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- SAIB -

XL Annual Meeting

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XL Reunión Anual

SOCIEDAD ARGENTINA DE INVESTIGACIÓN BIOQUÍMICA Y BIOLOGÍA MOLECULAR

December 5-8, 2004

Iguazú, Misiones
República Argentina

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International Union of Biochemistry and Molecular Biology (IUBMB)

European Molecular Biology Organization (EMBO)

Panamerican Association for Biochemistry and Molecular Biology (PABMB)

SAIB 2004 CONGRESS OVERVIEW

Sunday, December 5th	Monday, December 6th	Tuesday, December 7th	Wednesday, December 8th
	9:00-10:30 **Posters I**	9:00-10:30 **Posters II**	9:00-10:30 **Posters III**
	✳ **Symposium** ✳ Protein Phosphorylation and Bioregulation I	✳ **Symposium** ✳ Protein Phosphorylation and Bioregulation II	
	10:30-11:30 **Lecture** Dr. George Thomas	10:30-11:30 **"PABMB" Lecture** Dr. Tony Hunter	10:30-11:30 **Lecture** Dr. Francesco Blasi
	11:30-12:00 *Coffee Break*	11:30-12:00 *Coffee Break*	11:30-12:00 *Coffee Break*
12:00-16:00 Registration (Secretary of the Congress)	12:00-13:00 **Lecture** Dr. Alessandro Capponi	12:00-13:00 **Lecture** Dr. José Lemos	12:00-13:00 **Lecture** Dr. Jorge Martín Perez
	13:00-13:30 **Selected Abstracts** ST-C13 y ST-C14	13:00-13:30 **Selected Abstracts** ST-C15 y ST-C16	13:00-13:30 **Selected Abstracts** ST-C17 y ST-C18
	13:30-15:00 *Lunch*	13:30-15:00 *Lunch*	13:30-15:00 *Lunch*
	13:30-14:45 **Technical Workshop** "Proteomics"	13:30-14:45 **Technical Workshop** "Proteomics"	13:30-14:45 **Technical Workshop** "Microarrays"
16:00-18:00 **Oral Communications** Sala A MI (C1-C8); Sala B ST (C1-C4); LP (C1-C3); Sala C PL (C1-C7)	15:00-17:00 **Oral Communications** Sala A CB (C1-C7); Sala B MI (C9-C16); Sala C PL (C8-C14)	15:00-17:00 **Oral Communications** Sala A ST (C5-C7); LP (C4-C7); Sala B CB (C8-C15); Sala C ES (C1-C7)	15:00-17:00 **Oral Communications** Sala A CB (C16-C21); BT (C1-C2); Sala B ST (C8-C12); LP (C8-C10); Sala C MI (C17-C24)
	17:00-18:00 **"Alberto Sols" Lecture** Dr. Carlos López Otín	17:00-18:40 ✳ **Symposium** ✳ Plant Biochemistry and Molecular Biology Dr. Estela M. Valle Dr. Miguel A. Ballicora Dr. Joseph C. Polacco Dr. William C. Plaxton	17:00-18:00 **Lecture** Dr. Dennis E. Vance
18:00-18:45 *Coffee Break*	18:00-18:30 *Coffee Break*		18:00-18:30 *Coffee Break*
18:45-19:00 ◆ Open Ceremony ◆	18:30-20:00 ✳ **Symposium** ✳ Cell Biology Dr. Bruno Goud Dr. Keith Burridge Dr. Alfredo Cáceres	18:40-19:10 *Coffee Break*	18:30-19:30 **Forty years of SAIB** Dr. Rodolfo Brenner
19:00-20:00 **"Opening Lecture"** Dr. James L. Maller		19:10-20:10 **Lecture** Dr. Bernard Schimmer	19:30-20:30 **Closing Lecture** Dr. R. Dalla Favera
20:00-21:00 **"EMBO" Lecture** Dr. Chris Lamb	20:00-21:00 **Lecture** Dr. Roberto Kolter	20:30 **SAIB Assembly**	
21:00 ◆ *Welcome Party* ◆			22:00 ◆ *Closing Dinner* ◆

****BT**: Biotechnology; **CB**: Cell Biology; **ES**: Enzymology and Structural Biology; **LP**: Lipids; **MI**: Microbiology; **PL**: Plant Biochemistry and Molecular Biology; **ST**: Signal Transduction and Protein Phosphorylation;

PROGRAM

SUNDAY, December 5, 2004

12:00 – 16:00 **Registration** (Secretary of the Congress)

16:00 – 18:00 **ORAL COMMUNICATIONS**
MICROBIOLOGY
(SALA A)

Chairpersons: Dr. Juan Diaz Ricci (Instituto Superior de Investigaciones Biológicas, Tucumán).
Dr. Fernando C. Soncini (Universidad Nacional de Rosario).

16:00-16:15 MI-C1 NOVEL ROLE OF THE TRANSCRIPTION FACTOR SPO0A FOR THE MULTICELLULAR BEHAVIOUR OF *BACILLUS SUBTILIS*.
Rovetto A., Saball E., Salvarrey M., Goñi A. and Grau R.

16:15-16:30 MI-C2 TEMPERATURE REGULATION OF THE ANTHRAX TOXIN GENES IN *BACILLUS SUBTILIS*
Mansilla, M. C. and De Mendoza, D.

16:30-16:45 MI-C3 YojI OF *ESCHERICHIA COLI* FUNCIONTS AS A MICROCIN J25 PUMP.
Delgado Mónica, Vincent Paula, Farías Ricardo N., and Salomón Raúl A.

16.45-17:00 MI-C4 EL TOR HAEMOLYSIN FROM *VIBRIO CHOLERAE* INDUCES APOPTOSIS AND INCREASESINTRACELLULAR Ca^{2+} LEVELS IN HUMAN INTESTINAL CELLS.
Saka, Héctor A.; Bidinost, Carla; Bonacci, Gustavo; Chiabrando, Gustavo and José L Bocco.

17:00-17:15 MI-C5 CHARACTERIZATION OF F0.F1-ATPASE MUTANTS AND ANALYSIS OF THEIR CONTRIBUTION TO THE ACID TOLERANCE MECHANISM IN PNEUMOCOCCUS.
Cortes P., Piñas G., Regueira M., Albarracin A., Echenique J.

17:15-17:30 MI-C6 MEMBRANE TOPOLOGY ANALYSIS OF CYCLIC GLUCAN SYNTHASE, A VIRULENCE DETERMINANT OF *BRUCELLA ABORTUS*.
Ciocchini, Andrés E.; Roset M. S., Iñón de Iannino N. and Ugalde R.A.

17:30-17:45 MI-C7 PhoP/PhoQ REGULON: THE RstA/RstB SYSTEM AND A NOVEL REGULATORY CASCADE.
Cabeza, M. Laura, Aguirre, Andrés, Spinelli, Silvana, Soncini, Fernando C. and García Véscovi, Eleonora.

17:45-18:00 MI-C8 *mgtA* AND MAGNESIUM HOMEOSTASIS IN *Salmonella*. THE ROLE OF THE 5' UNTRANSLATED REGION ELEMENTS.
Spinelli Silvana, Pontel Lucas, García Véscovi Eleonora and Soncini Fernando C.

SIGNAL TRANSDUCTION AND PROTEIN PHOSPHORYLATION and LIPIDS
(SALA B)

Chairpersons: Dra. Norma Sterin de Speziale (Universidad de Buenos Aires).
Dra. Ana Russo de Boland (Universidad Nacional del Sur, Bahia Blanca).

16:00-16:15 ST-C1 SIGNAL COMPLEXES FORMED BY PRESYNAPTIC CALCIUM CHANNELS AND ORL1 RECEPTORS.
Altier, Christophe, Khosravani, Houman, Beedle, Aaron, McRory, John, and Zamponi, Gerald W.

16:15-16:30 ST-C2 SIGNAL TRANSDUCTION VIA SMALL REGULATORY RNAS IN A PSEUDOMONAS FLUORESCENS STRAIN.
Valverde, Claudio; Heeb, Stephan and Haas, Dieter.

16:30-16:45 ST-C3 EXTRACELLULAR INOSINE MODULATES ERK ½ AND P38 PHOSPHORYLATION IN CULTURED SERTOLI CELLS: POSSIBLE PARTICIPATION IN TNF-α MODULATION OF ERK ½.
Souza, Luiz F.; Horn, Ana P.; Gelain, Daniel P.; Jardim, Fernanda R. and Bernard, Elena A.

16.45-17:00 ST-C4 DIFFERENTIAL PHOSPORYLATION OF CNBP DURING ZEBRAFISH EARLY DEVELOPMENT.
Lombardo, Verónica A.; Weiner, Andrea; Armas, Pablo and Calcaterra, Nora B.

17:00-17:15 LP-C1 CHANGES IN LIPIDS CONTAINING VERY LONG CHAIN POLYUNSATURATED FATTY ACIDS (VLCPUFA) IN EXPERIMENTALLY CRYPTORCHID RAT TESTES.
Furland, N.E.; Maldonado, E.N. and Aveldaño, M.I.

17:15-17:30 LP-C2 EFFECTS OF DOXORUBICIN ON RAT TESTICULAR LIPIDS WITH VERY LONG CHAIN POLYUNSATURATED FATTY ACIDS (VLCPUFA).
Zanetti, S.R.; Maldonado, E.N. and Aveldaño, M.I.

17:30-17:45 LP-C3 EFFECT OF FENOFIBRATE AND INSULIN ON THE BIOSYNTHESIS OF UNSATURATED FATTY ACIDS IN THE STREPTOZOTOCIN RAT.
Montanaro, Mauro A.; González, María S.; Bernasconi, Ana M. and Brenner, Rodolfo R.

PLANT BIOCHEMISTRY AND MOLECULAR BIOLOGY
(SALA C)

Chairpersons: Dra. Maria Elena Alvarez (Universidad Nacional de Córdoba).
Dra. María L. Tomaro (Universidad de Buenos Aires).

16:00-16:15 PL-C1 ALTERING *ASR*1 GENE EXPRESSION HAS DRAMATIC EFFECTS ON METABOLISM IN DIFFERENT PLANT SPECIES.
Frankel N., Balbo I., Mazuch J., Iusem N., Fernie A.R. and Carrari F.

16:15-16:30 PL-C2 *HAHB-10*, A SUNFLOWER HOMEOBOX-LEUCINE ZIPPER GENE, IS INVOLVED IN THE RESPONSE TO DARK/LIGHT CONDITIONS.
Rueda, Eva C., Dezar, Carlos A., González, Daniel H., Chan, Raquel L.

16:30-16:45 PL-C3 FUNCTIONAL CHARACTERIZATION OF A POTATO TRANSCRIPTIONAL COACTIVATOR (StMBF1) UNDER DIFFERENT STRESS CONDITIONS.
Arce, Débora; Tonón, Claudia; Godoy, Verónica and Casalongué, Claudia.

16.45-17:00 PL-C4 A COMPREHENSIVE ANALYSIS OF PLOIDY-REGULATED GENE EXPRESSION IN *Paspalum notatum.*
Martelotto, Luciano G.; Ortiz, Juan Pablo A.; Espinoza, Francisco; Quarin, Camilo L. and Pessino, Silvina C.

17:00-17:15 PL-C5 DISRUPTION OF GAMMA CARBONIC ANHYDRASE 2 GENE CAUSES DRAMATIC CHANGES IN COMPLEX I ASSEMBLY AND RESPIRATION IN ARABIDOPSIS CELL CULTURES.
Perales, Mariano, Colaneri, Alejandro, Villarreal, Fernando, Braun, Hans-Peter and Zabaleta, Eduardo.

17:15-17:30 PL-C6 HEME OXYGENASE EXERTS A PROTECTIVE ROLE AGAINST OXIDATIVE STRESS IN SOYBEAN LEAVES.
Guillermo O. Noriega, Karina B. Balestrasse, Alcira Batlle and Maria L. Tomaro.

17:30-17:45 PL-C7 NITRIC OXIDE IMPROVES THE HEALING RESPONSE IN POTATO LEAVES.
París, Ramiro; Lamattina, Lorenzo and Casalongué, Claudia.

18:00-18:45 **Coffee Break**

18:45-19:00 **OPEN CEREMONY** (SALA A)

19:00-20:00 **OPENING LECTURE**
Dr. James L. Maller
(Howard Hughes Medical Institute and Department of Pharmacology
University of Colorado School of Medicine, Denver. USA)
"*The Role of Aurora Kinases in Mitotic Entry and Exit*"
Chairperson: Dr. Norma Sterin de Speziale (Universidad de Buenos Aires).

20:00-21:00 "**EMBO" LECTURE**
Dr. Christopher J. Lamb
(John Innes Centre, Norwich, U.K.)
"Plant diseases and pest resistance mechanisms"
Chairperson: Dr. Ricardo Wolosiuk (Fundación Instituto Leloir, Buenos Aires).

21:00 **Welcome Party**

MONDAY, December 6, 2004

9:00-10:30 **POSTER SESSION I**

10:30-13:30 **SYMPOSIUM**
"PROTEIN PHOSPHORYLATION AND BIOREGULATION I"
Sponsored by "**International Union of Biochemistry and Molecular Biology"**
(SALA A)

10:30-11:30 **LECTURE**
Dr. George Thomas
(Department of Biochemistry and Cell Biology. University of Cincinnati. USA)
*"**The mTOR/S6K Signaling Pathway: The Role of the TSC1/2 Tumor Suppressor Complex and the Proto-oncogene Rheb**"*
Chairperson: Dr. Ernesto Podestá (Universidad de Buenos Aires).

11:30-12:00 **Coffee break**

12:00-13:00 **LECTURE**
Dr. Alessandro Capponi
(Division of Endocrinology and Diabetology, University Hospital, Switzerland)
"Control of a mitochondrial cholesterol transport (StAR) expression. Role of Chromatin remodelling-ChIP, ERK1/2 activation of CEH and MKP-1 regulation"
Chairperson: Dr. Ricardo Boland (Universidad Nacional del Sur-Bahía Blanca).

13:00-13:30 **Selected Abstracts**

13:00-13:15 ST-C13 N-TERMINAL c-FOS TYROSINE DEPHOSPHORYLATION IS REQUIRED FOR c-FOS/ER ASSOCIATION AND PHOSPHOLIPID SYNTHESIS ACTIVATION.
Portal M.M., Ferrero G.O., and B.L. Caputto.

13:15-13:30 ST-C14 TYR-374 AND 395 WTHIN PKC-ζ ARE POTENTIAL AP-2 INTERACTION SITES REQUIRED FOR NA^+,K^+-ATPase ENDOCYTOSIS IN RESPONSE TO DOPAMINE.
Mendez Carlos F., Efendiev Ryad, Pedemonte Carlos, Podestá Ernesto, Bertorello Alejandro.

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

13:30-14:45 **Technical Workshop** (SALA B)
Proteomics Club Meeting
Dra. Andrea Llera (Fundación Instituto Leloir)

15:00-17:00 **ORAL COMMUNICATIONS**
CELL BIOLOGY
(SALA A)

Chaipersons: Dr. Jose Luis Daniotti (Universidad Nacional de Córdoba).
Dr. Alberto Kornblit (Universidad de Buenos Aires).

15:00-15:15 CB-C1 SCAFFOLDING PROTEINS AND RHO SIGNALING.
Rafael García-Mata and Keith Burridge.

15:15-15:30 CB-C2 STUDYING ACTIN DYNAMICS IN Y1 ADRENAL CELLS CONTROLLED BY ACTH: PARTICIPATION OF PROTEIN KINASE A AND TYROSINE KINASE .
Colonna Cecilia and Podestá Ernesto J.

15:30-15:45 CB-C3 EFFECT OF BRADYKININ IN FOCAL CONTACT (FC) ORGANIZATION OF IMMATURE RAT RENAL PAPILLARY CELLS.
María Laura Gagliano, Gabriela Marquez, Norma Sterin-Speziale.

15:45-16:00 CB-C4 PHOSPHOLIPASE C (PLC) IS INVOLVED IN BRADIKYNIN (BK)-INDUCED FOCAL CONTACT (FC) RESTRUCTURATION.
G. Márquez, L. Gagliano, D. Serrano, N. Sterin-Speziale.

16:00-16:15 CB-C5 STOP AND MBP ARE EFFECTORS OF MICROTUBULE COLD STABILITY IN OLIGODENDROCYTES.
Galiano, Mauricio; Bosc, Christophe; Andrieux, Annie; Job, Didier and Marta E. Hallak.

16:15-16:30 CB-C6 INTRACELULAR DISTRIBUTION OF THE GTPase Rab24 DURING MITOSIS.
Munafó, Daniela, Militello, Rodrigo, Monier, Solange, Goud, Bruno and Colombo, María Isabel.

16:30-16:45 CB-C7 STARVATION INDUCES FUSION BETWEEN AUTOFAGIC VACUOLES AND MVBs IN K562 CELLS.
Fader CM, Sanchez D and Colombo MI.

MICROBIOLOGY
(SALA B)

Chaipersons: Dr. Hugo Lujan (Universidad Nacional de Córdoba).
Dra. María Teresa Tellez-Iñon (INGEBI, Buenos Aires).

15:00-15:15 MI-C9 REGULATION OF SPORULATION AND ENTEROTOXIN PRODUCTION OF *Clostridium perfringens.*
Valeria Philippe, Lelia Orsaria, María E. Pedrido and Roberto Grau.

15:15-15:30 MI-C10 EFFECTS OF LEPTOSPIRAL PROTEINS ON HUMAN ENDOTHELIAL CELLS.
Gómez, Ricardo M; Gamberini, Marcia; Negrotto, Soledad; Schattner, Mirta; Von Atzinger, Marina; Leite, Luciana; Martins, Elizabeth; Ho, Paulo; Nascimento, Ana Lucia

15:30-15:45 MI-C11 PRO APOPTOTIC ACTIVITY OF *BORDETELLA BRONCHISEPTICA* LIPOPOLYSACCHARIDE IN EPITHELIAL AND IMMUNE HOST CELLS.
Sisti F., Fernández J., Bottero D., Gaillard E., Graieb A. y Hozbor D.

15:45-16:00 MI-C12 INFLUENCE OF EXOPOLYSACCHARIDE COMPOSITION AND N-SOURCE LIMITATION ON INFECTIVITY OF *BRADYRHIZOBIUM JAPONICUM.*
Quelas, J.I., S.L. López García, A. Casabuono, M.J. Althabegoiti, E. Mongiardini, J. Pérez Giménez, A. Couto, A.R. Lodeiro.

16:00-16:15 MI-C13 ISOLATION AND CHARACTERIZATION OF AN ACID SENSITIVE Tn*5* MUTANT OF THE ALFALFA NODULATING Or191-LIKE RHIZOBIA.
Del Papa, M.F.; Draghi, W.O.; Pistorio, M.; Lagares, A.

16:15-16:30 MI-C14 HIGHER BACTERIAL MOTILITY ENHANCES INFECTIVITY OF *BRADYRHIZOBIUM JAPONICUM.*
Althabegoiti, M.J. S.L. López García, E. Mongiardini, J. Pérez Giménez, J.I. Quelas, , A.R. Lodeiro.

16:30-16:45 MI-C15 TWO SALT TOLERANT RHIZOBACTERIA BELONGING TO OCHROBACTRUM GENUS PROMOTE MAIZE GROWTH
Príncipe Analía and Mori Gladys.

16:45-17:00 MI-C16 SEVERAL POLYSACCHARIDES ARE INVOLVED IN CELL TO CELL INTERACTIONS AND BIOFILM FORMATION IN RHIZOBIUM LEGUMINOSARUM BV. VICIAE.
Russo, Daniela M.; Williams, Alan; Downie, J. Allan and Zorreguieta, Angeles.

PLANT BIOCHEMISTRY AND MOLECULAR BIOLOGY
(SALA C)

Chaipersons: Dr. Lorenzo Lamattina (Universidad Nacional de Mar del Plata).
Dra. Raquel Chan (Universidad Nacional del Litoral).

15:00-15:15 PL-C8 NITRIC OXIDE EFFECT ON LIGHT-DEPENDENT STOMATAL OPENING PROCESSES.
Carlos García-Mata and Lorenzo Lamattina.

15:15-15:30 PL-C9 ANALYSIS OF THE CYTOCHROME C PROMOTERS FROM ARABIDOPSIS.
Welchen, Elina; Viola, Ivana; Chan, Raquel and Gonzalez, Daniel.

15:30-15:45 PL-C10 EFFECT OF CADMIUM STRESS ON PROTEIN SYNTHESIS AND DEGRADATION IN SUNFLOWER (*Helianthus annuus* L.) LEAVES.
L.B. Pena, L.A. Pasquini, M.L. Tomaro and S.M. Gallego.

15:45-16:00 PL-C11 CHLOROPLAST 2-CYS PEROXIREDOXIN. MODULATION OF CHAPERONE ACTIVITY BY DITHIOTHREITOL AND ATP-MG^{2+}.
Martin Aran, Patricio Craig, Daniel Caporaletti, Alejandro Senn and Ricardo A. Wolosiuk.

16:00-16:15 PL-C12 BABA AS CHEMICAL INDUCER OF POTATO RESISTANCE TO Phytophthora infestans.
Olivieri, Florencia P.; Guevara, María G.; Wolski, Erika; Gonzalez Altamiranda, Erika; Lobato, Candela; Daleo, Gustavo R. and Andreu Adriana B.

16:15-16:30 PL-C13 AN EXTRACELLULAR LIPID TRANSFER PROTEIN IS PARTIALLY BOUND TO MEMBRANES.
Pagnussat, Luciana, Regente Mariana, de la Canal Laura.

16:30-16:45 PL-C14 REVERSE ALTERATIONS OF THE GENOME DURING SERIAL PLOIDY LEVEL MODIFICATION IN Eragrostis curvula.
Mecchia, Martín A.; Martelotto, Luciano G.;Polci, Pablo; Cardone, Susana; Selva, Juan Pablo; Echenique Viviana and Pessino, Silvina C.

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

Dr. Carlos Lopez Otin

(Departamento de Bioquimica y Biologia Molecular, Facultad de Medicina Universidad de Oviedo. Oviedo, Spain)

"Exploring the human and mouse degradomes: new roles of proteases in cancer"

Chairperson: Dr. Hugo J.F. Maccioni (Universidad Nacional de Córdoba).

18:00-18:30 **Coffee Beak**

18:30-20:00 **SYMPOSIUM**

CELL BIOLOGY

(SALA A)

Chairpersons: Dra. Marisa Colombo (Universidad Nacional de Cuyo).
Dra. Beatriz Caputto (Universidad Nacional de Córdoba).

Speakers:

18:30-19:00 CB-S1 **Dr. Bruno Goud** (Centre National de la Recherche Scientifique -CNRS- Institut Curie, Paris, France).
«Role of the Golgi-associated Rab6 GTPase during cell cycle».

19:00-19:30 CB-S2 **Dr. Keith Burridge** (Department of Cell and Developmental Biology and Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, USA).
"Rho GTPases in cell adhesion and migration".

19:30-20:00 CB-S3 **Dr. Alfredo Cáceres** (Instituto Mercedes Martín Ferreira, Córdoba).
"Regulation of Golgi dynamics and vesicle trafficking by LIMK1".

20:00-21:00 **LECTURE**
Dr. Roberto Kolter
(Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, USA).
"Bacterial Biofilms: The Matrix Revisited"
Chairperson: Dr. Diego de Mendoza (Universidad Nacional de Rosario).

TUESDAY, December 7, 2004

9:00-10:30 **POSTER SESSION II**

Cell Biology	CB P25-CB P45	(pp. 60-65)
Enzymology and Structural Biology	ES P15- ES P27	(pp. 75-78)
Lipids	LP P15- LP P25	(pp. 82-84)
Microbiology	MI P30- MI P58	(pp. 92-99)
Plant Biochemistry and Molecular Biology	PL P19- PL P37	(pp. 111-115)
Signal Transduction and Protein Phosphorylation	ST P1 - ST P11	(pp. 119-122)

10:30-13:30 **SYMPOSIUM**
"PROTEIN PHOSPHORYLATION AND BIOREGULATION II"
Sponsored by "**International Union of Biochemistry and Molecular Biology"**
(SALAA)

10:30-11:30 **"PABMB" LECTURE**
Dr. Tony Hunter
(Molecular and Cellular Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, California USA)
"Signal transduction by protein phosphorylation"
Chairperson: Dr. Juan José Cazzulo (Universidad Nacional de San Martin).

11:30-12:00 **Coffee break**

12:00-13:00 **LECTURE**
Dr. Jose Lemos
(Worcester Foundation, University of Massachusetts Medical School. USA)
"Intracellular mechanisms of opioids on central nervous system terminals"
Chairperson: Dr. Armando Parodi (Fundación Instituto Leloir, Buenos Aires).

13:00-13:30 **Selected Abstracts**

13:00-13:15 ST-C15 PROTEIN TYROSINE PHOSPHATASES INVOLVED IN TRANSCRIPTION AND TRANSLATION OF KEY PROTEINS IN THE HORMONAL REGULATION OF STEROID SYNTHESIS.
Cornejo Maciel Fabiana; Castilla Rocío; Castillo Fernanda; Maloberti Paula; Duarte Alejandra; Poderoso Cecilia; Neuman Isabel; Gorostizaga Alejandra; Paz Cristina; Podestá Ernesto J.

13:15-13:30 ST-C16 SHP-1 PHOSPHATASE IS ACTIVATED BY ANG II AT_2 RECEPTORS IN RAT FETAL MEMBRANES.
Leonardo R. Seguin, Rodrigo S. Villarreal Sergio E. Álvarez, and Gladys M. Ciuffo.

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

13:30-14:45 **Technical Workshop** (SALA B)
Proteomics: advances and news; 2D/MS Platform
Dra. Marisa Dinocenzo (GE Healthcare Biosciences)

15:00-17:00 **ORAL COMMUNICATIONS**
SIGNAL TRANSDUCTION AND PROTEIN PHOSPHORYLATION and LIPIDS
(SALA A)

Chaipersons: Dr. Marta I. Aveldaño (Universidad Nacional del Sur, Bahia Blanca).
Dra. Gladys M. Ciuffo (Universidad Nacional de San Luis).

15:00-15:15 ST-C5 A POSSIBLE ROLE OF REDUCED PROLIDASE PHOSPHORYLATION IN FASTING – INDUCED INHIBITION OF COLLAGEN BIOSYNTHESIS.
Bankowski,Edward,;Cechowska-Pasko, Marzanna and Palka, Jerzy

15:15-15:30 ST-C6 ANTI-TRYPANOSOMA CRUZI RECOMBINANT ANTIBODIES WITH PARTIAL AGONIST EFFECTS ON CARDIAC RECEPTORS.
Smulski C, Grippo V, Labovsky V, Levy G, Ansaldi S, Gómez K, Levin M.

15:30-15:45 ST-C7 GLUCOCORTICOIDS REPRESS BCL-X_L EXPRESSION IN MOUSE THYMOCYTES THROUGH THE RECRUITMENT OF STAT5B TO THE P4 PROMOTER REGION.
L. Rocha Viegas, G.P. Vicent, M. Beato and A. Pecci.

15:45-16:00 LP-C4 PGD_2 DIFFERENTIALY REGULATES PC SYNTHESIS BY PLD AND PKC→MAPK INDEPENDENTLY-ACTIVATED PATHWAYS.
Favale N, Sterin-Speziale N, Fernández-Tome M.

16:00-16:15 LP-C5 AN ACYL-COA SYNTHETASE AS OBLIGATORY PROTEIN IN THE REGULATION OF ARACHIDONIC ACID RELEASE AND STEROIDOGENESIS.
Castillo Fernanda, Maloberti Paula, Castilla Rocío, Duarte Alejandra, Cornejo Maciel Fabiana, Paz Cristina and Podestá Ernesto J.

16:15-16:30 LP-C6 MALONYL-CoA IS A KEY METABOLITE SENSED BY GRAM POSITIVE BACTERIA TO AJUST FATTY ACID AND PHOSPHOLIPID BIOSYNTHESIS.
Schujman, Gustavo E. and de Mendoza Diego.

16:30-16:45 LP-C7 ANALYSIS OF GENES OF *Bacillus subtilis* THAT CODE FOR ENZYMES INVOLVED IN PHOSPHOLIPID BIOSYNTHESIS.
Paoletti Luciana, Schujman Gustavo E. y de Mendoza Diego.

CELL BIOLOGY
(SALA B)

Chaipersons: Dr. Luis S. Mayorga (Universidad Nacional de Cuyo, Mendoza).
Dr. José Luis Bocco (Universidad Nacional de Córdoba).

15:00-15:15 CB-C8 AUTOPHAGY INDUCTION STIMULATES THE DEVELOPMENT OF THE COXIELLA-REPLICATIVE VACUOLE.
Vázquez Cristina L; Gutierrez Maximiliano G; Munafó Daniela, Zoppino Martín; Berón Walter; Rabinovitch Michel; Colombo María I.

15:15-15:30 CB-C9 LIGAND-DEPENDENT AND INDEPENDENT INTERNALIZATION OF THE GPI ANCHOR PROTEIN uPAR FOLLOW TWO DIFFERENT MECHANISMS.
Sahores M, Cortese K, Madsen C, Chiabrando G, and Blasi F.

15:30-15:45 CB-C10 INFLAMMATION MEDIATORS REGULATE THE LRP-1 EXPRESSION IN J774 CELLS.
Ceschin D, Cáceres Leandro, Sánchez MC, Alvarez C, Chiabrando G.

15:45-16:00 CB-C11 Rab22a REGULATES TRANSFERRIN SORTING IN CHO CELLS.
Magadán Javier G, Barbieri Alejandro M, Mesa Rosana, Stahl Philip D and Mayorga Luis S.

16:00-16:15 CB-C12 AUTOREGULATION OF ZFHEP-1 IS MEDIATED BY THE BINDING TO A SPECIFIC E2-BOX.
Manavella P.A, Roqueiro G, Darling D and Cabanillas AM.

16:15-16:30 CB-C13 THE RGG BOX OF CNBP IS REQUIERED FOR RNA BINDING AND NUCLEIC ACID CHAPERONE ACTIVITY.
Armas, Pablo and Calcaterra, Nora B.

16:30-16:45 CB-C14 GENE EXPRESSION PROFILING ON THE ERBB4 MURINE MODEL OF DILATED CARDIOMYOPATHY.
Cecilia M. Hertig, Emiliano Buitrago-Emanuel, Mariana Malvicini, Henning Witt, Patricia Ruiz.

16:45-17:00 CB-C15 THE OLIGOSACCHARYLTRANSFERASE STT3P SUBUNIT IS INVOLVED IN DOLICHOL-P-P-GLYCAN STRUCTURE RECOGNITION.
Castro Olga, Movsichoff Federico and Parodi Armando J.

ENZYMOLOGY AND STRUCTURAL BIOLOGY
(SALA C)

Chaipersons: Dr. Néstor Carrillo (Universidad Nacional de Rosario).
Dra. Cristina Nowicki (Universidad de Buenos Aires).

15:00-15:15 ES-C1 SPECIFIC FEATURES OF HUMAN RNA POLYMERASE II ASSEMBLY PROCESS.
Vigneron M., Benga J., Grandemange S., Shpakovski G. and Kedinger Claude.

15:15-15:30 ES-C2 STRUCTURE-FUNCTION STUDIES OF ISOFORMS OF THE SPECIFIC SUBUNIT hRPB11 OF HUMAN RNA POLYMERASE II.
Shpakovski G.V., Shematorova E.K., Shpakovski D.G. and Proshkin S.A.

15:30-15:45 ES-C3 CARACTERIZATION OF OLIGOMERIC RAT LIVER MITOCHONDRIAL GLYCEROL-3-P ACYLTRANSFERASE.
Pellon-Maison M., Pereyra A., Coleman R.A. and González-Baró M.R.

15:45-16:00 ES-C4 SOLUTION STUDIES OF HPV-16 E2C, ITS DNA BINDING SITE AND THE COMPLEX.
Nadra A.D.; Tommaso E.; Morino V.; Trotta E.; Ferreiro D.U.; Paci M.; De Prat Gay G. and Cicero D.O.

16:00-16:15 ES-C5 SURFACE BEHAVIOR OF POLYGODIAL, AN UNCOU-PLER OF THE MITOCHONDRIAL ATP SYNTHESIS.
Castelli M.V., Ambroggio E.E., Zacchino S.A.S., Fidelio G.F., Roveri O.A.

16:15-16:30 ES-C6 A C-TERMINAL AMIDATED MICROCIN J25 DERIVATIVE HAS ANTIMICROBIAL ACTIVITY INDEPENDENT OF RNA POLYMERASE INHIBITION.
Vincent Paula A., Bellomio Augusto, F. de Arcuri Beatríz, Farias Ricardo N., Salomon Raúl A. and Morero Roberto D.

16:30-16:45 ES-C7 CLONING AND BIOCHEMICAL CHARACTERIZATION OF THREE DIFFERENT MALATE DEHYDROGENASE (MDH) ISOZYMES FROM *LEISHMANIA MAJOR*. *Fleming-Cánepa X, Leroux A, Aranda A, Cazzulo JJ and Nowicki C.*

17:00-18:40

SYMPOSIUM
PLANT BIOCHEMISTRY AND MOLECULAR BIOLOGY
(SALA A)

Chaipersons: Dr. Alberto A. Iglesias (Universidad Nacional del Litoral).
Dra. Estela M. Valle (Universidad Nacional de Rosario).

Speakers:

17:00-17:25 PL-S1 **Dra. Estela M. Valle** (Instituto de Biología de Rosario, Facultad Ciencias, Bioquímicas y Farmacéuticas, Universidad Nacional Rosario).
"Oxidative Stress and Signaling Pathways in Arabidopsis".

17:25-17:50 PL-S2 **Dr. Miguel A. Ballicora** (Dept. Biochemistry and Molec. Biology, Michigan State University, Lansing, Michigan, USA.
"Structural and Functional Insights into the Evolution of the Plant ADP-Glucose Pyrophosphorylase".

17:50-18:15 PL-S3 **Dr. Joseph C. Polacco** (Department of Biochemistry and Interdisciplinary Plant Group, University of Missouri-Columbia, USA)
"Ureide Degradation Pathway(s) in Soybean- a clue to drought-resistant N-fixation?".

18:15-18:40 PL-S4 **Dr. William C. Plaxton** (Dept. Biology and Biochemistry, Queen's University, Kingston, Ontario, Canada).
"Phosphite Blocks Phosphate Sensing in Plants and Yeast".

18:40-19:10 **Coffee Break**

19:10-20:10 **LECTURE**
Dr. Bernard Schimmer
(Banting and Best Department of Medical Research, University of Toronto. Canada)
"The global contributions of protein kinases A and C to hormone action as determined from genome-wide profiles of transcription"
Chairperson: Dr. Marcelo A. Dankert (Fundación Instituto Leloir).

20:30 **SAIB Ordinary General Assembly**

WEDNESDAY, December 8, 2004

9:00-10:30 **POSTER SESSION III**

Biotechnology	BT P1 - BT P14	(pp.51-54)
Cell Biology	CB P46-CB P69	(pp.65-71)
Microbiology	MI P59- MI P87	(pp.99-106)
Plant Biochemistry and Molecular Biology	PL P38- PL P52	(pp.115-119)
Signal Transduction and Protein Phosphorylation	ST P12- ST P22	(pp.122-124)

10:30-11:30 **LECTURE**
Dr. Francesco Blasi
Molecular Genetics Unit, Department of Molecular Biology and Functional Genomics, Universita Vita Salute San Raffaele and DIBIT, Milan, Italy.
"Molecular Mechanisms in the TALE class Transcription Factors in control of embryonic development"
Chairperson: Dr. Ricardo N. Farías (Universidad Nacional de Tucumán).

11:30-12:00 **Coffee Break**

12:00-13:00 **LECTURE**

Dr. Jorge Martín Perez

Instituto de Investigaciones Biomedicas, CSIC. Madrid, Spain

"Role of Src kinases on prolactin cell signaling"

Chairperson: Dra. María Teresa Tellez-Iñón (INGEBI-Universidad de Buenos Aires).

13:00-13:30 **Selected Abstracts**

13:00-13:15 ST-C17 THE DESK-DESR PHOSPHORYLATION CASCADE CONTROLS THE MEMBRANE LIPID FLUIDITY IN *BACILLUS SUBTILIS.*
Larisa E. Cybulski, Daniela Albanesi, María C. Mansilla and Diego de Mendoza.

13:15-13:30 ST-C18 NON PHOSPHORYLATED CREB REPRESSES GENE TRANSCRIPTION BY RECRUITING HDAC1.
Sirkin, Pablo; Ceruti, Julieta; Scassa, María and Cánepa, Eduardo.

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

13:30-14:45 **Technical Workshop** (SALA B)

Microarrays: advances and news. Codelink System.

Dra. Regina Maki (Ge Healthcare Biosciences).

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

CELL BIOLOGY and BIOTECHNOLOGY

(SALA A)

Chaipersons: Dra. Susana Genti (Universidad Nacional de Córdoba).
Dr. Gustavo Chiabrando (Universidad Nacional de Córdoba).

15:00-15:15 CB-C16 A REAPPRAISAL OF THE ROLE OF NUCLEOSIDE DIPHOSPHATASES ON YEAST *N*- AND *O*-GLYCOSYLATION.
D´Alessio, Cecilia and Parodi, Armando J.

15:15-15:30 CB-C17 REGULATORY ROLE OF ARGININE DEIMINASE IN THE BIOLOGY OF THE PRIMITIVE EUKARYOTE GIARDIA LAMBLIA.
Touz, María C and Nash, Theodore E

15:30-15:45 CB-C18 ALTERNATIVE SUBSTRATES OF THE INSECT β-ALANINE-CATECHOLAMINE LIGASE.
Pérez Martín; Curilovic Roberto; Inzillo Lorena; Mufato Jorge; Aguirre José M.; Quesada-Allué Luis A

15:45-16:00 CB-C19 HEME OXYGENASE AND OXIDATIVE STRESS IN ADRENAL CELLS.
Pomeraniec Yael; Pannunzio Vanesa; Cymeryng Cora.

16:00-16:15 CB-C20 ABIOTIC STRESS AND ANTIOXIDANT ENZYMES EXPRESSION IN SUNFLOWER LEAF DISCS.
G.G. Yannarelli, C.E. Azpilicueta, S.M. Gallego, M.P. Benavides, M.L. Tomaro.

16:15-16:30 CB-C21 CHEMOTAXIS AND HYDROCARBON BIODEGRADATION BY HALOARCHAEA.
Herrera Seitz, Karina, Sánchez, J.J. y De Castro, R.

16:30-16:45 BT-C1 EFFICIENT PRESERVATION IN SILICON OXIDE MATRIX OF ESCHERICHIA COLI PRODUCER OF RECOMBINANT PROTEINS.
Desimone M.F.; De Marzi M.C.; Copello G.J.; Bertinatto J.A.; Fernández, M.M.; Malchiodi E.L.; Diaz L.E.

16:45-17:00 BT-C2 MICROBIAL IMMOBILIZATION APPLIED TO THE SYNTHESIS OF NUCLEOSIDE ANALOGUES.
Trelles J.; Valino A.; Lewkowicz E. and Iribarren A.

SIGNAL TRANSDUCTION AND PROTEIN PHOSPHORYLATION and LIPIDS

(SALA B)

Chaipersons: Dra. Norma M. Giusto (Universidad Nacional del Sur, Bahía Blanca).
Dr. Eduardo T. Canepa (Universidad de Buenos Aires).

15:00-15:15 ST-C8 ACTIVATION OF MITOCHONDRIAL ERK1/2 IS REQUIRED FOR PKA-DEPENDENT STEROIDOGENESIS IN MA-10 CELLS.
Poderoso Cecilia, Converso Daniela, Rodríguez Victoria, Paz Cristina, Poderoso Juan J and Podesta Ernesto J.

15:15-15:30 ST-C9 SILENCING THE EXPRESSION OF MITOCHONDRIAL ACYL-COA THIOESTERASE I INHIBITS HORMONE-INDUCED STEROIDOGENESIS
Castilla Rocío, Maloberti Paula, Castillo Fernanda, Duarte Alejandra, Cornejo Maciel Fabiana, Paz Cristina and Podestá Ernesto J.

15:30-15:45 ST-C10 CHARACTERIZATION OF PYRUVATE KINASE 1 OF *SACCHAROMYCES CEREVISIAE* AS PKA SUBSTRATE.
Portela P., Moreno S. and Rossi S.

15:45-16:00 ST-C11 MODULATION OF TRACHEAL DEVELOPMENT BY THE OXYGEN SENSING PATHWAY IN *DROSOPHILA MELANOGASTER.*
Lázaro Centanin, Maximiliano Irisarri and Pablo Wappner.

16:00-16:15 ST C12 A NEW ROLE FOR THE KRÜPPEL-LIKE TRANSCRIPTION FACTOR KLF6 AS AN INHIBITOR OF c-JUN PROTO-ONCOPROTEIN FUNCTION.
Slavin, Daniela A.; Andreoli Verónica, and José Luis Bocco.

16:15-16:30 LP-C8 SPHINGOLIPID METABOLISM IN MDCK CELLS: ITS ROLE IN CELL PROLIFERATION AND SURVIVAL.
Leocata Nieto F., Sterin-Speziale N.

16:30-16:45 LP-C9 HOW IS PHOSPHOCHOLINE BIOSYNTHESIS REGULATED DURING THE CELL CYCLE?
Claudia Banchio and Dennis Vance

16:45-17:00 LP-C10 ALTERNATIVE PATHWAY FOR POLYUNSATURATED FATTY ACIDS (PUFAs) BIOSYNTHESIS IN TRYPANOSOMATIDS.
Karina Tripodi, Laura Buttigliero, Guillermo A. Petrini, Silvia G. Altabe and Antonio D. Uttaro.

MICROBIOLOGY
(SALA C)

Chaipersons: Dr. Raúl A. Salomón (Universidad Nacionalde Tucumán).
Dr. Carlos E. Argaraña (Universidad Nacional de Córdoba).

15:00-15:15 MI-C17 REGULATION OF SUCROSE METABOLISM IN *ANABAENA* SP. PCC 7120 UNDER DIAZOTROFIC GROWTH BY A GLOBAL NITROGEN REGULATOR.
Marcozzi, Clarisa; Cumino, Andrea C.; Giarrocco, Laura and Salerno, Graciela L.

