synthesis in *Arabidopsis* plants. The analysis of a knock down mutant *atfh-1* and antisense *as-atfh* plants revealed that both lines have similar amounts of catalase proteins compared to wt, but reduced heme content and also a decrease in total catalase activity (an heme-containing protein) compared with wt. Indeed, the addition of exogenous hemin rescues the catalase activity in leaves and flowers homogenates. Taken together, the results suggest that AtFH could be involved in the biogenesis of hemoproteins in *Arabidopsis* plants.

PL-P39.
GENETIC AND MOLECULAR CHARACTERIZATION OF TWO NOVEL PUTATIVE S-RNASES IN *Nicotiana alata*
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We are characterizing the molecular allelic diversity of S-RNases at the self-incompatibility (SI) locus in a natural population of *Nicotiana alata*. RT-PCR reactions were conducted on style RNA of eight plants, using degenerate oligonucleotides based on the conserved domains C1 and C5 of solanaceous S-RNases. Amplification products of expected size were cloned and sequenced, rendering two S-RNases previously characterized as SI alleles and three ribonucleases not reported yet as S-alleles, tentatively named D63, S5 and S7m. Genomic PCR with S5, S7m and D63 homologous primers revealed that the two first alleles were specific in six and one plants, respectively. By contrast, D63 was present in all plants examined, including a set of plants coming from a laboratory population. Spatial and temporal relative expression of S5 and S7m showed these alleles in developing and mature styles but not in floral tissues or leaves, paralleling the expression of well-known S-RNases. Conversely, expression of D63 ribonuclease was similar in different tissues and showed earlier expression respect to S5 and S7m in developing styles. Taken together these results strongly suggest that S5 and S7m are S-RNases and that D63 is a constitutive ribonuclease, not involved in SI. Genetic crosses and segregation analysis are in progress to demonstrate whether S5 and S7m are functional S-alleles.

PL-P40.
QUANTITATIVE DETERMINATION OF SUPEROXIDE IN *Strawberry* LEAVES USING A MODIFIED NBT STAINING METHOD
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The interest of studying the reactive oxygen species (ROS, i.e. H_2O_2, O^{2-}) in plants has increased lately for they are involved in different physiological and stress mechanisms. Although a variety of methods have been developed to determine the production of ROS in plants, its evaluation in leaves of certain species is difficult and scarcely reliable due to a great number of interferents. During the study of the defense reponse in strawberry we were interested in the determination of the anion superoxide (O^{2-}). The determination of O^{2-} is usually carried out by direct histochemical staining, but these methods allow only to a visual observation of O^{2-} production unless special computer programs are used to quantify the histological preparations. However, the accuracy of this prodecure of evaluation is always debatable. In this work, we present a protocol for the quantitative determination of the anion O^{2-} based on a modification of a classic histological method reported earlier by Jabs *et. al.* (1996) that uses a tetrazolium salt (NBT). The modification consists on the selective solubilization of the reduced Formazan produced during the O^{2-} formation, allowing to make reliable spectrophotometrics readings (Muñoz *et. al.* 2000). The method was tested and validated on strawberry leaves subjected to biotic and abiotic stresses produced by infection and an herbicide, respectively.

PL-P41.

EXPRESSION OF PHENYLALANINE AMMONIA LYASE DURING RIPENING OF DIFFERENT *Strawberry* CULTIVARS

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Ripening fruit undergo a complex developmental process that involves changes in the morphology and metabolism of their tissues. In some cases, one of the most important trait that make fruit attractive to the consumers, are the accumulation of anthocyanin pigments. In strawberry fruit (*Fragaria x ananassa*, Duch), development of fruit colour is associated to the phenylpropanoid pathway. Phenylalanine ammonia lyase (PAL) is a key enzyme in this pathway, determining strawberry fruit quality. In this work, we analysed the levels of anthocyanins during fruit ripening of cultivars with different colour development (Camarosa, intense red colour, and Toyonaka, light red). Toyonaka showed a lower accumulation of anthocyanins compared to Camarosa. In addition, we have designed specific primers to generate a 249 bp FaPAL gene fragment, which was used as a probe for expression analysis. FaPAL expression was detected in all stages analysed except in white stage in both cultivars, but the mRNA levels were higher in Camarosa. Finally, both cultivars showed similar rates of increment in PAL activity at the beginning of ripening. However, at the end of ripening PAL activity diminished in Toyonaka, while it rose markedly in Camarosa. Therefore, the higher FaPAL expression and PAL activity detected in Camarosa could be associated to the enhanced anthocyanin accumulation found in this cultivar.

PL-P42.

CHARACTERIZATION OF -XYLOSIDASE ACTIVITY IN TOMATO AND ANALYSIS OF ITS HORMONAL REGULATION

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Tomato (Solanum lycopersicum), a fleshy climacteric fruit, has long-served as a model for fruit development and ripening studies. During tomato ripening, significant modifications of the cell wall properties were reported and include both depolymerisation and solubilisation of polyuronides and depolymerisation of hemicelluloses. -xylosidases are enzymes involved in the breakdown of xylans and/or arabinoxylans. In this work, we study the -xylosidase activity during ripening of wild type (Rutgers and VF36), mutant (rin, nor and nr) and ACC-synthase antisense fruits. We also analyze the possible COOH-terminal processing of the primary translation products of LeXyl1 and LeXyl2 isoenzymes. By using the pericarp disc system from wild type and ACC-synthase antisense fruits, we evaluate the effect of ethylene and other biological regulators on -xylosidase activity. Our results demonstrate that -xylosidase activity would be negatively regulated by ethylene and positively regulated by auxins and gibberellins, whereas abscisic acid would not affect it.

PL-P42.

POLYGALACTURONASE IN *Strawberry* FRUIT RIPENING. EFFECTS OF PLANT GROWTH REGULATORS

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Strawberry (*Fragaria x ananassa* Duch.) is a fleshy fruit that undergoes extensive softening during ripening as a consequence of cell wall disassembly caused by a coordinated action of cell wall hydrolases, including polygalacturonases (PGs). Nowadays there is not a global understanding about the hormonal regulation of strawberry ripening and softening. In this work, the immuno-detection, by Western blot assays, of PG protein (FaPG1) during ripening of strawberry cultivars with contrasting firmness is reported. The purified antibody recognized two proteins with molecular mass around 45-50 kDa. The accumulation of FaPG1 in the softest cultivar (Toyonaka) began at white stage y remained high until the end of ripening. In the firmer cultivar (Camarosa) the accumulation of FaPG1 was low at 25% red stage and increased at 50% and 100% red. The presence of two immunoreactive bands is apparently due to differential glicosylation of one polypeptide. In addition, the effect of different plant growth regulators on FaPG1 protein accumulation was analyzed. The treatment with naphtalen acetic acid and gibberelins delayed ripening and anthocyanin accumulation, and reduced the accumulation of FaPG1 proteins. On the other hand, it was detected a higher level of anthocyanins and FaPG1 synthesis in fruit treated with abscisic acid, ethylene and NO.

PL-P44.

A SEQUENCE HOMOLOGUE TO HIS-KINASE IDENTIFIED FROM *Aspidosperma quebracho blanco*

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With the aim to obtain SCAR markers by sequencing of RAPDs markers from *Aspidosperma quebracho blanco* Schltdl., bands from three populations in Arid Chaco were selected because of its differential presence. Three experimental sites were studied: Quebracho de la Legua (QL), Chancaní (C) and Bajo de Véliz (BV) They were selected according to a humidity gradient, with mean annual precipitation of 250, 450 and 600 mm, respectively. A band of approximately 1200 pb obtained with *primer* OPA 7 (B-1200-OPA7) was purified from agarose gels and cloned in *pGem-T-Easy* vector. The sequence (1257 pb) showed homology with the family of the "Two Components System", involved in signal transductions in plants. The translated sequence (464 aa) exhibited two highly conserved domains: the REC domain (Signal Receiver Domain) and the HPT domain (Histidine Phosphotransfer Domain). Plants have multiple physiological and biochemical systems that enable them to tolerate environmental stresses. Both domains, REC and HPT, are highly conserved in His-kinase proteins, an osmosensor that detects water stress and initiates downstream responses. Marker B-1200-OPA7 was found in 28 of 29, 7 of 15 and 19 of 33 individuals from QL, BV and C respectively. Our observations suggest a prevalence of the putative osmosensor sequence in populations from lower humidity environments.

PL-P45.

FUNCTIONAL STUDIES OF *Arabidopsis* Mago Nashi AND Y14 GENE PROMOTERS

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We have previously shown that expression of Arabidopsis *COX5c* genes, encoding a cytochrome oxidase subunit, depends on a leader intron that produces an increase in transcription and translation efficiency. This effect may be mediated by the exon junction complex (EJC), a multiprotein assembly deposited by the spliceosome upstream of mRNA exon–exon junctions. The EJC is thought to provide a link between splicing and postsplicing mRNA metabolism, influencing mRNA export, stability, and translation. Two proteins of the EJC, Y14 and Mago, remain associated with the mRNA after its export to the cytoplasm. In this work, we tried to gain insight into the expression patterns of Arabidopsis Mago and Y14. For this, we analyzed GUS activity in plants that express the gus (ß-glucuronidase) gene under the control of AtY14 and AtMago promoter sequences. Both promoters direct GUS activity in the root meristem, cotyledon and leaf veins, and pollen grains. For Y14, expression was also observed in the root cortex, vascular bundle and hairs, in cotyledon blades, and the shoot apical meristem. The results indicate that both promoters produce partially overlapping expression patterns. Cells/tissues of preferential expression are coincident with those observed for the *COX5c* genes, suggesting that these proteins may be involved in the intron-mediated enhancement of the expression of these genes.

PL-P46.

CARBOHYDRATE METABOLISM DURING THE DEVELOPMENT OF ORANGE FRUIT *(Citrus sinensis var.* Valencia Late*)*

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Orange fruits accumulate citric acid from the beginning of its development. Further on, towards maturation, deassimilation produces a net decrease in organic acids and t an increase in the concentration of soluble sugars. The soluble sugars/acids ratio determines fruit quality, and its evolution is affected by chilling stress, which alters the commercial quality. Therefore, the study of the metabolism of carbohydrates during ripening is crucial for the understanding of the mechanisms involved in attaining commercial quality and in the fruit tolerance to chilling injury. Fruits of *Citrus sinensis* var. Valencia Late were periodically harvested throughout the development period to evaluate changes in the metabolism of carbohydrates. We have determined the enzymatic activities of the main glycolytic and gluconeogenic enzymes. Our results show that i) there is a higher metabolic activity during stage I, then the fruit´s metabolic activity decreases and remains fairly constant and ii) there is a net transition from a glycolytic to a gluconeogenic metabolism during stage III (ripening).

PL-P47.

ASSESSING THE MECHANISMS OF ATRAZINE TOLERANCE OF *Lolium multiflorum:* A PHYTOREMEDIATION APPROACH

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As a new technology, phytoremediation is a low cost, environmental friendly biotechnological solution for atrazine (ATZ) pollution. Hence, the finding of ATZ tolerant plants and elucidation of the mechanisms involved in tolerance are crucial for designing remediation projects. Due to its agronomical properties and physiology, *Lolium multiflorum* (ryegrass) is one of the main candidates. The objective of this work is to assess the ATZ tolerance of ryegrass and the mechanisms involved in the process. Thus, ryegrass ATZ tolerance was assessed in MS agar medium with increasing herbicide levels. Then, the mechanisms involved in ATZ tolerance such as mutation *psb*A gene, enhanced enzymatic detoxification via P_{450} or chemical hydrolysis by benzoxazinones (Bx) were evaluated. Mutation in the psbA gene, encoding the ATZ target site D1 protein was evaluated by sequencing. The enzymatic detoxification was assessed in hydroponics cultures, exposing plants to ATZ and 1-aminobenzotriazole (P_{450} inhibitor). For Bx, the hydrolytic capacity of plant extracts on ATZ was tested. *L. multiflorum* was found as new tolerant specie able to grow in presence of 1 mg Kg^{-1} of ATZ. The sequence of *psb*A gene was registered on GenBank. Atrazine tolerance is conferred in this specie by enzymatic detoxification via P_{450}. Ryegrass is an excellent option for phytoremediation of agricultural ATZ contaminated soils

PL-P48.

ANTHRAQUINONE PRODUCTION IN *M. citrifolia* SUSPENSION CULTURES TREATED WITH PROLINE ANALOGS

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Anthraquinones (AQs) are secondary metabolites produced by the Rubiaceae family that exhibit biological interesting properties. Different routes are involved in AQs synthesis: chorismic acid, produced by shikimate pathway (SP) is converted into isochorismic acid (IC) by the enzyme isochorismate synthase. IC reacts with alfa-ketoglutaric acid to form o-succinylbenzoic acid, the precursor of two rings of the AQs structure. The other ring is derived from an isoprene unit produced by the 2-C-methyl-D-erythritol-4-phosphate pathway. It was proposed that the proline cycle could be linked to the pentose phosphate pathway (PPP) by generating two $NADP^+$ which are cofactors of the two first enzymes in the PPP. PPP supplies the SP with erithrose-4-phosphate. The aim of this work was to study a possible link between proline cycle and AQs production. *Morinda citrifolia* cell suspension cultures were treated with two proline analogs: azetidine-2-carboxylic acid (A2C; 25 and 50 µM) and thiazolidine-4-carboxylic acid (T4C; 100 and 200 µM). AQs content was higher ($p<0.05$) than the control line after 6 days of culture in all treated cells except from A2C 25 µM treatment, whereas total phenolics (TP) production was increased only with T4C 200 µM. After a 10 day-culture, both T4C treatments increased AQs and TP accumulation compared to control. These findings could be correlated with PPP stimulation.

PL-P49.

DXS EXPRESSION IN _Morinda citrifolia_ CELL SUSPENSION CULTURES TO INCREASE ANTRHAQUINONES PRODUCTION

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Plant cell suspension cultures are attractive alternatives for large scale production of plant-derived natural products. *Morinda citrifolia* is able to produce Anthraquinones (AQs) that have a potential therapeutic use. AQs are anthracene derivatives whose basal structure, 9, 10-dioxoanthracene, can be substituted resulting in a diversity of structures. The A and B rings of AQs are synthetized from shikimic and a-ketoglutaric acids via the ischorismate/o-succinylbenzoate pathway. The C ring is originated from the 2-C-methyl-D-erythritol 4-phosphate pathway (MEP). 1-deoxy-D-Xylulose-5-phosphate synthase (DXS), the first enzyme in the MEP pathway converts pyruvate and glyceraldehyde 3-phosphate into 1-deoxy-D-Xylulose-5-phosphate. The aim of this work was to overexpress DXS protein in *M. citrifolia* suspension cultures. *Catharantus roseus* DXS cDNAwas inserted into the binary vector pMOG22-GUS, with hygromycin resistance, *GUS* reporter gene and the left and right T-DNA borders from *A. tumefaciens*. *M citrifolia* cell cultures were transformed by biolistic and *A. tumefaciens* strain LBA4404 methods. Transformation was confirmed by analysis of the *GUS* reporter gene. Transgenic cells lines showed significantly higher levels of AQs (21% and 30% after 3 and 6 days of culture; $p>0.01$) and higher DXS activity (60% in a 6 day-culture; $p>0.05$) compared to wild type cell lines.

PL-P50.

EFFECT OF _Opuntia salagria_ CONSUMPTION ON HEPATIC LIPIDS FROM _Streptozotocin_-INDUCED DIABETIC RATS

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A notorious increase in the medicinal use of plant natural products has been observed in the last years. The number of people with Diabetes mellitus increases on a daily basis. *Cactus opuntia* has been reported to display hypoglycemic and hypolipidemic effects but the mechanisms involved have not been elucidated to date.Our previous studies in *O. salagria*, an Argentinean autochthonous species, evidenced that the oral administration of cladodes and seed powders reduces plasmatic cholesterol, triacylglycerides (TAG), LDL-c, HDL-c and VLDL-c and glycemia in streptozotocin (STZ)-induced diabetic rats. In this work we analyzed the effect of *O. salagria* administration on hepatic lipids from diabetic rats. Cladodes and fruits were collected from Bahía Blanca, dried and milled. Male Wistar rats were made diabetic by intraperitoneal injection of STZ and received a daily dietary supplementation with cladodes and seeds over one month. Hepatic lipids were extracted, separated by thin-layer chromatography and derivatized by methanolysis. Phospholipid and cholesterol levels were not affected by cladode and seed treatments while several changes in free fatty acid composition were recorded. As to TAG and diacylglycerides, an important decrease in their amount and significant changes in TAG composition were observed, thus suggesting a potential application of this cactus to diabetes dyslipidemia.

SB-P01.

ANTIGENIC CROSS-REACTIVITY OF A NATURALLY OCURRING HYPOALLERGENIC AG5 WITH AN ALLERGENIC HOMOLOG

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Antigen 5 (Ag5) is the main allergen found in vespid venoms. However, Ag5 from Polybia scutellaris (Pol s5), a South American wasp, is a hypoallergenic variant. In this work, we studied its cross-reactivity with Ag5 from Polistes annularis (Pol a5), a well-known allergen, by indirect and inhibition ELISA. A significant cross-reactivity was found at the IgG level. Nevertheless, both proteins have a different immunogenicity and at least one different immunodominant epitope. This behaviour makes Pol s5 a suitable tool for immunotherapy.
As only low yields of the native Pol s5 are obtained, we previously cloned and expressed it in Pichia pastoris. Although the native protein is not glycosylated, most of the recombinant protein is. Due to the fact that glycosylation could affect the immunologic response to an antigen, we modified the putative glycosylation motif (Asn-Tyr-Ser) by site-directed mutagenesis (Ser-Tyr-Ser). Only the non-glycosylated isoform was expressed. Further studies are being conducted to characterize this mutant and confirm whether it maintains the hypoallergenicity and cross-reactivity of the native protein.

SB-P02.

MOLECULAR MARKERS IN CHILDREN PAROTID SALIVA

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The "normal" protein pattern of physiological fluids can be used as reference for differential diagnosis of pathologies. Saliva proteome is composed by a number of proteins involved in the homeostasis of the oral cavity.
The main objectives of this work are: a) to describe the protein profile of parotid saliva of healthy children; b) to identify molecular markers for juvenile rheumatoid arthritis (JRA). Ten samples of parotid saliva of healthy children and twelve samples of children with juvenile rheumatoid arthritis were collected with a Lashley cup following stimulation with a 2 % citric acid solution. After molecular exclusion chromatography, each fraction was submitted to HPLC-ESI-MS. The peak containing 10-23 kDa molecular species was analyzed by RP-HPLC. A further ESI-MS analysis allowed detection of an 18.455 Da protein present in most of JRA samples studied and absent in all control samples. Further studies are being performed to confirm whether such protein could be a new tool for the diagnosis of this disease.

SB-P03.