15:15-15:30 MI-C18 MAPPING OF MOLECULAR DETERMINANTS OF PATHOGENICITY OF COXSAKIEVIRUS B1.
Quintana, Silvina; Romanowski, Víctor and Gómez, Ricardo M.

15:30-15:45 MI-C19 STUDIES ON DEGRADATION OF *Crithidia fasciculata* ODC OBTAINED "in vitro" WITH A TRANSCRIPTION-TRANSLATION SYSTEM.
González, N. S. and Algranati, I. D.

15:45-16:00 MI-C20 THE COMPLETE STRUCTURE OF VIPER, AN UNUSUAL LTR RETROELEMENT PRESENT IN TRYPANOSOMAS BUT NOT IN *LEISHMANIA MAJOR.*
Mariano J. Levin, Hernan Lorenzi, Veronica dos Santos Ferreira, German Robledo

16:00-16:15 MI-C21 IDENTIFICATION AND PARTIAL PURIFICATION OF QUINONE OXIDOREDUCTASE (QOR) ACTIVITIES PRESENT IN *TRYPANOSOMA CRUZI.*
Cannata Joaquin J.B.; Podestá Dolores; Maugeri Dante; Agüero Fernán; Hellman Ulf and Cazzulo Juan J.

16:15-16:30 MI-C22 DIRECT MOLECULAR TYPING OF *T. CRUZI* LINEAGES INVOLVED IN CONGENITAL CHAGAS DISEASE.
Burgos JM; Bisio M; Duffy T; Altcheh J; Freilij H; Levin MJ and Schijman AG.

16:30-16:45 MI-C23 UNIQUE STRUCTURAL AND FUNCTIONAL FEATURES OF *TRYPANOSOMA CRUZI* RIBOSOMES AS DETECTED BY YEAST TWO-HYBRID, SEQUENCE COMPARISON AND CRYO-ELECTRON MICROSCOPY.
Maximiliano Juri Ayub, Haixiao Gao, Benson Nyambega, Sebastian Tanco, Joachim Frank and Mariano Levin.

16:45-17:00 MI-C24 PROTEIN INTERACTION MAP AND FUNCTIONAL ANALYSIS REVEAL THE UNIQUE ORGANITATION OF THE TRANS-SPLICEOSOMAL E COMPLEX IN TRYPANOSOMES.
Catalina Atorrasagasti, Natalia Bercovich, Florence Caro, Benson Nyambegah, Mariano Levin and Martin Vazquez.

17:00-18:00 **LECTURE**

Dr. Dennis E. Vance

(Heritage Medical Research Centre, University of Alberta, Edmonton, Alberta, Canada)

"Metabolic Insights from Murine Knockouts in Hepatic Phosphatidylcholine Biosynthesis"

Chairperson: Dr. Rodolfo R. Brenner (Universidad Nacional de La Plata).

18:00-18:30 **Coffee Break**

18:30-19:30 **Forty years of SAIB**
(Argentine Society of Biochemistry and Molecular Biology)
Dr. Rodolfo R. Brenner
(Facultad de Ciencias Médicas, Universidad Nacional de La Plata).

19:30-20:30 **CLOSING LECTURE**

Dr. Riccardo Dalla Favera

Institute for Cancer Genetics, Columbia University. New York, USA

"Molecular genetics of B cell lymphoma"

Chairperson: Dr. Néstor Carrillo (Universidad Nacional de Rosario).

21:00 **CLOSING DINNER**

Cell Biology Symposium

CB-S1.
ROLE OF THE RAB6 GTP-ASE DURING CELL CYCLE
Goud B.
UMR CNRS 144, Institut Curie, Paris, France. E-mail: bruno.goud@curie.fr

Two Rab6 isoforms termed Rab6A and Rab6A' are ubiquitously expressed in cells and tissues. They result from alternative splicing of a duplicated exon and differ in only three amino acids. We have recently designed double-stranded siRNAs that allow selective silencing of each isoform. We find that Rab6A', but not Rab6A, regulates retrograde transport of Shiga toxin B-subunit to Golgi, in good agreement with previous reports. Surprisingly, Rab6A function does not seem to be required for Golgi to ER retrograde transport. In addition, Rab6A' may collaborate to the modulation of retrograde pathways at the Golgi/endoplasmic reticulum interface. Our observations establish that a functional cross talk likely exists between Rab6 isoforms in interphase.

Rab6 also plays an important role during mitosis. Impairing GAPCenA, a Rab6 GTPase-activating protein, or Rab6 functions lead to an arrest in metaphase. Rab6A and Rab6A' act in two different pathways to control the metaphase/anaphase transition and cytokinesis. One pathway relies on the interaction of Rab6A' with the subunit of the dynactin complex $p150^{Glued}$ and is Mad2-dependent. Rab6A' would be involved in the activation of the dynein/dynactin complex at the kinetochores and the inactivation of the Mad2-spindle checkpoint. The other pathway relies on the interaction of Rab6A with Rabkinesin-6 (RK6) and is independent of Mad2 activity. RK6 is shown to be involved in the delivery of the passenger proteins Aurora B and TD-60 to the telophase disk, suggesting that the Rab6A/GAPCenA/RK6 pathway monitors the building of the telophase disk.

CB-S2.
RHO GTPASES IN CELL ADHESION AND MIGRATION
Burridge K, Garcia-Mata R, Sastry S[1].
Cell & Dev. Biology, UNC Chapel Hill, NC 27599, USA, [1]UTMB, Galveston, TX77555, USA. E-mail: keith_burridge@med.unc.edu

Cell migration and adhesion are regulated by Rho, Rac and Cdc42, members of the Rho family of GTPases. We are interested in how these are regulated by guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs). One mode of regulation of GEFs and GAPs is by tyrosine phosphorylation, leading us to investigate the role of tyrosine phosphatases with respect to GEF and GAP activity. PTP-PEST is a ubiquitously expressed tyrosine phosphatase, whose over-expression or deletion inhibits cell migration. Exploring the basis for these effects, we have found that over-expression elevates Rho activity, but decreases Rac activity. In contrast, cells lacking PTP-PEST exhibit elevated Rac and low Rho activity. We have used catalytically inactive trapping mutants of PTP-PEST to identify its potential substrates. This has revealed that PTP-PEST binds and acts on the GEF Vav2 and the GAP p190RhoGAP, thereby affecting the activities of Rac and Rho respectively. Many GEFs have C-terminal consensus sequences for binding PDZ domains, suggesting that PDZ proteins may recruit GEFs and GAPs to specific sites and provide a scaffold for their activity. Using SGEF as a model, we have shown that this binds multiple PDZ proteins. We have found that the interaction of SGEF with PDZ proteins suppresses its exchange activity. This result suggests that GEF-PDZ interactions may serve not only scaffolding and recruitment functions, but also to regulate GEF activity.

Plant Biochemistry and Molecular Biology Symposium

PL-S1.

OXIDATIVE STRESS AND SIGNALING PATHWAYS IN ARABIDOPSIS

Scarpeci TE[1], Zanor MI[2], Carrillo N[1], Valle EM[1].
[1]IBR (CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR, Suipacha 531, S2002LRK Rosario, Argentina; [2]Max-Planck Institute of Molecular Plant Physiology, Am Mühlenberg 1, 14476 Golm, Germany. E-mail: evalle@arnet.com.ar

Molecular mechanisms involved in the early antioxidant response of plants are for the most part still unknown. We examined the expression profile of 22,000 genes of Arabidopsis seedlings subjected to oxidative challenge. Approximately 600 genes showed significant expression changes within the first hours of treatment with methyl viologen, a redox cycling herbicide. The differentially expressed genes included few known proteins implicated in redox processes such us glutaredoxin, thioredoxin, NADH-dependent monodehydroascorbate reductase and ascorbate peroxidase. However, a much larger number of the identified genes have not been previously associated with oxidative stress. Among the highly induced genes were a number of genes related with receptor kinases, calcium regulation, protein modification and transcription factors. Our study revealed the induction of gene expression of signalling pathways common to processes other than oxidative stress, and points to an interplay among the different stress responses of plants at early events.

PL-S2.

STRUCTURAL AND FUNCTIONAL INSIGHTS INTO THE EVOLUTION OF THE PLANT ADP-GLUCOSE PYROPHOSPHORYLASE

Ballicora MA, Dubay JR, Devillers CH, Preiss J.
Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824, USA. E-mail: ballicor@msu.edu

Different evolutionary paths are theoretically possible for the loss or acquisition of enzyme function. In this work we studied the ancestral role of a model non-catalytic modulatory subunit. Plant ADP-glucose pyrophosphorylases comprise homologous but distinct catalytic (S) and modulatory (L) subunits. To explore the evolutionary origin of the L subunit function, we designed an L subunit in which we "resurrected" its ancestral activity. As few as two mutations turned the L subunit from *Solanum tuberosum* catalytic, showing that L and S subunits share a common catalytic ancestor, rather than a non-catalytic one. We show that the L subunit evolved to have a regulatory role, lost catalytic residues more than 130 million years ago before monocots and dicots diverged, and preserved, possibly as a byproduct, the substrate site architecture. Gene duplication of a catalytic subunit, divergence leading to new roles (modulatory), additional divergence of modulatory genes, and further establishment of differential tissue expression seems to be the strategy used by nature to control and regulate the synthesis of starch.

PL-S3.

UREIDE DEGRADATION PATHWAY(S) IN SOYBEAN—A CLUE TO DROUGHT-RESISTANT N-FIXATION?

Polacco JC, Todd CD.
Department of Biochemistry, University of Missouri; Columbia, MO 65211 USA. E-mail: polaccoj@missouri.edu

Two soybean cultivars, 'Williams 82' and 'Maple Arrow', were reported to use different pathways to degrade the ureides allantoic acid and ureidoglycolate. This purported difference is the basis of a model explaining the different sensitivities of their N-fixation to drought. $^{14}CO_2$ evolution from [2,7-^{14}C]allantoate was catalyzed by a leaf $AmSO_4$ fraction of both cultivars. This activity was inhibited by acetohydroxamate but not by phenylphosphorodiamidate (PPD), a more specific urease inhibitor. Thus, both cultivars exhibited an NH_3-generating allantoate amidohydrolase; its sensitivity to EDTA is consistent with our previous reports that the enzyme requires Mn^{2+}. However, leaf homogenates of both cultivars evolved [^{14}C]urea from [2,7-^{14}C]allantoin, likely from [^{14}C]ureidoglycolate. Plants of both cultivars accumulated urea when grown in the presence of PPD, especially under N-fixation. But PPD did not significantly impact total seed nitrogen. Thus, there is no evidence for two ureide degradation pathways— other explanations are needed for the drought tolerance of Maple Arrow. Structural gene disruptions of the putative ureide degrading enzymes of *Arabidopsis* are being analyzed. We are using yeast to test the function of the soybean orthologs.

PL-S4.

PHOSPHITE BLOCKS PHOSPHATE SENSING IN PLANTS AND YEAST

Plaxton W.
Dept. of Biology, Queen's University, Kingston, Ontario, Canada K7L 3N6. E-mail: plaxton@biology.queensu.ca

Phosphite ($H_2PO_3^-$, Phi) is a controversial agricultural commodity that is being widely marketed as both a fungicide and a superior P fertilizer. Phi-based fungicides effectively control crop pathogens such as *Phytophthora sp*. Although plants rapidly absorb and translocate Phi, it does not appear to be oxidized or metabolized *in vivo*. While Phi has little effect on the growth of phosphate (HPO_4^{2-}, Pi) sufficient plants and yeast, it is highly toxic to their development under Pi deficient conditions. Phi prevents the acclimation of plant and yeast to suboptimal [Pi] by specifically obstructing the derepression of genes encoding proteins characteristic of the Pi starvation response (*e.g.,* acid phosphatases, high-affinity Pi transporters, etc.). Phi treatment of Pi-deprived *Brassica napus* (canola) suspension cells also caused marked differential protein phosphorylation *in vivo*, and accelerated the onset of Pi-starvation-mediated programmed cell death by about 3 weeks. In the yeast *Saccharomyces cerevisiae*, Phi appears to target PHO84, a high-affinity Pi transporter and putative component of a Pi sensor complex. Although Phi cannot replace Pi for fulfilling the nutritional P requirements of plants/yeast, Phi can substitute for Pi in repressing their typical molecular and developmental responses to Pi deficiency. Phi thus represents a valuable tool for investigating the signaling pathway by which plant cells perceive and coordinate appropriate responses to Pi deprivation. We also need to critically assess the long-term consequences of the significant input of Phi into food products and the environment that is arising from its extensive use in agriculture and industry.

BT-C1.
EFFICIENT PRESERVATION IN SILICON OXIDE MATRIX OF *ESCHERICHIA COLI* PRODUCER OF RECOMBINANT PROTEINS

Desimone MF[1], De Marzi MC[2], Copello GJ[1], Bertinatto JA[1], Fernández MM[2], Malchiodi EL[2], Diaz LE[1].
[1]Cátedra de Química Analítica Instrumental. [2]Cátedra de Inmunologia. IDEHU-CONICET. [1-2]Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires. Junin 954 Piso 3, (1113) Buenos Aires, Argentina. E-mail: desimone@ffyb.uba.ar

The aim of this work was to study the use of silicon oxide matrix for immobilization and preservation of bacteria producers of recombinant proteins. *E. coli* BL21 cells were transformed with a plasmid encoding β chain T cell receptor hVβ5.2mCβ1, expressed as inclusion bodies, while others were transformed with plasmids encoding two superantigens, streptococcal superantigen SSA and staphylococcal enterotoxin G, and expressed as soluble protein in the periplasm. The properties of immobilization and storage stability in inorganic matrix prepared from two precursors, tetraethoxsilane (TEOS) and silicon dioxide (SiO_2) were studied. Immobilized *E. coli* were stored in hermetically closed tubes at 4ºC and 20ºC and the number of viable cells and recombinant protein production were studied weekly. We found that bacteria in SiO_2 derived matrix remain at 10^9 cfu/ml before immobilization for 45 days while those in TEOS decrease to 10^4 cfu/ml after two weeks. Immobilized bacteria conserved intact their biochemical characteristics and could be used as starter to produce recombinat proteins in the same level as glycerol stock.

BT-C2.
MICROBIAL IMMOBILIZATION APPLIED TO THE SYNTHESIS OF NUCLEOSIDE ANALOGUES

Trelles J[1], Valino A[1], Lewkowicz E[1], Iribarren A[1,2].
[1]Laboratorio de Biotransformaciones, Universidad Nacional de Quilmes (Argentina). [2]INGEBI (CONICET), Bs. As., Argentina. E-mail: jtrelles@unq.edu.ar

Modified nucleosides are being extensively utilized in antitumoral and antiviral therapies, and more recently for antisense strategies, with a high impact. Therefore, their optimized production is an important aim of the pharmaceutical industry.
Traditionally, nucleosides are prepared by chemical methods that often involve difficult, inefficient and time-consuming multistage processes. Microbial preparations are an alternative methodology, which offer regio- and stereoselectivity and simple experimental conditions.
In this work, we discuss the results obtained using microoganisms (*E. coli*, *Citrobacter*, *Aeromonas* and *Enterobacter*) immobilized on different supports (agarose, polyacrylamide, agar, alginate and modified poliethylene) for the synthesis of differents nucleoside analogues (adenine arabinoside, didesoxinosine, 6-chloro purine riboside, etc.).

CB-C1.
SCAFFOLDING PROTEINS AND RHO SIGNALING.

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Rho GTPases control many aspects of cell behavior through regulation of multiple signal transduction pathways. Rho activation is mediated by guanine-nucleotide exchange factors (GEFs), which catalyze the exchange of GDP for GTP. Rho-GEFs comprise a highly diverse family with more than 60 members in humans. We performed a bioinformatics analysis of the Rho-GEFs in the human genome and found that 27 out of 64 analyzed (42%) contain a putative PDZ-binding motif at the C-terminus. PDZ domains are protein-protein interaction domains that act as scaffolds to concentrate signaling molecules at specialized regions in the cell. We used a peptide pull down approach coupled to mass spec analysis to identify PDZ-containing proteins binding to Rho-GEFs. Our results using the C-terminal sequences of six different Rho-GEFs show that these binding domains are functional and can bind specifically to different PDZ proteins. Each Rho-GEF binds to a different subset of PDZ- proteins in different tissues. We confirmed some of these interactions boiochemically and showed that they are important for Rho-GEF targeting. In addition, our results suggest that these interactions not only play a role in targeting Rho-GEFs to their appropriate location in the cell but also function in modulating their activity. Specifically, binding to a PDZ protein can inhibit the exchange activity of Rho-GEFs. In summary, we propose a novel mechanism of regulating Rho-GEF localization and activity by the regulated interaction with a PDZ protein.

CB-C2.
STUDYING ACTIN DYNAMICS IN Y1 ADRENAL CELLS CONTROLLED BY ACTH: PARTICIPATION OF PROTEIN KINASE A AND TYROSINE KINASE

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Y1 adrenocortical cells respond to ACTH with a characteristic change in cell morphology (rounding-up) that facilitates subsequent cAMP signalling, critical for cholesterol transport to mitochondria and steroid secretion. Further insights about effector proteins and mechanisms involved in coupling activation of PKA to the control of cell shape were investigated in the present study, Previous results showed that ACTH-induced caveolin tyrosine phosphorylation is related to podosome assembly. After ACTH/8-Br-cAMP stimulation a ring-like structure formed by an F-actin core surrounded by phosphocaveolin-1 is formed at the periphery; confocal analysis and electron microscopy confirmed them as podosomes. Podosome assembly was dependent on both PKA and tyrosine kinase activity since their formation was impaired after treatment with either myristoilated PKI or PP2, respectively, previously to ACTH or 8-Br-cAMP stimulation. Also the role of the small RhoGTPase was assessed by transfection of RhoA or cdc42 GFP-tagged mutants, which resulted in inhibition of ACTH-induced rounding-up and podosome formation suggesting the participation of RhoGTPases in the control of actin dynamics. We demonstrate here that several pathways are involved in the control of cellular morphology triggered by ACTH and phosphocaveolin-1 can represent an early target/effector protein of the steroidogenic cascade, involved in the control of cell shape.

CB-C3.
EFFECT OF BRADYKININ IN FOCAL CONTACT (FC) ORGANIZATION OF IMMATURE RAT RENAL PAPILLARY CELLS

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FC are structures of cell attachment to extracelular matrix. Vinculin (V) and talin (T) are markers of FC, and bind to phosphatidylinositol 4,5 biphosphate (PIP2). We previously demostrated that BK modulates assembly of FC in adult rats. Since BK receptor (B2) is highly expressed in newborn rats, in the present work the effects of BK on FC assembly were studied in cultured papillary collecting duct cells from 10 days-old rats by immunofluorescence. Cells from untreated cultures showed two different kind of FC. One type was an elongated FC, peripherically located and the other centrally-located was wide short stick FC. V staining was concentrated in both FC and colocalized with PIP2. T staining was mainly localized in stick-like FC. Colocalization of V-PIP2 in FC was lost after BK treatment. After 1 minute of BK, V and PIP2 dissipated from FC while T staining increased in both FC. After 5 minutes, V and PIP2 staining reappeared in FC, and T staining dissipated. Within 10 minutes of BK, PIP2 and T showed the same pattern as control cells while V staining was increased on both FC, and had a net-like distribution, which was form by long wide axis from nucleus to the edge with transversal parallel lines interlacing them. These results show that BK by affecting cytoskeletal organization induce a cell migratory phenotype thus suggesting stimulation of cell migration.

CB-C4.
PHOSPHOLIPASE C (PLC) IS INVOLVED IN BRADIKYNIN (BK)-INDUCED FOCAL CONTACT (FC) RESTRUCTURATION

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FC are structures of cell attachment to extracelular matrix. We previously found that FC proteins vinculin (V) and and talin are associated with DRMs-detergent-resistant membrane domains, that DRMs-phosphatidylinositol 4,5 biphosphate (PIP2) binding to V is essential for FC formation, and that BK modulates FC assembly. Since BK stimulates PIP2 hydrolysis by PLC activation, we tried to determine whether BK can modulate FC assembly by this mechanism. We treated cultured renal papillary cells with U73122 -PLC inhibitor- before BK stimulation and analyzed FC and actin cytoskeleton by confocal microscopy analysis. In control cells, intense V staining localized to FC and colocalized with PIP2, and actin stress fibers were observed throughout the cell body terminating at the FC. After 1 and 5 min of BK, V and PIP2 immunostaining dissipated from FC, and actin accumulated at the cell periphery with a concomitant loss of stress fibers. BK did not mobilize talin in FC. Pretreatment of cells with U73122 blocked BK-induced loss of FC-V staining and provoked actin stress fibers reorganization into dense filamentous actin network. An intense FC-PIP2 immunostaining was also observed, accompanied by an increase of PIP2 staining with a granular pattern throughout the cell body. Since talin, but not V, remains localized to FC after BK stimulation, BK could be inducing a restructuration rather than a dissipation of FC by a mechanism that involves DRMs-PIP2 hydrolysis by activation of PLC.

CB-C5.
STOP AND MBP ARE EFFECTORS OF MICROTUBULE COLD STABILITY IN OLIGODENDROCYTES

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We described that oligodendrocytes (Olgs) express a major STOP variant of 89 kDa and two minor variants of 48 and 42 kDa which induce microtubule cold-stability and nocodazole resistance. In these cells, MBP is found associated to the network of microtubules (Mts). MBP has also been shown to function as a calmodulin regulated microtubule stabilizing protein *in vitro*. Herein, we analyse microtubule stability in Olgs cultures derived from STOP knock out (STOP-/-), MBP deficient (shiverer) and wild type (WT) mice. In WT and shiverer Olgs, which express STOP from early stages of differentiation, Mts are cold stable throughout maturation. In STOP -/- Olgs, Mts are initially cold labile until later stages of maturation when MBP is expressed and localized throughout the cell. To directly assay the microtubule stabilizing activities of MBP and STOPs in Olgs, we performed knock down assays of MBP or STOP expressions by transfection with specific siRNAs. The MBP suppression in STOP -/- Olgs or STOP suppression in shiverer Olgs leads to a complete suppression of microtubule cold-stability. Thus, the oligodendrocyte STOP variants are functional in cells whereas MBP functions as a microtubule stabilizing protein in Olgs. These results suggest that MBP is directly involved in regulation of microtubule stability and may be relevant for oligodendrocyte morphology.
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CB-C6.
INTRACELULAR DISTRIBUTION OF THE GTPase Rab24 DURING MITOSIS

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Rab24 is a member of the Rab GTPases family which regulates different intracellular transport events. We have recently described that Rab24 colocalized with the autophagosomal protein MAP-LC3 (Microtubule Associated Protein-LC3). In order to elucidate the function of Rab24 we have generated a policlonal antibody against the specific carboxi-terminal region to study its endogenous distribution. In interfase Rab24 showed a perinuclear reticular localization that often encircled the nucleus. In methanol fixed cells endogenous Rab24 localized on the centrosome, showing a clear colocalization with GAPCenA (GAP Centrosome Associated protein of Rab6). We have also shown interaction of these two proteins by “pull down” and immunoprecipitation assays. During mitosis, Rab24 localized on the mitotic spindle, whereas in cytokinesis was observed on the contractile cleavage furrow but not on the midbodies. This distribution correlates with the distribution of β-tubulin. In cells treated with vinblastine, a microtubule despolimerizing agent, Rab24 accumulated in structures around the nucleus that were also labeled with β-tubulin and MAP-LC3. Using short interfering RNA in HELA cells we decreased the expression of Rab24 and cells were accumulated in metaphase. These results suggest a role for Rab24 in controlling the cell cycle.

CB-C7.
STARVATION INDUCES FUSION BETWEEN AUTOFAGIC VACUOLES AND MVBs IN K562 CELLS
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During maturation reticulocytes loose some membrane proteins and organelles that are not required on the mature red cell. The proteins are released into the extracellular medium associated with vesicles present in multivesicular bodies (MVBs). Fusion of MVBs with plasma membrane results in secretion of the small internal vesicles, termed exosomes. It has been postulated that during erythroid maturation, autophagy was required to eliminate organelles such as ribosomes and mitochondria. The autophagic pathway is a normal degradative process that involves the sequestration of cytoplasmic components and organelles in a vacuole called autophagosome which finally fuses with the lysosome. We have previously shown that in K562 cells Rab11 decorates MVBs and regulates MVBs generation. In the present report, we have examined by confocal microscopy how starvation, a physiological inducer of autophagy, affects the development of MVBs in cells overexpresing GFP-Rab11 and RFP-LC3 (an autophagosomal marker). Starvation caused an enlargement of the vacuoles decorated with GFP-Rab11wt and RFP-LC3, and an increased colocalization between both markers that was abrogated by the autophagy inhibitor wortmannin. A similar effect was observed when cells were incubated under full nutrient conditions but in the presence rapamycin an agent that induces autophagy by inhibiting the kinase TOR. Our results suggest that autophagy contributes to the elimination of MVBs from the maturing cell.

CB-C8.
AUTOPHAGY INDUCTION STIMULATES THE DEVELOPMENT OF THE *COXIELLA*-REPLICATIVE VACUOLE
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Many bacteria have developed different mechanisms to invade and survive in the host cell, evading degradation. *Coxiella burnetii*, the etiologic agent of Q fever, is an obligate intracellular bacterium. Once inside the cell this microorganism multiplies in acidic vacuoles with lysosomal characteristics. We have previously observed that *C. burnetii* localizes in a compartment labeled by LC3, a protein that specifically localizes in autophagic vacuoles. Therefore, we decided to explore in more detail the relationship between *C. burnetii* and the autophagic pathway. Our results indicate that amino acid starvation, a physiological stimulus for autophagy, and treatment of cells with rapamycin, a drug that induces autophagy, increased the number of *Coxiella* infected cells. Furthermore, in cells overexpressing LC3 or Rab24, proteins that localize on autophagic vacuoles, an increase in the development of *Coxiella*-replicative vacuoles was observed. In contrast, in cells overexpressing mutants of these proteins both the vacuole number and size decreased during the first hours of infection. Taken together, these results indicate that interaction of *Coxiella*-containing vacuoles with the autophagic pathway is required for the normal development of its replicative niche.

CB-C9.
LIGAND-DEPENDENT AND INDEPENDENT INTERNALIZATION OF THE GPI ANCHOR PROTEIN uPAR FOLLOW TWO DIFFERENT MECHANISMS
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uPAR is a GPI anchored protein known to be internalized by the uPA-PAI-1 complex via a clathrin- and LRP-dependent pathway. We employed HT1080 cells, that express the en-dogenous receptor, uPA and PAI-1, and HEK293-uPAR cells, expressing uPAR but not its ligands. In HT1080 cells, uPAR was detected in the plasma membrane, in ruffles, co-localizing with clathrin, LRP and cholera-toxin. And also intracellularly, associated with early endosomes. In HEK293-uPAR cells, uPAR still located in early endosomes, but no-colocalization was observed with LRP or clathrin. Biochemical studies demonstrated that in these cells uPAR is internalized in the absence of ligands. EM identified uPAR in endocytic vesicles devoided of clathrin. FACS analysis showed that the endocytosis was partially inhibited by a dominant negative of Rac1. On the other hand no effect of LRP pathway inhibitors was observed. These results suggest the existence of at least two endocytic routes for uPAR internalization. One is ligand- and LRP-mediated and occurs via clathrin-coated pits. The second is ligand-independent, constitutive and appears to be connected to the ruffling mechanism mediated by Rac1. Despite the presence of the GPI anchoring, both mechanisms do not require the presence of uPAR in lipid rafts.

CB-C10.
INFLAMMATION MEDIATORS REGULATE THE LRP-1 EXPRESSION IN J774 CELLS
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In atherosclerosis, angiogenesis and tumoral metastasis occur a focal degradation of extracellular matrix components by a broad spectrum of proteinases. Anyway, anti-proteolytic molecules, including proteinase inhibitors and endocytic receptors, play key roles to control these processes. In this sense, LRP-1, a multifunctional endocytic receptor that belongs to the LDL receptor gene family, has been reported to modulate the extracellular proteolytic activity in inflammation. Although it is known that certain inflammatory mediators, including Insulin and Interferon-γ (INF-γ) or bacterial lipopolysacharides (LPS), could affect the biological function of LRP-1, the molecular mechanisms are not clearly understood. In this work we study the regulatory effect of inflammatory mediators and Insulin on LRP-1 expression in J774 macrophage-derived cell line. By western blotting and RT-PCR we demonstrate that both insulin and inflammatory mediators - obtained from a conditioned media of NIH.3T3 fibroblast-derived cell line (MCN) – down-regulate LRP-1 to protein and RNA level. In addition, by immunofluorescence assay we observe that insulin and MCN modify the endocytic activity of LRP-1 in J774 cells. In conclusion in this work we demonstrate that inflammatory mediators can affect the function of LRP-1 by regulating both the expression and endocytosis activity of this receptor.

CB-C11.

Rab22a REGULATES TRANSFERRIN SORTING IN CHO CELLS

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Internalized macromolecules are delivered to early/sorting endosomes, from where they are delivered to various intracellular destinations or recycled back to the cell surface. Rab proteins have key regulatory roles in most membrane-transport steps. We study the effect of Rab22aWT and Rab22aQ64L expression on the recycling pathway of Transferrin (Tfn) in TRVb-1 CHO cells. GFP-tagged Rab22a proteins caused enlargement of early endosomes that remained accessible to internalized fluorescently labeled Tfn. The Tfn Receptor accumulated in large Rab22a-positive vesicles and became depleted from the recycling center and from the cell surface. When a pulse of Tfn was chased for different periods of time, the protein was not recycled to the cell surface and remained in the Rab22a-positive endosomes. We then measure the effect of both proteins on the recycling of ^{125}I-Tfn. The expression of Rab22aWT and Rab22aQ64L caused a significant inhibition of ^{125}I-Tfn recycling and diminished the ^{125}I-Tfn binding on the cell surface. Altogether, these results indicate that Rab22a causes the accumulation of Tfn in enlarged endosomal structures. We propose that increased homotypic fusion between early endosomes may interfere with the spatial organization of membrane domains that mediate transport to the recycling pathway.

CB-C12.

AUTOREGULATION OF ZFHEP-1 IS MEDIATED BY THE BINDING TO A SPECIFIC E2-BOX

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Zfhep (Zinc Finger Homeodomain Enhancer-binding Protein) is a transcription factor expressed as two isoforms, Zfhep-1 and Zfhep-2. Zfhep-2 lacks the N-terminal DNA-binding domain of the larger Zfhep-1. Zfhep is involved in lymphopoiesis, neurogenesis and myogenesis. We previously demonstrated that
Zfhep-1 repressed its own promoter. Sequence analysis of the Zfhep-1 promoter (TRANSFAC database), revealed the presence of two potentials Zfhep binding sites. Our goal was to identify the sequences involved in Zfhep gene regulation by the Zfhep isoforms. Band shift assays were performed with Jurkat nuclear extracts and Zfhep-2 programmed rabbit reticulocyte lysates in the presence of [32P]-labeled DNA probe. Probes include the sequence of the putative binding site 1 (BS1), binding site 2 (BS2) or both binding sites (BS1+2). Both Zfhep isoforms bound either BS2 or BS1+2 probes, but none of the isoforms bound BS1 probe. Retardation complexes were competed by either anti-Zfhep antibodies or an excess of cold probe. The mutation of the E2-Box present in the BS2 region (CACCTG→CAtaTG) disrupted the binding of both Zfhep isoforms to the BS2. Competitive band shift assays shown that Zfhep-2 is able to compete and displace Zfhep-1 from its Binding site. Our data indicate that Zfhep-1 autoregulation is mediated by the binding of the protein to an E2-Box present between 216 and 228 of the Zfhep-1 promoter. We also demonstrate that Zfhep-2 is able to compete with Zfhep-1 for this binding site acting as a negative dominant isoform.

CB-C13.

THE RGG BOX OF CNBP IS REQUIERED FOR RNA BINDING AND NUCLEIC ACID CHAPERONE ACTIVITY

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Cellular nucleic acid binding protein CNBP has been identified as an eukaryote cellular factor that interacts with single-stranded nucleic acids. It has been associated with diverse gene expression control mechanisms that involve from transcription to translation. The main goal in this work was to identify and characterize *Bufo arenarum* CNBP (bCNBP) biochemical activity in order to approach to its biological function.
Electrophoretic mobility shift assays were performed to analyze the capability of bCNBP and mutant forms to bind single-stranded nucleic acid probes, in order to establish the protein motifs involved in the binding. Two mutant bCNBPs that lack the glycine and arginine rich motif (RGG-box) present in the primary structure do not bind to RNA probes, but they bind to ss-DNA probes with the same sequence.
Nucleic acid chaperone activity was determined by electrophoretic oligonucleotide hybridization assays. bCNBP showed a significant annealing accelerating activity, while RGG-box lacking mutants showed lower annealing rates compared with controls.
Thus, CNBP shows a dependence on the RGG-box to exert its biochemical activities, one of which is the nucleic acid chaperone, a function not previously described for CNBP, that may explain the multiple processes where it has been found.

CB-C14.

GENE EXPRESSION PROFILING ON THE ERBB4 MURINE MODEL OF DILATED CARDIOMYOPATHY

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Dilated cardiomyopathy (DCM) is a leading cause of heart failure that is believed to result from abnormal remodeling of cardiac tissue in response to a variety of stressors. Receptor protein-tyrosine kinase erbB4 activities are essential for the maintenance of normal heart function as the significant loss of erbB4 in ventricular muscle leads to a severe DCM.
We performed gene expression analyses in ventricular samples from the conditional erbB4 knockout mouse (erbB4 CKO) utilizing DNA micro-arrays. Differentially expressed genes, comprising more than 1.000, were functionally grouped and compared to gene expression from other forms of mouse DCM. Taken together these results showed significant changes in the expression pattern of genes involved in the hypertrophic response (re-expression of the fetal cell program), and required for the maintenance of the cardiomyocyte architecture and junction. Modifications in these structures are representative of a pathological remodeling of the myocardium. We have extended this analysis to examine the expression pattern of candidate genes in models of acquired DCM. For example, the severe cardiomyopathy displayed in a subset of breast cancer patients undergoing combined treatments with antibodies blocking the neuregulin signaling pathway and anthracycline derivatives.

CB-C15.
THE OLIGOSACCHARYLTRANSFERASE STT3P SUBUNIT IS INVOLVED IN DOLICHOL-P-P-GLYCAN STRUCTURE RECOGNITION

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In most eukaryotes *N*-glycosylation is initiated by the *en bloc* transfer of $Glc_3Man_9GlcNAc_2$ from a dolichol-P-P derivative to selected asparagine residues in nascent polypeptide chains. This reaction is catalyzed by the oligosaccharyltransferase (OT), composed by eight subunits in *S cerevisiae* and all other eukaryotes, with the exception of trypanosomatid protozoa in which the enzyme is composed by only one subunit (Stt3p). Whereas multisubunit OTs preferentially transfer the triglucosylated glycan over similar compounds lacking the full complement of glucose units, that of trypanosomatids do not discriminate between glucose-containing from glucose-free glycans. To determine whether glycan structure recognition is determined by OT subunits accompanying Stt3p or if, alternatively, this last subunit proper is involved in such recognition, we substituted *S. cerevisiae* Stt3p with that from *Trypanosoma cruzi*. The resulting chimera, composed by *S. cerevisiae* Ost1p, Ost2p, Ost3p, Ost4p, Ost5p, Swp1p, Wbp1p and *T. cruzi* Stt3p was shown, in both live yeast cell and cell-free experiments not to discriminate between glucose-containing and glucose-free glycans. It was concluded that Stt3p and not accompanying subunits, is responsible for glycan structure recognition.

CB-C16.
A REAPPRAISAL OF THE ROLE OF NUCLEOSIDE DIPHOSPHATASES ON YEAST *N*- AND *O*-GLYCOSYLATION

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It is currently assumed that nucleoside diphosphatases (NDPases) must reside in the same secretory pathway compartments (ER, endoplasmic reticulum and G, Golgi apparatus) as glycosyltransferases to relieve inhibition of the latter by nucleoside diphosphates and to provide nucleoside monophosphates that are the antiporters in nucleotide sugar entrance into the ER and G lumens. We have functionally expressed *Schizosaccharomyces pombe* UDP-Glc:glycoprotein glucosyltransferase in *Saccharomyces cerevisiae* ER and by analysis of *in vivo* synthesized monoglucosylated *N*-glycans in a variety of *S. cerevisiae* mutants (devoid of either one or of both secretory pathway NDPases -Gda1p and Ynd1p- or in the anterograd (ER-G) or retrograd (G-ER) vesicular traffic -sec12 or ufe1, respectively) we concluded that neither NDPase activities nor both types of vesicular traffic are required for UDP-Glc entrance into the yeast ER lumen. By analysis of *N*- and *O*-mannosylated glycans synthesized in above mentioned mutants we concluded that: a) entrance of GDP-Man into the ER and the ER-G intermediate compartment (ERGIC) and/or *cis* G cisternae does not require the presence of NDPases; b) nucleotide sugar-dependent extension of *O*- and *N*-glycans is initiated in the ER and in the ERGIC/*cis* G cisternae, respectively; c) NDPases are absolutely required for GDP-Man entrance into the *cis* to *trans* G cisternae where further extension of *N*- and *O*-glycans takes place.

CB-C17.
REGULATORY ROLE OF ARGININE DEIMINASE IN THE BIOLOGY OF THE PRIMITIVE EUKARYOTE *GIARDIA LAMBLIA*

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Giardia lamblia undergoes antigenic variation, a process where a single variant-specific surface protein (VSP) is replaced by an antigenically-different VSP. All VSP genes (~150) encode type I integral membrane proteins that have a conserved five-amino acid cytoplasmic tail, CRGKA. To identify molecules interacting with this tail, the peptide His_6-CRGKA was employed in pull-down assays. Surprisingly, arginine deiminase (gADI) bound specifically to CRGKA as identified by mass spectroscopy. In *Giardia* and prokaryotes, arginine deiminase is known to catalyze the conversion of free arginine into citrulline. In contrast, higher eukaryotes possess peptidil-arginine deiminases (PAD), enzymes that post-translationally deiminates the guanidine group of arginine residues in peptides and proteins to generate ammonia and citrulline. Analysis of *Giardia* proteins using a citrulline-specific antibody showed that VSPs are citrullinated. Activity analysis using His_6-CRGKA as substrate and purified gADI, demonstrated that this peptide can be citrullinated *in vitro* (C**cit**GKA). Immunofluorescence analysis of HA-tagged gADI showed cytoplasmic localization and close association to the plasma membrane. When trophozoites were induced to differentiate into cysts, gADI translocated from the cytoplasm to the nuclei and the process of encystation was abolished. These results show that a post-translational modification of proteins (citrullination) plays an essential role in the biology of *Giardia*.

CB-C18.
ALTERNATIVE SUBSTRATES OF THE INSECT â-ALANINE-CATECHOLAMINE LIGASE

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The insect ATP-dependent enzyme known as NBAD synthase, conjugating â-alanine and dopamine to synthesize N-â-alanyldopamine (NBAD), was first characterized by our group. We found that it is able to accept norepinephrine and other catecholamines as alternative substrates and we considered it to be a catecholamine-â-alanine ligase (CBAL). We also demonstrated (Neuroscience Lett., (2004) 368: 186-191) that this enzyme is constitutively expressed in neural tissue. Here we show that CBAL is able to synthesize â-alanine derivatives of octopamine and tyramine. Since NBAD and other catecholamine derivatives are not available we had to chemically synthesize them, to be used as standards. Using [^{14}C] â-alanine as a tracer and HPLC analysis we first demonstrated that both octopamine and tyramine are competitive inhibitors of the synthesis of [^{14}C]NBAD. The direct synthesis of Octopamine and Tyramine derivatives, tentatively identified as N-â-alanyloctopamine (NBAO) and N-â-alanyltyramine (NBATA) was demonstrated (HPLC, TLC). The apparent synthesis of these substances by brain extracts is also shown. Mutants of *C. capitata niger* and *D. melanogaster ebony* were unable to synthesize NBAO and NBATA thus confirming the enzyme identity. This reinforces the idea that CBAL is an enzyme with wide range of substrates which, depending on the tissue, is induced or constitutively expressed.

CB-C19.
HEME OXYGENASE AND OXIDATIVE STRESS IN ADRENAL CELLS
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Carbon monoxide (CO) has been postulated as a modulator of endocrine physiology. Heme oxygenase catalyzes the catabolism of heme with the production of CO, biliverdin and the release of iron. In previous studies we identified HO isoforms in adrenal cells and demonstrated an increase in HO activity by ACTH. HO has also been involved in cellular defense mechanisms against oxidative stress. In this sense the aim of the present study was to analyze if HO induction by ACTH protects the cell from the oxidative stress generated by the stimulation of metabolism triggered by ACTH. Our results showed a significant increase in ROS production by ACTH (C: 105,8 ± 5,5; ACTH 2h: 163,8 ± 12,7 $p<0.01$), that is prevented by antioxidants such as bilirubin and melatonin. On the other hand, H_2O_2 dependent increase in TBARS and carbonyl levels (index of lipoperoxidation and protein oxidation respectively) was blocked by previous incubation of the cells with ACTH for 5 hs (TBARS in nmol/mg: C:109.6 ± 5, H_2O_2: 294 ± 51, ACTH: 92.8 ± 11, ACTH + H_2O_2: 115.5 ± 12, $p<0.01$ vs C; carbonyls in nmol/mg: C: 1.34 ± 0.2, H_2O_2: 2.91 ± 0.31, A: 1.18 ± 0.24, A H_2O_2: 0.54 ± 0.11, $p<0.01$ vs C). ACTH also increased superoxide dismutase and glutathione peroxidase activity in adrenal cells. Both melatonin and bilirubin inhibited HO-1 induction by ACTH. In summary, our results suggest that HO induction by ACTH correlates with an increase in oxidative stress that is attenuated as HO activity is increased.