AMYLOID POLYMORPHISM OF FAMILIAL MUTANTS OF á-SYNUCLEIN SENSED BY A DUAL-EMISSION FLUORESCENT PROBE

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Parkinson's disease (PD) is a movement disorder characterized by the presence in the mid-brain of amyloid deposits of the protein -synuclein (AS). Three familial mutants, A53T, A30P and E46K, are linked with early onset PD. Compared to the WT, the mutants exhibit faster aggregation *in vitro* and subtle differences in ultrastructural features. Using the extrinsic dual-color emission probe 4'-diethylamino-3-hydroxyflavone (FE) we demonstrate that amyloid fibrils formed by WT and mutant AS differ in their supramolecular fibrillar organization.
The environment-sensitive FE probe exhibits two intensive well-separated emission bands reflecting excited state redistributions. Its ratiometric response constitutes a sensitive and tunable reporter of microenvironmental properties such as polarity and hydrogen-bonding. The FE probe bound to the four AS variants exhibits distinctive fluorescence spectral signatures. Two-dimensional deconvolution of the excitation-emission spectra reveals binding site heterogeneity characterized by different dielectric constants and extents of hydration.
The sensitivity of this probe to structural alterations induced by point mutations is unprecedented and will contribute to the understanding of various phenomena associated with amyloid fibrils and their prefibrillar intermediates: plasticity, polymorphism, and structure-function relationships underlying toxicity.

SB-P04.

SIMPLE SYNTHESIS OF PROTEIN-OLIGOSACCHARIDE CONJUGATES AS TOOLS FOR DIVERSE BIO-APPLICATIONS

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Due to the haptenic nature of sugars, preparation of an immunogen with a carrier protein is often necessary. In the last years several approaches have been developed: coupling of a conveniently derivatized sugar to an amino acid followed by automatic peptide synthesis, coupling of sugars to gold nanoparticules, glycosides functionalized with an electrophilic thiol specific reagent for immobilization by disulfide bonds to the protein. However, all these methods involve several steps of organic synthesis prior to the coupling reaction making it time-consuming and rather expensive.
In this work we present a method of preparing amino derivative sugars and an easy way to couple them to commercial proteins. We have performed the coupling reaction to bovine serum albumin using different oligosaccharides. Conjugates were tested by SDS-PAGE and characterized by UV-MALDI-TOF mass spectrometry analysis. Different reaction conditions were tried changing the reaction time, temperature and protein/sugar molar ratio. Best conditions yielded a BSA-modified conjugate bearing eleven sugar units. This approach allows the fast and cheap preparation of a wide variety of pseudo-glycoproteins by changing the peptide moiety and/or the sugar unit making it a potential and useful tool for different bio-applications such as antibody production and immunoassays.

SB-P05.

CYCLODEXTRIN GLYCOSYLTRANSFERASE FROM *Bacillus circulans* DF 9R: NATURAL MUTATION AT -6 SUBSITE

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Cyclodextrin glycosyltransferase (CGTase; E.C.2.4.1.19) is a member of the GH13 family, also known as the -amylase family, which catalyses cyclodextrin (CD) production from starch and other related carbohydrates. Nine substrate binding subsites were observed in an X-ray structure of the CGTase from *Bacillus circulans* strain 251. The -6 subsite (Y167, G179, G180, N193, D196) is fully conserved in CGTases, suggesting its importance in enzyme structure and function. Substrate interactions at this subsite activate the enzyme via an induced-fit mechanism. All the CGTases whose primary structure is already known have Gly at both positions, 179 and 180. It has been suggested that the absence of side chains in both residues is a requirement for substrate binding favoring the induced-fit mechanism. In this work, we describe the presence of Gln instead of Gly at position 179 of the *Bacillus circulans* DF 9R CGTase. This is the first change reported for the -6 subsite in a natural CGTase so far – such change being determined by both Edman degradation and DNA sequencing. Moreover, the enzyme has all the features previously described for an - or /â-CD producer. This work is a contribution to the knowledge of CGTase structure-function relationship, particularly that dealing with product specificity.

SB-P06.

THE IMMUNOGENICITY IS RELATED TO STABILITY: THE BLS-OMP31 MODEL

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Brucella abortus Lumazine Sinthase (BLS) is a highly immunogenic and very stable decameric 180 KDa protein. We have used BLS for the multiple antigenic display of peptides and protein domains by replacement of the amino terminus of each monomer, generating different types of chimeric proteins. BLS-OMP31, where OMP31 is a 27-mer exposed loop of the Brucella abortus membrane protein OMP31, has been characterized in detail.

The objective of this work was to generate mutants of BLS-OMP31 with different stability to study the relationship between the stability of the carrier and the immunogenicity of the peptide.

We modified by site-directed mutagenesis residues located at the interface between pentamers. We selected Phe 121 and 130 residues to perturb as little as possible the BLS structure (and introduce bulkier Trp residues), obtaining the mutants BLS-OMP31-W22A-F130W and BLS-OMP31-F130W. We also generated the mutant BLS-OMP31-L105A. We analyzed the folding, unfolding and stability of the mutants through different techniques: far UV and near UV circular dichroism and fluorescence spectroscopy. We also characterized the immunogenicity of the mutants in Balb/c mice, showing that there is a clear relationship between stability and the capacity of eliciting anti-peptide humoral responses.

SB-P07.

STRUCTURAL FEATURES OF *St*ASP-PSIR AND SAPLIPS COULD EXPLAIN CYTOTOXIC SELECTIVITY

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We have reported the antimicrobial activity of a domain present in potato aspartic proteases, called plant-specific insert (*St*Asp-PSI). This domain has a high structural homology with Saposin-*like* proteins (SAPLIPs) with antimicrobial/antitumoral activities. The aim of this work was to analyse if similarities/differences between *St*Asp-PSIr and SAPLIPs, found in the cytotoxic activities can be explained by similar/different 3-D structures. Comparisons of NK-Lysin, Granulysin and SP-B with *St*Asp-PSIr model showed that antibacterial activity towards *E. coli* and *B. cereus* would be dependent on the helix 1-helix 5 structure, while the hydrolytic activity towards *S. aureus*, only present in SP-B and *St*Asp-PSIr, would be influenced by residues L97, C98 and A100 in the helix 4 which are similar in both polypeptides. In addition, we have identified residues in the helix 4 (K113, K118, K120) that may account for the antimicrobial activity against Gram- bacteria, as reported for SP-B. Helices 4 and 5 of *St*Asp-PSIr would be responsible for bacterial aggregation previously reported for *St*APs. The analysis performed here suggests that similarities and differences in structural features between *St*ASP-PSIR and SAPLIPS could explain cytotoxic selectivity. However, mutagenesis assays will be necessary to corroborate this hypothesis.

SB-P08.

NATIVE STATE STABILIZATION WITH SMALL LIGANDS INHIBITS LYSOZYME AMYLOIDOGENESIS

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Lysozyme (LYZ) has been extensively employed as an experimental model of amyloid formation. Mutant lysozymes displaying reduced thermodynamic stability are linked to massive fibril deposition in humans, leading eventually to renal failure. LYZ fibrillar aggregates are similar to those detected in several neurodegenerative diseases, such as Alzheimer, Parkinson, and Transmissible Spongiform Encephalopathies (TSEs). In this sense, to develop strategies that inhibit fibril formation is an issue of broad interest. Treatments aimed at breaking fibrils are uncertain; since it has been suggested that the most cytotoxic species are not the fibers themselves but the intermediate oligomers. In many cases, fibril initiation requires a conformational transition of the native state. Therefore, it is expected that native state stabilization by small molecules will inhibit fibril growth. Here we show that several natural inhibitor of LYZ activity (N-acetyl glucosamine, N-acetyl chitobiose and N-acetyl chitotriose) inhibit amyloid formation *in vitro*. Aggregation assays using thioflavine T show that this fibril growth inhibition correlates with ligand concentration and their affinities for LYZ.

SB-P09.
ACETYLATION EFFECT ON BIOLOGICAL ACTIVITY OF PPGALNAC-T2

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Polypeptide GalNAc-transferase, isoforme 2 (ppGalNAc-T2) is a member of the large family of polypeptide GalNAc-transferases involved in mucin-type O-glycosylation initiation. ppGalNAc-T are class II membrane protein with a small citoplasmatic domain (N-terminal), a transmembrane domain followed by the catalytic domain and a lectin domain (C-terminal). In the present work we study the influence of a post-translational modification, acetylation, on the biological activity of ppGalNAc-T2. The chemical acetylation of recombinant ppGalNAc-T2 reveals a critical reduction in catalytic activity on mucin-type O-glycosylation. MALDI-TOF mass spectrometry showed acetyl residues in Ser and Lys amine acids of catalytic and lectin domains of ppGalNAc-T2. Carbohydrate-binding ability of this protein is also influenced by acetylation, as observed by several approaches (dot blots, western blots, ELISAs, competitive assays), showing a major GalNAc recognition. The acetylation of Ser 529 and Lys 521 corresponding to high-conservative QKW domain of Ricin-type lectin family could be the motive of shift on the glycan-binding specificity of acetylated ppGalNAc-T2. The intracellular localization of acetylated ppGalNAc-T2, using specific antibody generated in our laboratory, in several conditions of different cell lines is in study.

SB-P10.
ATP-DEPENDENT OLIGOMERIZATION OF RAPESEED 2-CYS PEROXIREDOXIN

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2-Cys peroxiredoxin (2-Cys Prx) is a subfamily of peroxidases implicated in oxidative stress and signal transduction. The covalently linked homodimers (ca. 23 kDa subunit) have a significant propensity to form non-covalently decamers (pentamer of dimers). We have shown that the rapeseed 2-Cys Prx binds ATP at a region close to the conserved "resolving" Cys175 thereby impairing the capacity to reduce hydrogen peroxide. Therefore, using surface plasmon resonance (SPR) and dynamic light scattering (DLS), we analyzed whether ATP affects the oligomerization of the protein.

The dramatic increase in the SPR signal caused by the concerted action of ATP and Mg^{2+} (henceforth ATP/Mg) indicated the formation of large assemblies close to the sensor. Assuming a single equilibrium (ATP/Mg) + 2-Cys Prx — [(ATP/Mg)-(2-Cys Prx)], the apparent association and dissociation constants were 0.94^{-1} s^{-1} and 2.3 s^{-1}, respectively. Congruent with this study, DLS experiments revealed that ATP/Mg converts the soluble 2-Cys Prx (diam.: 13.8 nm) to large aggregates (diam.: higher than 600 nm) which return to the original size upon the addition of EDTA.

Taken together these results evince the capacity of the couple ATP/Mg for the modulation of the quaternary structure and the peroxidase activity of 2-Cys Prx and, in so doing, uncover a novel nucleotide-dependent control of the oxidative stress.

ST-P01.
DESCRIPTION OF THE INTERACTOME OF P8, A PROTEIN RELATED TO TUMOR PROGRESSION

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p8 is an 8 kDa protein. It was identified due to its induction during the acute phase of pancreatitis. Functions related to stress, cell growth control and tumor progression have been proposed for p8. Analysis of its sequence identified a conserved region corresponding to a NLS.
Immunocytochemistry experiments, show that p8's sub-cellular localization depends on cell culture density, cell cycle and acetylation state of the cells. Its nuclear import is energy dependent; the NLS of p8 is necessary and sufficient to retain a heterologous protein in the nucleus.
The fact that p8 is small enough to diffuse between nucleus and cytoplasm, but still possesses a NLS and a controlled localization suggests that it could associate to multiprotein complexes. Our aim is to identify these complexes. We generated a HEK 293 cell line that expresses p8 fused to HIS-FLAG tag. Tandem affinity purification was performed. The purified complexes were digested and analyzed by tandem mass spectrometry to identify proteins associated to p8.
We generated a list of proteins that interact with p8. This, combined with two-hybrid experiments and bibliographical data, allowed to illustrate the p8 interactome, which shows proteins related to DNA repair and mRNA processing, suggesting that p8 is a multifunctional protein that interacts with different proteins in different cellular compartments.

ST-P02.
CHARACTERIZATION OF SEVERAL P8 INTERACTING PROTEINS

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Human p8 is an 82 aminoacid protein expressed in response to stress. It is proposed to be involved in tumour development and acts as growth factor. Previous results show that p8 localises to the nucleus in subconfluent cell cultures and throughout the whole cell under superconfluence. p8 was found to have a nuclear localisation signal (NLS). Two-hybrid system permitted us to identify several of its partners, among which we chose to study the small GTPase Ran as well as one of its accessory proteins, RanBP1, since they are involved in nuclear transport. Pull down technique confirmed that both proteins directly interact with p8. The strength of the interaction with these partners as well as and XM_496217, was compared using a two-hybrid approach. Weprothymosin generated site-directed lysine p8-mutants that proved to be less prone to degradation. Consistent with this fact, they appeared to have a more stable interaction with all tested partners. Confocal microscopy confirmed co-localisation of p8 and Ran in the nucleus. Although p8 mutants also co-localised with Ran, its concentration was increased in the cytoplasm in subconfluent cultures. Preliminary results led us to speculate that p8 levels may respond to a balance of degradation by ubiquitination and stabilization by SUMOylation, two post-traslational modifications that require lysines.

ST-P03.
IDENTIFICATION AND EXPRESSION OF NOVEL PKA GENES IN *Mucor circinelloides*

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We are interested in the PKA (R2C2) of the fungus *Mucor circinelloides*. Until recently its genome was unknown. A sequence for the C subunit (*pkaC*) and R subunit (*pkaR*) from *M.circinelloides* was available. In a previous work we had disrupted the pkaR gene from *M.circinelloides* (MU R strain); however this K.O. resulted in a decreased but not null cAMP binding activity and in a PKA still dependent on cAMP. The genome of this fungus is now cloned and the sequence available to us. A BLAST search of the genome using the already known sequences from this fungus and from mammals, resulted in the identification of 4 genes for R and 6 genes for C. The already described pkaC appears not to be a bonafide PKA C. The protein sequences were predicted and bioinformatic comparisons performed. Sq-RT PCR of a wt strain, in different stages of growth indicated that all the subunits are expressed in at least one stage of growth, including pkaC; the introns were confirmed. Three of the four R were identified by MALDI-TOF MS in purified preparations of R. The analysis of the expression of C and R in MU R, in which only one R isoform is deleted, suggests that the other three R genes tend to compensate the lack of *pkaR1*. It is the first time that a fungal system with more than one R is described. The differential expression of some of the subunits could indicate specific functions for each stage.

ST-P04.
INTERACTION OF PKA IN *Saccharomyces cervisiae* WITH ITS REGULATED TARGET GENES

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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. We analyzed gene occupancy profiles from the raw data of a genome-wide ChIP-Chip analysis of Tpk1 and Tpk2 during growth on glucose, glycerol and oxidative stress (Pokholok et al Science 313:533, 2006). As described, Tpk1 was physically associated with actively transcribed genes and Tpk2 with the promoters of ribosomal protein genes. Occupancy of Tpk3 was not detected. Additionally, we found from the raw data that both Tpk1 and Tpk2 seem to be simultaneously associated to the same regions of some genes upon fermentative metabolism and oxidative stress. The nuclear co-localization of Bcy1 and Tpks upon environmental conditions in which active PKA is required, directs the question about the role of the regulatory subunit in the nucleus. Using ChIP assays we analyzed the role of Bcy1 in the regulation of gene expression and its function in the association with the target genes of Tpk1 and Tpk2. We found that Bcy1 and Tpk1 are associated in a carbon source-dependent manner while Tpk2 remains constitutively bound to genes. Our data suggest that Bcy1 might participate in gene regulation allowing the positioning of the Tpk near the substrate.

ST-P05.
ACTIVATION OF PKA BY SUBSTRATES FROM *Saccharomyces cerevisiae*
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The effectiveness of protein phosphorylation by kinases is believed to depend on the primary structure of the protein around the phosphorylation site. Our aim is to assess the best consensus sequence for yeast Protein kinase A and the participation of the substrate in yeast holoenzyme activation. We used the proteins Pyk1, Pyk2 and Nth1, which have been described as PKA substrates in yeast and have consensus RRXS sequence for PKA phosphorylation. Five synthetic peptides including the consensus phosphorylation sequences from these proteins were phosphorylated in vitro. Three of them behaved as good substrates. Small differences in peptide sequences resulted in important Km and Vmax differences. Peptide array was designed to verify the residues responsible for these differences. The substrate role in the activation of holoenzyme was also investigated. Activity of purified PKA in presence of different concentrations of cAMP and different peptides was assayed. The cAMP A0.5 was different with each substrate and substrate concentration, indicating that the primary structure of the substrate plays an important role in the activation mechanism. The better the substrate the higher the activation. The activation of PKA was also different when the activation assays were made using the whole protein as substrate and compared with the peptide as substrate.

ST-P06.
***Candida albicans* PKA CATALYTIC ISOFORMS TPK1 AND TPK2 HAVE DIFFERENT ROLES IN STRESS RESPONSE**
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Candida albicans PKA is coded by two catalytic subunits *TPK1* and *TPK2*, and one regulatory subunit *BCY1*. In this organism the cAMP/PKA signaling pathway mediates basic cellular processes such as the yeast-to-hyphae transition and the cell cycle regulation. In *Saccharomyces cerevisiae* it is known that low PKA levels are associated with carbohydrates accumulation and heat and oxidative stress resistance. Previous results from our laboratory showed that *C. albicans* strains devoid of one or both *TPK1* alleles were defective in glycogen storage, while strains lacking Tpk2p accumulated higher levels of the polysaccharide, indicating that Tpk1p and Tpk2p have opposite roles in carbohydrate metabolism. In the present study, we investigated the role of *C. albicans* PKA in response to saline, heat and oxidative stresses. In order to fine-tune the analysis, we performed the study on several *C. albicans* PKA mutants having heterozygous or homozygous deletion of *TPK1* or *TPK2* in a different *BCY1* genetic background. Interestingly, we found that *tpk1*? strains developed a lower tolerance to saline exposure, heat shock and oxidative stress, while wild-type and *tpk2* mutants were resistant to these stresses, indicating that both isoforms play different roles in the stress response pathway.
Supported by grants from Saarland University (Germany), CONICET and ANPCyT.

ST-P07.
RELEVANCE OF THE 3' UNTRANSLATED REGION (3'UTR) IN MAMMARY GLAND B1-INTEGRIN EXPRESSION
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Integrins belong to a super family of cell surface adhesion receptors that play a relevant role during normal development and tumor formation in the mammary gland. We examined gene products of b1 integrin in normal and neoplastic tissue by 3'RACE-PCR. By cloning and sequencing these reaction products, we found a new b1-integrin mRNA species, which arises from the use of an alternative polyadenylation site located 578 bp upstream from the previously reported one. Our analysis also revealed two AU-rich element (ARE) sequences implicated in the regulation of mRNA levels, localized between those sites and, therefore, absent in the shorter mRNA species.
Both mRNA forms are present in normal and neoplastic tissue, with a specific ratio corresponding to each physiological condition. We have sub-cloned the corresponding 3'UTR sequences for the two mRNAs into reporter vectors downstream of a CMV driven luciferase gene. Then, mRNA stability assays were performed. The results indicated that the longer form is less stable, suggesting that the ARE sequences may play a relevant role on the stability of this mRNA. Taken together, these data indicate that the presence of alternative 3'UTR regulating sequences may provide another regulatory instance to determine more precisely the b1-integrin abundance required in the different dynamic physiological situations occurring in the mammary gland.