CB-C20.
ABIOTIC STRESS AND ANTIOXIDANT ENZYMES EXPRESSION IN SUNFLOWER LEAF DISCS
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Overproduction of reactive oxygen species (ROS) occur in plants under abiotic stress conditions. Although ROS act as mediators of oxidative damage, a signalling role for O_2^- and H_2O_2 has been proposed. In the present work, the effect of cadmium (300 and 500 μM $CdCl_2$) or UVB radiation (30 KJ/m^2) on expression of Cu-Zn superoxide dismutase (*sod3*) and catalase (*cat1* and *cat3*) was evaluated in sunflower (*Helianthus annuus* L.) leaf discs. Samples were collected at 0, 4, 8, 12 and 16 h of Cd treatments or white light recuperation after UVB treatment. RNA extractions and semicuantitative RT-PCR analysis were performed. Treatment of 300 μM Cd induced 6.4, 2.9 and 6 fold the expression of *sod3*, *cat1* and *cat3* over the controls, respectively, after 8 h of treatment, but 500 μM Cd showed lesser induction levels. Immediately after UVB irradiation, the mRNA of the three enzymes decreased. After 8 h of white light recovery, *cat1* and *cat3* were induced (1.9 and 3.5 fold, respectively) and the maximum *sod3* expression was observed at 12 h (7 fold), respect to control. In conclusion, the balance between superoxide dismutase and peroxidases activities in cells is crucial for determining the steady-state level of O_2^- and H_2O_2. In our assay conditions, *sod3, cat1* and *cat3* were induced in response to abiotic stress at a late phase (8-12 h). The main induction of *cat3* suggests that core-catalases of peroxisomes might play a key regulatory role in controlling H_2O_2 level.

CB-C21.
CHEMOTAXIS AND HYDROCARBON BIODEGRADATION BY HALOARCHAEA
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As in bacteria, archaeal chemotaxis is based on monitoring the extracellular level of attractant or repellent substances. In this way cells can migrate towards the more favorable zones. There is scarce information about chemotaxis in Archaea and most studies have been performed in the haloarchaeon H.salinarium. On the other hand, the ability of Haloarchaea to grow using hydrocarbons as sole source of carbon is another unknown subject.
The objectives of this work were: To nalyze chemotactic responses of different collection strains of haloarchaea towards several organic compounds; to study the ability of these strains to grow using hydrocarbons as sole carbon source; to evaluate chemotactic response of these strains towards hydrocarbons.
In order to test chemotactic responses, soft agar plates (swarming assay) and agarose-in-plug assays were used.
Our results indicated that several collection strains show chemotactic responses towards casa amino acids, peptone, and yeast extract but not towards glucose. On the other hand, two haloarchaeal strains could grow using a mixture of hydrocarbons (gasoil) as sole carbon source and, besides, one of these strains presented a chemotactic response towards gasoil.
Although more experiments are needed, these results are of interest for further applications in bioremediation processes of extreme environments or high salt containing effluents.
Funding for this project were from UNMdP, ANPCyT and CONICET.

ES-C1.
SPECIFIC FEATURES OF HUMAN RNA POLYMERASE II ASSEMBLY PROCESS
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The eucaryotic DNA dependent RNA polymerase II(B) (RPB) is the nuclear enzyme responsible for the transcription of the vast majority of the genome. In Saccharomyces cerevisiae, it consists of a complex of 12 subunits.
The structure of the yeast enzyme has been established at 2.8Å resolution from X-rays analysis of crystal of purified protein (Cramer *et al*., 2001). No other eucaryotic RNA polymerase complex structure has been resolved so far. The assembly of the complex is yet poorly understood.
We have addressed the RPB assembly process by studying the initial step of the complex assembly in both yeast and human systems, namely the heterodimerization of two subunits, RPB3 and RPB11. We used the two-hybrids genetic tool as an assay together with other genetic means.
We show that this basic step involves distinct sets of contacts in both systems. We shall discuss the consequences of these observations in terms of evolution of the gene encoding these subunits in yeast and mammalian genomes.

ES-C2.
STRUCTURE-FUNCTION STUDIES OF ISOFORMS OF THE SPECIFIC SUBUNIT hRPB11 OF HUMAN RNA POLYMERASE II

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Our laboratory is studying the mechanisms of eukaryotic transcription both in unicellular (yeast) and multicellular (man) organisms. The main object of our current investigation is the specific subunit of human RNA polymerase II hRPB11. Earlier, in the collaborative work with French scientists we showed that this subunit is encoded in *Homo sapiens* by a multigene family, which expression give rise to at least three protein isoforms [BMC Mol. Biol., **2** (1), 2001]. At present we have identified in human genome four genes (*POLR2**J1**-POLR2**J4***) encoding different variants of the hRPB11 subunit. The genes contain from 4 to 16 exons, most of them were not previously described. Using yeast two–hybrid approach we for the first time have detected interactors of the two minor hRPB11 isoforms (hRPB11bα and hRPB11bβ) among components of the human proteome. Novel partners of the Rpb11 subunit, i.e. MTR2 and GIS2 (component of the Cdk8-module of the Mediator complex), were identified with the use of suppressor analysis of *S. cerevisiae* mutants defected in function of this subunit.

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ES-C3.
CARACTERIZATION OF OLIGOMERIC RAT LIVER MITOCHONDRIAL GLYCEROL-3-P ACYLTRANSFERASE

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Mitochondrial Glycerol-3- phosphate acyltransferase catalyzes the first and committed step in the *de novo* cellular glycerolipid synthesis, the formation of 1-acyl-*sn*-glycerol-3-phosphate (lysophosphatidic acid) from glycerol-3-phosphate and long chain fatty acyl-CoA substrates. To date, no studies have assessed the quaternary structure of the enzyme. It was therefore the aim of the present study to provide evidence for the existence of oligomeric mtGPAT complexes, using recombinant GPAT overexpressed in different eukaryotic systems. Using chemical crosslinking and SDS-PAGE dimeric, trimeric, tetrameric and a higher molecular mass complex were found. At inhibitory palmitoylCoA concentration the complex could also be observed. Using Triton X-100 combined with non-denaturing gel, mtGPAT was mainly found as a tetramer. mt GPATs trucated at the C-terminal domain were found to oligomerize normally. These results demonstrate that: 1) mtGPAT exists as oligomeric complexes. 2) high palmitoylCoA concentrations did not alter the oligomerization 3) The C-terminal domain of mtGPAT is not involved in the arrangement of the quaternary structure and 4) the possibility of mtGPAT interacting with other mitochondrial protein/s can not be overlooked.

ES-C4.
SOLUTION STUDIES OF HPV-16 E2C, ITS DNA BINDING SITE AND THE COMPLEX

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Papillomaviruses are DNA viruses that infect a wide range of mammals, and represent a serious health threat for humans. Gene transcription in papillomavirus is regulated by the E2 protein which has several binding sites. These have different affinities and their occupancy varies along viral cycle. We are interested in the DNA recognition mechanism of E2C, both as a means to understand its role in papillomavirus gene regulation and as a model for protein-DNA recognition. With these goals in mind, we tackled a thorough investigation of the structural plasticity of HPV-16 E2C by multidimensional NMR. We also compare this data with those of the bound protein. Finally, we present conformational differences between two binding sites for which E2 shows different affinity. The overall analysis of this data provides a picture of the structural plasticity of this protein and its binding sites in solution.

ES-C5.
SURFACE BEHAVIOR OF POLYGODIAL, AN UNCOUPLER OF THE MITOCHONDRIAL ATP SYNTHESIS

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Polygodial - a natural sesquiterpene exhibiting several biological activities- inhibits mitochondrial ATP synthesis and exerts its effect at the coupling of the F_1F_oATPase and the respiratory chain.
Accordingly, the energy-driven ANS fluorescent enhancement was collapsed by polygodial (IC_{50}=2.2 μM). However, the succinate-induced ΔpH was not dissipated by polygodial, clearly indicating that the latter does not increase the proton permeability of the inner mitochondrial membrane.
To test if the uncoupling effect of polygodial is produced by an alteration of the electric properties of the mitochondrial membrane, we studied its interaction with interfaces and model membranes. The surface behavior of polygodial was determined using the Langmuir monolayer technique. Polygodial shows: i) spontaneous adsorption at the water-air interface with changes in the surface pressure ($\Delta\pi_{max}$ = 21 mN m^{-1}, K = 23.1 μM, n = 2.9) and potential (ΔV_{max} = 530 mV , K = 17.2 μM, n = 1.9): and ii) ability to penetrate monolayers of POPC, POPE and cardiolipin (cut-offs were 55, 51 y 42 mN m^{-1}, respectively).
These results support the above-mentioned hypothesis. The question if the $\Delta\mu_H^+$ or the ΔpH ($\Delta pH^{surface}$ or ΔpH^{bulk}) is the Δp component responsible for driving the mitochondrial ATP synthesis, is still matter of debate.

ES-C6.
CLONING AND BIOCHEMICAL CHARACTERIZATION OF THREE DIFFERENT MALATE DEHYDROGENASE (MDH) ISOZYMES FROM *LEISHMANIA MAJOR*
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Recently we have shown that the expression of the MDH isozymes is developmentally regulated during the life cycle of *Trypanosoma brucei*. The cytosolic MDH is the only isozyme expressed in the bloodstream forms, and it seems to be even more abundant than in the procyclics. Like *T. brucei*, and unlike *Trypanosoma cruzi*, *Leishmania mexicana* also appears to possess at least three different MDHs. Due to the low abundance and stability of the natural enzymes we decided to investigate the functionality of the three ORFs identified in the *L. major* genome project. The nucleotide sequence encoding each of the putative MDHs was amplified by PCR and cloned into an expression vector, pET28. Sequence alignments and the presence of a PTS in one isoform suggest that one of the MDHs may be located in the mitochondrion, another in the glycosome and the third would be cytosolic. The functionality of the three MDHs was proved by heterologous expression in *Escherichia coli*. The potential gMDH had app. K_m values for oxaloacetate and NADH of 0.03 mM and 0.05 mM, respectively. These kinetic parameters are almost identical for the other isozymes. However, the app. V_{max} values obtained for cMDH and mMDH are nearly 20 fold higher than those obtained for the gMDH suggesting that the latter isoform is the least efficient for oxaloacetate reduction. Additionally, these results clearly distinguish *T. brucei* and *L. major* from *T. cruzi*, since in the latter parasite only mMDH and gMDH are present.

ES-C7.
A C-TERMINAL AMIDATED MICROCIN J25 DERIVATIVE HAS ANTIMICROBIAL ACTIVITY INDEPENDENT OF RNA POLYMERASE INHIBITION
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Microcin J25 (MccJ25) is a 21-amino-acid antimicrobial peptide secreted by *Escherichia coli*. It has a dual mechanism of action: on the one hand, it inhibits transcription by binding within and obstructing the RNA polymerase (RNAP) secondary channel; on the other hand, it inhibits cell respiration with superoxide radical production. We synthesized a C-terminal amidated MccJ25 derivative (MccJ25*) which does not have any effect on RNAP *in vivo* or *in vitro*. The *E. coli* strain AB259, which shows turbid inhibition halos with MccJ25 in a spot-on-lawn test, was resistant to MccJ25* on M9 medium, while *E. coli* AB1133, a hypersusceptible strain, which gives clear spots with MccJ250, was susceptible to MccJ25*. A RNAP mutation (*rpoC* T931I) in AB259 strain causes resistance to MccJ25. When this mutation was transduced to AB1133 the transductant strain PA232 showed a residual sensitivity to the antibiotic. Similar to MccJ25, MccJ25* inhibited the growth, oxygen consumption and respiratory enzyme activities on PA232 (pGC01) strain, which overexpresses FhuA, the outer membrane receptor for MccJ25. These results led us to conclude that the MccJ25 C-terminal region is very important for RNAP inhibition and that the MccJ25* residual antimicrobial activity on *E. coli* strain AB1133 could be explained by inhibition of cell respiration.

LP-C1.
CHANGES IN LIPIDS CONTAINING VERY LONG CHAIN POLYUNSATURATED FATTY ACIDS (VLCPUFA) IN EXPERIMENTALLY CRYPTORCHID RAT TESTES
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The aim of this work was to examine the effect of experimental criptorchidism, a well-known model of spermatogenic arrest, on rat testicular lipids rich in VLCPUFA. Cryptorchidism resulted in a gradual decrease in the weight of the cryptorchid testis due to reduction of the seminiferous epithelium. The concentration and the fatty acid composition of all VLCPUFA-containing lipids were dramatically affected by the treatment. The neutral lipids, triacylglycerols, alkyl-diacylglycerols, and cholesterol esters, showed a time-dependent accumulation, mostly due to a marked increase in their levels of 20:4n-6 and 22:5n-6, the species with VLCPUFA being much less affected. After 10 days of cryptorchidism, the spermatogenic cell line practically disappeared from the tubules, as observed in histological preparations. At this stage the VLCPUFA of the neutral lipids remained, whereas those of the sphingolipids ceramide (Cer) and sphingomyelin were negligible. A significant increase in the total content of Cer was observed in the cryptorchid testis 5 days after surgery. This peak of Cer was accompanied by an increase in the content of Cer species containing saturated and monoenoic fatty acids, the VLCPUFA-containing Cer being decreased later on. The neutral lipids may reflect metabolic changes taking place in the somatic cells, whereas the sphingolipids apparently change as a result of germ cell involution.

LP-C2.
EFFECTS OF DOXORUBICIN ON RAT TESTICULAR LIPIDS WITH VERY LONG CHAIN POLYUNSATURATED FATTY ACIDS (VLCPUFA)
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The anthracycline drug doxorubicin (DXR) is among the most active antitumor compounds used in clinical oncology, but its use is limited due to its adverse reactions. In the testis, DXR was reported to induce apoptosis of spermatogonia, which leads to the involution of the seminiferous epithelium, causing aspermatogenesis and thereby azoospermia and oligospermia. The aim of this study was to assess the effects of a single dose of DXR (i.p.) on rat testicular sphingolipids and neutral lipids containing VLCPUFA. The earliest histological changes were observed 7 days after DXR administration, with a reduction in the number of cells of the seminiferous epithelium in part of the tubules. The process slowly continued, with no cells of the spermatogenic line remaining after 44 days in some rats. Ceramide (Cer) levels increased to about 30% at day 7. This change was mostly due to an increase in the amount of Cer species containing 16:0 and 18:1, very long chain fatty acids like 24:0 and 24:1 being not affected. Both Cer and sphingomyelin showed a gradual decrease in their VLCPUFA. Triacylglycerols, alkyl-diacylglycerols and cholesterol esters tended to increase, especially the latter, mostly due to the accumulation of PUFA. In rats where the germinal line had disappeared under the effects of DXR, the neutral lipids continued to accumulate and the sphingolipids lacked VLCPUFA altogether.

LP-C3.

EFFECT OF FENOFIBRATE AND INSULIN ON THE BIOSYNTHESIS OF UNSATURATED FATTY ACIDS IN THE STREPTOZOTOCIN RAT

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We currently investigate the simultaneous effects of glargine insulin and fenofibrate treatment on fatty acid biosynthetic parameters in rats injected with streptozotocin (70 mg/kg). Diabetes was evoked by streptozotocin injection). Fenofibrate (100 mg/kg/day) and glargine insulin (5 U/kg/day) were administered to the diabetic rats along 9 days. Blood parameters were measured at the moment of animal sacrifice when the insulin effect had declined. In these conditions fenofibrate did not alter significantly the insulinemia, glycemia, cholesterolemia and plasmatic free fatty acids of diabetic and diabetic rats treated with insulin. Insulin increased mRNAs and enzymatic activity of Δ9 and Δ5 desaturases of diabetic rats and fenofibrate increased both in diabetic and insulin treated rats, but the Δ6 desaturase remained unaltered. These results were only correlative to changes of oleic acid in the fatty acid composition of liver, liver microsomes and phosphatidylcholine of diabetic rats treated with insulin. The effects correlate changes of PPARα activity estimated by measuring the extramitochondrial acyl-CoA oxidase. In consequence, fenofibrate apparently through the PPARα enhances the Δ9 and Δ5 desaturase transcription and enzymatic activity synergistically, but independently from the insulin activating effect. Fenofibrate increased palmitic,but decreased stearic acid.

LP-C4.

PGD_2 DIFFERENTIALY REGULATES PC SYNTHESIS BY PLD AND PKC→MAPK INDEPENDENTLY-ACTIVATED PATHWAYS

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Phosphatidylcholine (PC) is a main lipid of biomembranes with important structural and functional roles. We have previously demonstrated that renal papillary PC synthesis is regulated by endogenous-synthesized prostaglandin D_2 (PGD_2) and that such an effect involves MAPK activation which seems to modulate cytidylyltransferase activity (CCT). In the present work we studied the initial events leading to MAPK activation in its action on PC synthesis. As MAPK activation can be preceded by PLD and/or PKC activation. Papillary PC synthesis, evaluated by ^{32}Pi incorporation, and MAPK activation, determined by westernblot, were evaluated in the absence or in the presence of PGD_2 with or without the addition of PLD (1% ethanol, E) and PKC (0.6 μM cheleritrine, C) inhibitors. 1 min PGD_2-stimulated PC synthesis and MAPK activation were blocked by E, C and also by U0126 (MEK inhibitor). After 15 min, a late PGD_2 stimulatory effect on PC synthesis and MAPK activation. In this case, PGD_2-stimulated ^{32}P-PC was only blocked by E. In contrast, MAPK activation was abolished by C but not E, which overincreased MAPK suggesting that MAPK activation does not depends on PLD. Our results indicate that PGD_2 stimulatory effect involves both PLD and MAPK activation, and both pathways operate independently on PC synthesis homeostasis.

LP-C5.

AN ACYL-COA SYNTHETASE AS OBLIGATORY PROTEIN IN THE REGULATION OF ARACHIDONIC ACID RELEASE AND STEROIDOGENESIS

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We have previously described in adrenal cells an inhibition of steroid synthesis using a specific inhibitor of an Arachidonic acid (AA)- preferring acyl-CoA synthetase (ACS4) that participates in concert action with an acyl-CoA thioesterase (MTE-I). Here in order to give a definitive answer to the role of ACS4 in steroidogenesis we knock down its expression using small interfering RNA (siRNA) methodology. Transfection of Leydig and adrenal cell lines with specific siRNA for ACS4 inhibits the enzyme expression analysed by western blot. This inhibition produced a decrease in hormone induced steroid synthesis (52.1 ± 4.8%; 51.6 ± 12.4% in adrenal and Leydig cells respectively) that was over come by exogenous AA addition. In order to address that ACS4 is working in concert action with MTE-I, we transfected cells with specific siRNA for ACS4 and MTE-I together in a dose that *per se* is not able to inhibit steroid synthesis. In this case, an inhibition of hormone steroidogenesis was observed. There was not effect of ACS4 siRNA on steroid synthesis when the cells were stimulated with a permeable analogue of cholesterol which by-pass the AA action on the rate-limiting step of steroidogenesis. These results strongly support the obligatory role of ACS4 in a new pathway of AA release involved in steroidogenesis.

LP-C6.

MALONYL-CoA IS A KEY METABOLITE SENSED BY GRAM POSITIVE BACTERIA TO AJUST FATTY ACID AND PHOSPHOLIPID BIOSYNTHESIS

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Microbial fatty acid biosynthesis has emerged as an excellent target for the design of novel antibiotics. That is the reason why we have been studying the regulation of this pathway in Gram-positive bacteria. We have recently reported the isolation and characterization of FapR, a transcriptional regulator present in many Gram positive bacteria that controls the expression of most of the genes involved in fatty acid and the first steps of phospholipid biosynthesis (the *fap* regulon) (Dev. Cell 2003, 4:663-672). We determined that the expression of the *fap* regulon increases when fatty acid biosynthesis is blocked by specific inhibitors, suggesting that the FapR repressor leaves the operator regions of those genes. Here, we present experiments indicating that the intracellular levels of FapR are maximal when the *fap* regulon is fully expressed, suggesting the existence of another molecule that modulates FapR activity. We constructed conditional mutants for each gene of the initial steps of fatty acid biosynthesis and the study of the expression of the *fap* regulon in these strains pinpointed to malonyl-CoA as the modulator of FapR. This result was verified by *in vitro* transcription assays, which confirmed that malonyl-CoA is able to specifically derepress transcription of the *fap* regulon in the presence of FapR. Finally, we present a model in which the key intermediate malonyl-CoA modulates through FapR the expression of the fatty acid synthase of Gram-positive bacteria.

LP-C7.

ANALYSIS OF GENES OF *Bacillus subtilis* THAT CODE FOR ENZYMES INVOLVED IN PHOSPHOLIPID BIOSYNTHESIS

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In *E. coli* there are two genes that code for acyltransferases involved in the catalysis of the first two reactions of phospholipd synthesis: *plsB* and *plsC*. In addition, there is a third gene, *plsX* whose function is not clearly determinated but which is probably related to the acyltransferases activities. In *B. subtilis* there is a unique gene homolog to *plsC*, yhdO, and there is also a *plsX* homolog, but no *plsB* homolog could be identified.
To determine the functions of the enzymes encoded by the *yhdO* and *plsX* genes of *B. subtilis*, conditional mutants of those genes were constructed. The mutant strains only could grow when the medium contained the apropiate inducer. The analysis of lipids extracted from cultures labeled with [^{14}C]?acetic acid showed that, when the inducer was depleted from the medium, phospholipid biosynthesis was blocked. Moreover, long chain fatty acid were accumulated in this condition. Expression of *yhdO* in *E.coli* mutant strains complemented a *plsC* mutant but not a *plsB* mutant. Also *B. subtilis plsX* was able to complement an *E.coli plsX* mutant.
Our results demonstrate that YhdO is an acyl-glycerol-3-phosphate acyltransferase essential for *B. subtilis* and for the first time that *plsX*, a gene widely distribuited in bacteria, plays a role in phospholipid biosynthesis.

LP-C8.

SPHINGOLIPID METABOLISM IN MDCK CELLS: ITS ROLE IN CELL PROLIFERATION AND SURVIVAL

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Sphingolipid (SL) metabolism has been implicated in cellular processes like proliferation and differentiation. We have observed that SL metabolism differently branches depending on rat renal papilla developmental stage. Aimed at studying the influence of SL metabolism in proliferation and survival of renal cells, we blocked SL biosynthesis at different steps and analyzed cell proliferation and viability with reference to the metabolic products. By incubating MDCK cells with ^{14}C-palmitic acid, SL *de novo* biosynthesis branches to the synthesis of sphingomyelin (39.8% of labeled SL), ceramide (Cer; 30.9%) and sphingosine-1P (S1P; 8.2%). Serine-palmytoil tranferase inhibition with 0.5 mM L-cicloserine reduced cell number (79.1% of the control) without loosing cell viability, and decreased the whole metabolic pathway activity. Inhibition of acyl-CoA transferase with 50 µM Fumonisine B1 (FB1), also reduced cell number (55.2 % of the control) without loosing viability, but induced an accumulation of dihydrosphingosine (DHS; 84.8% of labeled SL) with a reduction in Cer synthesis (3.6%). Sphingosine kinase inhibition with 25 µM D,L-threo-DHS (tDHS) decreased cell number (32.5% of the control) and viability (16.3%), reduced S1P synthesis (4.2% of labeled SL) and caused Cer accumulation (49.6%). The effect of tDHS was attenuated by the addition of FB1 raising cell number and viability (65.7% and 53%) and decreasing Cer accumulation (15.6% of labeled SL). Our results suggest that while the integrity of the whole biosynthetic pathway maintains cell proliferation, survival depends on relationship between S1P and Cer.

LP-C9.

HOW IS PHOSPHOCHOLINE BIOSYNTHESIS REGULATED DURING THE CELL CYCLE?

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One of the essential steps during cell division is the induction of phosphatidylcholine (PC) biosynthesis that provides the principal lipid component for the synthesis of new membrane. Regulation of CTP: phosphocholine cytidylyltransferase alpha (CTα) expression is critical since it is the rate limiting enzyme in the PC biosynthetic pathway. We previously demonstrated that CTα mRNA expression is increased during S phase and that Sp1 is the major transcriptional factor involved in this regulation. When cells reach the S phase, Sp1 interacts with cyclinA, cyclinE and cdk2. To understand the physiological role of this interaction we over-expressed each of these proteins in fibroblasts and determined that CTα expression increased to a high and constant profile throughout the cell cycle. Using siRNA to inhibit the expression of each of the proteins present in the complex, we were able to block the induction of CTα. Immunoblots against Sp1 showed a change in the mobility of Sp1 in samples obtained in S phase. Cell labeling experiments showed that Sp1 became phosphorylated when the cell reaches the S phase. We confirmed that the phosphorylation of Sp1 is mediated by cdk2 using *in vitro* phosphorylation assays. We concluded that phosphorylated Sp1 is the active form that binds the CTαpromoter.

LP-C10.

ALTERNATIVE PATHWAY FOR POLYUNSATURATED FATTY ACIDS (PUFAs) BIOSYNTHESIS IN TRYPANOSOMATIDS

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Trypanosome brucei and *Leishmania major* are parasitic protozoa belonging to the trypanosomatid family, causative agents of human African sleeping sickness and leishmaniasis, respectively. They have a high proportion of long-chain PUFAs which appear to be generated by a different pathway than in their mammalian host. This fact and the assumption that unsaturation degree of membrane lipids is crucial for parasite viability, make desaturases a good target for chemotherapeutic drugs. At present it is known that desaturation beyond some 18:2 substrates occurs throughout "front end" desaturases and may follow *Euglena gracilis* or mammalian pathway. Our main goal was to identify the route of PUFA's biosynthesis in trypanosomatids. We cloned and expressed three "front end" desaturases from *L. major* (Δ^5, Δ^6 and Δ^8-desaturases) and one from *T. brucei* (Δ^6-desaturase) managing available information in the genome project of each organism. After expression in yeast with the addition of different substrates, we purified the fatty acids and analysed their profile by GC-MS. Sequence analysis led us to predict that Δ^6 desaturases were actually Δ^4 desaturases, and this result was corroborated by activity studies. Previous work in our lab indicated that *T. brucei* contains a Δ^{12} desaturase, whereas no information is still available about the occurrence of a Δ^{15} desaturase. Altogether, our results suggest that PUFA's biosynthesis in trypanosomatids follows the recently described *E. gracilis* pathway.

MI-C1.
NOVEL ROLE OF THE TRANSCRIPTION FACTOR Spo0A FOR THE MULTICELLULAR BEHAVIOUR OF *Bacillus subtilis*

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The spore-forming bacterium *Bacillus subtilis* is capable of assembling multicellular communities (biofilms and fruiting bodies) that display a high degree of spatiotemporal organization. Wild type (wt) strains, that have not undergone domestication in the laboratory, produce particularly robust biofilms and fruiting bodies with complex architectural features, in which it is observed appendixes and preferential sites of sporulation. Here we worked with two strains of *B. Subtilis: JH642* and RGAR1. The former is a domesticated strain and the later has been isolated from human-destined food made of fermented soybean by a Japanese strain of *B. subtilis* variety natto. After construction and analysis of diverse RGAR1-derived strains, we observed that wt and *sigF* strains, but not *spo0A* mutants, growing up in complex media formed long multicellular structures. Moreover, we found that natto mutants in *spo0A* (which encodes for the major early sporulation transcription factor) were deficient in biofilms and fruiting bodies formation as long as mutants in *sigF* (which are unable to sporulate but produce Spo0A) resembled the wt phenotype. Using spectrophotometric and optical techniques we observed that the wt and *sigF* natto strains showed strong adherence to extracellular matrix proteins such as fibronectine and collagen. The overall results point out novel roles, apart from sporulation, for Spo0A during biofilms, fruiting body formation and adherence.

MI-C2.
TEMPERATURE REGULATION OF THE ANTHRAX TOXIN GENES IN *BACILLUS SUBTILIS*

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Anthrax, a potentially fatal disease of animals and man, is caused by the Gram-positive endospore-forming bacterium *Bacillus anthracis*. The anthrax toxin, one of its major virulence determinants, is a tri-partite molecule composed of a cell binding protein, termed protective antigen, the lethal factor and the edema factor. The toxin genes, *pagA, cya* and *lef,* are coordinately regulated at the level of transcription and are induced by bicarbonate and increase in temperature, but the mechanism of thermal regulation remains unsolved. Using *pagA-lacZ* transcriptional fusions we determined that expression of the protective antigen responds to temperature and to signals of stationary phase in a *B. subtilis* background, in the presence of the *B. anthracis* regulator AtxA. We also confirmed that transcription of the ORFs 90, 126 and *lef* coded in pOX1 is atxA-dependent, as had been observed in microarrays experiments, but only ORFs 90 and *lef* show temperature-dependent expression in a *B. subtilis* background. Since this effect is not mediated by an increase of AtxA protein, some other factor must be involved, and an homolog of this putative co-regulator of toxin genes transcription must be present in *B. subtilis*. We proved that the DesKR two-component system that senses temperature in this bacteria is not playing this role.

MI-C3.
YojI OF *ESCHERICHIA COLI* FUNCIONTS AS A MICROCIN J25 PUMP

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Microcin J25 (MccJ25) is a plasmid-encoded, 21-amino-acid, antibacterial peptide produced by *Escherichia coli*. In the course of experiments aimed at cloning a MccJ25-resistant mutation, we found a recombinant plasmid able to confer resistance to the antibiotic but, unexpectedly, this plasmid did not contain the mutation. Its analysis led us to the identification of a previously described chromosomal locus, *yoj*I. In the present study, we show that YojI, an *Escherichia coli* open reading frame of unknown function, mediates resistance to the peptide antibiotic microcin J25 when expressed from a multicopy vector. To see if *yojI* is functional when present in a single copy, it was necessary to compare the resistance to MccJ25 of wild-type and *yojI* cells. To this end, the chromosomal *yojI* gene of strain DH5á was deleted This mutant, in a spot-on-lawn test, was approximately eightfold more sensitive than the control DH5á and showed completely clear zones of growth inhibition. YojI was previously assumed to be an ATP-binding-cassette-type exporter on the basis of sequence similarities. We demonstrate that YojI is indeed capable of pumping out microcin molecules and that it requires the multifunctional outer membrane protein TolC for its function. Thus, one obvious explanation for the protective effect against MccJ25 is that YojI would keep the intracellular concentration of the peptide below a critical level. MccJ25 appears as the first known substrate for YojI.

MI-C4.
EL TOR HAEMOLYSIN FROM *VIBRIO CHOLERAE* INDUCES APOPTOSIS AND INCREASES INTRACELLULAR Ca^{2+} LEVELS IN HUMAN INTESTINAL CELLS

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V. cholerae O1 biotype El Tor and most of *V. cholerae* non-O1 / non-O139 (VCN) produce El Tor Haemolysin (ETH), a pore forming toxin encoded by the *hlyA* gene. In previous studies we have shown that ETH could be involved in the diarrhea caused by a VCN clinical isolate lacking cholera toxin (VCN CT-). Additionally, we demonstrated ETH-induced apoptosis on Cos-7 cells. To explore the potential involvement of apoptosis in the pathogenesis caused by ETH on human intestinal cells, we exposed undifferentiated Caco-2 and differentiated C2BBe1 to sterile culture supernatants from VCN CT- or from its isogenic *hlyA* null mutant. At different times post-incubation, caspase-3 activation, internucleosomal DNA fragmentation and increased sub-G1 fraction of fragmented nuclei were detected by a Caspase-3 apoptosis detection kit, agarose gel electrophoresis/ ethidium bromide staining and flow cytometry/propidium iodide staining, respectively. To investigate the role of second messengers involved in response to ETH, changes in Caco-2 intracellular Ca^{2+} levels were measured after exposition to ETH by flow cytometry using Fluo3-AM. After 2 and 4 h of exposition to 200 ng/mL of ETH, intracellular Ca^{2+} increased 22,0% and 36,6%, respectively. These results demonstrate that when added to human intestinal cells, ETH is able of causing cell death via apoptosis and that an intracellular increase in Ca^{2+} concentration occurs in response to this toxin.

MI-C5.
CHARACTERIZATION OF F0.F1-ATPASE MUTANTS AND ANALYSIS OF THEIR CONTRIBUTION TO THE ACID TOLERANCE MECHANISM IN PNEUMOCOCCUS

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We have previously reported an acid tolerance response (ATR) in pneumococcus, a main human pathogen. It has been described that F0.F1-ATPase plays a role in ATR in bacteria. In this work, our aim was to analyse the contribution of this enzyme to the induction of pneumococcal ATR. F0 F1 ATPase is constituted by several subunits codified by *atp* genes, but insertional mutants could not be obtained due to they are essential for viability. Point mutations on *atpAC* genes, coding for two subunits of F0 complex, have been described to be responsible for optochin resistance. With the purpose to obtain these mutants, we analyzed nine optochin-resistant clinical strains. The *atpABC* genes were amplified individually from each strain, PCR products were transformed to an uncapsulated pneumococcal strain, and mutants were selected by optochin resistance. Once these mutants were obtained in the same genetic background, the ATR phenotype was examined and compared with wild-type strain. We found that modifications in the subunits *c* (V48L) and *a* (W206C) showed a higher lethal pH than wild-type strain. In contrast, another mutation in the subunit c (G47V) showed an increased tolerance to acidic stress. In this work, we present ATR phenotypes of these *atp* mutants that were not reported previously in bacteria. We suggest that F0.F1-ATPase is one of the main components of the ATR mechanism in pneumococcus, most likely by its H^+ extrusion activity that controls the intracellular pH.

MI-C6.
MEMBRANE TOPOLOGY ANALYSIS OF CYCLIC GLUCAN SYNTHASE, A VIRULENCE DETERMINANT OF *BRUCELLA ABORTUS*

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Brucella abortus cyclic glucan synthase (Ba-Cgs) is an integral inner membrane protein of 316 kDa (2,831 residues) responsible for the synthesis of cyclic b-1,2-glucan by a novel mechanism in which the enzyme itself acts as protein intermediate. Ba-Cgs uses UDP-glucose as donor-sugar and has the three enzymatic activities required for the synthesis of the cyclic polysaccharide, ie: *initiation, elongation* and *cyclization*. Cyclic glucan is required in *Brucella abortus* for effective host interaction and full expression of virulence. To gain further insight into the structure and mechanism of action of Ba-Cgs we studied the membrane topology of the protein using a combination of in silico predictions, a genetic approach involving the construction of fusions between the *cgs* gene and the genes encoding alkaline phosphatase (*phoA*) and b-galactosidase (*lacZ*) and site-directed chemical labeling of lysine residues. We found that Ba-Cgs is a polytopic membrane protein with the amino and carboxyl terminus located in the cytoplasm and containing six transmembrane segments; I (residues 419-441), II (residues 452-474), III (residues 819-841), IV (residues 847-869), V (residues 939-961) and VI (residues 968-990). The six transmembrane segments determine four large cytoplasmic domains and three very small periplasmic regions.

MI-C7.
PhoP/PhoQ REGULON: THE RstA/RstB SYSTEM AND A NOVEL REGULATORY CASCADE

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In *S. typhimurium* the PhoP/PhoQ two-component system governs the adaptation to Mg^{2+} limited-media, and participates in macrophage survival, epithelial cell invasion, among other pathogenic phenotypes. We determined that the expression of another two-component system, RstA/RstB is under Mg^{2+}-controlled, PhoP-dependent regulation in *Salmonella*. Besides, we determined that *rstA* expression was not autoregulated. We carried out a screening for RstA regulated genes using a Mud*J* insertional mutagenesis approach and we found that s*pvA*, *narZ*, and a pseudogen (STM2689) were under RstA transcriptional control when this regulator was overproduced from a plasmid in a *rstA* background. STM2689 shows high homology with *Salmonella* repetitive proteins involved in virulence. *narZ* codes for a nitrate reductase and *spvA* belongs to the *Salmonella* virulence plasmid. Both *narZ* and *spvA* are involved in *Salmonella* intracellular survival, and are RpoS-dependent. We verified the RpoS-dependent activation in our insertional mutants, and additionally showed that it was not due to a transcriptional regulatory effect of RpoS on *rstA*. These results lead us to predict that rstA/B should be implicated in sensing environmental cues triggered by stationary phase and/or in the intracellular milieu.

MI-C8.
***mgtA* AND MAGNESIUM HOMEOSTASIS IN *Salmonella*. THE ROLE OF THE 5' UNTRANSLATED REGION ELEMENTS**

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Magenisum intracellular levels are tightly regulated within the cell because it is required for a wide variety of cellular functions. In *Salmonella*, three transporters mediate Mg^{2+} uptake: the P-type ATPases MgtA and MgtB, whose expression is induced by the PhoP/PhoQ two-component system; and the constitutively expressed ion-channel, CorA. We have previously reported the existence of a novel checkpoint in the Mg^{2+} deprivation response. This mechanism can detect this cellular stress bypassing PhoQ, posing an additional control over *mgtA* and *mgtCB* expression.
In this work we demonstrate that this regulation modulates the intracellular levels of the magnesium transporters by controlling the transcription initiation step, trough a mechanism dependent of topological factors. Moreover, strains lacking the 5'UTR of *mgtA* did not show the PhoQ-independent regulation, suggesting that a transcription attenuation mechanism could be involved in the control of magnesium homeostasis. We further characterized the 5´untranslated region elements by testing the effect of deleting distinct stem loop structures. This study provides new insights into the mechanisms that allow *Salmonella* to proliferate within its host.

MI-C9.
REGULATION OF SPORULATION AND ENTEROTOXIN PRODUCTION OF *Clostridium perfringens*
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The Gram-positive and spore-forming bacterium *Clostridium perfringens* is the main cause of gas gangrene and one of the most common agents of food poisoning in humans and animals. The enterotoxigenic pathology is due to the production of a potent toxin (CPE), which is responsible for the symptoms of enterotoxemia, intestinal cramping, and diarrhea. Expression of *cpe* is strictly linked to the spore formation. Since sporulation would be under the control of nutritional and environmental signals, we wondered about their nature. In this work, we show that phosphate (Pi) (but not pH) constitutes one important signal for sporulation and CPE production in *C. perfringens*. The wild type and reference strain of *C. perfringens* SM101 was able to sporulate only when a conditioned sporulation medium was supplemented with Pi. The efficiency of spore formation of SM101 dropped from 100% to 0.00001% when Pi was omitted from the conditioned medium without affecting the final cellular yield. The â-glucuronidase activity of a SM101 strain harboring a reporter *cpe-gusA* transcriptional fusion was almost abolished when Pi was omitted from the medium suggesting than CPE was not produced. Experiments of fluorescence microscopy plus western blot analysis using anti-Spo0A and anti-CPE antibodies indicated that the Pi signal acts at the level of the onset of *Clostridium* sporulation. To our knowledge this is the first time that it is identified a natural signal regulating the onset of sporulation in Bacteria. The results are also relevant to the design of antidotes against intoxications in humans and animals due to *C. perfringens*.

MI-C10.
EFFECTS OF LEPTOSPIRAL PROTEINS ON HUMAN ENDOTHELIAL CELLS
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Endothelial disfunction is observed in leptospirosis patients. In order to investigate this phenomenon, two lipoproteins identifyed on the leptospira genome, LIC10091 (P94) and LIC11360 (P107), were cloned in *E. coli*, expressed and purified, and the effects of the recombinant proteins on human umbilical vein endothelial cell (HUVECs) were evaluated. After exposure, von Willebrand Factor (vWF) was measured in HUVECS supernatants by ELISA and ICAM-1 or ELAM expression was determined by flow cytometry. We found that levels of both ICAM-1 and ELAM (mean fluorescence intensity, n = 6) were augmented in the cells exposured to P107 (5 mg/ml) (control: 37 ± 8 and 16 ± 4 vs. 189 ± 24 and 101 ± 23 respectively, $p < 0.05$). Neither constitutive synthesis or release of vWF were modulated by P107 (n=3). Exposure of HUVECs to different concentrations of P94 (5-50ug/ml) failed to modify the content of cell adhesion molecules or vWF levels (n=3). These results suggest that P107 (LIC11360) could be involved in the endothelial dysfunction caused by leptospira infection.

MI-C11.
PRO APOPTOTIC ACTIVITY OF *BORDETELLA BRONCHISEPTICA* LIPOPOLYSACCHARIDE IN EPITHELIAL AND IMMUNE HOST CELLS
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Bordetella bronchiseptica is a gram-negative bacteria that cause respiratory tract infections characterized by a chronic period. In this period the bacteria presents some phenotypic variations that includes the lipopolysaccharide (LPS) structure. While in the first acute period the LPS has a smooth structure, in the chronic period a deep rough LPS is present. This change in the LPS structure could be essential for bacterial survival within the host and, more specifically within the host cell. Taking into account that the LPS from other Gram-negative pathogens are involved in apoptotic induction mechanisms in different host cells, we decided to analyze if those different LPS forms of *B. bronchiseptica* were able to induce apoptosis in the host cells that were in close contact with the bacteria during the infection. For this purpose we used the smooth and deep rough forms of LPS obtained from either *B. bronchiseptica* isolated from different stage of human infection or *B. bronchiseptica* collection strain and its deep rough mutant constructed by us. In epithelial cells (A549) all the LPS forms studied induce apoptosis (3 to 5 times higher than the control) but not necrosis. In neutrophils we observed that smooth as well as the deep rough LPS forms induce apoptosis (6 times higher than the control) and necrosis. An interesting result was obtained when monocytes cells were used. In this case we observed that the deep rough LPS form was the unique structure of LPS that induced apoptosis suggesting that this form is critical for the persistence of the bacteria within the host cell.

MI-C12.
INFLUENCE OF EXOPOLYSACCHARIDE COMPOSITION AND N-SOURCE LIMITATION ON INFECTIVITY OF *BRADYRHIZOBIUM JAPONICUM*
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The exopolysaccharide (EPS) of *B. japonicum* plays a key role in the rate of bacterial penetration (infectivity) of soybean roots for the development of N_2-fixing nodules. Since the composition and amount of these molecules may change according to the bacterial growth conditions, it is important to assess these effects on infectivity.
Here we analyzed the EPS composition in the wild-type strain USDA 110 and in an *exo*B mutant, both grown in defined N-sufficient or N-limiting media, and with mannitol or malate as sole C-source. The samples were obtained by ethanol precipitation of culture supernatants and TFA-hydrolysis followed by HPAEC-PAD analysis. Both strains produced more EPS (mg sugar/mg cell protein) under N-starvation. However, no changes were observed in the EPS composition of each strain when grown with different C-sources or N-starvation. The wild-type EPS was composed of 1 Glu : 0.5 (OMe/OAc)Gal : 0.5 (OAc)GalA : 0.17 Man. The mutant EPS lacked Gal, as expected from the absence of UDP-4-Glu epimerase activity, but contained GalA. Although the *exo*B mutant was less infective than the wild-type with both C-sources, each strain was more infective when grown under N-starvation in relation with the same bacteria from N-sufficient broths.