ST-P08.
CORRELATION BETWEEN STARD7, -CATENIN AND INOS TRANSCRIPT LEVELS IN RAT TISSUES
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StarD7 transcript encodes one of the 15 family members with START domain present in the human genome. Highly similar human StarD7 orthologous genes have been annotated in GenBank databases in different genomes, suggesting a conserved physiological function. We demonstrated that StarD7 gene expression in human cells is regulated by Wnt/ß-catenin signaling which is implicated in numerous aspects of development, cell biology and physiology such as proliferation, inflammation, and cancer. The aim of the present study was to evaluate StarD7 transcript expression in rat tissue samples and correlate them with ß-catenin and inducible oxide nitric synthase (iNOS) ones, which is also target of ß-catenin pathway. RT-PCR techniques for StarD7, ß-catenin and iNOS were performed in liver, brain and ovary isolated from normal rats. The transcripts were detected in all tissue samples although the StarD7 tissue distribution was 2-4 times elevated in brain compared to ovary and 60-80 times higher in liver than ovary. These data show a significant correlation between expression of StarD7 and ß-catenin mRNA level as well as with iNOS expression and set more evidences indicating that StarD7 gene is a target of Wnt/ß-catenin signaling pathway.
Supported by CONICET, FONCyT and SECyT-UNC.

ST-P09.

INVOLVEMENT OF NO IN THE MECHANISM OF HISTAMINE-INDUCED INHIBITION OF LEYDIG CELL STEROIDOGENESIS

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The present study was conducted to shed light on the unexplored intracellular mechanisms underlying negative modulation of Leydig cell steroidogenesis by histamine (HA). Using MA-10 cells and purified rat Leydig cells, we examined the effect of the amine on biochemical steps known to be modulated by HA, or involved in LH/hCG action. HA at 10 µM showed a potent inhibitory effect on hCG-stimulated steroid synthesis. Moreover, HA not only decreased hCG-induced cAMP production but also steroid synthesis stimulated by db-cAMP. Considering the post-cAMP sites of HA action, HA inhibited db-cAMP-stimulated acute regulatory (StAR) protein expression, as well as the conversion of cholesterol to pregnenolone by cytochrome P450 cholesterol side-chain cleavage enzyme (CYP11A). The antisteroidogenic action of HA was blocked by the PLC-inhibitor U73122, and HA augmented IP3 production, suggesting a major role for the PLC/IP3 pathway in HA-induced inhibition of Leydig cell function. Finally, HA increased nitric oxide synthase (NOS) activity, and the NOS inhibitor L-NAME markedly attenuated the effect of the amine on steroid synthesis. On the basis of our findings, HA antagonizes the gonadotropin action in Leydig cells at steps both pre- and post-cAMP formation. NOS activation is the main intracellular mechanism by which HA exerts its antisteroidogenic effects.

Grants: ANPCYT, CONICET, UBA

ST-P10.

INTRACELLULAR CALCIUM OSCILLATIONS IN SPERMATOZOA EXPOSED TO MOLECULAR GRADIENTS OF PROGESTERONE

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Recently, we observed that along the egg-cumulus complex there is a concentration gradient of progesterone (P), which is probably the sole chemoattractant secreted by the cumulus cells. When such a gradient is generated by a pM P concentration, a subpopulation (~10%) of capacitated spermatozoa are chemotactically oriented in parallel to be primed for the acrosome reaction, whereas at µM P concentrations the spermatozoa become hyperactivated. The occurrence of these physiological sperm processes need intracellular calcium variations, which are stimulated by different P concentrations. The aim of this work was to characterize the intracellular calcium mobilization in spermatozoa confronted to molecular gradients generated by different P concentrations. By means of fluorescence video microscopy and computer image analysis, capacitated human spermatozoa were stuck to a chemotaxis chamber and then confronted to gradients of different P ranges (fM to µM). A sperm subpopulation (~20%) showed calcium oscillations, which increased in amplitude and frequency as a function of P concentration, started at the fM P range. These preliminary results suggest that the P gradient levels that induce specific sperm processes (e.g., chemotaxis, acrosome reaction priming, and hyperactivation) also trigger differential calcium oscillations.

ST-P11.

CERAMIDE INDUCES RESUMPTION OF MEIOSIS IN *Bufo arenarum* OOCYTES

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Ceramides are generated in response to cellular stimulation by progesterone in amphibian oocytes. In this work, ceramide is analyzed for its ability to induce germinal vesicle breakdown (GVBD) indicative of maturation. Methyl-ß-cyclodextrin (MßCD) was used for cellular cholesterol modulation in order to assess membrane raft involvement. Oocytes arrested in meiosis I were incubated for 24 h in a saline solution with C_2-ceramide or the corresponding dihydroceramide solubilized in dimethylsufoxide (DMSO) at different concentrations. Progesterone-induced maturation was achieved by incubation of oocytes with the steroid for 24 h. To remove cellular cholesterol, oocytes were pretreated with 25mM MßCD and subjected to incubation with either progesterone or ceramide. A detergent-free protocol was used to isolate caveolae-like membranes. Treatment of oocytes with exogenous C_2-ceramide induced GVBD without evidence of cytotoxicity at 100 µg/ml concentration with a maximal response at 150 µg/ml. C_2-dihydroceramide and DMSO induced no GVBD at the concentrations assayed. When MßCD-treated oocytes were incubated with ceramide, maturation was significantly inhibited. In contrast to progesterone, ceramide incubation affected the distribution caveolin and c-Src among membrane fractions. Results suggest that ceramide induces maturation probably by a pathway different from that of the steroid hormone.

ST-P12.

CLONING AND CHARACTERIZATION OF 3-CHIMAERIN: A NOVEL DAG REGULATED RAC-GAP

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Chimaerins comprise a family of phorbol ester/diacylglycerol (DAG) receptors with specific Rac-GAP activity. Two genes are present in mammals (and), coding for the four isoforms described up to date (1, 2, 1 and 2). Chimaerins posses a C-terminal Rac specific GAP domain and a C1 domain that binds DAG and phorbol ester with high affinity, similar to that found in PKC isozymes. In addition, 2- and 2-chimaerins have an autoinhibitory N-terminal region containing an SH2 domain involved in the regulation of its activity by DAG. Screening several mouse tissues by Western blot we have recently discovered the presence in kidney, heart and adrenal gland of an isoform showing and extra 10 kDa apparent molecular weight. Searching for EST sequences in the NCBI database, several cDNAs from kidney tumors and amygdala were found to code for a novel isoform we named 3-chimaerin. This protein differs from 2-chimaerin on its N-terminal portion having an essential domain involved in the autoinhibitory mechanism replaced by a novel 90 aminoacid sequence coded by two exons 48kb upstream of the 2-chimaerin transcription start site. Our goal is to clone and characterize 3-chimaerin and unveil the role of its N-terminal region in the regulation of the activity.

ST-P13.
SECOND MESSENGERS ORIGINATED FROM PHOSPHATIDIC ACID IN RAT CEREBELLUM NUCLEI
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Evidence not only confirms the presence of lipids in the nucleus as a part of the chromatin structure but also demonstrates the existence of numerous enzymes which modulate changes in their composition. The aim of the present work was to evaluate how phosphatidic acid (PA) is metabolized in rat cerebellum nuclei. To this end, adult (4 mo) rat cerebellum was homogenized and highly purified nuclei were isolated by sucrose-density ultracentrifugation. Nuclear preparations were checked for purity by electron microscopy and DAPI stain. Our results indicate that PA is metabolized to diacylglycerol (DAG) by LPPs and it to monoacylglycerol (MAG) by DAGL. Furthermore, it was observed that when several modulators of these enzymatic pathways, such as NaF 50mM are used, PA is metabolized to lysophosphatidic acid (LPA) and water soluble products (WSP). This also indicates the presence of PLA/LPA phospholipases and phosphohydrolases. In addition, LPPs dephosphorylate LPA, sphingosine 1-phosphate (S1P) and ceramide 1-phosphate (C1P). At equimolecular concentrations (100µM) of PA/LPA, PA/S1P or PA/C1P, LPPs diminished DAG formation by 61%, 32%, and 42%, respectively. These results show that PA could be metabolized by different enzymes in rat cerebellum nuclei, thus indicating the presence of important signalling pathways in this fraction.

ST-P14.
INSULIN ACTION IN PHOSPHATIDIC ACID (PA) FORMATION IN SYNAPTIC TERMINALS FROM ADULT AND AGED RATS
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PA formation is stimulated by insulin in cerebral cortex (CC) synaptosomes from adult rats. DAGK acts in response to increased DAG generated by PIP2-PLC and PLD-PAP2 pathways, this action being reinforced by an additional DAGK activation mechanism exerted by the hormone. We carried out studies on CC and hippocampal (Hp) synaptosomes to evaluate basal and insulin-activated PA synthesis from exogenous di-16:0 (DPG), di-18:1 (DOG) and 18:0-20:4 (SAG) in adult (3 m of age) and aged (24-26 m of age) rats. DAGK assay shows detergent (OG) and substrate (SAG) preference as well as insensitivity to R59022 and R59949 (DAGK inhibitors), both of which indirectly indicate DAGK activity under our assay conditions. The majority of CC synaptosomes processed for immunofluorescence reveal the presence of DAGK rather than DAGK . Also, whereas DAGs content is increased, DAGK activity is inhibited in CC synaptosomes from aged with respect to adult rats. PA formation from SAG in CC and Hp synaptosomes is preferentially diminished. However, DAGK is stimulated by insulin in synaptic terminals from Hp and CC from aged rats and hormone preferentially stimulates PA formation from SAG in Hp synaptosomes. Our results suggest that 20:4-PA formation is potentially involved in inositol lipid homeostasis at the synapse. This molecular mechanism could be one of the beneficial effects of insulin on the brain.

ST-P15.
PARTICIPATION OF IRS-4 AND PI3-K IN ANGIOTENSIN II AND INSULIN CROSSTALK IN HEPG2 CELLS
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The aim of the present study was to characterize the cross-talk between the signalling pathways of Insulin (Ins) and Angiotensin II (Ang II). Until now, the proteins involved in cross-talk between Ins and Ang II involved IRS-1 and IRS-2 substrates. In a previous study we observed the participation of IRS-4 in this cross-talk. By means of western blot and immunoprecipitation we demonstrated the association between IRS-4 and PI3K following stimulation with Ins and Ang II in HepG2 cells. PI3-K activity was measured in IRS-4 immunocomplexes. Ins induces Tyr-.phosphorylation of IRS-4 in HepG2 cells, a response blocked by pre-incubation with the Ins antagonist AG1024. Ang II AT1 receptors potentiates Ins effect on Tyr-phosphorylation of IRS-4. PI3-K inhibitors prevented the modulation by Ang II of Ins-induced Tyr-phosphorylation of IRS-4. In IRS-4 immunocomplexes we observed PI3-K association and activation after Ins stimulation, increased by Ang II. In the long term, Ins induces an increase of IRS-4 protein and mRNA level, supporting a functional role of this substrate in cellular growth. The present results would indicate for the first time a physiological role of IRS-4 substrate in response to Ins stimulation in the short and long term, an effect modulated by Ang II. Participation of IRS-4 in this signalling pathway is totally novel as well as its potential physiological role.

ST-P16.
ATP STIMULATES THE PI3K/AKT SIGNALING PATHWAY IN OSTEOBLASTIC AND BREAST CANCER CELLS
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It is well established that extracellular nucleotides, acting through P2 receptors, elicit a wide range of responses in many cell types. In the present work we studied the effect of ATP on the PI3K/Akt signaling pathway in rat osteosarcoma ROS-A 17/2.8 osteoblasts and human breast cancer MCF-7 cells. Western blot analysis showed that ATP rapidly (5 min) stimulated the phosphorylation of the serine/threonine kinase Akt in a dose- and time-dependent manner. Moreover, the use of ATP?S, UTP, ADP and ADPßS and RT-PCR studies suggested the involvement of the P2Y2 receptor subtype in the phosphorylation of Akt. In both systems, the use of Ly294002, a PI3K inhibitor, suppressed the phosphorylation of Akt at Ser 473 in response to ATP. We established that ATP stimulated the phosphorylation of Src at Tyr 416 in ROS-A 17/2.8 osteoblast-like and MCF-7 breast cancer cells. Immunocytochemistry studies showed that ATP induced the translocation of Src from cytoplasm to nucleus. In addition, the use of PP1/PP2, Src inhibitors, suppressed the effect of ATP on Akt phosphorylation. The results obtained suggest that ATP leads to the phosphorylation of Akt in a PI3K- and Src- dependent manner through P2Y2 receptors in both cell lines. These data may underly mechanisms involved in the regulation of proliferation and apoptosis in cancer cells by extracellular ATP.

ST-P17.
FINE TUNNING OF DES TRANSCRIPTIONAL REGULATION
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Membrane fluidity is tightly regulated by the Des system in *Bacillus subtilis*. This system is composed of a membrane histidine kinase, DesK, that phosphorylates the response regulator, DesR, when the membrane becomes rigid. Only the phosphorylated form of DesR activates the transcription of the ?5-acyl-lipid desaturase gene. In this study we have examined the role of the N-terminal domain of DesR, that contains the phosphrylatable conserved residue Asp 54 by constructing a truncated DesR protein, lacking the first 128 amino acids, $DesR^{C}$. Our data demonstrate that $DesR^{C}$ is able to bind to the *des* promoter in EMSA, but unable to stimulate transcription in vivo. We propose that the mechanism by which DesR is activated by phosphorylation implies release of the inhibitory effects of the N-terminal regulatory domain over the C-terminal domain containing the HTH motif. Besides, the effect of deletion of 0.5;1 or 1.5 DNA-helix turn of the low affinity site of the *des* promoter was evaluated by EMSA and transcriptional fusions. From this study we can conclude that a dimer of phosphorylated DesR bound to the high affinity site element in phase and close enough to the -35 is sufficient to activate *des* transcription, suggesting that the low affinity site functions as a threshold barrier for basal expression.

ST-P18.
A MINIMAL SENSOR THAT RESPONDS TO MEMBRANE FLUIDITY
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The *B. subtilis* DesKR two component system has been identified as the first system that senses changes in membrane lipid fluidity and responds accordingly. It is composed of a membrane histidin-kinase, DesK, a cytoplasmic response regulator, DesR, and the effector enzyme, delta5-desaturase. Under conditions of restricted membrane fluidity DesK phosphorylates DesR, enabling its binding to the desaturase promoter. A tetramer of phosphorylated DesR positioned on P*des* recruits the RNAPolymerase to increase the transcription of the acyl-lipid desaturase. This enzyme introduces double bonds into the acyl chains of membrane lipids to decrease its phase transition temperature.
To obtain a simple system to study the sensing mechanism of DesK we constructed a series of fusion proteins composed of the cytoplasmic domain of DesK and one of several chimerical transmembrane segments (TMSs) envisioned as possessing the critical elements involved in sensing. One of these fusion proteins behaves as DesK wild type, so we called it DesK-Minimal Sensor (DesK-MS).
The unique TMS of DesK-MS seems to harbor the essence of the functioning of a biological sensor, having the required elements to perceive and transmit the signal. The DesK-MS give us the opportunity to test our hypothesis on its mode of action by the construction of several site-directed mutants and analyze its behavior *in vitro*.

ST-P19.
STUDY OF PHOSPHORYLATED RESIDUES OF LEPRK2 IN TOMATO AND THEIR FUNCTION IN POLLEN-PISTIL INTERACTION
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LePRK1 and LePRK2 are pollen-specific receptor kinases from *Solanum lycopersicum*, expressed in pollen tubes and potentially involved in pollen-pistil interactions. Transduction by their kinase activity of a pistil signal might initiate a signaling cascade in the tube that regulates its growth. LePRK2 is phosphorylated *in vitro* in pollen membranes. To know which LePRK2 residues are phosphorylated *in vivo*, we separated proteins from mature pollen by 2D gels. Potential phosphorylated isoforms were visualized with western blots against LePRK2 and isolated in silver stained 2D gels. Using a computational approach that predicts phosphorylation sites in LePRK2, we selected 8 serines or threonines of LePRK2: S277, S279, S282, S304, S307, T308, T358 and S396. To study their functional relevance in pollen tube growth, we substituted them by Alanine and Aspartic acid using for mutagenesis the pLAT52-LePRK2-eGFP construct, followed by transient expression in tobacco pollen. We previously showed pollen receiving LePRK2-eGFP displayed aberrant *ballon tip* morphology at germination. Pollen transfected with LePRK2(A277/A279/A282)-eGFP recovered the *wt* phenotype and are longer; neither of the constructs with isolated alanine residues showed this reversion. Contrary, pollen tubes receiving LePRK2(D277/D279/D282)-eGFP showed a *wt* phenotype. These results will be complemented by the proteomic approach.

ST-P20.
PHOTORECEPTIVE RETINAL GANGLION CELLS: PHOTOTRANSDUCTION MECHANISMS
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In Vertebrates, non-visual photoperception provides a measure of environmental lighting conditions that synchronizes biological clocks through retinal ganglion cells (RGCs) that send photic information to the brain. A subset of RGCs expressing the photopigment melanopsin (Opn4) is intrinsically photosensitive (ipRGC) acting as circadian photoreceptors. Our studies and others showed that the nature of the biochemical events in ipRGC phototransduction may resemble that in rhabdomeric (Rb) photoreceptors involving the phosphoinositide (PIP) cascade. Light activates Gq/11-class G proteins, the activity of phospholipase C with the increase in cytoplasmic Calcium and membrane depolarization. However, it remains unknown the complete mechanism of phototransduction and involvement of PIPs in ipRGCs. To investigate this, we measured the formation of inositol-P3 and other inositols (by Dowex chromatography columns) in chicken RGC cultures by anti-Thy-1 immunopanning exposed to cool white light at different times. Then we assessed the activity of DAG Kinase and PIP Kinases (PIK and PIPK) in homogenates from the same conditions. Our results showed a 1.3 fold increase of Inositol-P3 and inhibition of PIK, PIPK and DAGK activities during short light stimuli (1 min). These results indicate that chicken ipRGC phototransduction involves a PIP cascade similar to that in Rb photoreceptors.

ST-P21.
E2F1 TRANSCRIPTION FACTOR MODULATES TRANSLATION INITIATION
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E2F1 has been shown to induce both proliferation and apoptosis through transcription regulation of several essential genes involved in these processes. In this study, we demonstrated that E2F1 could also modulate the translation initiation by a mechanism via p70S6 kinase and eIF-4E binding protein-1. By using ER-E2F1 system, we demonstrate that translocation of E2F1 to the nucleus by the addition of tamoxifen induces the phosphorylation of p70S6 kinase and eIF-4E binding protein. PI 3-kinase inhibitor, LY294002, and mTOR inhibitor, rapamycin, repress this effect, suggesting the involvement of PI 3-kinase/mTOR pathway in this effect. However, activation of PI 3-kinase activity, measured as rate of PKB phosphorylation, was not detected after the addition of tamoxifen. Surprisingly, the phosphorylation of p70 S6 kinase does not depend on the transcription activity of E2F1, suggesting that E2F1 activates translation initiation by a protein/protein interaction mechanism. Works in progress is to study the involvement of TSC2-Rheb-mTOR pathway in this regulation. The results obtained in this study point out for the first time a role of E2F1 on cell growth.