MI-C13.
ISOLATION AND CHARACTERIZATION OF AN ACID SENSITIVE Tn*5* MUTANT OF THE ALFALFA NODULATING Or191-LIKE RHIZOBIA

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Soil acidity is one of the environmental factors that more strongly hampers the establishment of an effective symbiotic interaction between rhizobia and *Medicago sativa* (alfalfa). In the recent past years a new group of alfalfa-nodulating rhizobia (Or191-like rhizobia) has been identified and genetically characterized, mainly due to their remarkable tolerance to acidity, inefficiency to fix nitrogen, and strong ability to outcompete *S. meliloti* during root nodulation at low pH. To advance on the genetics of acid tolerance in the Or191-like rhizobia, we generated a collection of Tn*5*B20 mutants and we screen the collection for acid sensitive phenotypes. One mutant out of 1,500 clones failed to grow on minimal medium at pH 5.0 but not at neutral pH. The acid-sensitive mutant, namely LPU833, also showed to be sensitive to oxidative stress, and to the presence of 100mM Cu^{++} and 40mM Zn^{++}. Genetic analysis showed that mutant LPU833 carries a single transposon insertion within an ORF with sequence similarity to a family of bacterial oxidoreductases, located immediately upstream of an *ubiH* homolog involved in ubiquinone metabolism. The mutant LPU833 kept the ability to nodulate alfalfa. However, the symbiotic relevance of the affected locus was evident in coinoculation experiements where mutant LPU833 resulted fully outcompeted by the wild type strain at both acidic and neutral pH.

MI-C14.
HIGHER BACTERIAL MOTILITY ENHANCES INFECTIVITY OF *BRADYRHIZOBIUM JAPONICUM*

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Motility of *Bradyrhizobium japonicum* in soil or vermiculite has been shown as scarce in the vertical direction. When a naturalized plant-compatible rhizobial population is established in the soil, this scarce vertical motility may prevent the nodulation by an inoculated strain applied to the seeds, because most of the inoculant remains in non-infectable root areas. Here we evaluated whether an increased rhizobial motility may enhance infectivity and nodule occupation.
By means of a recurrent selection procedure we obtained a *B. japonicum* strain with higher motility than the wild-type, a phenotype that we called as Mot^{++}. Growth rate and nodulation ability of Mot^{++} mutants were not affected, and the Mot^{++} phenotype was stable after passage through nodules. As the parent strain, the Mot^{++} strain had single polar flagella. The Mot^{++} strain displayed a higher rate to infect the emergent root hairs of young soybean plants. When applied to soybean seeds in competition with an isogenic rhizobial population established in the vermiculite substrate since 30 days before (10^7 cfu/ml free solution), a small increment in nodule occupation by the Mot^{++} strain was observed in relation to the wild-type. These results suggest that the increase in motility may be applied to improve inoculant competitiveness in field situations.

MI-C15.
TWO SALT TOLERANT RHIZOBACTERIA BELONGING TO OCHROBACTRUM GENUS PROMOTE MAIZE GROWTH

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With the aim of obtain salt tolerant strains native of Córdoba soils able to promote the growth of important regional cultures, we isolated rizosphere and endorhizosphere bacteria from maize as tramp host. They were tested in order to establish their PGPR properties *in vitro* (N_2 fixation, siderophore and AIA production, phosphate solubilization) and characterized on their ability to promote maize growth in greenhouse conditions.
The inoculation of maize grown in sand:perlite (1:1) with the strain designed $ME_2$11a significantly ($p<0.05$) increased the shoot and root dry weights about 40% as compared to the control in both salinity and non salinity conditions.
Other salt tolerant bacteria selected designed ME3b produced a significant increment in root and shoot dry weight parameters under non salinity conditions.
From the 16S rDNA sequencing analysis of these strains, they were assigned to the genus Ochrobactrum (99 % homology).
Ochrobactrum sp. ME11a and ME*3b* were able to produce siderophore and to solubilize phosphate on respective media.
The AIA production was positive only in *Ochrobactrum sp. 3b* and N_2 fixation was showed in *Ochrobactrum sp. 11a*.
One of these strains showed a high resistence to antibiotic and heavy metal and was isolated from the endorhizosphere of seedlings maize.

MI-C16.
SEVERAL POLYSACCHARIDES ARE INVOLVED IN CELL TO CELL INTERACTIONS AND BIOFILM FORMATION IN *RHIZOBIUM LEGUMINOSARUM* BV. VICIAE

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Our previous results indicated that *Rhizobium leguminosarum* bv. *viciae* 8401 pRL1JI is capable of forming a three-dimensional structure known as biofilm on minimal medium. This bacterial behaviour could be advantageous for rhizobial survival and promoting root hair attachment. Confocal laser scanning microscopy was used to visualise the different events of biofilm formation with GFP-labelled bacteria in chambered coverglass slides. The PrsDE system is responsible for secretion of several proteins including the glycanases PlyB and PlyA and three adhesins. We have previously shown that mutations in *prsD*, *prsE* or *plyB* severely affect biofilm maturation. The exopolysaccharide (EPS) and cellulose are both substrates of PlyB and PlyA. A mutant in *pssA*, a crucial gene for the EPS synthesis was unable to develop a biofilm although tight cell-to-cell interactions were observed in bacterial clumping. An additional mutation in *celA*, which encodes for the cellulose synthase abolished clamp formation in the *pssA* mutant, indicating that cellulose synthesis is crucial in clamping. Interestingly, the O antigen region of LPS contributes to lateral cell-to-cell interactions and it is essential in biofilm formation. Our results indicate that in *Rhizobium leguminosarum* multiple surface and extracellular factors participate in modulation of biofilm structure.

MI-C17.
REGULATION OF SUCROSE METABOLISM IN *ANABAENA* SP. PCC 7120 UNDER DIAZOTROFIC GROWTH BY A GLOBAL NITROGEN REGULATOR

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Global nitrogen regulation is operated in cyanobacteria by the CRP family transcriptional regulator NtcA, which activates or represses expresion of genes whose products are involved in nitrogen assimilation. In heterocyst-forming cyanobacteria, such as *Anabaena* sp. PCC 7120, NtcA is also required for heterocyst development and diazotrophic growth. It has been showed a crucial role of sucrose in dinitrogen assimilation in that strain. Sucrose is synthesized in a two step pathway involving sucrose-phosphate synthase and sucrose-phosphate phosphatase, and cleaved by sucrose synthase. These enzymes are encoded by *sps*A and *sps*B, *spp,* and *sus*A, respectively. We have detected the presence of putative binding sites for NtcA in the promoter sequences of *sps*B and *sus*A genes that are found upstream and downstream from the transcription start site, respectively. In order to investigate whether the genes involved in sucrose metabolism are trancriptionally regulated by NtcA, we studied gene expression in the strains PCC 7120 and CSE2 (mutant impaired in NtcA synthesis). Primer extension and RT-PCR assays allowed us to estimate the level of transcription of each gene. In the mutant strain, we detected higher levels of *sus*A and lower levels of *sps*B transcripts when comparing with the wild-type strain. These results suggest that sucrose metabolism may be coordinated with nitrogen assimilation through the action of NtcA.
Supported by ANPCyT, CONICET, UNMdP and FIBA.

MI-C18.
MAPPING OF MOLECULAR DETERMINANTS OF PATHOGENICITY OF COXSAKIEVIRUS B1

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Coxsakievirus B (CVB) is an etiological agent of myocarditis, which precedes dilated cardiomyopathy. Our aim was to characterize the molecular determinants of the pathogenicity of CVB1 (CVB serotype 1). To this end, we intraperitoneally inoculated viruses derived from infectious cDNA clones into several inbred strains of weanling mice. We first studied the effect of clone CVB1o (provided by Dr. Nomoto; University of Tokyo) which showed no signs of pathology. By inoculation of SCID mice with CVB1o a variant (CVB1m) was obtained that induced a severe pancreatitis and myocarditis in weanling C3H/HeJ mice. The genome of this variant was amplified by RT-PCR in three partially overlapping segments comprising nts 1-3448, 1571-5135 and 4916-polyA (the CVB1 genome contains ~7400 nts plus a ~100 nt long polyA tail). These cDNA fragments were cloned into pGEM-T vector and used to construct chimeras. A chimera including the 5' UTR of CVB1m and has a phenotype similar to that of CVB1o. In contrast, a chimera including the first 4300 nts of CVB1m exhibited a behavior similar to that of CVB1m. These results indicate that determinants of pancreatitis and myocarditis are located between nts 800 to 4300. We are generating additional chimeras that will allow us to narrow down the genomic sequences associated with pathogenicity, as well as to verify if cardiotropism and pancreatropism determinants correspond to the same region.

MI-C19.
STUDIES ON DEGRADATION OF *Crithidia fasciculata* ODC OBTAINED "*in vitro*" WITH A TRANSCRIPTION-TRANSLATION SYSTEM

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Polyamines are essential for cell growth and differentiation. The first step in putrescine biosynthesis found in mammals, lower eucariots and protozoa is catalyzed by ODC which promotes the decarboxylation of ornithine. *T. cruzi* is a unique organism in Nature, being auxothrophic for polyamines as it lacks ODC and ADC genes. Digenetic trypanosomatids contain stable ODCs, whereas *C. fasciculata* as well as other monogenetic parasites, have ODCs with a rapid turn-over. However, when *C. fasciculata* ODC gene is expressed in *T. cruzi*, the enzyme becomes stable with a half-life longer than 24 hrs.

To analyse the mechanism probably explaining the different stability showed by ODC when expressed in *C. fasciculata* or in transfected *T. cruzi*, labelled and active ODC was obtained "*in vitro*" by coupled transcription-translation system from reticulocyte lysates. When incubated with a *C. fasc*. extract, the radioactive product showed no degradation but the enzymatic activity of ODC was strongly inhibited. On the contrary, *T. cruzi* lysates were able to process the protein producing shorter polypeptides retaining most of the decarboxylating activity in the presence of PLP-cofactor. These preliminary results showed that the degradation systems of both parasites have different properties.

MI-C20.
THE COMPLETE STRUCTURE OF VIPER, AN UNUSUAL LTR RETROELEMENT PRESENT IN TRYPANOSOMAS BUT NOT IN *LEISHMANIA MAJOR*

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VIPER was initially characterized as a 2,359 bp long retroelement of *Trypanosoma cruzi* associated with SIRE (Short Interspersed Repetitive Element). Sequence comparisons of its RT-RNase H domains determine its classification in the family of LTR. Analysis of different CL-Brener genomic sequences allowed the identification of a 4,480 bp long VIPER that includes an ORF for *gag* protein, next to it an ORF encoding for tyrosine recombinase and the previously mentioned RT-RNase H ORF. This structure is unusual for a LTR retrotransposon because it lacks the long terminal repeats that are replaced at the 5' end by the first 182 bp of SIRE and at the 3' end by the SIRE's last 226 bp. Using BLAST we were able to identify VIPER-like retroelements in *Trypanosoma brucei*, but not in *Leishmania major*. Further analysis demonstrated that in *T. brucei* VIPER is found within 5' and 3' sequences that bare homology with SIRE. Genome wide analysis of the recently sequenced trypanosomatid genomes strongly suggest that extinction and expansions of LTR and non LTR retroelement families correlate with parasite speciation.

MI-C21.
IDENTIFICATION AND PARTIAL PURIFICATION OF QUINONE OXIDOREDUCTASE (QOR) ACTIVITIES PRESENT IN *TRYPANOSOMA CRUZI*

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The cytotoxic efects of several lipophilic *o*-naphtoquinones such as b-lapachone present were attributed to the formation of "reactive oxygen species" (ROS) by the one or two electron reduction of the quinone to semiquinone or hydroquinone respectively catalyzed by the "quinone oxidoreductasas" (QOR). Previous work of our group applying digitonin treatment of intact cells suggested the presence of several QOR activities with different subcellular localization in epimastigotes of *T.cruzi.* One of them presented a unique localization since it was liberated from the cell at a concentration of digitonin of 0.16 mg/ml, considerably lower than the one necessary to liberate the cytosolic markers. Partial purification of the extract obtained by batch treatment of the epimastigotes with this concentration of digitonin rendered a preparation that analyzed by SDS-PAGE showed the presence of one band identified by MALDI-TOF MS as "The old yellow enzyme". On the other hand purification of the extract obtained by freezing and thawing of the pellet that remains after treatment of the cells with 0.4 mg/ml digitonin, allowed the partial purification of a " hypothetical protein" annotated in the *T.cruzi* genomic data base as a member of the family of D-isomer specific 2-hydroxyacid dehydrogenases, not detected before in Trypanosomatids, that presented a NADH dependant QOR activity.

MI-C22.
DIRECT MOLECULAR TYPING OF *T. CRUZI* LINEAGES INVOLVED IN CONGENITAL CHAGAS DISEASE

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Congenital Chagas disease (CgCD) affects 1 to 7% newborns of infected mothers. A factor in CgCD transmission may be the parasite strain. *Trypanosoma cruzi* has a multiclonal structure classified into 6 major phylogenetic lineages: I and IIa-e, based on culture depending biochemical and DNA techniques. This study aimed to set up and apply PCR methods based on polymorphic sequences (mini-exon, 18S, 24S rDNA) to type *T.cruzi* lineages directly from peripheral blood of mothers and their newborns, as well as from CgCD suspected infants (n=33). Their parasite entities were also profiled by RFLP-PCR and LSSP-PCR from minicircle DNA amplicons (kDNA). RESULTS: *T. cruzi* IId was found in all mothers and their offspring. The kDNA profiles were almost identical within each mother-newborn pair, but different among the tested pairs. Eight of 10 CgCD suspected cases were infected by *T. cruzi* IId and two by *T. cruzi* I. The last ones were brother and sister of 2 *T. cruzi* IId infected children; they also depicted differential kDNA profiles associated to each lineage, suggesting a different source of transmission in this family. The RFLP-PCR patterns of the patients´parasite populations showed lower kDNA diversity than the observed in culture stocks of the same lineages. A higher degree of genetic diversity was also detected in bloodstream parasites of immunosupressed chagasic patients, suggesting that the immunological system of the host somehow regulates the diversity of *T.cruzi* populations.

MI-C23.
UNIQUE STRUCTURAL AND FUNCTIONAL FEATURES OF *TRYPANOSOMA CRUZI* RIBOSOMES AS DETECTED BY YEAST TWO-HYBRID, SEQUENCE COMPARISON AND CRYO-ELECTRON MICROSCOPY

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Ribosomal P proteins form a conserved ribosomal structure, named the stalk, involved in the translocation step of protein synthesis. *T. cruzi* P proteins were initially cloned as major antigens recognized by the host immune system during the chronic phase of Chagas Disease. Sequencing of these proteins revealed specific features of the *T. cruzi* P protein system. The complete set of *T. cruzi* P proteins was cloned and studied using yeast two-hybrid. Comparison with yeast and mammals reveals a parasite-specific profile of protein interactions. Together with this, analysis of rRNA and protein ribosomal genes obtained from the *T. cruzi* genome databank show several parasite specific signatures. In order to confirm these observations, we used cryo-electron microscopy (CEM) to reconstruct the *T. cruzi* ribosome at 12.6 Å resolution. Notably, remarkable and unique features of the parasite ribosome correlate with experimental and data mining findings.

MI-C24.
PROTEIN INTERACTION MAP AND FUNCTIONAL ANALYSIS REVEAL THE UNIQUE ORGANITATION OF THE TRANS-SPLICEOSOMAL E COMPLEX IN TRYPANOSOMES

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Data mining of the trypanosome genome data banks (*T. cruzi* and *T. brucei*) allowed the identification and cloning of 18 ORFs involved in trans-splicing and 9 polyadenylation factors. Our initial analysis focused on the E complex composed by U2AF, an heterodimer formed by the U2AF 35 and 65 subunits, and the SFI/BBP protein. U2AF35 is a nuclear protein distributed in speckles, and lacking an essential Trp residue in position 134, which is essential for the "tongue in groove" heterodimerization with U2AF65. Accordingly, TcU2AF35 did not interact with the human U2AF65. The trypanosome version of U2AF65 is also largely modified with respect to the human counterpart. The SFI/BBP factor has a modified KH domain and lacks the Zinc finger motifs. A trypanosome specific profile of interactions among these proteins was mapped. Functional analysis suggests that U2AF35 is the "date hub" of the E trans-splicing complex of Trypanosomes.

PL-C1.

ALTERING *ASR*1 GENE EXPRESSION HAS DRAMATIC EFFECTS ON METABOLISM IN DIFFERENT PLANT SPECIES

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The *Asr* gene family was first cloned from commercial tomato. Since then it has been found widely spread throughout the plant kingdom with the exception of *Arabidopsis*. Four members have been found in tomato localised on chromosome IV. *Asr* genes have been shown to be regulated by various kinds of stresses and ABA. On the basis of *in vitro* experiments a recent report has demonstrated that ASR1 binds DNA in a Zn-dependent manner; in addition another report remarks that an ASR acts as a transcriptional regulator of a hexose transporter in grape. However, the physiological role of these proteins remains to be elucidated. Here we present results with tobacco and potato plants that express tomato *Asr*1 either in sense or antisense orientation. By means of a detailed metabolic analysis we have observed dramatic effects on growth and hexose metabolism, with phenotypes resembling those of plants displaying altered sugar transport. These features support a potential role for this transcription factor as a central contributor to a mechanism for sensing and/or distributing sugar within the plant.

PL-C2.

***HAHB-10*, A SUNFLOWER HOMEOBOX-LEUCINE ZIPPER GENE, IS INVOLVED IN THE RESPONSE TO DARK/LIGHT CONDITIONS**

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Homeodomain-leucine zipper (HD-Zip) proteins constitute a family of transcription factors found only in plants. Experimental evidence indicated that these proteins are involved in regulating developmental processes associated with the response of plants to environmental conditions.

We have performed functional studies on *Hahb-10* (*Helianthus annuus* homeobox-10), a member of the Hd-Zip II subfamily of Hd-Zip proteins. Expression studies showed that this gene is expressed primarily in mature leaves and is strongly induced in seedlings when seeds are germinated and grown in the dark. A similar high induction of *Hahb-10* expression was observed when seedlings were subjected to treatment with gibberellins. Transgenic *Arabidopsis thaliana* plants that express *Hahb-10* under the control of the 35S cauliflower mosaic virus promoter were obtained. These plants showed special phenotypic characteristics like darker cotyledons and a marked acceleration of development, reducing the life cycle about 25%. These plants also showed atypical responses when treated with different light conditions. We propose that *Hahb-10* may function in signalling cascade(s) that control(s) plant adaptive responses to dark/light conditions.

PL-C3.

FUNCTIONAL CHARACTERIZATION OF A POTATO TRANSCRIPTIONAL COACTIVATOR (StMBF1) UNDER DIFFERERNT STRESS CONDITIONS

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Multiprotein bridging factors 1 (MBF1) have been described as transcriptional coactivators that mediates transcriptional activation by bridging between an activator and TATA-box binding protein (TBP). We have reported the identification of a *Solanum tuberosum* transcriptional coactivator named *St*MBF1, which is up-regulated by biotic and abiotic stress in potato tubers. In this work we described a fast *St*MBF1 protein accumulation in potato cell suspensions treated with 20 mM H_2O_2 Hydrogen peroxide production is though to be increased under various biotic and abiotic stresses and to enhance gene expression of active oxygen scavenging (AOS) enzymes. To further study the possible role of MBF1 during stress, we persuade its functional characterization in *Arabidopsis thaliana*. MBF1 protein levels in flower and fruit were higher than in root and leaves. Furthermore, we have developed transgenic antisense lines of the *Arabidopsis* MBF1c gene and we are also characterizing a T-DNA insertion line in this gene from the SALK collection. Five day-old plants from these lines were treated with 15 mM H_2O_2 (30 min to 24 h) and analyzed cell death by Evans blue staining. We also tested if high salt concentrations (50-100 mM NaCl) affect root elongation. In both cases, transgenic plants were more susceptible to the stress treatments. All these findings reinforce the role of MBF1 in plant stress responses, although their regulation in the transcriptional mechanism remained unclear.

PL-C4.

A COMPREHENSIVE ANALYSIS OF PLOIDY-REGULATED GENE EXPRESSION IN *Paspalum notatum*

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Although the causes of novel variation in polyploids are not well understood, they could involve changes in gene expression through dosage-regulation and altered regulatory interactions, as well as rapid genetic and epigenetic changes. The wide-genome expression of ~ 10000 genes was monitored by using transcript profiling technology on flowers of a diploid genotype and its newly-formed autotetraploid derivative. Eighty total primer pair combinations were assayed in differential display experiments. Out of 129 polymorphic bands, 90 were succesfully reamplified and cloned. Differential expression of 64 clones was validated by reverse Northern blot. Sequence analysis showed that 42 clones corresponded to 26 distinct genes of known function (E values $< e^{-4}$) while the remaining 22 were classified as novel sequences. Genes detected were involved in processes of recombination, chromatine structure modification, regulation of transcription, proteolisis and its regulation, protein folding, carbohydrate metabolism and signal transduction. Curiously, two highly-adenylated mitochondrial-encoded genes were found to be differentially regulated. The genetic structure of both lines, examined with 565 RAPD markers, revealed 9.2% of polymorphisms.

PL-C5.
DISRUPTION OF GAMMA CARBONIC ANHYDRASE 2 GENE CAUSES DRAMATIC CHANGES IN COMPLEX I ASSEMBLY AND RESPIRATION IN ARABIDOPSIS CELL CULTURES

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Assembly of respiratory protein complexes has to follow especial routes in plants. The most mysterious features of respiration in plant cells today are the numerous nuclear encoded plant specific subunits, which form part of respiratory protein complexes and which partially introduce side activities into these enzyme complexes. Between several new discovered plant specific proteins, complex I has associated 5 subunits with high structural similarity to γ carbonic anhydrases (γCAs). Moreover, these *Arabidopsis* γCA proteins are able to interact *in vivo* forming a sub complex tightly associated to complex I.
In an attempt to better understand the function of these extra subunits of mitochondrial complex I, *Arabidopsis* knock-out lines were physiological and biochemically characterized. Here we show that disruption of γCA2 gene causes decreased growth (30%-50%) and oxygen consumption rates in *Arabidopsis* cell cultures. Abundance of singular complex I subunits is dramatically reduced in this mutant. This is strong evidence suggesting that γCA2 protein seems to be essential for assembly of complex I in plants.

PL-C6.
HEME OXYGENASE EXERTS A PROTECTIVE ROLE AGAINST OXIDATIVE STRESS IN SOYBEAN LEAVES

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We have previously demonstrated that the induction of heme oxygenase-1 (EC 1.14.99.3) plays a protective role for mammalian cells against oxidative stress. Here, we investigated for the first time the possible role of heme oxygenase-1 as an antioxidant defense in leaves of soybean plants. Treatment with 200 μM Cd during 48 h caused a 70% increase in thiobarbituric acid reactive substances, whereas GSH decreased 67%, guaiacol peroxidase and total superoxide dismutase were also inhibited 49% and 46%, respectively. 200 μM Cd produced the overexpression of heme oxygenase-1, as well as a 4.5 fold enhancement of its activity. Administration of biliverdin partially prevented the effects caused by Cd. Pretreatment with Zn protoporphyrin IX, a potent inhibitor of heme oxygenase, expectedly decreased heme oxygenase-1 activity to half. When the inhibitor was given before Cd, completely prevented the enzyme induction increasing the levels of oxidative stress parameters. Collectively these results indicated that although plant heme oxygenases share little homology to heme oxygenases from non-plant species, they also play an important protective role against oxidative cell damage.

PL-C7.
NITRIC OXIDE IMPROVES THE HEALING RESPONSE IN POTATO LEAVES

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Plant wounding response is a well-characterized event mediated by different hormones such as ethylene, ABA and JA. However, the basis of healing, the main wounding downstream event, has not been fully established.
It has recently been demonstrated that wounding causes a local accumulation of auxins (aux) and nitric oxide (NO). In plants, aux are involved in processes such as cell division, cell differentiation and morphogenesis. NO is a novel gaseous plant hormone and growth regulator. To study aux and NO as novel key molecules orchestrating plant-healing events, we evaluated the healing response on wounded potato leaves (*Solanum tuberosum* cv. Spunta) treated with the NO donor SNP. After 4 days of wounding, NO treated leaves shown a layer of brown material on the boundary of the wound sites, suggesting that the healing response is occurring. An increased accumulation of the wound-induced β 1-3 glucan callose was detected in such colored boundary. We also study the wound-regulated genes: PAL, ACCO and extensin. Northern blot analyses shown that these all transcripts are over induced by the treatments of wounded leaves with the NO donor (SNP 0.1 mM) or aux (AIA 0.01 mM), in comparison with wounded leaves treated with water. These evidences indicated that NO may be improving the healing response of potato wounded leaves.
Supported by UNMDP, Conicet and ANPCyT, Argentina.

PL-C8.
NITRIC OXIDE EFFECT ON LIGHT-DEPENDENT STOMATAL OPENING PROCESSES

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Nitric oxide (NO) gas is a very reactive free radical molecule reported to be present in myriad of biological systems. In plants NO has been reported to participate in different physiological and developmental processes such as germination, rooting, senescence, and stomatal closure, among others. Stomatal movement is regulated by three main factors: light, CO_2 and ABA. About light regulation, it is well known that high intensities of light induces a fast aperture of the stomatal pore. This response involves a fast proton extrusion through the activation of a H^+-ATPase. The resulting hyperpolarization of the plasma membrane activates a voltage-dependent ion uptake and the consequent aperture of the stomatal pore. As well as in stomatal closure, abscisic acid (ABA) also affect light-dependent stomatal movement, by inhibiting stomatal opening through the inactivation of the H^+ pump and K^+ uptake. In our lab we reported that NO is required for ABA-induced stomatal closure, but little is known about the participation of NO during light dependent stomatal opening. In order to see if NO affects light induced stomatal opening, we treat dark incubated *V.faba* epidermal strips with the NO donor SNAP and we shift them to light. In this work we demonstrate that SNAP-treated stomata were unable to open even 2 hours after the dark/light shift. In addition we show that the NO scavenger PTIO blocks ABA-inhibition of stomatal opening. These results open a new window toward the understanding of stomatal opening processes.
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PL-C9.

ANALYSIS OF THE CYTOCHROME *C* PROMOTERS FROM *ARABIDOPSIS*

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The plant mitochondrial respiratory chain ends up in two different pathways, the cyanide-resistant or alternative pathway and the cytochrome *c*-dependent one. Two nuclear genes encoding cytochrome c *(Cytc)* are present in *Arabidopsis thaliana*. To gain insight into the mechanisms of *Cytc* gene regulation, we have used regions upstream of the both *Cytc* genes (*Cytc-I* and *Cytc-II)* to drive the expression of the βglucuronidase (GUS) reporter protein in *Arabidopsis* plants. Histochemical staining indicated that the *Cytc* genes are differentially expressed in plant tissues. *Cytc-I* directs the expression in meristems and pollen, while *Cytc-II* is expressed in vascular tissues of cotyledons, roots and hypocotyls, as well as in pollen and the central leaf vein. The analysis of progressive upstream deletions of the promoters revealed an essential region for *Cytc-I* expression located between nucleotides –124 and –31. The importance of binding sites for TCP-domain proteins within this region was assessed by mutagenic analysis. For *Cytc-II*, expression was abolished when nucleotides –172 to –103 were removed. A comparison of upstream sequences between *Cytc* genes showed both common and different regulatory motifs. An exhaustive analysis of these elements will be helpful to understand the molecular mechanisms of expression of these genes.

PL-C10.

EFFECT OF CADMIUM STRESS ON PROTEIN SYNTHESIS AND DEGRADATION IN SUNFLOWER (*Helianthus annuus* L.) LEAVES

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The effect of oxidative stress induced by cadmium on growth parameters and the balance between protein synthesis and degradation in sunflower (*Helianthus annuus* L.) leaves were studied. Plants were germinated 10 d and then transferred to hydroponics medium devoid (control) or containing 100, 200 and 300 μM $CdCl_2$. Analysis were performed between 0 and 4 days of treatments. All Cd concentrations significantly reduced leaf area, fresh and dry weight, but leaf relative water contain only decreased with 200 and 300 μM Cd respect to control plants. Control and treated plants had similar soluble protein content and showed the same rate of soluble protein labeling under the assay conditions. Although protease activity increased with cadmium treatment, proteasome activity was significant inhibited respect to control values. Cadmium caused an increase of the ubiquitin-conjugated proteins and carbonyl groups content in treated leaves compared to control values. Cadmium induced an increase in protease specific activity, nevertheless it was not enough to avoid accumulation of oxidized proteins. These results suggest that 26S proteasome could be related to the secondary antioxidative defenses, degrading oxidized proteins in plant cells under oxidative stress generated by cadmium.

PL-C11.

CHLOROPLAST 2-CYS PEROXIREDOXIN. MODULATION OF CHAPERONE ACTIVITY BY DITHIOTHREITOL AND ATP-MG^{2+}

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Peroxiredoxins (Prxs) are peroxidases linked to diverse cellular functions that reduce H_2O_2, peroxinitrite, and organic hydroperoxide. These ubiquitous antioxidant proteins contain one and two conserved cysteine residues, 1-Cys and 2-Cys Prx, respectively, that are functional in the peroxidatic activity using reduced thioredoxin as electron donor. X-ray crystal structures of human erythrocytes and *Crithidia fasciculata* 2-Cys Prx evinced the noncovalent association of five homodimers whose constituent monomers are linked via a disulfide bond; i.e. $[(Prx)_2]_5$.

To estimate the chaperone activity of rapeseed chloroplast 2-Cys Prx, we followed the denaturation of citrate synthase and glyceraldehyde 3-P dehydrogenase by light scattering. 2-Cys Prx prevented the thermal aggregation of both enzymes in a process that was enhanced by reduction with dithiothreitol (DTT). Size exclusion cromatography and light scattering studies showed that chloroplast 2-Cys Prx adopted a decameric structure when DTT cleaved the disulfide bond of the dimer. Moreover, both ATP and Mg^{2+} inhibited the chaperone activity of chloroplast 2-Cys Prx. Apparently, the concerted action of ATP and Mg^{2+} caused the precipitation of chloroplast 2-Cys Prx and, in so doing, impaired the protective capacity. These results indicate that, *in vitro*, chloroplast 2-Cys Prx is a chaperone whose activity could be modulated via changes in the quaternary structure by ATP and the reduction of the intrinsic disulfide bond.

PL-C12.

BABA AS CHEMICAL INDUCER OF POTATO RESISTANCE TO *Phytophthora infestans*

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Several strategies for controlling leaf and tuber diseases have been introduced over the years, but serious losses in potato harvest still occur. The use of chemicals that induce resistance was tried as an alternative. The aim of this work was to investigate the biochemical mechanisms by which the BABA (â-amino-butyric acid) increases resistance in potato leaves against *P. infestans*.

Plants of two potato cultivars: cv. Pampeana (resistant) and Bintje (susceptible) were treated with BABA, as chemical inducer. Three or twenty days after treatment, leaves were inoculated with *P. infestans*. The highest level of protection against *P. infestans* was observed after 3 days of treatment with BABA, although this protection decreased in foliage at 20 days of treatment in both cultivars. The levels of phytoalexin and phenols were increased only at 20 days in treated leaves from plants of both cultivars.

The expression of two PR proteins (basic chitinases and aspartic proteases) was analyzed at 24 and 48 hs after inoculation with *P. infestans* for both times of treatment with BABA (3 and 20 days). In cv. Pampeana, the expression of these enzymes was increased in plants with 3 days of treatment; this induction decreased at 20 days. In cv. Bintje, the effect of BABA on the induction of these enzymes was less evident. Future efforts will focuse in the analysis of these enzymes in tubers from treated plants.

PL-C13.

AN EXTRACELLULAR LIPID TRANSFER PROTEIN IS PARTIALLY BOUND TO MEMBRANES

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Lipid Transfer Proteins (LTPs) are low-molecular-mass pro-teins extensively studied in higher plants. We have previously characterized Ha-AP10, a sunflower antifungal protein homologous to members of the LTP family. Ha-AP10 has been de-tected in the extracellular fluids of sunflower seeds and its cDNA presents a typical secretory signal peptide sequence. Since previous experiments suggested that Ha-AP10 might be associated to membranes, the objective of this work was to de-termine if a fraction of Ha-AP10 was effectively membrane bound. To this aim, we separated membrane-associated and soluble proteins by centrifugation. The absence of cytoplasmic contamination in the membrane fraction was confirmed by tes-ting the presence of glucose-6-phosphate-dehydrogenase acti-vity. Western blot analysis revealed that Ha-AP10 is present in similar amounts both in the membrane and soluble fractions and that it is released from the membranes by non-ionic de-tergents. Bioinformatic analysis revealed that Ha-AP10 does contain neither transmembrane domains nor myristoylation signal sequences. After treatment of the membrane fraction with 0.1M Na_2CO_3 or 0.6M NaCl, Ha-AP10 was found in the supernatant, indicating that is loosely bound to membranes. This result was confirmed by isolating perispheric proteins by phase separation in Triton X-114. Assays are in progress to understand the biological significance of this finding.

PL-C14.

REVERSE ALTERATIONS OF THE GENOME DURING SERIAL PLOIDY LEVEL MODIFICATION IN *Eragrostis curvula*

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Eragrostis curvula (2n = 4x = 40) is an exotic grass widely cultivated in the semiarid regions of Argentina. It reproduces by obligate pseudogamous diplosporous apomixis of the *Antennaria* type. The objective of this work was to analyze the genomic structure of a tetraploid-diploid-tetraploid euploid series of the species. We examined an apomictic tetraploid natural genotype (Tanganyka) (T), a sexual dihaploid derivative obtained from T by immature inflorescences *in vitro* culture (D) and two tetraploid plants of unknown reproductive mode originated by colchicine treatment from D (C and M). RAPD analysis using 210 markers followed by similarity analysis (Jaccard) and clustering (UPGMA) showed a dendrogram displaying two main clusters: T, C and M grouped together with 0.96 similarity while D was placed at 0.72 with respect to the rest of the plants. By the other hand, a progeny test using 60 RAPD markers showed that M reproduces by sexuality. Surprisingly, our results showed that most of the genomic features altered during the tetraploid-diploid conversion are restituted in the diploid-tetraploid shift.

ST-C1.

SIGNAL COMPLEXES FORMED BY PRESYNAPTIC CALCIUM CHANNELS AND ORL1 RECEPTORS

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We investigated the modulation of voltage-gated calcium channels by opioid-receptor like (ORL1) receptors. In DRG neurons and in tsA-201 cells, receptor activation mediated a pronounced inhibition of N-type channels, whereas other calcium channel subtypes were not affected. This inhibition had both voltage-dependent (I.e., G beta gamma mediated) and voltage independent components. Intriguingly, a tonic voltage-dependent G protein inhibition was induced by ORL1 receptors even in the absence of agonist, and became increased with increasing receptor density. Biochemical characterization revealed the existence of a physical N-type calcium channel/ORL1 receptor signaling complex which results in colocalization of channels and receptors and efficiently exposes N-type channels to constitutive ORL1 receptor activity. In response to prolonged agonist application, an additional voltage-independent inhibition of the channels occurred. Confocal fluorescence imaging reveals that this effect results from an internalization of the channel-receptor complex, thus effectively removing calcium channels from the plasma membrane. Overall, our data show that calcium channel activity is regulated by physiological or pathological changes in ORL1 receptor expression levels, and provide the first demonstration of internalization of a voltage-dependent calcium channel by a G protein coupled receptor.

ST-C2.

SIGNAL TRANSDUCTION VIA SMALL REGULATORY RNAS IN A *PSEUDOMONAS FLUORESCENS* STRAIN

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In the biocontrol model strain *Pseudomonas fluorescens* CHA0 the synthesis of antibiotics and exoenzymes is under the control of the GacS/GacA two-component system, which up-regulates its target genes at a post-transcriptional level during the entry into stationary phase. Upon activation by an uncharacterized cell-to-cell signal, the Gac system relieves the translational repression exerted on the RBS of target mRNAs by a couple of RNA binding proteins, RsmA and RsmE. We found that two small untranslated RNAs (sRNAs), RsmY (118-nt) and RsmZ (127-nt), are the effectors of the Gac system, which activates their transcription. Overexpression of RsmY or RsmZ reverts Gac mutations, whereas a double *rsmY rsmZ* mutant behaves as a Gac mutant. The secondary structure of both sRNAs shows several stem-loops with GGA repeats in single stranded regions of the molecules. Both sRNAs bind multiple copies of RsmA and RsmE proteins, but RsmY mutants lacking the GGA motifs can no longer interact with RsmA or RsmE, suggesting that these trinucleotides are the binding sites of RsmA/RsmE proteins. These observations add up to the rapidly growing list of bacterial processes that are directly controlled by small regulatory RNAs.

Valverde *et al*. 2003. Mol. Microbiol. 50:1361-1379.
Valverde *et al*. 2004. J. Biol. Chem. 279:25066-25074.

ST-C3.

EXTRACELLULAR INOSINE MODULATES ERK ½ AND P38 PHOSPHORYLATION IN CULTURED SERTOLI CELLS: POSSIBLE PARTICIPATION IN TNF-α MODULATION OF ERK ½

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Extracellular ATP and adenosine modulation of MAPKs is well described in different cells, but extracellular inosine action it is not known. Previous reports showed that H_2O_2 and TNF-á increases extracellular inosine concentration in cultured Sertoli cells. This nucleoside protects Sertoli cells against H_2O_2 induced damage and participates in TNF-á induced nitric oxide production on these cells. Considering that MAPKs are key mediators of cellular response to a great variety of stimulus, in this work it was investigated the effect of extracellular inosine in the phosphorylation of ERK ½ and p38 MAPKs in cultured Sertoli cells. Also it was investigated if this nucleoside could be involved in TNF-á modulation of ERK ½ phosphorylation. Extracellular inosine increased the phosphorylation of ERK ½ and p38, as well as a selective A1 adenosine receptor agonist. Selective A1 adenosine receptors antagonists inhibited extracellular inosine effects on ERK ½ and p38. These antagonists also inhibited TNF-á increase in the phosphorylation of ERK ½. Additionally, TNF-á rapidly augmented extracellular inosine concentration in cultured Sertoli cells. These results shown that extracellular inosine modulates ERK ½ and p38 in Sertoli cells, possible trought A1 adenosine receptor activation. Besides, this nucleoside participates in TNF-á modulation of ERK ½.

Grants: CNPQ and CAPES

ST-C4.

DIFFERENTIAL PHOSPORYLATION OF CNBP DURING ZEBRAFISH EARLY DEVELOPMENT

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Cellular nucleic acid binding protein (CNBP) is widespread throughout the animal kingdom. This protein shows an striking sequence conservation and general structural organisation. Zebrafish CNBP (zCNBP) contains seven retroviral CCHC zinc knuckle motifs, an RGG box, a PEST-type proteolysis sequence and four putative phosphorylation sites in Ser_4, Tyr_{41}, Tyr_{85} and Tyr_{145}. We determined that CNBP is *in vitro* phosphorylated by zebrafish embryo extracts and that this phosphorylation is differential during early development. Phosphorylation levels were low and uniform during the first stages, reached a maximum between 24 and 48 hours post-fertilisation and deceased later. After incubation with alkali, no phosphorylation was observed in over-exposed SDS-PAGE. Because of this result we presume that the amino acid phosphorylated is Ser_4. We have also observed that zCNBP is differentially cleaved during embryonic development and this cleavage starts when the phosphorylation begins to raise, leading us to think that both phenomena could be related. Moreover, in proteolysis experiments, we have observed that the fragment containing the first 29 amino acid residues of zCNBP retains the phosphorylation signal, again suggesting that Ser_4 is the phosphorylated residue. The implications of this phosphorylation in nucleic acid binding and oligonucleotide annealing promoting are analysed.

ST-C5.

A POSSIBLE ROLE OF REDUCED PROLIDASE PHOSPHORYLATION IN FASTING - INDUCED INHIBITION OF COLLAGEN BIOSYNTHESIS

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Fasting is accompanied by a decrease in collagen biosynthesis. It involves inhibition of prolidase activity, an enzyme that plays a key role in regulation of collagen metabolism. Phosphoenolpyruvate (PEP) is known as a strong inhibitor of prolidase activity. It exerts this effect by inhibition of prolidase phosphorylation. Unphosphorylated prolidase is inactive. One may expect that fasting-associated increase in PEP content in tissues may be a factor, which inactivates prolidase and makes it inactive in collagen biosynthesis. We measured the levels of PEP, pyruvate, and pyruvate kinase in the skin of control and fasted rats and correlated these parameters with prolidase expression, prolidase activity and collagen biosynthesis in this tissue. An increase in PEP concentration (30%) was found in the skin of fasted rats. In the same time prolidase activity and collagen biosynthesis decreased by about 50% and 30%, respectively. Western immunoblot analysis did not show a decrease in the amount of prolidase but a reduction in enzyme phosphorylation was observed. It is known that PEP is converted to pyruvate by pyruvate kinase. Fasting-associated reduction in the activity of pyruvate kinase decreases utilisation and promotes accumulation of PEP in tissues. The accumulated PEP may exert an inhibitory effect on prolidase phosphorylation, preventing the formation of active enzyme and resulting in decrease of collagen biosynthesis.

ST-C6.

ANTI-*TRYPANOSOMA CRUZI* RECOMBINANT ANTIBODIES WITH PARTIAL AGONIST EFFECTS ON CARDIAC RECEPTORS

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Sera from patients with chronic Chagas´ Heart Disease (cChHD) present antibodies directed to the carboxyl terminal regions of the *Trypanosoma cruzi* ribosomal P2β protein, defined by the epitope named R13 (EEEDDDMGFGLFD). These antibodies have the ability to induce chronotropic alterations in cardiomiocytes cultures due to the cross reactivity with the acidic regions of the second intracellular loop of the cardiac beta-1 adrenergic (βAR) and muscarinic cholinergic receptors. Mice immunization with recombinant TcP2β protein allowed us to obtain an anti-R13 monoclonal antibody, called 17.2 that proved to have functional effects on cardiac rhythm upon passive transfer. Anti-R13 svFc derived from a cChHD combinatorial antibody library and from 17.2 monoclonal antibody producing cells (APC) have been obtained and characterized. Recombinant svFc constructs derived from 17.2 APC, and expressed in a pHEN 2 vector recognized the 5 parasite ribosomal P proteins, bound to ribosomes of fixed parasite cells, cross-reacted with the β1-AR of transfected CHO-K1 cells inducing an increase in cAMP levels, and bound to their surface as assessed by indirect immunofluorescence. Passive transfer of this recombinant antibody to naïve mice provoked arrhythmias in recipients heart, as recorded by electrocardiograms. These functional effects are currently under pharmacological characterization.