ST-P22.
INVOLVEMENT OF SIGNALING PATHWAYS IN THE PHOSPHORYLATION OF ZEB1 IN EPITHELIAL CELLS
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The activity of a transcription factor (TF) can be controlled by phosphorylation. ZEB1 (Zinc Finger E-box Binding Homeobox) is a TF involved in differentiation of mesoderm derived cells and cancer metastasis. ZEB1 exists in two phosphorylated forms (P-ZEB1). Our goal is to uncover which signaling pathway/s can modify ZEB1 status of phosphorylation and in consequence its function. We showed that the hypophosphorylated ZEB1 binds to target genes stronger than the hyperphosphorylated one. Cell lines expressing one or both P-ZEB1 were treated with TPA/ionomycin (PKC activators), and the inhibitors CalphostinC (PKC's), LY294002 (PI3K's), PD98059 (ERK's) and SB203580 (p38MAPK's). EMSAs made with target gene promoters of ZEB1 as probes showed an increased binding capacity to the nuclear extracts (NE) treated with CalC, PD98059, a lower binding with TPA/IO and LY294002 and no change with SB203580. Accordingly, TPA/IO and LY reverted ZEB1 repression of E-cadherin gene in transfection assays. Western blots in NE showed increased ZEB1 expression with TPA/IO which was not due to change in location of ZEB1 as seen by immunofluorescence microscopy and WB but to transcriptional level (cycloheximide reverted effect). Multiple pathways seem to be involved in ZEB1 phosphorylation. They cross-talk in different models of cell differentiation. In our case PI3K and PKC wouldn't be part of the same pathway

ST-P23.
CARBOXY-TERMINAL ZINC FINGER DOMAIN (ZD2) OF ZEB1 CONTAINS ITS MAIN PHOSPHORYLATION SITES
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The activity of a transcription factor (TF) can be controlled by phosphorylation. ZEB1 exists as two phosphorylated forms. ZEB1(Zn Finger E-box Binding Homeobox) is a TF involved in cell differentiation and metastasis of tumors. ZEB1contains two Zn finger domains (ZD1, ZD2) flanking a homeodomain (HD) and an acidic domain (E). The isoform ZEB1-b lacks ZD1.We showed that the hypophosphorylated ZEB1 binds to its target genes stronger than the hyperphosphorylated one. Our aim here is to delimit which of the ZEB1 domains are involved. Rabbit reticulocyte lysates (RRL) were programmed with expression vectors that encode the ZEB1 protein fragments ZEB1-b, HD, ZD2E and ZD2. RRL were incubated with phosphatase (CIP) or CIP+phosphate. EMSAs were done with the RRLs and 32P-oligonucleotides harboring ZEB1 binding sites from a4integrin, CD4 and p73 promoters. CIP treatment increased ZEB1 binding capacity to all the probes assayed with all the RRLs but HD. Binding was competed by either anti-ZEB1 antibodies or an excess of cold oligonucleotides. COS/CHO cells were transfected with same vectors and CD4/p73-luciferase promoters. Then, cells were incubated with TPA/ionomycin. As expected, luciferase activities (normalized by ßGal) were lowered by ZEB1/ZD2E/ZD2. TPA/IO treatment reverted that repression to the target genes. The results suggest that ZD2 contains main phosphorylation sites of ZEB1.

ST-P24.
TYROSINE PROTEIN PHOSPHORYLATION DURING SPERM CHEMOTAXIS MEDIATED BY PROGESTERONE
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Sperm chemotaxis is a cell guidance mechanism that orients spermatozoa towards the egg. Recently, we observed that: I) a concentration gradient of progesterone (P) is formed along the cumulus, II) a subpopulation (~10%) of capacitated spermatozoa chemotactically respond to pM P gradients, III) the steroid is probably the unique attractant secreted by the cumulus cells, and IV) the molecular pathways involved in the chemotactic response mediated by P are mAC-cAMP-PKA and sGC-cGMP-PKG, in addition to calcium mobilization. The aim of this work was to further study the sperm chemotaxis mediated by P by verifying at which step of the signal transduction pathway, tyr-protein phosphorylation (if any) takes place. Capacitated human spermatozoa were stuck to a chemotaxis chamber and then confronted to pM concentration of P. By means of immunohistochemistry, we observed that only acrosome intact capacitated spermatozoa expressed a specific pattern of tyr-protein phosphorylation in the sperm equatorial region and tail, which was activated during chemotaxis, by mAC-cAMP-PKA pathway. These results suggest that during sperm chemotaxis mediated by P, the proteins are phosphorylated in tyr in the sperm equatorial region and tail, at early steps of the signal transduction pathway.

ST-P25.
EXTRACELLULAR NUCLEOTIDE SIGNALING IN THE HUMAN INTESTINAL CELL LINE CACO-2
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The objective of this study was to examine the effects of exogenous ATP on the intracellular mitogen-activated protein kinase (MAPK) signaling pathways, in the human intestinal cell line Caco-2, which express functional P2Y and P2X receptors. The current study demonstrated that ATP activated ERK1/2, JNK1/2 and p38 MAPK in a dose- and time-dependent manner. PP2 (a c-Src inhibitor), Ro318220 (a PKC inhibitor), RpcAMP (a PKA inhibitor), significantly attenuated the ATP-induced activation of MAPKs. In Caco-2 cells, ATP rapidly modulates cytosolic calcium levels ([Ca2+]i), the cell Ca2+i response to the nucleotide was rapid and transient. In addition, activation of the MAPKs cascades by ATP was suppressed by the intracellular Ca2+ chelator BAPTA-AM, 2-APB, an IP3R inhibitor, or a Ca2+-free medium (EGTA). Furthermore, a marked inhibition of ATP-dependent JNK and p38 MAPK phosphorylation was observed in cells pretreated with the EGFR antagonist, AG1478. However, ERK activation by ATP, that was followed by its nuclear translocation, was slightly affected by the EGFR antagonist.
All these findings indicate that extracellular ATP through purinergic stimulation or EGFR transactivation are effective regulators of the MAPK signaling pathways in the intestinal cell line Caco-2.

ST-P26.
17ß-ESTRADIOL INHIBITS APOPTOSIS THROUGH ACTIVATION OF MAPKS AND ESTROGEN RECEPTOR IN MUSCLE CELLS
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17ß-estradiol (E2) can sustain survival or induce apoptosis depending on the biological context of cells. We showed that E2 abrogates apoptosis in C2C12 cells involving estrogen receptors ERa and ERß at least by two different ways. One of them involves PI3K/Akt/BAD activation, which depends on both ERs. The other relates to a protective effect on mitochondria and involves mainly ERß. ERK1/2 and p38 MAPK were implicated in both mechanisms. In view of this evidence, we studied the role of ERs in the activation of MAPKs by E2. First, we evaluated by Western blot assays the activation of ERK1/2 and p38 MAPK by E2 in presence of the antagonist ICI182780. We found that ER participates in ERK1/2 but not in p38 MAPK phosphorylation. The same experiments performed with C2C12 cells transfected with siRNAs against the ER a and ß isoforms showed that E2 activates ERK1/2 through ERa. In addition, studies with E2-BSA indicated that E2-induced phosphorylation of MAPKs is not related to the participation of receptors localized in the plasma membrane. To validate the use of the C2C12 cell line, we evaluated the function of MAPKs in the antiapoptotic action of E2 in primary cultures of mouse skeletal muscle cells. Comparable results were obtained. Altogether, these findings raise the possibility that abnormal regulation of apoptosis may play a role in estrogen-dependent sarcopenia.

ST-P27.
1,25(OH)$_2$-VITAMIN D$_3$ACTIVATION OF MAPKS IN PROLIFERATING SKELETAL MUSCLE CELLS
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1,25(OH)$_2$-vitamin D$_3$ [1,25] is a steroid hormone that rapidly elicits intracellular signals in various tissues. ERK1/2 and p38 are MAPK signaling pathways which mediate fast responses to extracellular stimuli. Upstream stimulation of MAPKs through c-Src by 1,25 in skeletal myoblasts has been previously demonstrated in our laboratory. In this work, the role of caveolae components in the fast effects of the hormone was investigated in the skeletal muscle cell line C2C12. We show by Western blot that 1,25–dependent ERK1/2 and p38 phosphorylation and c-Src activation are suppressed by disassembling caveolae with methyl-beta cyclodextrin (MßCD). Caveolin-1 (cav-1) is one of the main molecules of caveolae. Therefore, we studied the relationship between cav-1 and c-Src. Immunocytochemical assays showed a basal cav-1 and c-Src colocalization at the plasma membrane. 1,25 abolished colocalization of cav-1 and c-Src only in absence of MßCD. Experiments on the localization of the vitamin D receptor (VDR) indicated that VDR translocation to the plasma membrane occurs in cells treated with 1,25. In cells exposed to MßCD the VDR is present only in the nucleus. These data suggest that caveolae/cav-1 are involved in c-Src–dependent MAPK activation by 1,25(OH)$_2$D$_3$ through the VDR in skeletal muscle cells, providing information on the mechanism of initiation of the non-genomic hormone signal.