ST-C7.
GLUCOCORTICOIDS REPRESS *BCL-X_L* EXPRESSION IN MOUSE THYMOCYTES THROUGH THE RECRUITMENT OF STAT5B TO THE P4 PROMOTER REGION

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Bcl-X is one of the members of Bcl-2 family proteins involved in the control of apoptosis. Five different isoforms, produced by alternative splicing and with opposite effects, have been described. We have demonstrated that glucocorticoids induce *bcl-X* expression and increase the ratio *bcl-X_L* (antiapopototic)/*bcl-X_S* (apopototic) in mammary epithelial cells. The 5'-UTR region of the mouse *bcl-X* gene contains five different promoters, that exhibit a tissue-specific promoter usage. The region located upstream of Promoter 4 (P4) contains two hormone response element (HREs) which confer *in vivo* induction of *bcl-X_L* expression upon binding of the glucocorticoid receptor (GR) in epithelial mammary cells. Here we show that in mouse thymocytes glucocorticoids inhibit *bcl-X_L* expression and P4 activity, favouring the apoptosis. According to ChIPs assays, a decrease in the RNA polymerase II occupancy of the P4 and no recruitment of GR to the HREs is observed, while STAT5B binds to the P4 in a process dependent on the integrity of a putative STAT5 binding site. Inhibition of the JAK pathways reverts this effect to an activation of *bcl-X_L* transcription accompanied by recruitment of GR, and inhibition of STAT5B binding to the P4. Theses results suggest that different transcription regulatory complexes may be assembled in response to glucocorticoids. As a consequence the relative abundance of *bcl-X* isoforms changes in opposite directions.

ST-C8.
ACTIVATION OF MITOCHONDRIAL ERK1/2 IS REQUIRED FOR PKA-DEPENDENT STEROIDOGENESIS IN MA-10 CELLS

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Early steps of steroidogenesis occur in mitochondria and involve activation of Steroidogenic Acute Regulatory protein (StAR) leading to cholesterol import to the inner mitochondrial membrane, the rate-limiting step in steroid biosynthesis. Recently, our group and others reported that members of MAP kinases family, the extracellular signal-regulated kinases 1/2 (ERK1/2), are activated by phosphorylation in mitochondria. To investigate a putative non-genomic ERK mitochondrial effect on steroidogenesis, MA-10 cells were arrested in G1 phase and stimulated with 8Br-cAMP (0.5 mM) in the presence or absence of two inhibitors of MEK1/2 (MAP kinase kinase, upstream ERK kinase), PD98056 and U0126. Both compounds inhibited 8Br-cAMP stimulated-steroid production without affecting hormonal basal level or PKA activity. MEK1/2 and ERK1/2 resulted phosphorylated with maximal activity after 5 min of stimulation that decreased up to 1 h, without modifying total kinase expression. A similar time-course activation of constitutive ERK1/2 was found in purified non-contaminated mitochondria as assessed by Western blot or by co-localization in immunocytochemistry with mitotracker red, a mitochondrial dye. This pattern of cAMP activation was inhibited by H-89, a permeable PKA inhibitor. It is surmised that sustained mitochondrial ERK activity *via* PKA is required to StAR-dependent cholesterol transport and steroidogenesis in cAMP-stimulated MA-10 cells.

ST-C9.
SILENCING THE EXPRESSION OF MITOCHONDRIAL ACYL-COA THIOESTERASE I INHIBITS HORMONE-INDUCED STEROIDOGENESIS

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Arachidonic acid (AA) plays a fundamental role in the hormonal regulation of steroidogenesis. We have proposed the involvement of mitochondrial acyl-CoA thioesterase I (MTE-I) as important regulators of AA release in the mechanism of action of trophic hormones. Knocking down the expression levels of MTE-I by antisense or small interfering RNA (siRNA) produced a marked reduction in steroid output of cAMP-stimulated Leydig and adrenal cells. This effect was blunted by a permeable analogue of cholesterol that bypasses the rate-limiting step in steroidogenesis, the transport of cholesterol through the inner mitochondrial membrane. The inhibition of steroidogenesis was overcome by addition of exogenous AA, indicating that the enzymes are part of the mechanism responsible for AA release involved in steroidogenesis. Knocking down the expression of MTE-I leads to a significant reduction in the expression of Steroidogenic Acute Regulatory protein (StAR), a protein that is induced by AA and that controls the rate-limiting step. Overexpression of MTE-I resulted in an increase of cAMP-induced steroidogenesis. In summary, our results demonstrate a critical role of MTE-I in the hormonal regulation of steroidogenesis as a new pathway of AA release.

ST-C10.
CHARACTERIZATION OF PYRUVATE KINASE 1 OF *SACCHAROMYCES CEREVISIAE* AS PKA SUBSTRATE

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In this work we characterized a *S.cerevsiae* PKA substrate, through the identification of the phosphorylation site, and the study of how this phosphorylation affects its activity and how a natural substrate participates in yeast holoenzyme activation. Analysis of the Pyk1 sequence revealed three potential phosphorylation sites (Ser^{22}, Thr^{94} and Thr^{478}) within a PKA sequence motif. Three peptides containing these sites were synthesized .*In vitro* assays using catalytic subunit from bovine heart PKA (C_b) indicate that only one peptide, the LRRTSIIGTR, including the Ser^{22}, was substrate for the kinase. The specificity constant (k_{cat}/K_m) is 6.6 pmol $(min.unit)^{-1}\mu M^{-1}$, one order lower than the one for kemptide. The peptides behavior was the same when assayed *in vitro* with crude extracts from a TPK1*tpk2Δtpk3Δbcy1Δ* strain as kinase source. *In vivo*, phosphorylation using permeabilized yeast cells, indicates too that the only peptide Ser^{22} was substrate. $Pyk1^{S22A}$ and $Pyk1^{T94A}$ mutant proteins were expressed in a yeast strain whith a mutation that prevents endogenous Pyk1 expression (*PYK1-5*) and were subjected to phosphorylation with C_b and TPK1. 0.5 and 0.075 mol of phosphate were incorporated in Pyk1 respectively and it was reduced by more than 90% in $Pyk1^{S22A}$. The k_{cat}/K_m of C_b for Pyk1, and $Pyk1^{T94A}$ were in the same order. The phosphorylation in the Ser22 is important in the regulation of the activity of Pyk1.Preliminary results indicates a participation of the substrate in the mechanism of activation of yeast PKA holoenzyme. Peptide Ser22 produced a decreased in the A0.5 and is a better activator as compared withpyk whole protein which is similar to kemptide.

ST-C11.

MODULATION OF TRACHEAL DEVELOPMENT BY THE OXYGEN SENSING PATHWAY IN *DROSOPHILA MELANOGASTER*

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Animals respond to low oxygen by changing the expression of a large number of genes. The molecules involved in transducing the hypoxic signal were recently identified. Oxygen dependent prolyl hydroxylases (HPH) regulate the level of the hypoxia inducible heterodimeric transcription factor (HIF). In normoxia, HPHs hydroxylate two key proline residues in HIF-α subunit, allowing its destruction by the ubiquitin-proteasome pathway. Reduced oxygen levels prevent HPHs function, resulting in HIF-α stabilization and induction of its transcriptional target genes. We have demonstrated in the lab the occurrence of a homologous system in *Drosophila Melanogaster*, being Sima and Fatiga the HIF-1α and the HPH homologues. We found that disruption of *fatiga* gene results in Sima stabilization and upregulation of the transcriptional response to hypoxia. Here we describe different phenotypes of *fatiga* mutants, including defects in tracheal system formation and changes in tracheal cell fate. We demonstrate a functional interaction of signal transduction pathways involved in tracheal development with the oxygen sensing machinery, suggesting an environmental regulation of a "hard wired" developmental process.

ST-C12.

A NEW ROLE FOR THE KRÜPPEL-LIKE TRANSCRIPTION FACTOR KLF6 AS AN INHIBITOR OF c-JUN PROTO-ONCOPROTEIN FUNCTION

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Krüppel-like transcription factors (KLFs) represent one of the most diverse set of regulators in vertebrate organisms. KLF family members are involved in cell proliferation and differentiation control in normal as well as in pathological situations. Here, we demonstrate that KLF6 behaves as a functional antagonist of the c-Jun proto-oncoprotein. Thus, KLF6 overexpression downregulated c-Jun-dependent transcription and a physical interaction between c-Jun and KLF6 was detected. Moreover, cell proliferation induced by c-Jun was significantly decreased by KLF6. The inhibition of c-Jun functions correlates directly with c-Jun protein degradation induced by KLF6. We also show that all KLF6 effects on c-Jun were largely dependent on phorbol ester (TPA/ionomycin) extracellular stimulation, which enhanced KLF6 nuclear translocation and transcriptional activity and modified its phosphorylation status. Our data are consistent with a novel mechanism of KLF6's role as an inhibitor of cell proliferation by counteracting the function of the c-Jun proto-oncoprotein involving enhanced c-Jun degradation by the proteasome-dependent pathway, and further reinforces KLF6 as a potential tumor suppressor gene product.

ST-C13.

N-TERMINAL c-FOS TYROSINE DEPHOSPHORYLATION IS REQUIRED FOR c-FOS/ER ASSOCIATION AND PHOSPHOLIPID SYNTHESIS ACTIVATION

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c-Fos is a component of AP-1 transcription factor family that regulates genes involved in proliferation and differentiation. c-Fos is not a constituent component of cells but its expression is rapidly and transiently induced by trophic factors. We disclosed an additional activity of c-Fos, that is its capacity to associate to the ER and activate phospholipid synthesis. Herein we report, the molecular constraints that determine c-Fos/ER association and consequently its phospholipid synthesis activation capacity. In quiescent cells, the small amounts of c-Fos present are phosphorylated in Tyr residues, a novel phosphorylation site for c-Fos. Induction of cells to re-enter the cell cycle results in the loss of Tyr phosphoryl groups present in preformed c-Fos, and additional c-Fos expression. Dephosphorylated c-Fos now associates to the ER and activates phospholipid synthesis. If dephosphorylation is impaired, phospholipid synthesis activation is not observed and proliferation rates diminish. Recombinant c-Fos associates to ER and activates phospholipid synthesis whereas purified Tyr-phosphorylated c-Fos does not. Using c-Fos deletion mutants, it was found phosphorylation/ dephosphorylation in all C-terminal deletion mutants. Previous studies have shown Ser/Thr phosphorylation as a delicate mechanism to regulate c-Fos transactivation capacity. Herein we postulate that c-Fos Tyr-phosphorylation is a regulatory event that determines the rate of phospholipid synthesis by regulating c-Fos/ER association, thus being a key event in cell cycle progression.

ST-C14.

TYR-374 AND 395 WTHIN PKC-ζ ARE POTENTIAL AP-2 INTERACTION SITES REQUIRED FOR NA^+,K^+-ATPase ENDOCYTOSIS IN RESPONSE TO DOPAMINE

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Clathrin-dependent endocytosis of Na^+, K^+-ATPase (NK) molecules in response to dopamine (DA) requires phosphorylation of the NK α-subunit via a PKC-ζ-dependent mechanism. We sought here to further analyze the possible interaction of PKC-ζ with the clathrin network, i.e., its interaction with the adaptor protein-2 (AP-2) and to determine whether PKC-ζ is essential for recruiting the cargo at the plasma membrane (phosphorylation of the NK α-subunit), for the formation of clathrin vesicles, or for both. We thus mutated three potential tyrosine-base motifs within the PKC-ζ molecule and examined the ability of DA to inhibit NK activity and subunit endocytosis. Cells expressing these mutants were exposed to 1μM DA or its vehicle and NK activity/subunit phosphorylation were determined. NK activity was significantly inhibited by DA and this effect was abolished in the presence of Y374F and Y395F mutants ($p = 0.0054$ by ANOVA). However, expression of these mutants did not prevent the phosphorylation of NK by dopamine. Our results indicate that Tyr 374 and 395 within PKC-ζ are potential interaction sites with the AP-2 molecule and that PKC-ζ is crucial for the formation of clathrin vesicles during membrane endocytosis.

ST-C15.
PROTEIN TYROSINE PHOSPHATASES INVOLVED IN TRANSCRIPTION AND TRANSLATION OF KEY PROTEINS IN THE HORMONAL REGULATION OF STEROID SYNTHESIS

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It is very well accepted that phospho-dephosphorylation mechanisms in Ser/Thr residues play an important role in transcription and translation of key proteins in different cellular types. In this report we describe the participation of dephosphorylation processes in the control of transcription and translation in steroidogenic cells. Cells pre-treated with cycloheximide (CHX), actinomicin D (AD), phenylarsine oxide (PAO) or benzylphosphonic acid (BPA) were stimulated with 8Br-AMPc for different times. CHX, PAO and BPA inhibit steroidogenesis (ST) at all periods of time studied. However, AD only affected ST after 1 h of stimulation. The results showing the inhibition of both phases of ST by tyrosine phosphatase inhibitors (PAO and BPA) suggest that these compounds are acting similarly to CHX, indicating that tyrosine dephosphorylation is needed for translation. However, the analysis of two key proteins of the regulation of ST such as the acyl-CoA synthetase 4 (ACS4) and the steroidogenic acute regulatory (StAR) protein indicates that protein tyrosine phosphatases are involved in the translation of the ACS4 and in the transcription of StAR. These results show for the first time that tyrosine dephosphorylation is involved in transcription and translation in steroidogenic systems.

ST-C16.
SHP-1 PHOSPHATASE IS ACTIVATED BY ANG II AT_2 RECEPTORS IN RAT FETAL MEMBRANES

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Ang II participates in growth control and organogenesis by interacting with its receptors. We described that Ang II elicit protein tyrosine dephosphorylation in rat fetal membranes (E20) while induces tyr-phosphorylation of the phosphatase SHP-1. SHP-1 is tyr-phosphorylated within 1 min, in a dose-dependent form by Ang II via AT_2 receptor. SHP-1 phosphorylation is followed in time by Tyr dephosphorylation of different proteins, suggesting a sequence of events. Immunocomplexes obtained with anti-AT_2 receptor antibody contained SHP-1 phosphatase and exhibits selective AT_2 binding. Similarly, anti SHP-1 antibody immunoprecipitates the AT_2 receptor. Following Ang II stimulation, SHP-1 activity was measured by using a peptide substrate in both immunocomplexes, and correlates in time with the maximal level of SHP-1 phosphorylation. The response is blocked by the selective AT_2 antagonist PD 123319. Since AT_2 receptor has no intrinsic PTK activity, we assayed the presence of Src in the immunocomplexes. Src was present in Ang II stimulated immunocomplexes, thus suggesting that this PK is responsible of SHP-1 tyr-phosphorylation. These results suggest a potential a role of AT_2 in growth and differentiation by activating PTPase SHP-1 and the possible involvement c-Src in SHP-1 activation.

ST-C17.
THE DESK-DESR PHOSPHORYLATION CASCADE CONTROLS THE MEMBRANE LIPID FLUIDITY IN *BACILLUS SUBTILIS*

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The Des pathway of *Bacillus subtilis* regulates the synthesis of the cold-shock induced membrane-bound enzyme Δ5-fatty acid desaturase (Δ5-Des), which introduces double bonds into phospholipids acyl chains. This pathway is uniquely and stringently regulated by a two-component system composed of a membrane-associated kinase, DesK, and a soluble transcriptional activator, DesR. We demonstrated that DesK is a bifunctional enzyme with both kinase and phosphatase activities, that could assume different signaling states in response to changes in membrane fluidity. We also studied the mechanism by which the sensor protein DesK controls the level of phosphorylation of its cognate partner, DesR. We found that only the phosphorylated form of protein DesR is able to bind to a regulatory region immediately upstream of the promoter of the Δ5-Des gene (P*des*). Phosphorylation of the regulatory domain of dimeric DesR promotes, in a cooperative fashion, the hierarchical occupation of two adjacent, non identical, DesR-P DNA binding sites, so that there is a shift in the equilibrium toward the tetrameric active form of the response regulator. Subsequently, this phosphorylation signal propagation leads to the activation of the *des* gene through recruitment of RNA polymerase to P*des*. This is the first dissected example of a phosphorylation-activated cascade for a cold-shock gene, allowing the cell to optimize the fluidity of membrane phospholipids.

ST-C18.
NON PHOSPHORYLATED CREB REPRESSES GENE TRANSCRIPTION BY RECRUITING HDAC1

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Transcription factor CREB, phosphorylated in S133, induce the activity of a great variety of CRE-containing gene promoters. On the contrary, unphosphorylated CREB cause an inhibitory effect on transcription in several genes like 5-aminolevulinate synthase (ALAS). The molecular basis of this inhibitory effect remains unclear. Transfection experiments in HepG2 cells show that CREB overexpression inhibits basal activity of ALAS/CAT fusion gene and that this effect is blocked by cotransfection with CBP. As we show using a ΔHAT-CBP mutant, HAT activity is crucial for CBP action. Overexpression of HDAC1 counteracts the CBP rescue effect. Similar results were observed on endogenous ALAS mRNA in HepG2 cells as assessed by Northern blot. Coimmunoprecipitation reveals a CREB-HDAC interaction in HepG2 cells that is strongly impaired after cAMP stimulation. We utilize a chromatin reconstitution approach on ALAS promoter using *Drosophila* extracts and recombinant proteins to further explore the CREB effects. Promoter activity were analysed by *in vitro* transcription and primer extension. We show that P-CREB and CBP increase ALAS expression. Excess of recombinant CREB diminishes the transcriptional activity and HDAC co-addition enhanced this inhibitory effect. We propose that CREB affects gene expression in a phosphorylation status-dependent manner. Non phosphorylated CREB recruits HDAC in order to maintain a repressive condition. Phosphorylation at S133 disrupts HDAC association and allows CBP engagement to render a productive transcriptional initiation complex.

BT-P1.

GENETIC DIVERSITY AMONG ISOLATES OF *Phaeoisariopsis griseola* BY MEANS OF ISSR MARKERS

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Phaeoisariopsis griseola (*PG*) is a fungus that causes considerable reductions of bean (*Phaseolus vulgaris* L.) yields. This pathogen, like others, coevolved with the host, so that fungal isolates cluster as Mesoamerican and Andean. The development of a strategy to control the disease requires a prior knowledge of genetic diversity, distribution and compatibility with cultivated genotypes. In this work, Inter-simple sequence repeat (ISSR) markers were used to characterize the genome of *PG*. ISSR were evaluated for their capacity to discriminate between Andean and Mesoamerican isolates and within these two groups. Forty five representatives of *PG* isolated from Northern Argentina were analyzed by means of 7 ISSR primers. The total number of bands amplified was 93, 68% of them polymorphic. Multivariate analysis was used to generate a fenogram that clustered isolates in Andean and Mesoamerican and based on the place of isolation. Statistical analysis resulted in the identification of 17 haplotypes of the fungus, that belong to both Mesoamerican and Andean type. Considering that the fungus has asexual reproduction diversity among them is considerable and related with the place of origin of the materials suggesting that the places are rather unconnected in terms of fungal spore dispersal. Finally, ISSR proved to be reliable tools not only to identify but also to cluster *PG* isolates and to detect diversity.

BT-P2.

ERYTHROMYCIN BIOSYNTHESIS IN *E. coli*

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Many polyketide synthetases (PKSs) have been genetically and biochemically characterized during the last years, leading to the construction of combinatorial polyketide libraries generated by genetic modification of macrolide PKSs. However, these libraries were constructed in heterologous hosts which lack the post-PKS "tailoring" steps (glicosylations, hydroxylations, methylations, etc), so only the corresponding aglycones were produced. We wished to expand the capabilities of the combinatorial biosynthesis strategies to incorporate these post-PKS steps, in particular the addition of deoxysugar moieties, essential for macrolides activity. Since *E. coli* is the most widely used host for heterologous gene expression, we decided to genetically engineer this species to perform both the synthesis of the polyketide 6-dEB and the tailoring steps that lead to the formation of the antibiotic erythromycin C. This implied the construction of two operons including the genes from *Micromonospora megalomicea* involved in the biosynthesis of the deoxysugars TDP-L-mycarose and TDP-D-desosamine and their corresponding glycosyltransferases, two hydroxylases and an erythromycin resistance gene. The expression of these two operons in *E. coli* strain K207-3, together with the 6-dEB synthetase genes from *Saccharopolyspora erythraea*, allowed us to obtain erythromycin C and other intermediate compounds from batch cultures fed with propionate.

BT-P3.

MONOOXYGENASE-MEDIATED DEGRADATION OF TDTMA BY *Pseudomonas putida*

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Tetradecyltrimethylammonium (TDTMA) is a quaternary ammonium compound commonly released into the environment. *P. putida* tolerated high concentrations of TDTMA and used it as sole carbon and nitrogen source. We report here the complete TDTMA degradation and the initial characterization of inducible monooxygenase activity involved in the first degradation steps. This enzyme, which catalyzes cleavage of N-R bound of TDTMA producing trimethylamine (TMA) and an alkyl, was measured by two methods: i) formation of tetradecanoic acid, analyzing the methyl acid ester by GC?MS, or ii) production of TMA by GC-MS in a reaction mixture containing 14 mM phosphate buffer pH 7.4, 0.5 mM NAD(P)H, 0.5 mM TDTMA and cell free-extract corresponding to 0.01-0.35 mg of protein. The activity was O_2- and NAD(P)H-dependent, and showed sigmoidal velocity curves with respect to TDTMA, with n_{app} (Hill coefficient) and $[S]_{05}$ values of approximately 2.24 and $4.26.10^{-4}$ M, respectively. FAD addition (20-100 μM) led to 20% enzyme activation. No effect was found with Al^{3+}, Zn^{2+}, Mg^{2+} and Cu^{2+} or with metal-chelating agents as EDTA and *o*-phenanthroline. Taking into account that other bacteria cannot grow with TDTMA as a sole C / N source, the presence of a monooxygenase activity revealed the potential of *P. putida* to metabolize quaternary ammonium compounds.

BT-P4.

BIOREMEDIATION OF HYDROCARBON-POLLUTED SOIL USING PSYCHROTOLERANT BACTERIA. A MESOCOSMS ASSAY UNDER ANTARCTIC CONDITIONS

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Temperature strongly limits biodegradation rate. Only cold-adapted microorganisms are suitable for bioremediation processes in cold areas, such as hydrocarbon-contaminated soils near Antarctic stations. We analysed here how biodegradation activity is affected by biostimulation (N and P) and bioaugmentation with previously isolated Antarctic hydrocarbon-degrading bacterial consortia. We have carried out a field assay in Antarctica, using 1 m^2 land plots containing chronically diesel-oil polluted soil. Heterotrophic and hydrocarbon-degrading aerobic bacterial counts, total hydrocarbon content (FT-IR) and some individual compounds (GC) were determined. Biostimulated autochthonous microflora from chronically-polluted soils showed high degradation activity (69.6% of removal at day 48) and non-significant differences were observed between this system and those bioaugmented with M10 or J13 consortia at the end of the assay (62.61% and 58.01%, respectively). Hydrocarbon amounts were significantly reduced in the autochthonous and bioaugmented systems compared to the untreated controls. The results show the effectiveness of biostimulation of autochthonous microflora to improve field bioremediation in Antarctica.

BT-P5.

ISOLATION AND FUNCTIONAL CHARACTERIZATION OF A NOVEL *cry* GENE FROM A NATIVE *Bacillus thuringiensis* STRAIN

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Bacillus thuringiensis (*Bt*) is a gram-positive endospore forming bacterium that produces an insecticidal parasporal crystal inclusion (Cry toxins). We have isolated and characterized naturally occurring Bt strains collected in Argentina. The aim of this work was the isolation and characterization of a novel *cry*-type gene present in the FCC41 strain. The full-length encoding sequence (2,061 bp) was obtained from genomic DNA using PCR methods such as TAIL-PCR. The deduced protein (687 aminoacid residues) displayed 52% identity with Cry24Aa toxins. The coding sequence was expressed in a BL21 *Escherichia coli* strain. Cell extracts from the transformed strain showed high insecticidal activity against *Aedes aegypti*. Therefore, bioassays against third instar larvae of this dipterous were carried out with the transformed *E. coli* strain, FCC 41, against the commercial *Bt* sp. *israelensis* strain, toxic against dipterans. The 50% lethal concentration (LC_{50}) was determined by Probit analysis, showing that the novel Cry24-type protein displayed a high insecticidal activity against *A. aegypti*. Sequence and structural analysis of the novel Cry toxin revealed that it has conserved aminoacids present in other Cry proteins active against dipterans, such as Cry2Aa, Cry4Aa, Cry10Aa and Cry11Aa toxins.

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BT-P6.

SHOTGUN SEQUENCING OF PLASMIDS FROM ARGENTINEAN ISOLATES OF *Bacillus thuringiensis*

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Bacillus thuringiensis (*Bt*) is a well known bacterium classified in many subspecies, which might produce proteins with different insecticidal activities. The genes coding for insecticidal proteins are usually in plasmids that display large variation in size and copy number, and little is known about coded genes. We have analyzed the plasmid profiles of several Argentinean isolates of *Bt* by pulsed field gel electrophoresis (PFGE) and DNA sequencing. The plasmids changed their migration patterns at different run conditions, as expected for circular plasmids. A shotgun library from isolate INTA-FR7-4 was constructed and random clones were sequenced with universal primers at both sides of the insert. The total number of bases sequenced exceeds 200 kb. The contigs assembled correspond to more than 90 kb. None of the contigs showed large similarities when comparing with nucleotide databases. Preliminary annotation was performed searching protein databases with translated ORFs. Similarities found permit assignation of functions to several genes, including replicases and mobilization proteins. This approach allowed differentiation of Argentinean isolates from other known classical strains, not only based on their plasmid profile but also on their sequence.

BT-P7.

CHARACTERIZATION OF TRANSGENIC PLANTS EXPRESSING A POTATO VIRUS X (PVX) RNA

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Plants are currently being used as a cost-effective and safe heterologous system for the expression of functional biomolecules. We have developed a PVX vector that allows expression of a cDNA encoding the complete genome of PVX under control of the 35S CaMV promoter. This vector was expressed in a transient system by agroinfiltration of *Nicotiana tabacum* leaves. We have obtained two different transgenic lines named PVX1 and PVX2, both confirmed by PCR amplification and Southern blot. PVX2 displays PVX infection symptoms while PVX1 has a healthy appearance. Western blot analysis revealed expression of viral capsid and the movement protein p24 only in PVX2, while no viral proteins were detected in PVX1. In spite of that, both transgenic lines were able to produce infective virions when the infectivity of PVX1 and PVX2 plant extracts was analyzed. PVX1 plants infected with PVY, a virus that encodes a strong silencing suppressor, developed severe symptoms of infection and produced high levels of virions, suggesting that the low level of PVX in this line is due to post-transcriptional silencing. To our knowledge, this is the first time that a PVX transgenic plant (the PVX2 line) produces high levels of virions, resembling an infected plant. Further studies will determine whether these results can be generally applied for high efficiency transgene expression in plants.

BT-P8.

LEPIDOPTERAN CELL GROWTH AND BACULOVIRUS REPLICATION IN A CHOLESTEROL-FREE MEDIUM

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Unlike mammalian cells that require cholesterol to survive and divide, lepidopteran insect cells can be cultivated in cholesterol-free media. However, efforts to infect insect cells and produce baculovirus progenies in serum-free media lacking cholesterol had been so far unsuccessful. Infection of the UFLAg-286 cell line with *Anticarsia gemmatalis* multicapsid nuclepolyhedrovirus (AgMNPV) in a new serum-free, cholesterol-free medium, where essential lipids are provided through a microemulsion of soybean oil, was used as a model to study the influence of cholesterol on baculovirus infection and replication. Cultures adapted to growth in the same medium, but additionally supplemented with cholesterol, were used as controls. Cholesterol-free cells were able to attach the virus, although at a significantly lower rate than cells cultivated in cholesterol-supplemented medium. Surprisingly, cholesterol-free UFLAg-286 cells could replicate AgMNPV. The kinetics of production of both viral progenies was substantially different in cholesterol-free and cholesterol-supplemented cultures, but final yields of enveloped virus and polyhedra were similar in both cultures. These results show that cholesterol-free UFLAg-286 cells are fully permissive to virus replication, but have limited susceptibility to baculovirus infection.

BT-P9.

INTRACELLULAR TRANSIT OF SULFADIAZINE IN DENDRIMERS: NANOTEC VS T.GONDII?

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Dendrimers (D) are infinitely hydrosoluble unimolecular nano-micelles. The use of D for incorporation of non-hydrosoluble drugs which remain retained in hydrophobic pockets or associated to the hydrophilic surface is a powerful tool for increasing their concentrations in aqueous media and/or modify their biodistribution. The aim of this work was to design strategies leading to maximise incorporation of the non-hydrosoluble antifolate sulfadiazine (SD) in D, to determine cytotoxicity, and to register uptake and intracellular transit in cultured fibroblasts and macrophages. PAMAM G4 (cationic) and G4,5 (anionic) D were incubated with SD in methanol at 1:70 and 1:140 (D:SD, mol:mol) ratios for 3 or 48 h at room temperature in the dark. Methanol was eliminated by N_2 stream at 40°C and the dry residues suspended in 150 mM Tris-HCl /NaCl pH 7,4; supernatants were then separated and D and SD quantified by colorimetric methods (fluorescamine, SD-*ad-hoc* and UV absorption). The best incorporation strategy resulted in 20-fold higher solubility of SD. In addtion, the D G4 was labelled with the dye FITC and its endocytic uptake as well as intracellular transit in J774 cells were followed by fluorescence microscopy. The absence of cytotoxicty in a wide range of D G4 and G4,5 amounts was determined as formazan by the MTT assay.

BT-P10.

INCORPORATION AND CHARACTERIZATION OF ANTI-HELMINTIC DRUGS INTO SOLID LIPID NANOPARTICLES

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Solid lipid nanoparticles (SLNs) are able to capture, retain and release lipophilic drugs. Albendazole (ABZ) is an anti-helmintic drug, effective against hydatidic disease (echinococcosis) and neurocysticercosis. ABZ is water insoluble, with low systemic bioavailability and efficacy due to scarce adsorption after oral or intrarruminal administration to bovine and ovine. The sulfoxide derivative (ABZSO) is soluble at pH 2-3, but i. m. administration is highly harmful due to its high tissue permeability and diffusion through blood-brain barrier. We prepared SLNs loaded with ABZ and ABZSO by diluting a micro-emulsion of ABZ and ABZSO dissolved in different lipidic phases (palmitic, myristic and stearic acids) with soy phosphatidylcholine and taurocholic acid. The solubility of the drugs increased several-fold through their incorporation into the SLN, as measured by HPLC. Negatively stained SLN were observed by transmission and scanning electron microscopy and detected by differential scanning calorimetry. Cytotoxic assay on Vero and J774 cells showed their safety over a wide range of concentrations. Finally, we studied the interaction of the fluorescently labelled SLN with both cell lines by fluorescence microscopy and the internalization pathway (endo or phagocytosis) was determined by phagosome isolation.

BT-P11.

ARCHAEOSOMES: STRUCTURAL STABILITY OF ALBUMINE IN PHAGOSOMES

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The main interest of archaeosomes (vesicles formed by polar lipids extracted from archaebacteria) resides in their adjuvancy to humoral and cellular immune responses. However, since their bilayers show high mechanical and chemical structural resistance, conventional methods for loading proteins as well as disruption methods of vesicles are useless for archaesomes. To test the occurrence of those phenomena inside cultured cells, archaeosomes prepared with total polar lipids extracted from halophilic archaeas, loaded with the fluorescent pH-sensitive dye HPTS quenched with DPX and containing albumin, were incubated with J774 macrophages. Structural stability was quantified as the permanence of intact albumin in phagosomes. Efficient internalisation of archaeosomes and overall retention of their aqueous contents during processing were assessed by fluorescence microscopy. Phagosomes were isolated, their contents extracted and subjected to denaturing gel electrophoresis. The exceptional structural stability of archaeosomes inside the cells, revealed by a plot of intact albumin as a function of time, was several orders of magnitude over conventional liposomes.

BT-P12.

DECOLORIZATION OF SYNTHETIC DYES BY THE *Pestalotiopsis guepinii* CLPS NO. 786 STRAIN (DEUTEROMYCETES)

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The ability of the *Pestalotiopsis guepinii* CLPS no. 786 strain (*Deuteromycetes*) compared to a *Phanerochaete chrysosporium* (*Basidiomycetes*) strain, to grow on and to decolorize an agar medium with dyes of varied structure was analyzed. Strains were inoculated on modified Czapek Dox agar medium supplemented with different chromophore types (0.01%, w v^{-1}). The dye-decolorizing ability was analyzed by the naked eye after 15 days of incubation at 25ºC in the dark. Both fungi tested decolorized some but not all the dyes investigated under the test conditions. However, the medium supplemented with congo red, fuchsin, methylene blue B or neutral red was only decolorized by *P. guepinii*. In this way, it was also analyzed the ability of *P. guepinii* for transforming and/or bounding to its mycelium dyes in liquid culture. Absorbance at the maximum wavelengths for each dye used was measured on appropriately diluted (using McIlvaine buffer, pH 7.0) extracellular supernatants from the incubation of live and dead 3-days-old cultures with each dye immediately after its addition, as well as after 4 and 8 days. *P. guepinii* transformed both cristal violet and methylene blue B dyes, and adsorbed different chromophores onto its mycelium.

BT-P13.
EVALUATION OF ANTIOXIDANT CAPACITY FROM *ROSMARY PLANTS*

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This work reports on the study of antioxidant (AOX) capacity from rosemary plants grown in Argentina's northwest. The AOX status of biological systems is implicated in several degenerative diseases. There are two basic categories of AOX: synthetic and natural. Restriction of the use of synthetic AOX because of their carcinogenicity motivated a considerably new interest in natural plant AOX. *Rosmarinus officinalis* L. exhibits potent AOX activity. Although different analytical methods are employed to determine natural AOX, a kinetic behavior of the antioxidant activity of rosemary plant extract was not reported. We used two methods: a model lipid system (linoleic acid emulsion) and the scavenging of free radical ability. With both methods, rosemary extracts exhibited an *in vitro* antioxidant effect, which was similar to those of vitamin C or BHT. The kinetic behavior of rosemary extracts revealed the presence of slow and fast AOX. Their activity was related to phenolic compounds of plant extracts such as caffeic acid derivates and phenolic diterpenes. Distribution and quantification by HPLC, revealed a high content of AOX in leaf extracts relative to flowers and stems. A good correlation between AOX activity and active compounds in several rosemary extracts was found.

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BT-P14.
ENZYMATIC BROWNING INHIBITION IN SOLANUM TUBEROSUM BY POST-TRANSCRIPTIONAL GENE SILENCING

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Enzymatic browning is one of the most important colour reactions that affect fruits, vegetables and seafood. It is catalyzed by the enzyme polyphenol oxidase (1,2 benzenediol; oxygen oxidoreductase, EC1.10.3.1; PPO) which is also referred to as phenoloxidase, phenolase, monophenol oxidase, diphenol oxidase or tyrosinase. There are known at least five PPO genes in potato plants, each with a specific spatial and temporal expression pattern. The Pot 32 gene is strongly expressed in tubers and roots whereas the Pot 33 gene is mainly expressed in the outer cortex of tubers. In our laboratory, we have designed a post-transcriptional gene silencing vector against the 5' region of Pot 32 gene in order to silence it only in tubers. The engineered construct was cloned in the pZP200HYG vector, introduced into *A. Tumefaciens* and used to transform potato plants.
At the moment, we are studding the transgene presence by PCR assays and inducing the tuber formation to study the phenotype. The recombinant Pot 32 and Pot 33 enzymes were expressed in *E. coli* as a 6xHis fusion protein using pRSET A vector and confirmed by Western Blot.

CB-P1.
INTERACTION OF TUBULIN WITH H^+-ATPase OF PLASMA MEMBRANE IN *SACCHAROMYCES CEREVISIAE*. INFLUENCE OF GLUCOSE UPTAKE

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It was previously reported that Na^+,K^+-ATPase in membranes of brain and cultured astrocytes interacts with tubulin and that this interaction inhibits the enzyme activity. Dissociation of the tubulin/Na^+,K^+-ATPase complex leads to activation of the enzyme and to a lesser amount of hydrophobic tubulin. In the present study we investigated the eventual interaction of tubulin with H^+-ATPase in yeast membranes. We found that the acetylated tubulin is present in plasma membrane of yeast and interacts with H^+-ATPase. We demonstrate that this interaction inhibits the enzyme activity. In effect, when isolated membranes were treated with tubulin purified from brain, we found inhibition of H^+-ATPase in a degree that was higher when the tubulin preparation contained higher proportion of the acetylated isoform. It is known that H^+-ATPase is activated by the addition of D-glucose into the culture medium. As expected, after treatment of the cells with glucose, we found an inverse correlation between the activation of H^+-ATPase and the amount of acetylated tubulin associated with the enzyme. Addition of 1 mM 2-deoxy-D-Glucose (inhibitor of D-Glucose transport) after D-glucose treatment reversed the effects of D-Glucose. These results indicate that the H^+-ATPase activity of *S. cerevisiae* is modulated by the association/dissociation of (acetylated tubulin-H^+-ATPase) complex.

CB-P2.
REGULATION OF ACETYLATED TUBULIN/Na^+,K^+-ATPase INTERACTION BY L-GLUTAMATE IN NON-NEURAL CELLS: INVOLVEMENT OF MICROTUBULES

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A subpopulation of membrane tubulin consisting mainly of the acetylated isotype is associated with Na^+,K^+-ATPase and inhibits the enzyme activity. We found recently that treatment of cultured astrocytes with L-glutamate induces dissociation of the acetylated tubulin/Na^+,K^+-ATPase complex and increases enzyme activity. We now report occurrence of this phenomenon in non-neural cells. As in the case of astrocytes, the effect of L-glutamate is mediated by its transporters and not by specific receptors. In COS cells, the effects of L-glutamate were reversed by its elimination from culture medium, provided that D-glucose was present. Na^+ ions were involved in dissociation of acetylated tubulin/Na^+,K^+-ATPase complex, and the effect of L-glutamate was not observed in the absence of Na^+. The ionophore monensin, in the presence of Na^+, had the same effect as L-glutamate. Treatment of cells with taxol prevented the dissociating effect of L-glutamate. Nocodazole treatment of intact cells or isolated membranes reduced the amount of acetylated tubulin bound to Na^+,K^+-ATPase and increased enzyme activity. These results indicate a close functional relationship among Na^+,K^+-ATPase, microtubules, and L-glutamate transporters, and a possible role in cell signaling pathways.

CB-P3.
M6A IS A STRESS-REGULATED GENE THAT INDUCES FILOPODIAL FORMATION IN CULTURED NEURONES
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Chronic stress affects several cellular, biochemical and molecular parameters over different brain areas. Among these, the hippocampus is particularly sensitive to the stress effects. Morphological alterations in this area include dendritic atrophy of pyramidal neurons and reduced spine density, events related to connectivity and plasticity. We have previously shown that hippocampal expression levels for the neuronal membrane protein M6a are decreased in chronic psychosocially stressed tree shrews. Now we found that transcript levels for this gene are also downregulated in mice subjected to chronic restraint. In the present study we analyzed M6a protein in primary hippocampal cultures and cell lines. Immunocitochemistry assays in hippocampal neurons showed that endogenous M6a is concentrated in soma, dendritic and axon membranes. Moreover, it co-localizes with the postsynaptic marker PSD-95, a protein highly enriched in spines. Overexpression of M6a fused to green fluorescent protein resulted in increased density of dendritic filopodia. Furthermore, we also found an induction of filopodial formation in PC12 and neuroblastoma N2a cell lines; as well as in the non-neural cells COS-7. In view that spine formation is originated by filopodial maturation, our findings suggest that M6a might be involved in spinogenesis. Its decrease in chronic stress could be, at least in part, responsible for the diminished spine density reported in the hippocampus of stressed animals.

CB-P4.
THE SOLUBLE MYOSIN II ISOTYPE ASSEMBLES IN FILAMENTS DURING THE CONTRACTION OF PERITUBULAR MYOID CELLS
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Peritubular myoid cells contract the seminiferous tubules (ST) in order to facilitate the transport of spermatozoa and testicular fluid. These cells contain a myosin II isotype (T-myosin) which is found soluble in the cytoplasm. In order to determine whether this soluble T-myosin is able to participate in cellular contractile processes, rat ST were isolated in MEM medium at 32ºC and treated with 50 nM of endothelin-1 (ET-1) for 20 seconds, and the myoid cell contraction patterns and the degree of T-myosin assembly were analyzed. A fraction of the ST were fixed in 3% glutaraldehyde and analyzed by scanning electron microscopy. The remaining fractions of the ST were frozen in liquid nitrogen, homogenized in PMEE buffer, centrifuged, and the pellets and supernatants analyzed by 7.5% SDS-PAGE in order to identify T-myosin. The scanning electron microscopy showed that 90% of peritubular myoid cells were contracted in the ST treated with ET-1. The degree of T-myosin assembly was 30 ± 5% in the control ST and 60 ± 3.5% in those incubated with ET-1.
From these results we conclude that T-myosin is able to assemble in filaments and participate in the mechanisms of cell contraction.

CB-P5.
***Coxiella burnetii* TRANSITS THE ENDOCYTIC PATHWAY AT EARLY TIMES AFTER INFECTION**
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C. burnetii, the etiological agent of human Q fever is an intracellular obligate parasite that inhabits in phagolysosome-like vacuoles. Previously, our group has demonstrated that *Coxiella* interacts with the autophagic pathway as a strategy for its survival. However, very little is known about the first steps during *Coxiella* infection and the molecular mechanisms that control the replicative vacuole development. In this work we analyzed the role of the small GTPases Rab5 and Rab7, markers of early and late endosomes respectively, on *C. burnetii* trafficking. CHO cells overexpressing GFP-Rab5, GFP-Rab7 or their mutants, were infected for different periods of time with *C. burnetii* phase II. The bacterium was then localized by immunofluorescence using a specific antibody, and the samples were visualized by confocal microscopy. Our results indicate that *Coxiella* phagosomes acquire Rab5 and Rab7 sequentially during phagosome maturation. Interestingly, the dominant-negative form of Rab5 (Rab5S34N) reduced *Coxiella* entry at least by 50% whereas the Rab7 mutant T22N altered the development of the *Coxiella* replicative vacuole. Taken together our results suggest that at early times after infection *Coxiella* follows the classical phagosome maturation pathway.

CB-P6.
THE SMALL GTPASE RAB1 COLOCALIZES WITH AUTOPHAGIC VACUOLES
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Autophagy is a process by which portions of cytoplasm and/or organelles are sequestered by membranes to form vesicles called autophagosomes, which finally fuse with lysosomes. The origin of the sequestering membrane has not been clearly determined. Intracellular tubulovesicular trafficking is regulated by members of the Rab GTPase family. It is well known the function of Rab1 promoting the anterograde transport between ER and Golgi membranes. In this study we have analyzed by fluorescence and confocal microscopy, the behavior of GFP-Rab1b wt and mutants, during autophagy by using autophagosome markers such as GFP/RFP-LC3 and monodansylcadaverine (MDC). Cells were incubated under different conditions that stimulate autophagy such as starvation or rapamycin treatment. Our data suggest that Rab1-labeled vesicles partially colocalized with RFP-LC3, whereas a marked colocalization was observed in cells overexpressing the GTPase deficient mutant Q67L. The Rab protein remained associated to these vacuoles until a later stage (e.g. autophagolysosome), as indicated by the acquisition of cathepsin D, a lysosomal enzyme. Moreover, Rab1 requires its active conformation to anchor on autophagic membranes, since the mutant S22N with impaired capacity to bind GTP, did not colocalize with MDC marked vesicles. Our results suggest that transport vesicles containing Rab1 may contribute to the generation of autophagic vacuoles.

CB-P7.
ROLE OF RAB1 IN THE MATURATION OF TRANSPORT INTERMEDIATES EXPORTED FROM ENDOPLASMIC RETICULUM
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Recruitment of type I coat protein complex (COPI) is required for maturation of vesicles exported from the Endoplasmic Reticulum (ER) to the Golgi complex. COPI is recruited to membranes by a small GTPase of the ARF family. To become active, ARF must interact with GBF1, a guanine nucleotide exchange factor that stimulates the exchange of GDP for GTP. Previous works indicate that the small GTPase Rab1 modulates COPI recruitment; however, the molecular mechanism that links Rab1 activity and COPI recruitment is unknown. In this report, GBF1 was identified as a Rab1 effector protein. GST pull down assays show that GBF1 binds preferentially to Rab1-GTP. Additionally, double-immunofluo-rescence assays show that over-expression of the Rab1 GTP-restricted mutant (Rab1-Q67L) increases membrane association of COPI proteins and GBF1, preferentially at ER exit sites. Finally, in order to characterize *in vivo* the effect of the Rab1-Q67L on COPI dynamic, Fluorescence Recovery After Photobleaching (FRAP) was performed in GFP-ε-COP expressing cells co-transfected with or without Rab1-Q67L. Fluorescence recovery was significantly slower in Rab1-Q67L expressing cells, indicating that COPI association from membranes is dependent on Rab1-GTP hydrolysis. Taken together our results suggest that Rab1 recruits GBF1 to membranes, which in turns activate ARF1, which then recruits COPI and allows COPII/COPI exchange on transport intermediates derived from the ER.

CB-P8.
GANGLIOSIDE GD3 TRAFFICS FROM *TRANS*-GOLGI NETWORK TO PLASMA MEMBRANE BY A BREFELDIN A-INSENSITIVE EXOCYTIC PATHWAY
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Gangliosides, complex glycosphingolipids containing sialic acids, are synthesized in the lumen of the Golgi complex and appear unable to translocate from the lumenal toward the cytosolic surface of Golgi membrane to access the monomeric lipid transport. As a consequence, they can only leave the Golgi complex via the lumenal surface of transport vesicles. In this work, we analyzed the exocytic transport of the disialo ganglioside GD3 from *trans*-Golgi network (TGN) to plasma membrane in CHO-K1 cells, by immunodetection of endogenously synthesized GD3. We found that ganglioside GD3, unlike another luminal membrane-bounded lipid (glycosylphosphatidylinositol-anchored protein), trafficked from TGN to plasma membrane by a BFA-insensitive exocytic pathway. Moreover, dominant negative form of Rab11, which prevent exit of vesicular stomatitis virus glycoprotein (VSVG) from the Golgi complex, did not influence the capacity of GD3 to reach the cell surface. Our results strongly support the notion that most ganglioside GD3 traffics from TGN to plasma membrane by a non-conventional vesicular pathway where lateral membrane segregation of VSVG and GPI-anchored proteins from GD3 is required before exiting TGN.
Supported by: Fundación Antorchas, CONICET, SECyT-UNC.

CB-P9.
SUBGOLGI LOCALIZATION OF GAL-T1/SIAL-T1/SIAL-T2 MULTIENZYME COMPLEX DEPENDS ON EXPRESSION LEVELS OF SIAL-T2
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Ganglioside glycosyltransferases form multienzyme complexes in the Golgi apparatus. Here we studied whether the proximal Golgi localization of Gal-T1 and Sial-T1 complex is affected when another member of this complex, Sial-T2, is co-expressed. For this, CHO-K1 cells, which lack endogenous Sial-T2, were stably transfected with full-length Sial-T2. The subGolgi location of these activities was determined by measuring the effect of Brefeldin A on I), the metabolic labeling of glycolipids from ^{14}C-galactose; II), the subcellular localization of chimeras of Gal-T1 and Sial-T1 and spectral variants of the GFP and by III), isopicnic ultracentrifugation. It was found: in I) that in parental cells BFA imposed a block in the synthesis of glycolipids beyond GM3, while in Sial-T2 expressing cells the block was imposed beyond GlcCer. In II), that in parental cells BFA redistributed Gal-T1-GFP and Sial-T1-GFP to the ER, while in Sial-T2 expressing cells these chimeras mostly remained associated to a distal Golgi (post BFA) compartment. In III) that in parental cells BFA displaces Gal-T1-GFP and Sial-T1-GFP to fractions enriched in ER membranes while in Sial-T2 expressing cells these chimeras were displaced to fractions floating slightly above Golgi membranes. These results suggest that subGolgi localization of these complexes is dynamic and may change according to relative levels of expression of the participating enzymes.

CB-P10.
DISRUPTING LIPID RAFTS LEADS TO A REDUCTION IN POLIOVIRUS INFECTIVITY
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Lipid microdomains rich in glycosphingolipids and cholesterol which are present in the plasma membrane (rafts), play a critical role in several biological processes, including viral infection. The data presented in this communication describe the importance of these domains in the infection of HeLa cells by poliovirus (PV). To study the role of rafts in PV infection we depleted cellular cholesterol using two different methodologies. 1) HeLa cells were cultured in the presence of simvastatin/mevalonate in lipoprotein deficient medium. The results show that when cellular cholesterol was reduced by 50%, there was a 90% reduction in the titer at MOI 3. The synthesis of viral proteins was greatly reduced as well, whereas there was no effect of low cholesterol on synthesis of cellular proteins in non-infected cells. This effect could not be overcome by increasing MOI to 100. 2) HeLa cells were treated with methyl-ß-cyclodextrine to reduce cholesterol. In this case there was also a 90% reduction in PV titer upon exposure of cells to MOI=3. However, control titer could be recovered in these treated cells by increasing the MOI to 100 which suggests that this cholesterol-lowering technique affects pathways different than the ones affected by simvastatin/mevalonate treatment. Our results indicate that rafts play an important role in PV infectivity *in vitro*. Further, they show that different methodologies used to disrupt rafts can be important tools to investigate the different steps involved in viral infection.

CB-P11.

APOLIPOPROTEIN A-I DOMAINS INVOLVED IN CHOLESTEROL MOBILIZATION AND EFFLUX FROM MAMMAL CELLS

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Apolipoprotein A-I (apoA-I) plays a key role in promoting cholesterol (Ch) efflux from peripheral tissues. Its interaction with specific sites at the cell membrane triggers Ch mobilization from particular pools like those available for esterification by acyl-CoA acyl transferase (ACAT).

We demonstrated previously that apoA-I interaction with artificial membranes is mediated by a central domain containing two amphipatic helices class Y. We study here the role of the protein central helices in cell Ch removal and mobilization from ACAT-available pools. We used CHO and CCD27 SK cells and compared: a) plasma apoA-I, b) a central peptide A-I 77-120, c) a natural single amino acid deletion apoA-I (A-I 0_K107), and d) a variant in which two central helices were replaced by two Y helices of the C-terminus (AI H10_H4).

Our results suggest that the two central helices would be sufficient to mediate the mobilization of Ch available for ACAT and its efflux from CHO cells. The mutant A-I 0_K107 was not effective to produce such effect, probably due to the lack of a positive charge, or to the disruption of the appropriate orientation of the amphipatic α helices. On the other hand, A-I H10_H4 was efficient to activate these mechanisms, suggesting that, more than a specific sequence, a charge distribution type Y would be needed in the polar face of the central domain. Only apoA-I and AI H10_H4 were active to remove Ch from CCD27 SK cells.

CB-P12.

H-RAS LOCALIZES AT RECYCLING ENDOSOMES IN CHO-K1 CELLS. ROLE OF PROTEIN ACYLATION

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Ras proteins were described localizing at the plasma membrane (PM) where they activate diverse signal transduction pathways. There are three isoforms of Ras proteins, H-, N- and K-Ras, which undergo different lipid modifications at the C-terminus. These lipid modifications confer them the capacity to associate with PM.

To better characterize the intracellular distribution and sorting of Ras proteins, two constructs were engineered to express the C-terminal domain of H- and K-Ras fused to GFP (GFP-H-Ras^{C20} and GFP-K-Ras^{C14}). By using confocal microscopy, we found that GFP-H-Ras^{C20} (palmitoylated and farnesylated) localized mainly at the PM, recycling endosomes (RE) and in minor extent at late endosomes. In contrast, GFP-K-Ras^{C14} (farnesylated and nonpalmitoylated) localized at the PM and cytoplasm. Interestingly, the subcellular distribution of GFP-H-Ras^{C20} and GFP-K-Ras^{C14} completely colocalized with the full length version of these proteins. We also demonstrate that another unrelated palmitoylated protein, ^{N13}GAP43-GFP, also localized, like H-ras, both at PM and RE.

In conclusion, we demonstrate for the first time that H-Ras localizes, in addition to PM, at RE. Furthermore, our results suggest that palmitoylation at the C-terminus region of H-Ras might be a dominant sorting signal for proper subcellular localization of this protein in CHO-K1 cells.

Supported by: Fundación Antorchas, CONICET, SECyT-UNC.

CB-P13.

ACTIVATED ALFA-2 MACROGLOBULIN (α^2M^*) MEDIATES INTRACELLULAR SIGNALING PATHWAYS VIA THE ENDOCYTIC RECEPTOR LRP-1

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Alfa-2-macroglobulin (α_2M) is the most important human proteinase inhibitor, upon binding to proteinases undergoes a conformational change, activated α_2M (α_2M^*), that expose recognition receptor site allowed its interaction with LRP-1 receptor. LRP-1 is a member of the LDL receptor gene family that binds to a variety of ligands, some of which trigger signalling transduction. Previously we have demonstrated that α_2M^* has proliferative effects and increase intracellular calcium in J774 macrophage derived cell line LRP positive. In this work we evaluate the α_2M^* effect down-stream to LRP-1 interaction measuring MAPK phosphorylation in J774 cell cultured in the presence and absence of LPS (a down regulator factor of LRP-1). By Western blotting we observe that α_2M^* 20 nM and 70 nM promote ERK-1/2 phosphorylation at different times (15, 30, and 60 min.). On the other hand, when J774 were treated with LPS 30 μg/ml for 24 hs we demonstrate that intracellular signalling pathways were not activated in the presence of α_2M^*. In addition, we analyse the α_2M^* effect on ERK-1/2 phosphorylation in HT-1080 fibroblast derived cell LRP-1 positive. Like J774, α_2M^* 20 nM and 70 nM promotes ERK-1/2 phosphorylation. In conclusion, in this work we demonstrate that α_2M^* can activate intracellular signalling pathways mediated by LRP-1.

CB-P14.

FUNCTIONAL CHARACTERIZATION OF *HAHB-4* (*HELIANTHUS ANNUUS* HOMEOBOX 4) PROMOTER REGION

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We have analyzed the promoter region of the sunflower gene *Hahb4*, encoding an homeodomain-leucine zipper protein involved in water stress responses. For this purpose, we have obtained plants stably transformed with different promoter fragments fused to the β-glucuronidase reporter gene. Histochemical staining indicated that the *Hahb4* promoter directs expression in cotyledons, roots and leaves during the entire plant life cycle. The analysis of progressive upstream deletions of the promoter indicated that a 400 bp minimal fragment is required for basal expression, the presence of positive regulatory elements between nucleotides -600 and -800 from the transcription initiation site and of a specific root central cylinder expression sequence between -800 and -1000. Plants bearing a construct with only the first 300 nucleotides did not show any expression. Drought, ABA or NaCl treatments induced *Hahb4* promoter-dependent β-glucuronidase expression through elements putatively located at distal locations, as observed by northern blot hybridization experiments. We propose that this promoter constitutes a biotechnological tool for the inducible expression of this or other genes in transgenic plants.

CB-P15.
CONVERGENCE OF PUTATIVE PSG5-SPECIFIC REPRESSORS AND GENERAL ACTIVATORS IN THE REGULATION OF PSG5 GENE

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Pregnancy-specific glycoprotein 5 human gene (PSG5) belongs to a family of highly similar and active genes. High levels of PSG biosynthesis are restricted to the placental syncytiotrophoblast and are essential for the maintenance of normal gestation. PSG5 promoter contains a GC-like box recognized by the ubiquitous Sp1 transcription factor. Now, we have characterized the regulatory region that represses PSG5 expression. EMSA and UV-crosslinking assays revealed that the -543/-517 *cis* element, included in a negative regulatory region, is specifically recognized by proteins of 115, 85 and 72 KDa present in PSG-non-expressing cell lines and absent in human term placenta extracts. Dissection of the negative regulatory region in PSG5-CAT constructs showed that at least two elements, including -543/-517, mediate repressed transcriptional activity in transient-expression assays. These negative acting modules are specific of PSG5 promoter since no repression was observed on heterologous promoters.
In addition, we examined the role of transcriptional activators of placenta-specific genes in PSG5 regulation. SNURF co-activator and c-Jun transcription factor were able to enhance transcription even in the presence of the repressive region, while GCM transcription factor did it only when this module was absent.
We propose that placenta specific expression of PSG5 gene is mainly achieved through an impairment of the repressor functions that allows high levels of expression through the action of the identified transcriptional activators.

CB-P16.
POLAR EFFECT IN ALTERNATIVE SPLICING REGULATION

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About 60% of human genes are alternatively spliced and ~30% of them have more than one alternative region. The fibronectin gene, comprises three alternative regions (EDII, EDI & IIICS). To evaluate if there is a link between the way in which two alternative events are processed in the same transcript, we transfected mammalian cells with minigenes carrying two alternative exons separated by three constitutive exons and analyzed the rate of exon inclusion into mature mRNA by RT-PCR. In a tandem construct with two EDI exons we found that the proximal splicing event influences the distal one. Disruption of the proximal EDI splicing enhancer (ESE) not only prevents its own inclusion, but favors skipping of the distal EDI exon. The effect shows polarity, since disruption of the distal EDI ESE does not affect the proximal one. The polar effect was observed in the endogenous fibronectin gene: in cultured murine embryonic fibroblasts (MEFs) that constitutively include EDI, the isoform of total inclusion of IIICS was underepresented in comparison to MEFs which always exclude EDI. Using promoter swapping, we demonstrated that the effect is dependent on the promoter structure. These results provide the first evidence of regulation of one alternative splicing event by another-one located upstream in the same transcript, and the possible coordination between this regulation and transcription.

CB-P17.
CONTROL OF ALTERNATIVE SPLICING IN A NEURONAL DIFFERENTIATION SYSTEM

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Alternative splicing is one of the most important ways of generating protein diversity in vertebrates. The nervous system is a clear example of fine regulation of protein expression by this mechanism, with several cases of genes with splicing variants that differ in their function. The neural cell adhesion molecule NCAM is a membrane bound protein, which mediates cell-cell interactions. The alternatively spliced-exon 18 is included mostly in differentiated neurons but not in their precursors. This change is important for proper development and for neuronal plasticity in adults. However, no molecular mechanism has yet been proven to be responsible for the regulation of this exon during differentiation. We used the murine neuroblastoma cell line N2a as a model to study a possible influence of transcriptional activity on exon 18 skipping. Inhibiting histone deactylases with Trichostatin A (TSA) raises the proportion of transcripts excluding exon 18, showing that acetylation has a negative effect on exon inclusion in these cells. The effect is not dependent on protein synthesis and is neither seen with a non-neuronal fibroblast cell line nor with a non-neural specific exon. Moreover, treating the cells with TSA after DMSO-induced differentiation still caused the inhibition of exon 18 inclusion. Given that histone acetylation is asociated with more efficient and processive transcription, this is compatible with an elongation model where slower RNA pol II transcription favours alternative exon inclusion.

CB-P18.
STAUFEN INHIBITION BY siRNA PREVENTS STRESS GRANULE ASSEMBLY

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Stress granules (SGs) are stress induced organelles, consisting on dynamic, perinuclear structures thought to transiently harbor housekeeping mRNAs when translation is aborted due to stress (Anderson and Kedersha, 2002). Several functions like mRNA recruitment, silencing, stabilization and degradation occur in SGs. However a relatively reduced number of component RNA-binding proteins were described. We have recently found (Thomas *et al*., 2004) that the double stranded-RNA binding protein Staufen is a general component of SGs. We study the role of Staufen in oxidative stress-induced SGs by analyzing the effect of Staufen inhibition by siRNAs. Distinct siRNA sequences for Staufen 1 and Staufen 2 diminished the expression of Staufen GFP reporters to 10-80 %. Isoform specificity was confirmed. Silencing of endogenous Staufen 1 in HeLa cells was assesed by Real Time-PCR and inmunofluorescence. RNA levels were reduced to 25% and protein levels to 50-70 %. Preliminary results showed a reduction in the number of cells bearing arsenite-induced SGs after depletion of Staufen 1. Our results suggest that Staufen is required for SG formation, likely by mediating stability of RNA-protein aggregates or by recruiting molecular motors required to achieve the perinuclear distribution of otherwise disperse mRNPs.

CB-P19.

STAUFEN FORMS DISTINCT RNPs IN NORMAL AND STRESS CONDITIONS

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The double-stranded RNA-binding protein Staufen is a quite ubiquitous RNA granule-forming factor required for mRNA localization in different organisms. We have recently found that in rodent oligodendrocytes Staufen 1 and Staufen 2 are present in two independent sets of RNA granules located at the distal myelinating processes and associated to cytoskeleton and polysomes (Thomas *et al.*, 2004). Under oxidative stress, both homologues are recruited into stress granules (SGs). SGs are stress-induced organelles that contain transiently silenced messengers and have perinuclear localization. Staufen SGs also include Poly(A)-binding protein (PABP), the RNA-binding proteins HuR and TIAR, and small but not large ribosomal subunits. Our results indicate that Staufen 1 and Staufen 2 are novel and ubiquitous SG components and suggest that Staufen RNPs are involved in repositioning of most polysomal mRNAs during the stress response. Currently, we are trying to isolate Staufen SGs from rat oligodendrocytes and transfected cell lines via cell fractionation and purification strategies based on immunoprecipitation and Staufen-tagged constructs. To gain insight into Staufen SG function we will study the protein and RNA composition of the isolated SG by mass spectrometry and microarray analysis respectively.

CB-P20.

SMAUG, A NOVEL RNA BINDING PROTEIN AND ITS ROLE IN POSTRANSCRIPTIONAL REGULATION IN THE POSTSYNAPSIS

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Cytoplasmic events contribute to the fine-tuning of gene expression. These mechanisms depend on several families of RNA binding proteins (RBPs) that are less known than transcription factors, in spite of their comparable importance in regulating gene expression. SAM-containing RBPs constitute a novel family that function as post-transcriptional regulators. The SAM domain in these proteins binds a sequence motif known as SRE (Smaug Recognition Element). Drosophila Smaug is involved in translational repression of maternal mRNAs, contributing to define embryo polarity. In contrast, the yeast member, Vts1, stimulates degradation of SRE-containing messengers. Two homologous genes are present in the mammalian genome. Here we show that one of the Smaug homologue (*mSmaug1*) is expressed in the CNS and is abundant in synaptoneurosomes, where translation is tightly regulated. Biochemical analysis indicated that mSmaug1 forms small RNPs, and is absent from polysomes. When expressed in non-neural cell lines, Smaug1 forms cytoplasmic granules that contain polyA+ mRNAs. Smaug1 granules are in dynamic equilibrium with polysomes and can be converted into stress granules upon induction of cellular stress. Our results suggest a role for mammalian Smaug in translation regulation at post-synaptic sites.

CB-P21.

INHIBITION OF *TRYPANOSOMA BRUCEI* TRANS-SIALIDASE EXPRESION BY RNA INTERFERENCE

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Trypanosoma brucei is the agent of sleeping sickness in humans and Nagana in animals. Unable to sinthetize sialic acids, trypanosomes use a trans-sialidase to scavenge the monosaccharide from the host glycoconjugates and sialylate acceptor molecules present on the surface. The trans-sialidase in *T. brucei* (TbTS) is present in the procyclic form, the stage of the parasite that replicates in the tsetse fly midgut. We have described the molecular and biochemical characterization of TbTS. Evidence from cloning and Shouthern blot analysis suggests that the genes coding for the active TbTS are present in a small number of copies. Therefore, we decided to silence TbTS gene by RNA interference using a construction inducible by tetracycline. The expression of the gene in procyclic trypanosomes could be efficiently silenced. The trans-sialidase activity in induced cells decreased to about 3% of that in control cells and the total sialic acid content was significantly decreased to about 10% of wild type trypanosomes. To test the function of TbTS in the tse-tse fly insect vector, a second aproach was performed in order to obtain cells expressing RNAi constitutively. These mutants also shown reduced levels in trans-sialidase (5% compared to the control) activity and in the sialic acid content (9% compared to the control).

CB-P22.

POST-TRANSCRIPTIONAL REGULATION OF *TRYPANOSOMA CRUZI* TRANS-SIALIDASE EXPRESSION

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Trans-sialidase (TS) is an enzyme unique of trypanosomatids that allows the incorporation of sialic acid to surface parasite molecules. In *Trypanosoma cruzi*, two different TS are expressed from two gene families, the insect vector TS (iTS) and the vertebrate host TS (vTS). In the parasite stage in which only iTS is expressed, iTS and vTS transcripts have a half-life of about 5hs and 1hs respectively. Analysis of the 3'-untranslated regions (3'UTRs) showed that iTS and vTS mRNAs have different 3'UTRs. However, these sequences were almost identical among members of the same family. While studying the mechanism through which vTS transcripts are degraded, a small and stable product derived from the mature mRNA was detected by Northern blotting. This result suggests that vTS mRNAs decay involves an endonucleolytic activity. In addition, the use of a cat reporter gene fused to both 3'UTRs showed that these sequences influence translation efficiency. Finally, different regions of the 3'UTRs were found to bind putative RNA-binding proteins, including the previously identified TcUBP1, a protein involved in mRNA stabilization. We conclude that the 3'UTRs from TS transcripts are likely to be involved in the modulation of both, mRNA stability and translation efficiency.

CB-P23.
EXPRESSION OF mRNAs OF ANG II RECEPTORS BY RT-PCR AND NORTHEN BLOT IN CEREBELLUM

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Angiotensin II (Ang II) exerts its physiological effects through binding to membrane receptors, classified by their pharmacological characteristics as AT_1 and AT_2 receptors. Ang II receptor expression is highly modulated during development suggesting a role of these receptors in growth and organogenesis. With the aim to explore the expression pattern of Ang II receptors, we subcloned PCR fragments of AT_1 and AT_2 receptors in the p-GEM T easy vector. The identity of the amplification products and subcloned inserts was verified by RFLP. For pGem-AT_1 we used Ssp I, Pvu II, Dde I and Not I and for pGem-AT_2 the restriction endonucleases used were EcoR I, Ssp I and Pvu II. The inserts were extracted from the vector with restriction enzymes and the band excised from the gel and purified. The subcloned fragment was labeled with ^{35}S ATP by random priming and used as a probe for Northern blot analysis. Rat cerebellum mRNAs of different ages were analyzed. The expression pattern was compared with RT-PCR analysis of the same samples. The highest expression of AT_2 receptor was observed at the age of 15.

CB-P24.
EXPRESSION AND LOCALIZATION OF STARD7 PROTEIN

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We have previously reported the cloning and characterization of a new gene up-regulated in the choriocarcinoma JEG-3 cell line, denominated StarD7. This gene encodes a protein that belongs to the StAR-related lipid transfer proteins involved in intracellular lipid transport pathways. It was demonstrated that purified overexpressed StarD7 interacts differentially with phospholipid monolayers. In the present work, we transiently transfected Cos-7 cell line with pcDNA/TO/myc plasmid containing a wild type StarD7 cDNA and both NH2- and C-terminal deleted versions. Western blot and immunofluorescence assays of the transfected cells were performed with two different antibodies obtained against overexpressed StarD7 and the StarD7-C-terminal amino acids, respectively. Antibody specificity was verified with antibodies against epitope-c-Myc. The results obtained demonstrate that StarD7-c-myc wild type protein as well as the mutants ones are localized in the endoplasmic reticulum. Furthermore, it was demonstrated that antibodies recognize a 40 kDa protein preferentially present in JEG-3, HepG2 and, HTR-8/SVNeo cell line.

CB-P25.
THE EBAF/lefty2 GENE IS EXPRESSED IN THE PORCINE OVIDUCT

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We have previously isolated an oviduct-uterus specific EST (Expressed Sequence Tag) (Accession No. AF202268) corresponding to the 3' UTR (Untranslated Region) of the rat homolog of human *ebaf* (endometrial bleeding associated factor) gene and cloned its cDNA sequence (Accession No. AY758558). Ebaf, a secreted protein member of the Transforming Growth Factor β (TGF-β) superfamily, was described as being expressed specifically in the stromal cells of human uterus, where stimulates the production of matrix metalloproteinases (MMPs) during menstruation. In normal human endometrium, this gene is transiently expressed before and during menstrual bleeding.
In this work we show that *ebaf* is expressed in the porcine oviduct. Total RNA was isolated and a reverse-transcription polymerase chain reaction (RT-PCR) was performed using primers designed based on the rat, mouse and human cDNA sequences. A 740 bp cDNA containing exon 1 to exon 4 of the porcine ebaf mRNA was obtained; the cDNA fragment was cloned and sequenced. Ebaf mRNA expression was detected in epithelial oviductal cells from ampulla, isthmus and in stromal tissue. The finding of ebaf mRNA in the rat and porcine oviducts strongly suggests a role of this gene in the oviduct. Moreover, the epithelial expression of *ebaf* also indicates that the protein is secreted to the lumen and could be important for the developing embryo.

CB-P26.
EXPRESSION OF THE *BUFO ARENARUM* OVIDUCTIN

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The unfertilizable vitelline envelope surrounding the *Bufo arenarum* (*B.a.*) egg is converted to a fertilizable form during the passage through of the oviduct. A protease from the first portion of the oviduct secretions produces the ultrastructural and biochemical changes of the vitelline envelope. The enzyme has characteristics of a serine protease with trypsin-like specifity. The sequence analysis of the cloned *Xenopus laevis* (*X.l.*) and *Bufo japonicus* (*B.j.*) cDNA oviductins reveals that the protease is translated comprising an arrangement by two protease domains that are interrupted by CUB1 and CUB2 domains, followed by CUB3 domain in the more C-terminal side.
Taken into account that no direct evidence of *B.a.* oviductin expression has yet been reported, the aim of this work was to know if the oviductin gene is expressed in the *B.a.* oviductal *Pars Recta* (PR). Total RNA was isolated from PR of stimulated oviduct after injection of homologous pituitary. Based on the sequence of nucleotides from *X.l.* (Acc. No. U81291) and *B.j.* (Acc. No. AB070367) oviductins, a set of primers directed against the conserved sequences were designed. We amplified by RT-PCR a 530 bp cDNA, harboring the N-terminal protease domain and the CUB1 domain, which was cloned and sequenced (Acc. No. AY704215). The deduced amino acid sequence was determined to share 92% identity with the reported domains of *B.j.* oviductin (with four cysteine residues conserved in CUB1 domain) and 46% identity with the reported domains of *X.l.*

CB-P27.

IDENTIFICATION OF A NEW ISOFORM OF MTUS1 GENE OVER-EXPRESSED IN NORMAL GESTATIONAL TISSUES

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We performed Differential Display tecniques to search genes involved in the phenotypic alterations present in gestational trophoblastic diseases. This strategy resulted in the isolation of one transcript upregulated in normal trophoblastic tissues, called NEP. A clone containing a cDNA insert of 2951 bp was isolated and sequenced (Accession number AY363099). This clone encodes an 837 aa truncated protein called NEP (AAQ24172). The AY363099 nucleotide sequence is included in the GenBank database as a novel MTUS1 (Mitochondrial Tumor Suppressor 1) transcript isoform. This sequence adds 332 pb in the 5´-end to the longest sequence described in GenBank database and lacks 161 bp belonging to exon 4. The 683 bp of the 5´ terminal region of cDNA was cloned into the high-expression vector pET28a. Over-expression of the NH2-terminal region of recombinant AAQ24172 protein in *E. coli* BL21 (DE3) allowed it to obtain an antibody by rabbit immunization. This antibody recognizes a 45 kDa protein present in both first and third trimester placenta and complete hydatidiform mole, one 50 kDa protein mainly expressed in JEG-3, HTR-8/SVNeo, HepG2, HeLa and COS-7 cell lines. Proteins of 17, 18 and 94 kDa were particularly detected in HTR-8/SVNeo cell line. Monkey derived COS-7 cell line, displays particular proteins of 35 and 67 kDa, suggesting NEP expression in different species.
Future analysis will be necessary to define the functional role of AAQ24172 protein (NEP) codified by AY363099 transcript isoform of MTUS1 gene.

CB-P28.

RETINOID RECEPTORS REGULATE THE TRANSCRIPTION OF PREGNANCY SPECIFIC GLYCOPROTEIN GENES

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Expression of PSG genes is increased upon differentiation of cytotrophoblast to syncytiotrophoblast by an unknown mechanism. Retinoid receptors, RXRα and RARα, regulate the differentiation of cytotrophoblast, activating gene transcription mainly by a ligand-induced manner. In addition, they interact with Sp1, enhancing its binding to CACCC-boxes and therefore increasing the Sp1-mediated activation of genes lacking canonical RAREs. Here we show that endogenous PSG expression in trophoblast-derived cells is induced by 9-cis retinoic acid (9cRA). Moreover, RXRα, RARα and Testicular Receptor (TR)4 activates the PSG5 promoter, which is recognized through a composite RARE/CACCC motif by RXRα and TR4, thereby confirming the potential role of the RARE. Most importantly, RXRα activates the PSG5 promoter in a ligand-dependent or independent manner at different expression levels. Accordingly, RXRα enhances Sp1 binding to the CACCC-box, but increasing concentrations of RXRα impairs this interaction and the Sp1-mediated activation, independently of its own DNA binding. These results suggests that the RARE/CACCC motif can integrate different regulatory signals, being able of switching from a Sp1-inducible to a retinoid-responsive state, suggesting for the first time, a mechanism explaining how is reached the high expression of PSG genes during trophoblast differentiation.

CB-P29.

ANALYSIS OF CNBP EXPRESSION PATTERN IN ZEBRAFISH EMBRYO AND CHARACTERISATION OF SOME BINDING TARGETS

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Cellular nucleic acid binding protein (CNBP) is a strikingly conserved single-stranded nucleic acid binding protein containing seven CCHC zinc knuckle domains. CNBP function is not clearly understood yet, however it has been associated with diverse gene expression control mechanisms involving from transcription to translation and related to cell proliferation events. Considering that early development shows a high cell division rate and making use of the advantages of zebrafish as animal model, we carried out whole-mount *in situ* hybridization and immunohistochemistry assays to analyze the z*CNBP-mRNA* and protein expression pattern in early embryos. Both of them, mRNA and protein, are evenly distributed in zebrafish embryo. However, we observed that CNBP translocates to the nucleus once the zygotic transcription starts, suggesting a role in transcription regulation or in newly synthesised RNA folding assistance.
Electrophoretic mobility shift assays were performed to analyze the capability of recombinant zCNBP to bind to single-stranded nucleic acid probes which have different sequences and secondary structures. zCNBP binds specifically to several oligonucleotides with sequences corresponding to 5'UTR from certain maternal mRNAs that are translationally regulated during early development.

CB-P30.

PPARs AND TGF-β1 EXPRESSION IN VITAMIN A DEFICIENT RAT AORTA

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We have previously shown that Vitamin A deficiency is associated with an increase of oxidative stress and inflammation in rat aorta and alteration in the lipid content of serum and aorta tissue. PPARs and TFG-beta play an important role in vascular events during progression of atherosclerosis. The aim of the present study was to examine the effects of Vitamin A deficiency on the expression of the genes of PPARalpha and PPARgamma and TGF-beta1 protein expression in rat aorta. Wistar male 21 days old rats were fed during three months with free vitamin A diet (-A) and the same diet plus 8 mg of Retinol palmitate/kg of diet (+A). Total RNA was isolated with TRIzol. Rat aorta proteins were resolved by SDS-PAGE (10%). TGF-beta1 was identified by Western blot with a specific antibody and bands were revealed using Vectastain ABC detection kit. Analysis of mRNA expression with reverse transcription-polymerase chain reaction (RT-PCR) for PPARs and protein levels for TGF-beta1 revealed higher expressions in -A aortas when compared to +A. In the present study, we show that Vitamin A deficiency regulates positively the transcription of PPARs and TGF-beta1 expression in rat aorta. These results suggest that the balance between these mediators is involved in the mechanism that probably modulates inflammatory responses in this experimental model.

CB-P31.
EXPRESSION OF THE ZFHEP GENE DURING NEUROGENESIS AND MYOGENESIS

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Zfhep (Zinc Finger Homeodomain Enhancer-binding Protein) is a transcription factor involved in differentiation processes such as myogenesis, neurogenesis and lymphopoiesis. The promoter has been recently isolated and so far, there is no direct assessment of its regulation during cell differentiation. Our goal was to evaluate the activity of Zfhep promoter during myocyte and neuron development. The embryonal carcinoma (P19) and a mouse myoblast (C2C12) cell lines were differentiated for inducing neurogenesis (by pretreatment with retinoic acid) or myogenesis (by incubation with 2% horse serum), respectively. Western blot and immunocytochemistry methods were used to validate the model. Human Zfhep promoter was cloned into pGL3-basic and several mutant deletions were made. The reporter chimeras and CMVβ-gal (to normalize transfection efficiency) were transfected into C2C12 and P19 cells by calcium phosphate and FuGENE6, respectively; then they were induced to differentiate. Luciferase and β-galactosidase activities were assessed in cell lysates by standard methods. The promoter was active in all of the constructs tested in both, undifferentiated C2C12 and P19 cells. The activity of the Zfhep promoter, decreased significantly after differentiation of P19 to neurons. The activity was also lowered by 30 fold in differentiated C2C12 cells. These results first show that the Zfhep gene is dynamically regulated during myogenesis and neurogenesis. The repression of the gene could be related to a lack of function of Zfhep during the differentiated cell state.

CB-P32.
EFFECT OF ZINC DEFICIENCY ON THE EXPRESSION OF PARAMETERS OF LUNG INJURY

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Zinc is a micronutrient whose deficiency is associated with oxidative stress. Besides, nitric oxide (NO) plays an important role in the pulmonary physiology, but the expression of the inducible isoform of the enzyme that synthesizes NO (iNOS) is presented in lung injury. It is known that PPARγ and TGF-β1 are factors whose expression is related to inflammatory situations.
To evaluate the effect of lung injury in the chronic Zinc deficiency we studied the expression of iNOS, PPARγ and TGF-β1 in rat lung. Wistar male rats (200 ± 20 g) were fed with AIN-93 diet (zinc deficient, ZD) or with 30 mg Zn/ kg (Control, Co) during 2 months. Total RNA was isolated from lung by using TRIzol. 1 μg of RNA was transcribed to cDNA at 42°C using random hexamers as primers and RT-MMLV (Moloney Murine Leukemia Virus). Aliquots of 2 μl of cDNA were used in the amplifications by PCR using specific primers for the following genes: iNOS and PPARγ. Beta actin was used as an internal control. 100 mg of lung were homogenized in the presence of protease inhibitors and 40 μg of proteins were separated in an 10% SDS-PAGE, transferred to PVDF membranes and incubated with an antibody against TGF-β1. The bands were quantified by densitometry.
We detected a significant increase in iNOS ($p< 0.001$) and TGF-β1 ($p<0.01$) while PPARγ decreased significantly ($p< 0.05$). These results confirm an inflammatory situation under chronic Zinc deficiency.

CB-P33.
EXPRESSION, SUBCELLULAR LOCALIZATION AND GENOTYPE ANALYSIS OF THE TRANSCRIPTION FACTOR KLF6 IN NORMAL AND CANCER CELLS

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The Krüppel-like transcription Factor 6 (KLF6) is involved in mechanisms controlling normal cell proliferation and tumor formation. To determine the impact of KLF6 on potential target genes that regulate cell cycle, the genotype, expression level and subcellular localization of KLF6 was analyzed in different cell lines, normal and tumor tissues. RT-PCR, immunohistochemistry, flow cytometry and Western blot techniques were applied to analyze different tissues and cell lines (breast cancer, n=60, others, n=25). We investigated: (1) KLF6 expression level; (2) subcellular localization of the protein; (3) the relationship with expression of tumor markers (erbB2), (4) the genotype of KLF6 in breast cancer cells and (5) the impact of KLF6 on proteins regulating cell cycle. KLF6 expression and its subcellular localization have a direct correlation with the erbB2 expression in breast cancer. Also allelic variations of KLF6 gene were determined in breast cancer cells (MCF7 and T47D) by sequencing. The inducible expression of KLF6 in HeLa cells increased some cell cycle regulators such as p27, p21, cycling D1, D3 while others were not modified. e.g. c-myc, cdc25, DNA-polymerase δ. Results indicated that KLF6 was localized in the nucleus of breast cancer cells where can interact to its target genes, thereby positioning KLF6 as a promising indicator of an aggressive outcome of certain types of cancer.

CB-P34.
LPS-INDUCED INCREASE OF THYROGLOBULIN (TG) GENE EXPRESSION INVOLVES TTF-1 AND PAX-8 TRANSCRIPTION FACTORS IN RAT THYROID CELLS

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Bacterial lipopolysaccharide (LPS) induces multiple gene expression in several cell types. LPS action on thyroid function has not been studied. We previously demonstrated an LPS-induced stimulation of iodide uptake and iodide transporter expression in rat thyroid cell line FRTL-5. Here we analyse the LPS effect on TG gene expression. An increase of TG level was observed after 24-48h of LPS treatment. LPS induced an increase in TG mRNA after 3-6h which was reduced at 48h. In cells transfected with minimal TG promoter containing thyroid transcription factor-1 (TTF-1), TTF-2 and Pax-8/TTF-1 (C-site) binding sites, LPS increased transcriptional activity at 18-24h and decreased it at 48h. A lack of response to LPS was observed transfecting a Pax-8-site mutated promoter. LPS activated transcription of a construct with 5 C-sites. The binding of TTF-1 and Pax-8 to a C-site oligonucleotide was increased by LPS (EMSA). LPS increased TTF-1 and Pax-8 mRNA (3h) and protein (12h) expression. These results indicate that the LPS-induced increase of TG involves transcriptional activation of TG gene by mediation, at least in part, of TTF-1 and Pax-8. An increase of transcription factors binding to the TG promoter and the induction of TTF-1 and Pax-8 expression could be involved. In conclusion, these findings revealed that LPS is able to stimulate TG gene expression, a fact of possible pathophysiological significance.

CB-P35.
CO-IMMUNOPRECIPITATION AND FRET BETWEEN c-FOS AND ENZYMES OF PHOSPHOLIPID SYNTHESIS THAT IT ACTIVATES
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We have previously demonstrated that the protooncoprotein c-Fos has the capacity to associate to the endoplasmic reticulum (ER) and activate phospholipid biosynthesis (PLB) necessary for the genesis of membrane required for cell growth. This activation is independent of its transcription factor capacity (FASEB J,15:556,2001;M. Biol Cell,15:1881, 2004). Using c-Fos deletion mutants, we determined that the protein domain called BD of c-Fos (aa139-159)is relevant for this activation: only mutants containing BD associate to the ER and activate PLB. Herein, we examined a possible direct interaction between c-Fos and some of the enzymes it activates. For this, pcDNA3.1myc was used to express Phosphatidylinositol synthase (PIS1), CDP-DAG synthase (CDS1), CDP-choline cytidylyltransferase (CPT), Choline transferase (CT) and the α and ß subunits of the kinase PI4KII in NIH 3T3 cells. PI4KII, CPT and CT co-immunoprecipitate with c-Fos as determined by Western blot analysis, indicating a direct interaction between the enzymes and c-Fos. To confirm this,we measured FRET(Fluorescence Resonance Energy Transfer)on a confocal microscope between the α or the ß subunits of PI4KII fused to the CFP protein and c-Fos fused to YFP protein, expressed in NIH 3T3 cells. When measured as percentage of efficiency of FRET, only the α subunit, but not the ß subunit of PI4KII, was found interacting with c-Fos, in agreement with the results obtained by co-immunoprecipitation assays.These results strongly support that a direct interaction between c-Fos and the PLB enzymes that it regulates is required for its cytoplasmic activity.
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CB-P36.
UNRAVELING THE PARTICIPATION OF CYTOPLASMIC c-FOS IN NERVOUS SYSTEM TUMORIGENESIS
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c-Fos is rapidly induced in response to a plethora of stimuli. It heterodimerizes mainly with the protein c-Jun. These dimers constitute the AP-1 family of transcription factorsand they regulate the expression of genes involved in processes such as mitosis and differentiation. We have previously demonstrated that c-Fos has the capacity to associate to the endoplasmic reticulum (ER) and activate the synthesis of phospholipids for the genesis of membrane required for cell growth by a non-genomic mechanism.
Herein, we examined the expression and sub-cellular localization of c-Fos in 147 human brain tumors; c-Fos was found in 100% of the specimens co-localizing with ER markers. No detectable expression of c-Fos was found in 7 non-pathological specimens. T98G cells were inoculated intracraneally in Balb c/c nude mice. At day 6 post-inoculation an osmotic pump (for continuous drug delivery) was implanted in the inoculation site. During the following 30 days, the pumps delivered either vehicle, c-Fos mRNA antisense oligonucleotide or sense oligonucleotide. Tumor development was found in 9 out of 10 animals receiving vehicle, 6 out of 7 sense oligo and 0 out of 9 c-Fos antisense oligonucleotide. c-Fos expression was also examined in brain and peripheral tumors (neurofibromas) occurring spontaneously in B6 mice heterocigotes for the *p53* and *Nf1* genes (NPcis mice). Both in brain tumors and in neurofibromas, high levels of c-Fos expression were observed. In neurofibromas c-Fos is mainly found in Schwann cells. These results disclose a relevant role for cytoplasmic c-Fos in nervous system tumor growth.