ST-P28.
INTEGRATION OF MULTIPLE STIMULI BY THE MAPK NETWORK IN S CEREVISIAE
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Cells are usually exposed to multiple signals, which often act together to affect cell decisions. Here we studied how budding yeast integrates information when MAPK pahtways are stimulated at the same time. We chose the mating pheromone response and the high osmolarity (HOG) response pathways, which share some components. In the presence of constant input, while response to pheromone is stable over time, the response to high osmolarity is transient, since the output of this pathway increases the internal glycerol concentration to match the external osmolarity. To study their interaction, we measured the activity of each pathway in cells expressing specific fluorescent protein-based transcriptional reporters. Co-stimulation with high osmolarity and pheromone showed that cells respond to both stimuli independently, with no correlation in their level of activation in single cells. Surprisingly, a 90 min pheromone pretreatment changed the HOG transcriptional response from transient to sustained. This change required the activation by pheromone of another MAP kinase pathway, the cell wall integrity pathway (CWI). Our data suggest that the CWI pathway counteracts the HOG pathway by opening of the glycerol transporter Fps1. Loss of glycerol causes an osmotic imbalance that in turn activates Hog1. Thus, the HOG pathway seems to be able to integrate inputs from other cellular pathways.

ST-P29.

DYNAMICS OF G-PROTEIN MEDIATED MEMBRANE RECRUITMENT OF A MAPK SCAFFOLD PROTEIN IN BUDDING YEAST

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We are interested in understanding how cells process external information to make decisions. Here we study the yeast mating pheromone response, a prototypic signaling pathway comprised of two modules, a G protein coupled receptor, and a MAP kinase cascade, which activates downstream targets necessary for mating. At the interface between these modules lies the scaffold protein Ste5, which simultaneously binds all three MAPK cascade components and membrane-tethered Gß?, released from Ga by pheromone bound receptor. The aim of this work is to understand the initiation of signal transmission. To do that, we quantified recruitment of YFP-Ste5 to the membrane in live cells under the confocal fluorescence microscopy, with fast time resolution within the first few minutes after pheromone stimulation. Using our software Cell-ID to extract quantitative information from images we analyzed the time and dose dependency of recruitment. Our results show that the level of Ste5 recruitment matches quantitatively the fraction of occupied receptor and the level of pheromone-induced gene expression (Dose Response Alignment). Paradoxically, Ste5 recruitment reaches steady-state before pheromone reaches equilibrium with its receptor. Modeling efforts are under course to explain these features of the system.

ST-P30.

SIGNALING PATHWAYS INVOLVED IN c-FOS mRNA DECAY

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In the signal transduction field, molecular events involved in the activation of cellular responses are far better understood than its inactivation. It is also common to assume that phosphorylation is a synonym to activation. We are interested in the mechanisms by which the cell keeps its integrity not only activating, but also deactivating these same pathways. We have been studying the signal transduction pathways that regulate mRNA and protein expression for years and lately we have focused on c-fos, an immediate early responsive gene of the AP-1 family as a model. We have observed that endogenous c-fos mRNA levels peak shortly after cells are stimulated with growth factors and sharply decrease afterwards. Certain proteins known as AUBPs bind to specific motifs (AREs) in the 3' untranslated (3'UTR) region of mRNAs changing its stability. With the objective of dissecting the primary elements that lead to c-fos mRNA decay, we have subcloned the c-fos 3'UTR into a reporter vector downstream of a luciferase gene with a tetracycline responsive promoter. We observed that stability of both recombinant luciferase-fos mRNA and endogenous c-fos mRNA is dependent on SAPK activity. In addition we have identified some proteins that bind to the 3'UTR of c-fos as possible targets for phosphorylation by SAPKs.

ST-P31.

ACTH ACTIVATES THE PROTEIN TYROSINE PHOSPHATASES PTP1D AND PTP-PEST IN ADRENOCORTICAL CELLS

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We have already demonstrated that ACTH activates protein tyrosine phosphatases of 80 and 115 kDa (PTP80, PTP115) in the cytosol of rat adrenal zona fasciculata (rZF). Since PTP activity exerts a crucial role in steroidogenic cells, the aim of this work was to identify/characterize these enzymes. An antibody against PTP1D, a 72 kDa PTP, revealed a protein of 80 kDa when proteins of rZF and Y1 adrenocortical cells were analyzed by Western blot. In-gel PTP assay of rZF proteins precipitated with the anti-PTP1D antibody revealed a band of activity corresponding to a 80 kDa protein, being the activity of the samples obtained from ACTH-treated rats higher than controls. Immunoprecipitation with anti-PTP1D antibody of in vitro PKA phosphorylated rZF cytosolic proteins rendered an 80 kDa phosphorylated protein, detected by autoradiography, and in-gel assay of immunoprecipitates revelead a phosphorylation-dependent increase of PTP activity. Regarding PTP115, an anti-PTP-PEST antibody recognized a 115 kDa protein in Y1 cells, in rZF and also in a partially purified sample of PTP115. This sample, that displayed only one band of activity (115 kDa) in the in-gel assay, showed changes in the kinetic parameters after in vitro PKA phosphorylation. We conclude that the ACTH-activated PTPs of 80 and 115 kDa are PTP1D and PTP-PEST and that both enzymes are regulated by PKA-mediated phosphorylation.

ST-P32.

TG1019, AN EICOSANOID RECEPTOR, IN STEROIDOGENIC CELL FUNCTION

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Hormonal regulation of steroidogenesis involves arachidonic acid (AA) release and metabolization through 5-lipoxygenase pathway. One of the products of this pathway, 5-hidroperoxyeicosatetraenoic acid (5-HpETE), acts as a modulator of the activity of the steroidogenic acute regulatory (StAR) protein promoter. Besides, the TG1019 is a membrane protein with high affinity and response to 5-HpETE, among other AA derivatives. The aim of our work was to elucidate whether this receptor may be involved in steroidogenesis. MA10 cells (murine Leydig testicular cell line) were used to evaluate the effect of docosahexaenoic acid (DHA), an antagonist of the receptor, on steroid production and StAR promoter activity. While the treatment of MA-10 cells with 8Br-cAMP (0.5 mM) during 5 hs increased the levels of progesterone 20-fold compared to control ($p<0.01$), simultaneous treatment with DHA (10 µM) reduced steroid production by 66% ($p<0.01$). This partial inhibition is due to the fact that AA per se or other of its metabolites would exert TG1019-independent effects. Similarly, StAR promoter activity increased 4-fold by 8Br-cAMP treatment ($p<0.01$) and DHA decreased this stimulatory effect by 50% ($p<0.01$). These results lead us to conclude that AA might modulate StAR promoter activity and steroidogenesis partially through 5-HpETE production and activation of a membrane receptor, such as Tg1019.

ST-P33.

BCL-X_L MEDIATES EGF DEPENDENT CELL SURVIVAL ON HC11 MAMMARY EPITHELIAL CELLS

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EGF activates signalling pathways associated with cell proliferation and apoptosis. The HC11 mouse mammary epithelial cell line is a useful model system for studying mammary cell proliferation and differentiation. We have previously demonstrated that EGF reverts apoptosis of confluent HC11 cells starved by serum deprivation and increases Bcl-X_L (antiapoptotic) mRNA and protein levels; being PI3K the main kinase involved on this regulation. *bcl-x* gene expression is controlled by five alternative promoters. Here, we studied the relevance of Bcl-X as mediator of EGF-dependent cell survival and analysed which promoter/s is/are involved on *bcl-x* induction. RNA interference experiments showed that viability felt down when cells were incubated with *bcl-x* siRNA independently of the presence of EGF. Quantification (by RT-qPCR) of *bcl-x* transcripts generated from the different promoters showed that only promoter 1 (P1) transcripts increase upon EGF addition. This induction is blocked by the PI3K inhibitor LY294002. HC11 stable cells for reporter vectors expressing the luciferase gene downstream each *bcl-x* promoter were generated and confirmed the EGF activation of P1*bcl-x* and its dependence of PI3K. Conclusion: Bcl-X_L is a key anti-apoptotic protein critical for mediating EGF cell survival on mammary epithelial cells. EGF-dependent PI3K activity increases Bcl-X_L expression by regulating P1.

ST-P34.

GANGLIOSIDE MODULATION OF GM-CSF-MEDIATED PROLIFERATION

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Granulocyte-macrophage colony-stimulating factor (GM-CSF) is one of the major hematopoietic cytokine involved in proliferation and differentiation of myeloid lineages. Gangliosides are sialic acid-containing glycosphingolipids largely related to modulation of growth factor receptors. Our previous work showed that gangliosides were required for optimal mielossuportive capacity of stromal cells. Here, we study the involvement of gangliosides on GM-CSF-mediated proliferation of the myeloid precursor cell lineage, FDC-P1. Cells previously treated with a glucosylceramide synthase inhibitor (10μM D-PDMP for 48h) to deplete endogenous gangliosides or treated with exogenously added gangliosides GM1, GM3 and GD1a (20μM) were stimulated with 2ng/ml GM-CSF for 24h and the proliferation monitored in both experimental conditions. While addition of GM3 or GD1a, but not GM1, enhanced the proliferation at 24h, ganglioside depletion reduced the proliferative response. Interestingly, addition of GD1a, but not GM3, was able to restore the proliferation in ganglioside-depleted FDC-P1 cells. Our ongoing study aims to elucidate whether ganglioside modulation of GM-CSF-mediated proliferation occurs by GM-CSF receptor activation, expression and/or association with regulatory molecules located at the plasma membrane.

Supported by CNPq, CAPES, CONICET, FONCYT, SECYT-UNC.

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