CB-P37.
hDlg HPV E6-MEDIATED DEGRADATION AND CARCINOGENESIS
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High risk HPVs play a causal role in the development of cervical cancer. HPV E6 oncoproteins target h-Dlg (Dlg) for ubiquitin-mediated proteolysis. Dlg oncosuppressor is associated with cell-cell interactions, cell polarity and regulation of proliferation. Dlg was expressed in cervical intraepithelial lesions (SIL), but it was absent in samples derived from invasive carcinoma. Although the presence of high risk HPV was detected in some SIL specimens, Dlg levels were high. E6 phosphorylation by protein kinase A (PKA) reduces its capacity for binding to Dlg and thereby reduces E6-stimulation of Dlg degradation. We analyzed PKA activity in cervical samples by immunohistochemistry. We demonstrated variations in PKA activity during the development of cervical tumors that could be altering E6 phosphorylation status, and this should explain the differences in Dlg expression observed in intraepithelial or invasive HPV-associated lesions. Taking into account Dlg functions in the cell, its down-regulation mediated by E6 may contribute to transformation and to the occurrence of some of the characteristics of the HPV-transformed cells, such as changes in cell morphology, loss of polarity and high migration ability. We obtained a cancer HPV-associated cell line over-expressing Dlg, that is being used as a model for studying the incumbency of Dlg degradation in such cell properties.

CB-P38.
ANTINEOPLASIC EFFECT OF SULPHONILUREAS. *IN VIVO* AND *IN VITRO* STUDIES
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The objective of this work was to evaluate the antitumoral action of a sulphonylurea, glibenclamide (Gli), in comparison with the effect of the antiestrogen tamoxifen (Tam). *In vivo* studies were performed on an experimental model of mammary carcinoma induced in Sprague-Dawley rats. Both Gli (0.2 mg/Kg/day oral) and Tam (1mg/Kg/day ip), were administrated every day along 30 days when tumors reached a diameter of 6 mm. Gli treatment produced a regression of 64% of tumors, while treatment with Tam provoked a regression of 55% of tumors ($P<0.0001$ vs control without treatment). Combined treatment (Gli+Tam) potentiated the effect of Tam ($P<0,01$). *In vitro*, we studied the effect of Gli on cellular proliferation employing two human mammary carcinoma cell lines: MCF-7 and MDA-MB-231. A significant dose-dependent inhibition on cell growth was observed (clonogenic assay) in both cell lines when Gli or Tam were added to culture. In MCF-7, combined treatments (Gli 50 uM+Tam 0.25 uM, and Gli 50 uM+Tam 0.50 uM) showed an additive effect on the inhibition of cell growth ($P<0,05$) whilst this effect was not observed in MDA-MB-231 cells. Cell cycle studies (by flow cytometry), showed that Gli induced cell cycle arrest in G0/G1in both cell lines. Our results show a promising perspective for the employment of Gli alone or combined with Tam in breast cancer patients.

CB-P39.
MUTATION OF *ING1* TUMOR SUPPRESSOR DETECTED IN HUMAN NEOPLASIA ABROGATES DNA REPAIR

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The tumor suppressor ING1 inhibits cell growth in G1 phase by transactivating the CDK inhibitor p21waf1. Its biological functions have been studied in the last years and have been reported to mediate senescence, apoptosis and DNA repair. In this study, we investigated the involvement of p33ING1 (one of ING1 isoforms) in the modulation of DNA repair and the effect of point mutations on this p33 activity. We first examined whether p33ING1 responds to UVC in HeLa cell line. By northern blot, we found that UV light induces expression of p33 in a dose- and time-dependent manner. Western blots reveal a concomitant protein induction. To determine if p33 mediates DNA repair in HeLa cells, we used the host-cell-reactivation assay (HCR) where a UV-damaged plasmid is contransfected with p33 expression vector. Our data show that cells overexpressing p33 increase the rate of repair of the UV-damaged plasmid. To investigate whether *ING1* mutation affects DNA repair, we assessed HCR assays using expression vectors codifying point mutations C215S or K270N. While C215S did not affect p33 ability to improve DNA repair, K270N reduced CAT activity. Similar results were obtained by means of unscheduled DNA synthesis assay, that evaluates the extent of repair of genomic DNA in UV irradiated cells. These results demonstrate that p33ING1 mutation, detected in human neoplasia, reduces the NER capacity, leading to an increased genomic instability, a condition that favors tumor progression.

CB-P40.
ANTI-INSULINIC AND ANTITUMORAL ACTION OF METFORMIN ON MICE

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Our objective was to evaluate the anti-insulin and antitumoral effect of metformin in a mouse mammary tumor model. Female BALB/c mice were transplanted with MPA induced mammary tumors. Metformin was applied at the moment of subcutaneous tumor transplant, as a sub-cutaneous slow releasing pellet (1000 mg/day). We meassured: glycemy, cholesterolemy and lactic acid, insulin and IGF-I serum levels, mice weight was controlled, tumor size was determined and also the content of estrogen (ER) and progesterone (PR) receptors in tumors. Anatomo-pathologic studies were performed. Treatment with metformin during 35 days significantly decreased: the insulinemy in fasting condition ($p<0.001$); the PR tumor content ($p<0.001$); the number of mitosis per field ($p<0.01$) and significantly increased ($p<0.01$) the picnotic-nucleus number per field in the histological preparations. No modification were observed for the other analyzed para-meters. The significant statistical decrease of insulinemy seen in these cases could be considered as one of the causes of the more benign histological pattern. Even if the tumors of treated mice did not shown a significant diminution of size, the microscopic observation revealed numerous tumoral nucleous with condensed chromatin, which is characteristic of apoptotic cells.

CB-P41.
HORMONAL ALTERATIONS IN OBESE PATIENTS BEARING BREAST CANCER

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The objective of this paper was to evaluate hormonal levels in obese breast cancer patients and verify a possible prognostic relation with the disease evolution.
The following hormones were determined in 53 patients by RIA assays: Insulin (I), IGF-I, Growth Hormone (GH), Estradiol (E_2), progesterone (P), free T4, and total T4. Obesity degree was determined by the Body Mass Index (BMI), in accordance with the reference values: normal 18.5-24.9; pre-obese 25-29.9; and obesity, over 30. 12 of the 53 patients (23%) resulted obese; 18 (34%) pre-obese and the remainders (43%) normowheight. 38% of patients are pre-menopausal. Normoweight patients shown low, normal and high levels of IGF-I; pre-obese and obese patients showed low levels of IGF-I ($p<0.05$). 41% of post-menopausal patients showed higher than normal E_2 values. 28% showed insulinemies higher than normal. T4 levels, free as well as total, were positively correlated with the BMI ($p=0.023$). A non-significant age-related increment of GH values was observed. We propose that the augmented values of I, E_2, and GH together with obesity should be understood as an association of bad prognosis for disease evolution and that a more detailed follow-up should be performed in patients with these associations.

CB-P42.
GLUCOCORTICOID RECEPTOR IN TOAD TESTIS

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Glucocorticoids (GC) are the hormonal mediators of stress. In mammals, high levels of GC have negative effects on reproductive physiology. For instance, GC can suppress testicular testosterone synthesis by acting via glucocorticoid receptors (GR), the extent of the inhibition being dependent on GC level. However, the effect of GC on testicular function and even the presence of GR in amphibians are still unclear. The purpose of this work was to characterise testicular GC binding sites in *B. arenarum* as well as to determine the seasonal changes in GR kinetic parameters in correlation with plasma corticosterone (B) concentration. The presence of GR was assayed in testis cytosol with ^{3}H-Dexametasone (Dex) in 10 mM Hepes buffer with 5 mM EDTA, 10% Glicerol, 20 mM Na_2MoO_4 and 0.1 mM PMSF. Total and free plasma B was determined by RIA. Results indicate that in toad testes GR express pharmacological properties similar to rat receptor. Competition studies with different steroids showed that the order of displacement of ^{3}H-Dex specific binding is Dex~RU486~DOC>Aldosterone> RU28362>Progesterone>>>11-dehydroB. The affinity of GR for Dex (Kd = 8.16 ± 1.02 nM) and the capacity (R = 276.02 ± 37.90 fmol/mg protein) remained constant all year long. Therefore, testis sensitivity to GC action depends mainly on testicular inactivating mechanisms (11β-hydroxysteroid dehydrogenase type 2) and B plasma levels. Since total (145 ± 18 nM) and free B (94 ± 12 nM) are higher in the reproductive than in non-reproductive period (Total: 82 ± 8 nM; Free: 62 ± 5 nM), the magnitude of GC actions could be higher during the breeding season.

CB-P43.
INSULIN EFFECT ON GELATINASES ACTIVITY IN DIFFERENT CELL LINES
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The main biological role of insulin is to control the glucose level but this hormone also can regulate the cellular growth and differentiation by binding to its specific cell surface receptor (IR) which down-stream activate multiple signaling pathways.
Matrix metalloproteinases (MMPs) are a family of proteases with diverse substrate specificity and their primary targets are extracellular matrix components. The gelatinases, MMP-2 and MMP-9, are a subgroup of MMPs with ability to degradate native collagen type IV, the major constituent of basement membranes, and therefore they have a key role in cancer, promoting the extravasation and migration of tumor cells.
In this work we investigate the activity of the gelatinases by zymography analyzing the effect of insulin at different doses (1.10^{-4} to 1.0 U/ml) and times of incubation (2 to 24 hs) in the human fibrosarcoma cell line HT1080 and the chinese hamster ovary cell line CHOK1.
We demonstrate that insulin stimulates the gelatinase activity in both cell lines. At short incubation times MMP-2 activity increased with high doses of insulin (1.10^{-1} to 1.0 U/ml), whereas at long incubation times the MMP-9 activity was maximum with low concentration of insulin (1.10^{-4} to 1.10^{-2} U/ml).
In conclusion our results indicate that the insulin has different effects on gelatinases activity, which is dose and incubation time dependent in both cell lines analyzed.

CB-P44.
ROLE OF GLUCOCORTICOIDS AND ITS RECEPTOR IN THE GLUCONEOGENIC BLOCKADE OBSERVED IN PORPHYRIA CUTANEA TARDA MODEL INDUCED BY HEXACHLOROBENZENE (HCB)
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The role of glucocorticoids (GCs) and their receptors (GCr) in the gluconeogenic blockade induced by HCB was studied. GCs are important regulators of glucose synthesis and GCr are clearly modified in TCDD-induced experimental porphyria. In Wistar rats, HCB has a clear effect in the activity of the gluconeogenic enzyme phosphoenolpyruvate-carboxykinase (PEPCK). Thus, the level of plasma corticosterona (B) in rats was determined after 2, 4, 6 and 8 weeks of treatment with HCB. Additionally, the effect of HCB in the number and affinity of hepatic GCrs as well as in the activity of PEPCK was studied in adrenalectomized porphyric rats after 8 weeks of treatment. Plasma B levels in intoxicated animals were significantly reduced (40%) after two-week of treatment and remained relatively constant during the other 5 weeks. After 8 weeks, a reduction in the number of hepatic GCrs (40%) as well as in the activity of PEPCK (30%) was observed. Taking these results into account, it is possible to speculate that the decrease in the activity of the gluconeogenic pathway could be a consequence of the hormonal disturbance caused by the chlorinated pesticide reported in this work. Also, a significant diminution in the biosynthesis of B in adrenals from intoxicated animals was observed.

CB-P45.
STRESS RESPONSES TO CARBARYL AND ORGANOPHOSPHATES IN TOAD LARVAE
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Carbaryl (CB), a carbamate insecticide vastly used in Argentine, acts primarily on acetylcholinesterase (AChE), the same target as organophosphates (OP). However, secondary effects described for aquatic species, i.e. alteration of antioxidant system, differ in both families of pesticides. Our objective is to compare these targets in CB-exposed and OP-exposed toad larvae. *Bufo arenarum* larvae were exposed up to 48 h to 5-20 mg/l CB. The activity of AChE, aliesterase, antioxidant enzymes glutathion-S-transferase (GST), catalase (CAT) and glutathione reductase (GR), and reduced (GSH) and oxidized (GSSG) glutathione were analysed. Similar data were obtained for the OP malathion and azinphos methyl.
Esterases were inhibited by CB (60-90%) similarly as described for fish by us. GSH level was diminished after 24 h (38-72%) and oxidized to GSSG, but it recovered at 48 h. CAT and GST were induced by CB at 48 h, while GR remained unchanged. OP decreased both GSH and GSSG levels persistently at 48 h, induced GST and inhibited CAT and GR. CB induce a transient oxidative stress in *Bufo arenarum* larvae by depleting GSH, which is overcome at 48 h by induction of CAT and GST as antioxidant mechanisms. Our scope is now to evaluate if these responses are linked to aryl receptor AhR and up-regulation of XRE (xenobiotic)-dependent genes and secondary gene upregulation through Antioxidant RE. On the other hand, both OP are demethylated by GST via GSH, and the oxidative stress caused by ROS is high enough to inactivate CAT and downregulate GR.

CB-P46.
EFFECTS OF PARAQUAT ON THE FRESHWATER SNAIL *BIOMPHALARIA GLABRATA*. COMPARISON BETWEEN PIGMENTED AND NON-PIGMENTED ORGANISMS
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Paraquat is a widely used herbicide both in terrestrial and aquatic environments. The aim of this work was to evaluate the effects of this pesticide on several biological parameters, some of them related to oxidative stress, in *Biomphalaria glabrata* snails. Within its natural population, pigmented and non-pigmented organisms can be distinguished by spontaneous mutation. The snails were treated with 0.5 mg/L of paraquat using either the active compound or a formulated commercial product for 96 h. Non-pigmented organisms showed an increase in lipoperoxidation processes (180%) and a decrease in catalase activity (78%) after treatment with the commercial product in relation to control snails. Pigmented organisms did not show lipoperoxidation, but it was found a similar decrease in catalase activity in comparison with non-pigmented snails. In any case, significant differences were not observed in glycogen levels (7.34 ± 3.26 and 7.09 ± 4.09 mg/g soft tissue for pigmented and non-pigmented *B. glabrata*. Glutathione levels were very low: 2.55 ± 0.86 and 4.64 ± 2.41 nmol/mg protein for pigmented and non-pigmented snails respectively. According to the data, the commercial product was more toxic than the active compound to induce lipoperoxidation processes.

CB-P47.

IN VITRO PREVENTION OF LIPID PEROXIDATION BY EXTRACTS OF _BIOMPHALARIA GLABRATA_

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The assessment of the total scavenger capacity of oxyradicals by a tissue provides a well understanding of its resistance to toxicity caused by reactive oxygen species. In this study, we evaluated the antioxidant capacity of extracts derived from the snail *Biomphalaria glabrata* against *in vitro* hydroxyl radical-induced oxidative injury to lipids. Time course lipid peroxidation was assayed by the thiobarbituric acid test. Hydroxyl radicals were generated at 37°C by the iron plus ascorbate Fenton reaction. Homogenates of total soft tissue (TST) and of different anatomical regions of *B. glabrata* were made in phosphate saline buffer, pH 7.4 and centrifuged at 3000 x g for 15 min to precipitate insoluble pigments. No Fe^{++}/ascorbate induced lipoperoxidation could be observed in extracts of TST and hepatopancreas. Instead, a slight lipoperoxidation occurred when gonadal and pulmonary tissues were analyzed (0.4 and 0.6 fold of increase, respectively). Extracts of TST inhibited in a dose dependent mode the Fe^{++}/ascorbate induced lipoperoxidation of pig liver homogenate (highest inhibition observed: 78%). Considering the different anatomical regions analyzed, the highest antioxidant capacity was found in hepatopancreas. TST from non-pigmented organisms showed a higher antioxidant capacity than TST from pigmented ones. Neither endogenous levels of GSH nor the protein fraction of snail extracts would account for the lipid peroxidation inhibition observed.

CB-P48.

EFFECTS OF INGESTED PHLOXIN B IN PEST FLIES

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We previously demonstrated the chemical and photochemical toxicity of Phloxin B (PhB) (a red xanthene eosin) on immature stages of the Medfly, *Ceratitis capitata*. This dye represents an interesting option to traditional insecticides. We established insect cultures suitable for our experimental protocols. A new successful medium was established for *Ceratitis capitata*, based on orange squash. *Musca domestica* (house fly) was grown on powder milk and yeast medium. Since our protocol requires that the flies develop in the dark to be able to measure phototoxic effects after exposure to light, difficulties arise due to the illumination requirements of the insects. A special set of devices and conditions have to be implemented for growing *Haematobia irritans* (the horn fly) in the lab. We have been able to establish cultures using cattle blood that allowed normal development from egg to adult. However conditions for mating in artificial conditions were not attained yet. We have demonstrate the following: (1) Chemical toxicity of PhB: At 5mM mortality of house fly was higher (86.6%) than that of Medfly (18%). At 0.5 mM mortality of Horn fly was 73,3%, thus showing very high sensitivity to the dye. (2) After exposition to light, photochemical toxicity followed the same patterns, at much lower concentrations. (Horn fly 100% death after 90 min exposure to light in 0.1 mM PhB; House fly 96,8% death after 8 hs exposure to light in 5mM Ph.B). We show that PhB displays antibiotic activity against gut and fecal microflora.

CB-P49.

SACCHAROMYCES CEREVISIAE VANADATE-RESISTANT MUTANTS WITH DEFICIENT L-LEUCINE UPTAKE ACTIVITY

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In *Saccharomyces cerevisiae* several permeases mediate the transport through the plasma membrane of the amino acids present in the growth medium. Glycosylation of proteins plays an important cellular role in the steps necessary to obtain a functional protein after its movement through the secretory pathway. Assays made in our laboratory, using the antibiotic tunicamycin, which avoids N-glycosylation of proteins, showed: a) the growth of the wild type strain MMY2 is inhibited by tunicamycin or vanadate and b) in rich medium L-leucine uptake values in presence of tunicamycin are lower than in control cells. Considering these results, and in order to study the effect of glycosylation on L-leucine uptake, we decided to prepare glycosylation-defective mutants. Mutants were obtained by UV mutagenesis and isolated by their resistance to sodium vanadate. The phenotype of the mutants showed increased sensitivity to hygromycin B and to other aminoglicosidic antibiotics. This strategy has let us isolate a series of 22 putative vanadate resistant mutants, eleven from the wild type strain MMY2 and eleven from the *gap1* strain MMY2/H3. Uptake values for L-^{14}C leucine on the mutants decreased compared to the parental strains and yeast cells presented an altered sensitivity to Trifluorleucine (TFL), a toxic analogue of L-leucine.

CB-P50.

EFFECT OF DEHYDROLEUCODINE ON THE MALE MOUSE REPRODUCTIVE TRACT

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It has been shown that sesquiterpene lactones act *in vitro* as inhibitors of a group of steroidogenic enzymes, the aromatases. These enzymes transform testosterone into estradiol, and are localized in Leydig cells and in cells of the seminiferous epithelium. We tested the effect of dehydroleucodine (DhL, a sesquiterpene lactone isolated from matico, a medicinal herb widely distributed in the Cuyo area) in the male tract of adult male Balc D strain mice. Mice were treated with 7 mg of DhL/kg body weight per day for 30 days. After the treatment, the levels of serum steroid hormones were assayed and the cytology of the testes was analyzed. Testosterone levels in control animals were 1.65 ± 1.2 ng/ml, and in treated animals 0.44 ± 0.26 ng/ml, a diminution of 70% in the levels of testosterone. Estradiol values for control animals were 16 ± 7.9 pg/ml and for treated animals 12.67 ± 6.76 ng/ml, with no significant difference between the groups.
Histological analysis of the testes of treated animals revealed profound changes in the appearance of interstitial cells, where approximately 50% of the Leydig cells were picnotic, suggesting that they are completely inactive.
From these results we conclude that DhL is affecting serum testosterone levels - probably due to an inhibition of the activity of Leydig cells.

CB-P51.

NIFURTIMOX NITROREDUCTASE ACTIVITY IN THE CELLULAR FRACTIONS FROM MALE RAT PANCREAS

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Nifurtimox (Nfx) is a drug used in the treatment of Chagas' disease. It has shown toxic side effects and is believed to be related to its nitroreduction to reactive metabolites. The aim of this study was to analyze the ability of Sprague Dawley male rat pancreas cellular fractions to nitroreduce Nfx and to provoke some deleterious effects. Microsomes exhibited Nfx nitroreductase activity in the presence of NADPH and under N_2 atmosphere (493 ± 16 pmol/min.mg prot) that was fully inhibited under air but not altered when N_2 was replaced by pure CO. The mitochondrial fraction was not able to nitroreduce Nfx in the presence of NADPH. The cytosol nitroreduced Nfx in the presence of hypoxanthine and under N_2 (212 ± 49 pmol/min.mg prot), that was negligible under air and inhibited by allopurinol. Nfx levels in pancreatic tissue, at 1, 3, or 6 h after intragastric administration (100 mg/kg) were 4.01 ± 3.41; 5.95 ± 2.18; 2.63 ± 1.03 µg/g respectively. A significant increase in t-butylhydroperoxide promoted chemiluminiscence was detected 6 h after Nfx administration. Protein sulfhydryl content significantly decreased at either 1, 3, or 6 h after Nfx. Results evidence that Nfx reaches the pancreas and that might be nitroreduced there to reactive metabolites, to produce some deleterious effects which still remain to be fully established.
Supported by FONCyT (PICT00-5-9941) and UNSAM (PIDA UF014).

CB-P52.

CHEMOTAXIS TOWARD PENTACHLOROPHENOL (PCP) OF TWO PCP-DEGRADING STRAINS

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The involvement of chemotaxis in the degradation of chloroaromatic compounds is a field little explored, though it might be relevant for bioremediation processes. The objective of this work was to study the chemotaxis behavior toward pentachlorophenol of two PCP-degrading strains (*P. aeruginosa* and *Achromobacter* sp.) able to cope with high concentrations of this pollutant. The chemotactic response of the strains toward PCP was analyzed by three qualitattive assays (agarose, drop and swarming assays). Both strains were found to respond chemotactically to PCP, being the response of *Achromobacter* sp. much more stronger and reproducible. Microorganisms with this characteristic would have a competitive advantage on other microorganisms which degrade PCP but are not chemotactic toward the toxic compound. These results represent an interesting aspect, that might be useful to take into account, when assessing the adequacy of strains to participate in actual bioremediation processes.

CB-P53.

BIOCHEMICAL AND ULTRASTRUCTURAL ALTERATIONS DUE TO REPETITIVE ETHANOL ADMINSTRATION ON THE RAT VENTRAL PROSTATE

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In this work we report that rat prostate mitochondria (MIT) are able to bioactivate EtOH to AC and 1HEt. The enzymatic process leading to AC requires oxygen but not NADH or NADPH, is fully inhibited by 1mM diethyldithiocarbamate and is partially susceptible to 1mM deferoxamine. Incubation mixtures containing either microsomes or cytosol or mitochondria and (if necessary) cofactors plus EtOH lead to covalent binding of the reactive metabolites formed to their NaBH4-reduced proteins from the three fractions. Sprague-Dawley rats were fed with a nutritionally adequate liquid diet containing alcohol, for 28 days. This resulted in ultrastructural alterations in the epithelial cells, consisting of marked condensation of chromatin around their perinuclear membrane and of moderated dilatation of their endoplasmic reticulum. We also observed that this repetitive EtOH administration was accompanied of slightly increased peroxidability of prostatic lipids as detectable by the t-butylhydroperoxide promoted chemiluminscence emission test. Rat ventral prostate has several metabolic pathways for EtOH bioactivation to AC and 1HEt, some of which remain to be fully characterized. The reactive metabolites formed covalent bind to cellular components and are involved in the cell injury observed.
Supported by CONICET (PIP02323) and UNSAM (PIDA UF013)

CB-P54.

OXIDATION OF ALCOHOL TO ACETALDEHYDE AND 1-HYDROXYETHYL RADICAL BY RAT TESTICULAR MICROSOMES

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In this work, we report studies on the biotransformation of ethanol (EtOH) to acetaldehyde (AC), 1-hydroxyethyl (1HEt) and hydroxyl by rat testicular microsomes and about the nature of the enzymatic processes involved in them. Microsomal fractions were able to oxidize enzymatically EtOH to AC and 1HEt and hydroxyl. The AC formation was NADPH and oxygen dependent and partially inhibited by 4-methylpyrazole, SKF 525A and diethyldithiocarbamate. This suggests the participation of CYP2E1. Diphenyleneiodonium strongly inhibited AC formation, suggesting the involvement of a flavoenzyme, probably P450 reductase. The remaining ability of the microsomal fraction to activate EtOH to AC can not be ascribed to prostaglandin endoperoxide synthase because of its resistance to indomethacin or acetylsalicylic inhibitory effects. The partial but significant inhibitory effects of dapsone; nordihydroguaiaretic acid and of gossypol would indicate the participation of peroxidases. The NADPH and oxygen dependent formation of hydroxyl and 1HEt during EtOH microsomal metabolism was detected by GC-MS of adducts of the radicals with the spin trap PBN. These processes of EtOH bioactivation to acetaldehyde and free radicals might be involved in the deleterious reproductive effects of EtOH.
Supported by CONICET (PIP02323) and UNSAM (PIDA UF013)

CB-P55.
ALCOHOL BIOACTIVATION TO ACETALDEHYDE IN MAMMARY TISSUE FROM RATS OF DIFFERENT AGE
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Previous results from our laboratory evidenced that the rat mammary tissue has the ability to bioactivate ethanol to acetaldehyde in the microsomal and cytosolic fractions. In this work we studied the mammary tissue from rats of different age for the ability to generate acetaldehyde. Three groups of Sprague Dawley female rats were used: immature (5 weeks) (IM), virgin adult (18 weeks) (VA) and post lactation young mothers (18 weeks) (YM). In the case of the microsomal mediated ethanol metabolism, the NADPH and oxygen dependent response was the lower for the IM and reached a maximum for the VA. No difference was observed among groups for the non-NADPH dependent metabolism. For the cytosolic activation of ethanol to acetaldehyde, we found a maximum in the group YM, and this correlated well with a maximum in the xanthine oxidoreductase activity, as established by histochemistry and the production of uric acid. This was in agreement with previous findings from our laboratory evidencing a key role of this enzyme for the ethanol oxidation to acetaldehyde in the cytosolic fraction of this tissue. The risk derived from the exposition in mammary tissue to a carcinogen like acetaldehyde is analyzed.
Supported by ANPCyT (PICT/99 05-06045) and UNSAM (PIDA UF015).

CB-P56.
PROTEIN OXIDATION IN RELATION TO HEPATIC GLYCOGEN METABOLISM IN AN ACUTE PORPHYRIA MODEL
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The administration of 2-allyl-2-isopropylacetamide (AIA) and 3,5-diethoxycarbonyl 1,4-dihydrocollidine (DDC) produces in rats an acute porphyria model that is characterized by the hepatic overproduction of 5-aminolevulinic acid (ALA), metabolite that generates reactive oxygen species (ROS) which oxidize proteins. In previous studies, in this model, we have observed diminution of the enzymatic activity of cytosolic proteins related to the glucose/glycogen metabolism such as phosphoenolpyruvate-carboxykinase (PEPCK, a gluconeogenic enzyme), pyruvate kinase (PK, a glycolytic enzyme) and glycogen phosphorylase (GP). The objective of the present work was to investigate in mentioned model, if there are any relation between the dose of AIA and the hepatic concentration of ALA, and to evaluate cytosolic protein oxidation, measuring protein carbonyl groups content in relation to GP activity. The hepatic glycogen level was also evaluated. The results indicate a: 1) relationship between AIA treatment (100 to 500 mg/kg bw) and hepatic ALA content (7 - 28 nmol of ALA/ g wet liver), 2) relationship between protein carbonyl groups content (1.3 - 4.5 nmol/mg protein) and decrease in GP activity (2.2 - 1.0 µmol Pi/g wet liver. min). The hepatic glycogen level increased 4-fold respect to the control at the highest dose. These oxidative disturbances of proteins may contribute to the pathogenesis of the altered glycogen metabolism present in this porphyria model.

CB-P57.
TRYPTOPHAN METABOLISM AND CHRONIC HEPATIC PORPHYRIA
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Hexachlorobenzene (HCB) produces in rats an experimental hepatic porphyria similar to human PCT. One of the routes of metabolization of dietary tryptophan is formation of serotonin (5-HT). In pineal gland, where neurochemically important indoles are widely found, it can be converted, only in the dark phase of an imposed light-dark cycle, to melatonin via N-acetyl-serotonin.. .5-hydroxyindoleacetic acid (5-HIAA) is formed by another route. The aim of our work is to find how alterations in tryptophan metabolism are related to clinical manifestations in chronic hepatic porphyria. Alterations in formation of melatonin and its intermediates are evaluated in pineal glands, using HPLC, in rats treated with HCB. Thus, variations respects to controls, in these contents are determined in glands taken during diurnal dark or light period. We previously reported that, in light phase, there is an increase in 5-HT and 5-HIAA contents in porphyric materials, although tryptophan level is maintained as in the dark phase. Now we are finding no variations in these three contents during the dark period, or in melatonin or N-acetyl-serotonin levels; these last two are not detected in the light phase. 5-hydroxytryptophol is not found in any case. These results could be related with hyperpigmentation and/or photosensitivity observed in PCT. Tryptophan can also be transformed via kynurenine by action of tryptophan pyrrolase, a haem enzyme. HCB treatment appears to have no effects on its holo- and apo-enzyme activities measured by use of added haem. These results suggest that regulatory haem pool would not be diminished and this would not act as a trigger in this hepatic porphyria onset.

CB-P58.
EFFECT OF CYCLICAL PROTEIN DEPLETION-REFEEDING ON THE CONTENTS OF FEMALE MOUSE LIVER PROTEINS
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Unlike moderate caloric restriction, either fasting or extreme caloric restriction severely change the whole body metabolism. Thus, a protein-free diet modifies the levels of several cytosolic proteins in the liver of female mouse. In addition, cycles of fasting -refeeding enhance the incidence of pre-cancer lesions in rat liver. This work tested the effect of 4 cycles of protein depletion-refeeding on the contents of liver cytosolic proteins of female mouse. The treatment diminished body weight, liver weight and cytosolic protein. By proteomics techniques, the treatment particularly decreased the levels of carbonic anhydrase III (50%), fatty acid synthase (36%), and catalase (30%) and increased those of glutathione S-transferase subunit GSTM1, while not changed that of glyceraldehyde 3-phosphate dehydrogenase. By means of 2D-NEPHGE, a decreased level of other proteins related to nitrogen metabolism, protein synthesis and oxidative stress was observed. The addition of methionine to the protein depletion diet partially prevented the general and particular changes observed, but not that of CAIII level. On the other hand, the treatment generates hepatocytes with heterogeneous glycogen deposits and acidophilic phenotype. In bulk, 4 cycles of protein depletion-refeeding tends to make permanent some changes caused by protein depletion on liver proteins associated with early pre-neoplasic status.
Supported by CONICET and UNMDP.

CB-P59.

TSH-INDUCED NITRIC OXIDE (NO) INHIBITS IODIDE UPTAKE AND THYROGLOBULIN (TG) mRNA EXPRESSION IN FRTL-5 THYROID CELLS

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NO is a free radical that mediates numerous physiological and pathological processes. NO mainly acts on soluble guanylyl cyclase with formation of 3´5´-cyclic guanosine monophosphate (cGMP) which activates a variety of effectors such as the protein kinase cGMP-dependent (PKG). The three isoforms (I-III) of the NO generating enzyme, NO synthase (NOS), have been demonstrated in thyroid tissue. This work aimed to study the action of thyrotropin (TSH) on the NO production and the role of the endogenous NO on functional thyroid parameters in the rat thyroid cell line FRTL-5. Stimulation with TSH (200-500 µUI/ml) for 24-48h increased NO production (^{14}C-citrulline). An increment of NOS III mRNA expression (in situ hibridization) was induced by TSH at 48h. The presence of the NOS inhibitor, L-NAME (1mM), induced an increase of the TSH-stimulated iodide uptake and TG mRNA expression (Northern Blot) at 48h. A significant increment of TSH-stimulated iodide uptake was also obtained when cells were incubated for 48h. with the PKG inhibitor, KT-5823 (5µM). In conclusion, these results indicate that the NO production could be induced by TSH in the thyroid cell. A possible role of NO as a negative signal in thyroid hormonogenesis was evidenced.

CB-P60.

HISTAMINE MODULATES CELL GROWTH, REACTIVE OXYGEN SPECIES PRODUCTION AND ACTIVITY OF ANTIOXIDANT ENZYMES IN HUMAN CELL LINES

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In the present work we investigated the capacity of Histamine (HA) to modulate cell proliferation and ROS production as well as the receptors involved in this response. We employed mammary normal (HBL-100) and malignant cell lines (MDA-MB-231, MCF-7). The levels of the free radicals superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) and intracellular HA content were determined by flow citometry. The activity of antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) were determined by kinetic spectrophotometric techniques. Cell proliferation was evaluated by the clonogenic assay.
Cell lines presented intracellular levels of HA directly correlated with their malignancy. In MDA-MB-231, HA via H_2R increased cAMP levels (EC^{50} 0.45 µM) producing in turn a significant inhibition on cell proliferation ($p<0.01$). On the contrary, HA at low doses (<0,1 µM) and via H_1R, stimulated cell growth. H_2O_2 levels resulted inversely correlated with cell proliferation while $O_2^{\cdot-}$ remained unchanged. Furthermore, HA significantly modified the activity of SOD and CAT. In normal HBL-100, HA modulated cell proliferation but in exactly the opposite range of doses. The effect on cell growth was inversely correlated with H_2O_2 levels.
The present data indicate that histamine can modulate cell proliferation via H_1R and H_2R. This effect is exerted in part by the modulation of antioxidant enzymes and ROS production.

CB-P61.

OXIDATIVE STRESS IN RAJIDAE ELECTROCYTES

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Psammobais extenta -Rajidae- is a weak electric fish with electric organs of myoblastic origin. Their electrocytes, which are cup-shaped cells, have a large system of caveolae. We have studied the elemental composition in these electrocytes via electron probe X-ray microanalysis-Scanning Electron Microscopy. In the present study, the energy dispersive spectra showed an intracellular accumulation of oxygen and their relative semi-quantitative weight % (K_α) was about 70. Nitrogen, Cu^{2+}, Zn^{2+}, and Al^{3+} were also present. In addition, *P. extenta* electrocytes exhibited a decomposition product of peroxidized polyunsaturated fatty acids such as malondialdehyde. Malondialdehyde production was estimated by the thiobarbituric acid (TBA) test on quantitative fractions. The distribution of malondialdehyde was detected in microsomes fraction after subcellular fractionation of the electric tissue. The relative specific activity (RSA) for microsomes was 4; nuclear fraction, large granules and final supernate 0.8, 0.9 and 0.6, respectively, with a recovery of % 105. These data are discussed in relation to electrocyte death.
Supported by Secretaría General de Ciencia y Técnica, Universidad Nacional del Sur, Bahía Blanca, Argentina.

CB-P62.

FATE OF THE EXOGENOUS ORNITHINE DECARBOXYLASE GENE IN TRANSFECTED *T. cruzi*

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Polyamines, a group of aliphatic cations, play essential roles in cellular proliferation and differentiation in *T. cruzi* and other eukaryotic cells. The biosynthesis of polyamines in most of the organisms begins with the conversion of ornithine into putrescine, catalysed by ornithine decarboxylase (ODC).
Previously, we have demonstrated that *T. cruzi* epimastigotes are auxotrophic for polyamines because they lack ODC gene. Then, we have transformed *T. cruzi* epimastigotes with the ODC gene form *C. fasciculata*, cloned into the vector p-Ribotex. The transgenic *T. cruzi* expressed ODC activity and becomes autotrophic for polyamines and sensitive to difluoro-methylornithine (DFMO). The presence of DFMO in transfected cultures elicited the selection of resistant parasites that showed ODC gene amplification, increased levels of specific mRNA and higher enzymatic activity.
We analyzed the fate of the exogenous ODC gene through the time post-transfection in both sensitive and resistant parasites by *PCR* and *PFGE*. We have found that ODC gene was initially free as an episome into the cell (1-3 hs post-transfection), partially integrated into the parasite genome at 48 hs, and mostly or totally integrated after a week of transfection (this last point was also suggested by miniprep and hybridization with a specific probe of ODC).
Restriction analyses of DNA from either sensitive or resistant *T. cruzi* cells indicated that one copy of the recombinant plasmid was integrated at each recombination event.

CB-P63.

TRANSGLUTAMINASE ACTIVITY AND POLYAMINE LEVELS IN MOUSE LIVER AFTER 3,5-DIETHOXYCARBONYL-1,4-DIHYDROCOLLIDINE (DDC) ADMINISTRATION

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Rodents treated with DDC are model of two hepatic toxic manifestations: porphyria and the apparition of hepatic cytoplasmic protein aggregates known as Mallory Bodies (MBs). Rodents unable to form MBs present more porphyrins than those that form them. Transglutaminase (TGase) catalyzes the cross-linking of proteins via γ-(glutamyl) lysine or N,N-bis (γ-glutamyl) polyamine cross links. The aim of the present work was to investigate hepatic TGase activity in CF1 male mice treated with DDC (0.1% in the food) for 3, 7, 30 and 45 days and to correlate TGase activity with polyamine and porphyrin levels. Polyamine levels were determined by HPLC and TGase activity was measured by the method of Folk and Cole. At day 3, statistically significant increases in TGase activity (75%), porphyrin content (6641%) and spermidine levels (73%) were observed. The highest TGase activity was observed at day 30 (522%), while pophyrin levels were still increasing by day 45. From day 7 of treatment and until the end of the experiment, putrescine levels remained increased (770%). Spermine levels were not affected by the treatment. Our results suggest that the early increase in TGase activity and polyamine levels after DDC treatment may contribute to the formation of protein aggregates that would lead to the appearance of MBs.

CB-P64.

IDENTIFICATION OF A NUCLEAR PUTATIVE GLUTAREDOXIN IN *TRYPANOSOMA CRUZI*

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Glutaredoxins (Grxs) are thiol-redox enzymes, which contain an active site with the sequence motif CXXC. Grxs are responsible for the reduction of intracellular disulfides *in vivo* and moreover, they contribute to a range of important cellular processes, as DNA synthesis, transcriptional control and cell growth and death control. We have cloned a novel gene codifying for an atypical Grx in the ethiological agent of Chagas' Disease, *Trypanosoma cruzi*. With the aim to identify the native protein in the parasite, a serum against the recombinant *Tc*Grx was raised in mice. The anti-*Tc*Grx serum recognized in western blot a protein with the expected molecular weight of 35 kDa in epimastigote, amastigote and tripomastigote stages. In epimastigotes, *Tc*Grx was present in nucleus enriched fractions, but not in soluble fraction. The sub-cellular location of *Tc*Grx was confirmed by immunogold electronic microscopy using the anti-*Tc*Grx serum. Labelling localized mainly in nucleus, specifically in the nucleolus, and in a lesser extent in the kinetoplastid DNA.
Since nuclear *Tc*Grx is present in non-soluble fractions, we are currently trying different solubilization protocols in order to further carry out its biochemical characterization and define its function.

CB-P65.

CONDUCTION SYSTEM DEFECTS IN THE VENTRICULAR SPECIFIC ERBB4 KNOCKOUT MOUSE

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Conditional inactivation of erbB4 in ventricular muscle induces cardiac dilation and conduction abnormalities, leading to heart failure in adult mice (erbB4 CKO). Electrocardiographic recordings of erbB4 CKO showed widened QRS complex and prolonged QT, which are usual conduction defects affecting individuals with diverse symptoms ranging from arrythmias to sudden death. We investigated the role of erbB4 in the formation of the conduction system by monitoring the cellular localization and expression of Connexin-40 (Cx40), the most accepted developmental marker of the His bundle-Purkinje fiber system, and of Desmin. The expression of Cx40 and Desmin were respectively reduced to 50% and 70% in erbB4 CKO as measured in Western blots. Cx40 protein was observed by immunohistochemistry in a cluster of smaller cells compared to wildtype embryos. Postnatal analysis showed a lack of targeting of Cx40 to intercalated discs, instead Cx40 was detected along the cardiomyocyte membrane. This result suggests a delayed or miss-differentiation of cells forming the His-Purkinje fiber system. We also examined changes in the expression of specific ion channels that may be underlying the observed conduction defects. We speculate that erbB4 plays a morphogenetic role, where it is required for normal differentiation of trabecular myocytes into structures of the conduction system.

CB-P66.

ANTHRACYCLINE-MEDIATED CARDIOTOXICITY IN VENTRICULAR SPECIFIC ERBB4 KNOCKOUT MOUSE

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An effective antibody-based therapy directed against erbB2 for the reduction of mammary tumors could lead to a severe cardiomyopathy when combined to anthracycline chemotherapy. We showed that conditional inactivation of erbB4 in ventricular muscle (erbB4 CKO) resulted in cardiac dilation, suggesting that Neuregulin signaling through erbB2 and erbB4 heterodimers may not only act as a modifier but as an essential component for normal cardiac remodeling. Although doxorubicin-mediated programmed cell death may induce cardiac dysfunction, the relative number of apoptotic cells was similar in wildtype and erbB4 CKO hearts as monitored by Tunel and Bax/Bcl-2 ratio. We therefore investigated possible targets for the potentiated cardiotoxicity in doxorubicin-injected erbB4 CKO compared to wildtype mouse. We examined changes in the subcellular targeting and activity of related membrane proteins in tissue sections by immunohistochemistry and in cell extracts by Western blots. ErbB2 was found miss-localized with a 2-fold reduction in membrane extracts from doxorubicin-treated erbB4 CKO. The significant ß-adrenergic response indicates that the overall integrity of the membrane is not affected in the absence of erbB4. We suggest that erbB4 links neuregulin signaling to scaffold proteins involved in the regulation of the cardiomyocyte architecture and myocardial contractility.

CB-P67.

IS IT POSSIBLE THE DIRECT ENERGY TRANSFER FORM THE LHII TO THE CR IN THE ABSENCE OF LHI ANTENNA COMPLEX?

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There are different results on the effectiveness of energy transfer from the peripheral light-harvesting (LH) complex 2 directly to the reaction center (RC) in mutant strains lacking the core LH1 complex. A LH1- mutant of *Rhodovulum sulfidophilum*, named rsLRI, was constructed by deleting the *puf* A and B genes, resulting in a Kanamycin resistant greenish-brown, photosynthetically positive clone. Under photosynthetic conditions, those cells grew only at high-light intensities (500 Wm^{-2}). Below this value the efficiency of photosynthetic growth decreases and at 50 Wm^{-2} no photosynthetic growth was observed. The wild type phenotype was completely restored by inserting in trans a DNA segment containing the *puf* A and B genes. Light-induced FTIR difference spectra of wild type and rsLRI showed to have only marginal differences, indicating no large structural changes of the RC due to the deletion of LH1. The amount of RC in each strain was similar, according the bleaching of the absorption band at 865 nm (ΔA865, is indicative for the P→P+ transition in the reaction center). These results are interpreted as indicating that energy transfer between LH2 and RC is inefficient. It is possible that most of the energy necessary for photosynthetic growth was provided by light harvested directly by the RC.

CB-P68.

IDENTIFICATION AND ANALYSIS OF AMINOACID CHANGES IN THE PROTEIN SEQUENCE OF THE CYTOCHROME OXIDASE-SUBUNIT II (CO II) OF SOUTH AMERICAN CAMELIDS

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To thrive at high altitude, South American camelids (SAC) have evolved strategies that have been suggested to be genetically determined. Mitochondrial DNA encodes 13 polypeptides involved in oxidative phosphorylation and their variation could potentially represent one of the genetic factors associated to animal adaptation to hypoxia. In particular, cytochrome c oxidase is a mitochondrial protein that plays a critical role in the terminal oxidative step of the energy metabolism, catalyzing the transfer of electrons from cytochrome c to oxygen to form H_2O. The aim of this project was to compare the aminoacid sequence of the subunit II of cytochrome c oxidase (CO II) from the SAC llama, alpaca, guanaco and vicuña with those from other artiodactyls living at the sea level. With this purpose, the complete CO II gene was amplified by PCR using primers anchored at the flanking genes coding for the ^{Asp}tRNA and ^{Lys}tRNA. Alignments of our inferred CO II sequences for the SACs against those of sheep, cow, and pig resulted in 18 aminoacid changes; which were always conserved within the later group. Four out of the 18 observed changes were non-conservative resulting in clear modifications in the polar properties of the residues. It remains to be investigated if any of the observed changes in the CO II are or not associated to -yet unidentified- functional changes that could improve adaptation of SACs to their natural environment.

CB-P69.

PVF1/PVR SIGNALING AND APOPTOSIS PROMOTES THE ROTATION AND DORSAL CLOSURE OF THE MALE TERMINALIA IN DROSOPHILA

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We show that the Pvf1 gene, encoding one of the three Drosophila homologues of the mammalian VEGF/PDGF growth factors, is required for rotation and dorsal closure of male genital disc. Males either mutant for Pvf1 or bearing a dominant negative form of Pvr or stasis (stai), the unique PVF receptor, do not complete these processes. Pvf1 expression in the genital disc is restricted to the A8 cells. Flies hemizygous for the apoptotic genes hid, reaper and grim, or mutant for puckered (puc) a member of the n-Jun-N terminal kinase pathway (JNK), lead to the same phenotypes as mutations in PVF1/PVR. Our results indicate that the PVF1/PVR function as well as apoptotic phenomena are required during rotation and dorsal closure.

ES-P1.

REVERSIBLE FOLDING OF THE K+ CHANNEL KcsA

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The potassium channel KcsA is an integral membrane protein from *S.lividans*. We studied the folding properties of the wild-type channel (1-160) reconstituted in the detergent dodecylmaltoside (DDM), and also the influence of the C-terminal domain on the stability of the protein, by means of a 1-125 construct. Using TFE as denaturing agent, we observed a biphasic behaviour by fluorescence and circular dichroism. The first phase was characterized by a decrease on tryptophan fluorescence intensity and α-helix content. In the second phase, a marked increase in helical structure was observed (from the TFE propensity to induce ordered structures). This process was KcsA concentration-dependent (typical from oligomeric proteins), but not DDM concentration-dependent. These data indicated that, at relatively low concentrations, TFE is able to disrupt the folded state of KcsA. The unfolding was reversible, as shown by dilution of KcsA/TFE mixtures with DDM 5 mM. The kinetic unfolding and refolding of KcsA in TFE was characterized following fluorescence changes. Mathematical fits suggested the presence of, at least, one kinetic intermediate. All the results obtained were similar for the two forms of KcsA, WT and 1-125, indicating that the citoplasmatic C-terminal domain is not relevant for correct protein folding.

ES-P2.
NOVEL GENETIC PARTNERS OF RPB4 SUBUNITS OF YEAST RNA POLYMERASE II

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Transcriptional regulation by RNA polymerase II and its associated proteins lies at the core of eukaryotic gene expression. *Saccharomyces cerevisiae* RNA polymerase II is a complex of 12 subunits, Rpb1-12, named in the order of decreasing size. Conserved from archaea to humans, Rpb4 is a non-essential subunit of RNA polymerase II. *Rpb4* forms a sub-complex with Rpb7 that easily dissociate from the polymerase under mild denaturing conditions and is involved in promoter dependent initiation of transcription. rpb4 yeast strains grow at moderate temperatures slowly, cannot survive at temperatures above 32°C or below 15°C. They are also defective for sporulation and exhibit a variety of stress response defects. Rpb4 might be implicated in the process of transcription regulation of RNA polymerase II. Recently was found strong genetic interaction of *RPB4* and other genes coding components of transcription machinery, *RPB9*, *SPT8* and *DST1*. These genes are coding RPB9, another non-essential subunit of RNA polymerase II, SPT8, a component of the SAGA co-activator and TFIIS, an elongation factor of RNA polymerase II, respectively. *C*-terminal part of Rpb4 is the most conserved part of this protein. It was demonstrated that this part of the polypeptide might play a role in activated transcription. To understand the function of *RPB4* in transcription and the mechanism by which this gene is required for cell viability, we searched for genes that being over-expressed could suppress the thermosensitive phenotype of the *rpb4* strain with a truncated form of Rpb4 (*rpb4Δ198* allele). Together with the known partner of Rpb4, subunit of RNA polymerase II Rpb7, we have identified as new possible Rpb4 interactors proteins encoded by *RTS1*, *CDC5*, *RSK2* and *YEL033W* genes.

ES-P3.
OLIGOMERIZATION OF MutS FROM *E. coli*. INFLUENCE OF THE C-TERMINAL REGION

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Fidelity of DNA replication in eukaryotes and prokaryotes depends on the accuracy of DNA polymerase and the proofreading activity of this enzyme. Errors that escape from this last activity are corrected by the Mismatch Repair System (MMRS). MutS, MutL and MutH are the main factors that constitute this system. MutS, an 853 amino acids (a.a.) protein, recognizes and binds to mismatched or unpaired bases present in the recently synthesized DNA. It has been shown that *in vitro*, MutS oligomerizes, being the tetramer the most active conformation. It has also been shown that oligomerization and the interaction with MutL are dependent on the 50 C-terminal a.a. since deletion of this region abolishes the oligomerization of MutS and diminishes its binding to MutL. In this work, the protein oligomerization was analyzed by turbidemitry at 350 nm and native gel electrophoresis, using a purified his-tag recombinant wild type MutS from *E. coli*. We found that the oligomerization process occurs only in the presence of Mg^{++}, is maximal at pH 7 and is inhibited by low concentration of glycerol. Using C-terminal deletion versions of *E. coli* MutS, we found that the deletion of the 24 C-terminal a.a. are sufficient to restrain both high order oligomerization and tetramer formation. A synthetic peptide containing the 50 C-terminal a.a. of MutS did not affect the protein oligomerization suggesting that the structure of this peptide is different to that present in the protein.

ES-P4.
ANALYSIS OF MutL-MutH INTERACTION

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Escherichia coli MutS, MutL and MutH proteins act sequentially in the mismatch repair system, which repairs DNA replication errors. We have previously shown that *mutL* from *Pseudomonas aeruginosa* complements an *E. coli* MutL-deficient strain, although *P. aeruginosa* does not have the corresponding *E. coli* MutH homologue. Here we use the *P. aeruginosa* MutL protein as a tool to further analyze the interaction MutL-MutH. We compared and analyzed the amino acids sequences and the predicted secondary structures of the *E. coli* and *P. aeruginosa* MutL proteins, and analyzed the *in vitro* and *in vivo* functioning of the wild type and chimeric MutL proteins (*P. aeruginosa*/*E. coli*). We also used protein affinity chromatography to analyze the interactions between the N- and C-terminal regions of *P. aeruginosa* and *E. coli* MutL proteins with the *E. coli* MutH protein. These analysis revealed that the C-terminal region of MutL proteins interact with MutH. Although the C-terminal regions of MutL proteins from *P. aeruginosa* and *E. coli* have only 18% of amino acid identity, they have 85% of identity in the predicted secondary structure. These results are in agreement and further support the hypothesis that a large surface of MutL, rather than single amino acids, is involved in the interaction with MutH, and that the C-terminal region of the MutL protein participates in the MutL-MutH interaction, although the N-terminal region is crucial for the stimulation of the MutH endonuclease activity.

ES-P5.
A NEW CYSTEINE PEPTIDASE FROM RIPE FRUITS OF *SOLANUM GRANULOSO-LEPROSUM* (*SOLANACEAE*)

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Cysteine endopeptidases have been found in viruses, bacteria, protozoa, plants and mammals and more recently were also discovered in fungi. Most of the plant cysteine peptidases belong to the papain family (Family C1). A crude extract possessing proteolitic activity was obtained by expressing the ripe fruits of *Solanum granuloso-leprosum* Dunal. This preparation was preliminary purified by acetone fractionation. The redissolved acetone precipitate contained 89% of proteins and 76% of total caseinolytic activity in relation to the crude extract. Isoelectric focusing followed by zymogram showed more of ten protein bands, most of them proteolytically actives, but only three of which (pI = 8.82, 9.03 and 9.15) proved to be important. On the basis of these results, cation exchange chromatography was selected for the next purification step. FPLC on CM-Sepharose FF allowed the separation of several fractions, of which the most basic (pI = 9.15) was homogeneous by IEF and SDS-PAGE. The enzyme had a molecular mass of 28.8 kDa, and maximum caseinolytic activity was achieved at pH 6.5-8.5. The enzyme was completely inhibited by E-64 and iodoacetic and activated by the addition of cysteine; these results strongly suggest that the isolated protease should be included within the cysteine group.

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ES-P6.
KINETIC CHARACTERIZATION OF PLANT ENDOPEPTIDASES ISOLATED FROM THE LATEX OF *ARAUJIA ANGUSTIFOLIA* (HOOK ET ARN.) DECAISNE (ASCLEPIADACEAE)

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Kinetic characterization of three new endopeptidases isolated from latex of *Araujia agustifolia* (Hook et Arn.) Decaisne fruits has been achieved. The peptidases, named *araujiain aI*, *aII*, and *aII*, were purified by differential centrifugation of the latex ("crude extract") followed by cation exchange chromatography (FPLC, SP-Sepharose Fast Flow). Homogeinity of the purified peptidases was confirmed by gradient electrophoresis (SDS-PAGE) developed by silver staining. Molecular masses (around 23 kDa) were assessed by SDS-PAGE (tricyne buffer) and mass spectrometry. The three peptidases have basic pI (IEF): 8.5, 8.9 and >9.3 for *araujiain aI*, *aII*, and *aIII*, respectively. Esterolytic activity of the peptidases was assayed on N-α-CBZ amino acids, showing the following order of preference: Ala, Phe, Gln, Asp, Tyr, Gly (*araujiain aI*), Gln, Ala, Gly, Tyr, Phe, Asp (*araujiain aII*), and Ala, Gln, Asp, Gly, Asn, Phe, Tyr, Val (*araujiain aIII*). Kinetic parameters (K_m, V_{max} and k_{cat}) were estimated for higher preference N-α-CBZ substrates, as well as for L-pyroglutamyl-L-phenylalanyl-L-leucine-*p*-nitroanilide (PFLNA), a specific substrate for thiol proteinases.
The present study was supported by grants from ANPCyT (PICT 9-9916), CONICET (PIP 2813) and CYTED (Project IV.22).

ES-P7.
CRYSTAL STRUCTURE AND LIGAND SELECTIVITY OF THE ANTINEOPLASTIC LECTIN FROM THE COMMON EDIBLE MUSHROOM (*AGARICUS BISPORUS*)

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The lectin from the common mushroom *Agaricus bisporus*, the most popular edible species in western countries, has potent antiproliferative effects on human epithelial cancer cells without any apparent cytotoxicity. This property confers to it an important therapeutic potential as an antineoplastic agent. The three-dimensional structure of the lectin was determined by X-ray diffraction. The protein is a tetramer with 222 symmetry and each monomer presents a novel fold with two beta sheets connected by a helix-loop-helix motif. Selectivity was studied by examining the binding of four monosacharides and seven disaccharides in two different crystal forms. The T-antigen disaccharide, Galβ1-3GalNAc, binds at a shallow depression on the surface of the molecule. The binding of N-acetylgalactosamine overlaps with that moiety of the T-antigen but surprisingly, N-acetylglucosamine binds at a totally different site on the opposite side of the helix-loop-helix motif. The lectin has thus two distinct binding sites per monomer that recognize the different configuration of a single epimeric hydroxyl.

ES-P8.
STEROIDOGENESIS IN TOAD TADPOLES

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Interrenal and gonadal steroidogenesis in amphibian adult males has been described in several species. However, little is known about steroidogenesis in early developmental stages. Therefore, we have studied steroid biosynthesis and the activity of 3β-hydroxysteroid dehydrogenase/isomerase (3βHSD) in *Bufo arenarum* tadpoles at stage 25 (complete operculum). 3βHSD activity was assayed in larvae homogenised in 10 mM Tris-HCl buffer, 20% sucrose, 0.4 mM 2-mercaptoethanol and 0.1 mM EDTA with ^{3}H-pregnenolone (P5). Proteins in the assay were equivalent to 8 larvae (pH 7.4, at 28ºC) and incubations were carried out with or without 0.5 mM NAD^+ and/or CNK (3βHSD inhibitor). Biosynthetic studies were performed with larvae slices and 1μCi ^{3}H-P5 or progesterone (P4). Activity of 3β-HSD/I was 14 times higher in the presence of NAD^+ than without the cofactor, this activity being strongly reduced in the presence of CNK. The inhibition by CNK confirmed the specificity of the determination. Steroidogenic studies corroborated the expression of 3βHSD. When larvae slices were incubated with ^{3}H-P5 or ^{3}H-P4 the following products were obtained: P4, DOC and corticosterone. However, neither P5 nor P4 were converted into androgens (androstenedione, testosterone, DHT) or estrogens (estradiol, estrone). These results demonstrate that at early developmental stages the only one active steroidogenic tissue is the interrenal. These results agree with previous histological studies suggesting that gonadal differentiation in *B. arenarum* takes place after metamorphosis.

ES-P9.
PROTEOMIC IDENTIFICATION OF THE BOVINE SPERM PROTEASE BSp66

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BSp66 is a glycoprotein with trypsin-like activity located in the acrosome of mammalian sperm. The aim of this work was to determine BSp66 sub-cellular localization and to characterize it by proteomic studies. After sub-cellular fractionation, BSp66 proteolytic activity was detected in the cytosolic fraction of bovine sperm. BSp66 was developed by silver staining and by immuno-detection using a polyclonal antibody against bovine BSp66 on 2D-IEF gel from cytosolic extracts. The spot corresponding to BSp66 revealed a Mr of 66 kDa and pI 5.27 which had homology to Testase 1 (ADAM 24). A 2D-functional analysis of bovine cytosolic proteases showed 10 proteolytic activities, with Mr 40-66 kDa and pI≤6.1. The gelanolytic spot corresponding to BSp66 was not observed suggesting the lost of activity under the assayed conditions. Then, we tested the presence of BSp66 on human (Ho) and hamster (Ha) total sperm extract using proteomic tools. A conserved major protein with Mr 66 kDa and pI 5.7 was observed in both Ho and Ha although it was not recognized by the heterologous anti-BSp66. However, the immunoblot assays revealed a spot of Mr 50 kDa and pI 4,9 in Ha sperm. In Ho sperm, the antibody revealed a set of spots with Mr around 36 kDa and pI near to 6,13 homologue to a testis-specific mammalian serine kinase-3. The results suggest that BSp66 in both Ho and Ha slightly differ from bovine BSp66, and that there are several acidic proteases within bovine cytosol. In order to confirm that BSp66 is conserved among species we must carry out the sequencing of this protease. *(Funded by UNMdP, CONICET)*

ES-P10.
COPROPORPHYRINOGEN-III: ENZYMATIC MECHANISM OF BIOSYNTHESIS

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Uroporphyrinogen Decarboxylase (URO-D) is the 5th enzyme in haem biosynthetic pathway, catalizing the sequential decarboxylation of Urogen III to Coprogen III. It can also transform Urogen I; II y IV and their intermediates to render Coprogen. During the Urogen III to Coprogen III transformation, theoretically, can exist 24 different routes involving 14 porphyrin structures [4 heptacarboxylic, (Heptagens a; b; c and d); 6 hexacarboxylic (Hexagens ab; ac; ad; bc; bd and cd) and 4 pentacarboxylic (Pentagens abc; abd; acd and bcd)]. Physiologically, there is no reason why a strict sequence should take place, since all the porphyrinogens could generate the same final product, Coprogen III. However, URO-D takes only one route out of 24 possible involving 3 intermediates (Heptagen d; Hexagen ad and Pentagen abd. Kinetics constants were determined for all the intermediates. From Kms, Vmax, and EEFs values, the following order of efficiency was established: exagen ad > Urogen III > Heptagen d > Pentagen abd. Urogen III to Coprogen III enzymatic decarboxylation showed a great accumulation of Heptagen d. Accordingly to our results, the great accumulation of Heptagen d in Urogen III decarboxylation could be attribute to spatial impediments of the substrate into the active site cleft.

ES-P11.
DISSECTING THE REPLICATION COMPLEX OF DENGUE VIRUS

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Dengue (DV) is an enveloped virus with a positive-stranded RNA genome of 10.7 kb. After translation, the genomic RNA is copied into a complementary negative strand, which in turn is used as template for amplification of the positive strand. Most of the nonstructural (NS) viral proteins have been involved in RNA replication. However, little is known about the mechanism by which viral polymerase (NS5) initiates synthesis of RNA. Some evidence suggests that RNA structures at the 5' and 3' non-coding regions play crucial roles during RNA amplification. In order to evaluate the specificity of NS5 binding to viral RNA, we analyzed RNA-NS5 interactions using gel shift assays. The viral RNA was radiolabeled and incubated with purified recombinant proteins. We found that NS5 specifically binds to an RNA structure present at the 3' end of viral RNA, however, protein binding shows higher affinity when structures from both the 3' and 5' of the genome were present. Interestingly, using tapping mode atomic force microscopy, we observed that DV genome circularizes through two complementary sequences present at the 3' and 5' ends. This result suggests that long-range RNA-RNA interactions could be important for NS5 binding probably acting as a promoter for RNA synthesis. Furthermore, to study molecular details of DV replication, we are developing a system to reconstitute the viral process using recombinant NS proteins and RNAs made *in vitro*.

ES-P12.
SITE-DIRECTED MUTAGENESIS OF *Pseudomonas aeruginosa* PHOSPHORYLCHOLINE PHOSPHATASE GENE EXPRESSED IN *E. coli*

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P. aeruginosa phosphorylcholine phosphatase, PChP, is synthesized when the bacteria are grown with choline or its derivatives. It catalyzes the hydrolysis of phosphorylcholine in choline and Pi. The gene PA5292 (*pchP*), responsible for the synthesis of PChP, was expressed in *E. coli* ER2566 and site-directed mutagenesis was carried out using a QuickChange XL Kit (Stratagene). DNA sequences of mutant plasmid were determined to confirm the mutation points. The expressed self-cleavage PChP?Intein tag proteins were purified by a chitin-binding column (IMPACT-CN, New England BioLabs). Bioinformatic data revealed that PChP contained three conserved motifs characteristic of the bacterial HAD hydrolase superfamily. A totally conserved motif I, and two other less well conserved motifs, II and III are found in the residues 53-57, 188-190 and 283?288, respectively. Motif I has affinity for divalent ions as Mg2+. Conservative mutants D53E, D55E and T57S and nonconservative D53A, D55A and T57A in motif I led to a complete loss of PChP activity. The gram positive bacterial choline binding domain, GW(V/L)(K/Q)D(N/K)(G/D)TWYYL (N/D)S(S/D)G (A/S)MAT, was not found in PChP, but probably the ^{104}YYY^{106} or ^{149}TYY^{151}residues from PChP may be involved in the binding of choline.

ES-P13.
MOLECULAR ARCHITECTURE OF THE GLUCOSE1-PHOSPHATE SITE OF ADP-GLUCOSE PYROPHOSPHORYLASES

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ADP-glucose pyrophosphorylase (ADP-Glc PPase) is a key enzyme in the metabolism of starch and bacterial glycogen. It catalyses the conversion of glucose 1-phosphate (Glc1*P*) and ATP to ADP-glucose (ADP-Glc) and PP_i. A three dimensional model of the *Escherichia coli* enzyme was obtained using the crystal structure of the *Solanum tuberosum* ADP-GlcPPase small subunit complexed with ADP-Glc as a template (unpublished). Lys^{195} is important in the Glc1*P*-enzyme interaction [Hill *et al*., (1991) J Biol Chem 266, 12455-12460]. The present model suggests that Glu^{194}, Tyr^{216}, Asp^{239}, Phe^{240}, Trp^{274}, and Asp^{276} are also located in a pocket interacting with Glc1*P*. These residues were replaced by Ala and other amino acids, and the recombinant mutant enzymes were purified and characterized. All mutants lowered the apparent affinity for the substrate Glc1*P* from one to two orders of magnitude. The analyzed set of residues are highly conserved among ADPGlc PPases and the putative Glc1*P* site of this model overlaps with other pyrophosphorylases, such as the dTDP-Glc PPase and the CDP-Glc PPase. This is a structurally conserved region and, therefore, the mutagenesis data can be extended to other members of the NDP-glucose PPase family.

ES-P14.
ADP-GLUCOSE PYROPHOSPHORYLASE FROM GRAM POSITIVE BACTERIA

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Glycogen synthesis in bacteria takes place by the pathway utilizing ADP-Glc as the glucosyl donor, being the reaction catalyzed by the enzyme ADP-Glc pyrophosphorylase (ADP-Glc PPase) the regulatory step. ADP-Glc PPase from Gram negative bacteria has been extensively studied, mainly from *Escherichia coli* and *Agrobacterium tumefaciens*. In these, the enzyme was characterized as a homotetramer allosterically regulated by key metabolites of the main pathway for carbon utilization in the respective organism. Conversely, studies on ADP-Glc PPases from Gram positive bacteria are scarce. Between the latter, and after analysis of genome projects, two types could be distinguished: those from the firmicutes (low G+C) group that contain two different genes (*glg*C and *glg*D) coding for ADP-Glc PPase; and those from the high G+C group having only the *glg*C gene, as occurs in all other bacteria. We performed the molecular cloning of the genes coding for ADP-Glc PPase from different Gram positive bacteria: *Mycobacterium tuberculosis* and *Streptomyces coelicolor* (high G+C); and from *Streptococcus mutans* (low G+C). The respective genes were expressed and the recombinant proteins purified and characterized. Results show that the ADP-Glc PPases with the different structures exhibit dissimilar regulatory properties that could be associated with the distinctive metabolic pathways occurring in each microbial organism.

ES-P15.
STUDIES ON THE DIVALENT CATION BINDING SITE IN ADP-GLUCOSE PYROPHOSPHORYLASE

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ADP-glucose pyrophosphorylase (ADP-Glc PPase) catalyzes the key regulatory step in the synthesis of glycogen and starch in bacteria and plants, respectively. The enzyme requires a divalent cation (physiologically Mg^{2+}) as an essential cofactor. Several studies have been performed to identify amino acid residues and structural domains in the protein involved in the binding of subtrates (Glc1*P* and ATP) and allosteric effectors, as well as in catalysis. The crystal structure of a small subunit of potato tuber ADP-Glc PPase is close to be solved. So far, little information is available concerning residues and structural domains involved in the binding of the divalent cation to the enzyme. We performed affinity cleavage by Cu^{2+} on the ADP-Glc PPase from *Escherichia coli*. The cation Cu^{2+}, in the presence of H_2O_2 and ascorbate, cleaved the enzyme mainly in two sites, which localization was determined by the use of a mutant enzyme and toward mass spectrometry analysis of the resulting peptides. The main cleavage occurred between residues D233 and E234, and a second cleavage (enhanced by ATP) took place around K39. Results agree with the hypothesis that the enzyme may have two metal binding sites located in opposite sides in the 3-D model of ADP-Glc PPase.

ES-P16.
THE INCORPORATION OF THE FIRST GLUCOSE INTO THE TYROSINE RESIDUE OF GLYCOGENIN

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To establish the glucosylation state of glycogenin (Gn) is relevant to perform studies for the understanding of its action mechanism. According to Alonso *et al*. (FEBS Lett., 352, 222, 1994), recombinant Gn is expressed in *E. coli galU*, lacking UDPG pyrophosphorylase, as carbohydrate-free apoGn. Evidence for the apo form was a faster SDS-PAGE mobility than glucosylated Gn expressed in the wt, and the failure to detect linked carbohydrate. Previously (SAIB 2003), we described a way to compare the glucosylation state under which recombinant Gn is expressed, by using the auto-[14C]glucosylation/trans-[14C]glucosylation index (ATI) of both, the intact and exhaustive amylolyzed forms. We now describe that Gn expressed in the *galU* mutant (U-Gn) shows the fast SDS-PAGE mobility ascribed to apoGn, but the ATI values indicate that U-Gn is expressed in a glucosylated form. Moreover, the *galU* strain shows both, UDPG- and CDPG-pyrophosphorylase activities (Upy and Cpy). The Upy was 1/3-1/5 the activity of the wt, which together with Cpy could sustain Gn glucosylation. No controls of the lack of Upy and Cpy were shown by Alonso *et al*., as to exclude any glucosylated Gn as the autoglucosylating species of U-Gn rather than apoGn incorporating also the first glucose to its tyrosine residue. We conclude that the Gn autoglucosylation of Tyr-194 deserves further evidence, the nature of the reaction still remaining an open question.

ES-P17.
A NEW PROTEASE ISOLATED FROM LATEX OF *ASCLEPIAS CURASSAVICA* L. (ASCLEPIADACEAE)

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A new cysteine endopeptidase isolated, purified and characterized from latex of *Asclepias curassavica* L is described. The latex collected on 0.1 M phosphate buffer (pH 6.5) containing 5 mM EDTA and cysteine was submitted to centrifugation and ultracentrifugation, yielding a "crude extract" containing soluble proteins. This crude extract was purified by FPLC (SP-Sepharose Fast Flow, buffer Tris-ClH 0.05 M, pH 8.25, linear gradient 0.0-0.6M NaCl), which produced two active fractions, both homogeneous by SDS-PAGE. The first one, *asclepain c I*, has been previously reported. The second protease, named *asclepain cII*, has a molecular mass of 23,590 Da (mass spectrometry) and the N-terminal sequence (LPSFVDWRQKGVVFPIRNQGQ CGSCWTFSA) showed a high similarity with other proteases from species of the *Asclepias* genus. The new protease showed a higher preference for the glutamine derivative when assayed on N-α-CBZ-aminoacid-*p*-nitrophenyl esters. N-α-CBZ-glutamine-*p*-nitrophenyl ester and PFLNA (L-Pyroglutamyl-L-phenylalanyl-L-leucine-*p*-nitroanilide) were chosen to determine kinetic parameters.

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ES-P18.
INTERACTION OF TETRADECYLTRIMETHYL AMMONIUM WITH *P. aeruginosa* PHOSPHOLIPASE C

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Hemolytic phospholipase C (PLcH) of *P. aeruginosa* is a multimeric protein composed of PLcH and one of two co-secreted chaperones, $PLcR_{1,2}$. The expression of PLcH is regulated by phosphate (Pi) and choline. Only the PLcH induced by choline is inhibited *in vivo* by 10 mg/l of tetradecyltrimethylammonium (TDTMA). Here we show the effect *in vitro* of TDTMA on *P. aeruginosa* PLcH when the bacteria were grown under different nutritional conditions. The PLcH was purified by precipitation from the supernatant of the culture medium with $(NH_4)_2SO_4$ in the presence of Celite 545 and by use of a reverse gradient of 70-0% $(NH_4)_2SO_4$. The $(NH_4)_2SO_4$ elution peaks were: 30% (PLcH of choline) and 10% (PLcH of low Pi). TDTMA at 10 mg/l inhibited only the peak of 30%. Peak 10% was incubated with 1% v/v of SDS and then this preparation was used on a Sephacryl S-200. Two peaks of protein corresponding to 70 kDa (PLcH) and 17 kDa ($PLcR_2$) were detected. Only the peak of 70 kDa maintained PLcH activity and was inhibited by 10 mg/l of TDTMA. In the presence of increasing amounts of $PLcR_2$ the loss in PLcH activity caused by TDTMA was drastically reduced. These results showed that $PLcR_2$ remains associated with PLcH after its secretion only when PLcH is induced under low-Pi without choline. When *P. aeruginosa* PLcH is induced by choline, no detectable $PLcR_2$ was found and the consequence of this was that TDTMA inhibited the PLcH activity.

ES-P19.
STRUCTURE-FUNCTION RELATIONSHIP STUDIES ON AMINOTRANSFERASES (ATs) WITH DUAL-SUBSTRATE SPECIFICITY

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We have selected *T. cruzi* tyrosine AT (TAT) and *T. brucei* alanine AT (ALAT) as models for structure-function relationship studies due to these enzymes plausible relevance in trypanosomes metabolism. TATs and ALATs, which transaminate amino acids with a non polar side chain and utilize varied 2-oxoacids as amino acceptors, have been poorly studied at the structural level. Despite the usually low sequence similarity within the AT family, *T. cruzi* TAT shows 40% and 25% overall identity with the rat TAT and *T. brucei* ALAT, respectively. In *T. cruzi* and rat TATs, the N-terminal region appears to be involved in catalysis. In the present work, a truncated rat TAT variant was constructed, the deletion of the first 60 amino acids rendered an inactive mutant however, able to reach the PMP form in presence of L-tyrosine. In ALATs, the residues responsible for activity are still unknown. Thus, the functionality of the conserved N-terminal motif: YAVRG was explored by mutagenesis. The Arg33Ala mutation lowered ten fold the App K_m value for 2-oxoglutarate. The substitution Tyr30Phe also lowered significantly the catalytic efficiencies (k_{cat}/K_m) towards the substrate pair alanine/2-oxoglutarate. These results suggest that the N-terminal region of ALATs and TATs is implicated in the catalytic mechanism.

ES-P20.
PROTEOMIC OF THE NUCLEOSIDE DIPHOSPHATE KINASE FROM *T. cruzi*: PRELIMINARY STRUCTURAL STUDIES, EXPRESSION AND PURIFICATION

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The enzyme nucleoside diphosphate kinase (NDPK) is a major component of the pathway for the synthesis of nucleosides triphosphates other than ATP. The mechanism of reaction involves the formation of a phospho-histidine intermediate. All known NDPK are oligomers made of small polypeptides of about 150 residues (17kDa) with a high degree of sequence similarity. *T. cruzi* NDPK (TcNDPK) has been characterized and could be involved in flagellar movement and therefore in the pathology of the parasite. *T. cruzi* NDPK (TcNDPK) has been cloned in *E. coli* DH5α strain. The gene has been cloned into a pRSET A plasmid and expressed in *E. coli* BL21-DE3 strain as a fusion protein containing TcNDPK preceded by an hexa-His-tag. The fusion protein has been purified with a HiTrap chelating column loaded with 0,1M $NiSO_4$ in different buffer conditions and its purification level visualized in SDS-PAGE. The conformational state of the purified TcNDPK has been observed by different methods such as native PAGE. We have obtained large quantities of TcNDPK to be used for crystallization and interaction assays. The objective of this work is the resolution of the three-dimentional structure of TcNDPK by X-ray crystallography as a first step for rational drug design based on the structure.

ES-P21.
CLONING, EXPRESSION AND CHARACTERIZATION OF *Trypanosoma cruzi* CYTOCHROME P-450 REDUCTASES

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Nitric oxide synthases (NOS) belongs to the large family of NADPH dependent flavoproteins domains containing cyt P-450 reductase enzymes *Trypanosoma cruzi*. NOS activity had been previously reported in our laboratory. Here we present two sequences, ORF 639 and ORF 819 encoding for homologues of FAD and FMN containing NADPH dependent cyt P-450 reductases. The predicted proteins have molecular masses of 67 kDa and 81 kDa respectively and their structural similarities to Cyt P-450 reductase include a NADPH, FAD, FMN, and BH4 domains. Classical L-Arginine binding domains homologous to those of mammalian, could not be found by sequence analysis, in spite of at least 70 % identity was found with *Physarum polycephalum* NOS. Southern blot analysis showed single gene copies for both sequences. Northern blot analysis of ORF 639 showed a single transcript of 1.8 kb. Apparent molecular weights for respective histidine tagged proteins determinated by Western blot were coincident with the predicted values. Both reductases were successfully expressed in BL 21(DE3) pLys E cells by using pRSET-A-639 and pRSET-A-819 recombinant vectors. Soluble cyt P-450 reductase activities were time and protein concentration-dependent and both absolutely required NADPH.

ES-P22.
BACTERIAL GLYCOGEN SYNTHASE: ROLE OF E376 AND OTHER AMINOACID RESIDUES IN CATALYSIS
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Bacterial and plant glycogen synthase (GS) belong to the GT5 Glycosiltransferases family responsible for the synthesis of glycogen and starch, the most widespread energy storage compounds. The intimate mechanism by which glycogen/starch is synthetized remains an unsolved issue. The recently determined crystal structure of GS revealed that the overall fold and the active site architecture of the protein are remarkably similar to those of glycogen phosphorylase, indicating a common catalytic mechanism and comparable substrate-binding properties. We have constructed a series of mutants of key GS amino acids residues that could be directly involved in the catalysis. The keyed residues were selected according to the following criteria: i) conservation between species; ii) chemical properties of the side chain residues; and iii) spatial orientation in the crystal structure. Mutants were obtained by different directed mutagenesis methods. Purification procedure of recombinant mutant proteins was optimized to achieve maximal recover and purity. Kinetic parameters of the wild type and mutant proteins were determined. The crystal structure predicted that E376 is the possible nucleophile residue during catalysis. The mutant E376/A showed an important inactivation with conservation of the affinity for ADP-glucose, thus confirming that this residue is a good candidate to carry out the first nucleophilic attack. This inactive mutant is being submitted to crystallization in order to obtain the closed conformation of the enzyme.

ES-P23.
THE ROLE OF CYS318 IN THE ENZYMATIC ACTIVITIES OF THE CU(II)-REDUCTASA NADH DEHYDROGENASE-2
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NADH dehydrogenase-2 (NDH-2) of *Escherichia coli* is a membrane flavoprotein coded by *ndh* gene. We described previously that it is Cu(II)-reductase and it has a Cu(I) atom bound per polypeptidic chain. The protein contains 4 cysteines: Cys_{315}, Cys_{318}, and Cys_{339} in a Heavy-Metal-Associated Motif, and Cys_{137} in a predicted flexible loop. To study the relevance of the Cys residues in NDH-2 we constructed single and combined-double mutants of the protein, changing Cys by Ser. We assayed the dehydrogenase and cupric-reductase activities of the enzyme either on membranes or on detergent solubilized extracts. Results demonstrated that the substitution of the Cys_{318} led to a 50-70% decrease of the activities compared with the wild-type protein. Also the double mutants $C_{315}S$-$C_{318}S$ and $C_{137}S$-$C^{318}S$ diminished the activities in comparable percentages to the simple mutant. These mutants presenting low activities are deficient to grow on mannitol as it is the *ndh*- strain. On the other hand, the mutant $C_{339}S$-$C_{318}S$ showed an increased of 20-50% in the activities respect to the wild-type and recovers the capacity to grow on mannitol. Previous results of the group demonstrated an increase in all the activities, for the single mutant $C_{339}S$. These results suggest that Cys_{318} residue in NDH-2 would be directly involved in the protein activities and/or the copper binding. We discuss the possible participation of Cys_{339} as redox-regulator.

ES-P24.
CHARACTERIZATION OF THE RHO PROTEIN OF THE FUNGUS *MUCOR ROUXII*
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Cell polarity is central to cell function, acting to organise the biochemistry and the structures that result in the development of cells with specialised physiology. The Rho family of GTPases, particularly RhoA, have emerged as central coordinators of a remarkable variety of cellular functions including cytoskeletal organization. *RHO1* gene from *M.rouxii* has been cloned and the deduced amino acid sequence has highest homology with *S. pombe* (88%) and human RhoA (80%). The 3D structure of *M.rouxii* Rho1p was obtained by molecular modelling through the Swiss-model protein modelling server using as template the coordinates of crystallized human RhoA.The overall structure is conserved. The expression of Rho1p was confirmed by ADP-ribosylation and cytoimmunofluorescence. Rho1p has a differential expression during germination as was detected by Western-blot and RT-PCR. The *M.rouxii RHO1* gene could rescue a *S.cerevisiae* strain containing a *rho1* deletion. Rho1p in *S.cerevisiae* was identify as the regulatory subunit of β–1,3-glucan synthase. Vegetative *M.rouxii* cell wall does not have a significant amount of glucans however caspofungin an antifungal agent which inhibits the synthesis of β–1,3-glucan has an important effect on morphology and growth. The MEC (minimum effective concentration necessary to observe morphological changes) was 10μg/ml similar to that in *Aspergillus spp* and *Fusarium spp* suggesting that Rho1p of *M.rouxii* could regulate β–1,3-glucan synthesis.

ES-P25.
EPIMASTIGOTE PROTEIN EXPRESSION OF THE *TRYPANOSOMA CRUZI* CYCLOPHILIN GENE FAMILY
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The immunosuppressive peptide Cyclosporin A (CsA) has anti-parasitic effect against several protozoans, and was also demonstrated for *Trypanosoma cruzi* (Búa *et al*, Bioorg. & Med. Chem. Lett., 2004, 14, 4633-37). The trypanocidal effect is probably exerted through the formation of a specific complex with Cyclophilins (CyPs), which are a multigenic family of enzymes that mediate cellular folding events, acting as chaperones. There are twelve genes that codify for CyPs in this parasite, though some of them are divergent in their CsA binding domain. Seven genes appeared to be orthologues of other trypanosomatid cyclophilins according to epimastigote Expressed Sequence Tags annotation in the *T. cruzi* Genome Project. By means of RT-PCR amplification experiments, these seven genes also proved to be transcribed in amastigotes and trypomastigotes. Expressed CsA binding proteins were purified by affinity chromatography. Four protein bands were detected by silver staining after running the eluate on an acrylamide gel. These spots were analysed by MALDI-TOF mass spectrometry after in-gel tryptic digestion. Database searching using peptide mass fingerprints allowed the identification of *T. cruzi* cyclophilins of 19, 21, 28 and 40 kDa, being the most expressed *Tc*CyP19. We conclude that these four cyclophilins are actively expressed in the epimatigote stage of *Trypanosoma cruzi*.
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ES-P26.
HEME BIOSYNTHESIS AND METAL INTERACTION *IN VITRO* AND *IN VIVO*
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A number of chemicals induce disturbances of heme synthesis. Some trace minerals are essential nutrients for normal growth but excessive amounts can be detrimental to the developing embryo. Environmental pollution feeds soil and foods with heavy metals and cause teratogenic defects. Chick embryo has been used to study porphyrin metabolism and toxicological and teratogenic effects of many chemical substances. The aim of this study is to evaluate Cd^{2+}, Pb^{2+}, Zn^{2+} and Cu^{2+} teratogenicity on chick embryo and to study *in vivo* and *in vitro* effects on yolk sac membrane (YSM) and liver Uroporphyrinogen Decarboxylase (URO-D) and Coproporphyrinogen oxidase (CPG-ox) activities. Chick embryos of 12 days of development were used. Cd^{2+}, Pb^{2+} acetates or Cu^{2+} sulfate were inoculated into the yolk at 2 different conc. (12-60 hs) and liver and YSM were removed. Enzymatic activities were determined and products analyzed by HPLC. CPG-ox activity in boths tissues studied did not change significantly, however URO-D YSM and liver enzyme are inhibited by Cu^{2+}. Different effects were observed *in vivo* and *in vitro* and they could be due to the different capacity to cross vitelline membrane, the tissue ability for mobilization, uptake and short-term storage for these trace minerals. The results reflect different coordination geometry and Lewis acid-base capacity of these cations. Pb^{2+} and Cu^{2+} induced much lower embryonic mortality rate than Cd^{2+}. The alterations were doses dependent. These three metals are teratogens but their embryopathic mechanisms remain uncertain.

ES-P27.
CLUSTERING EFFECT OF BENZYL ALPHA THOMSEN-FRIEDENREICH DISACCHARIDE ON ITS IMMUNOGENICITY
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Mucin-type O-glycans are upregulated and aberrantly glycosylated in many carcinomas. O-glycan Core 1 (Galβ1-3GalNAcα-O-), called Thomsen-Friedenreich disaccharide (TFD), is an example of a cryptic structure that is over-expressed in cancer cells by changing its glycosyltransferase profile. This molecule is an attractive model to study carbohydrate immunogenicity and a potential candidate for active specific immunotherapy of patients with cancer. The aim of present work is study the influence of synthetic clustered sugars with a terminal hydrophobic residue (benzyl) on the addressing of carbohydrate immunogenicity. As cluster arm was used Lys5, which is covalent linked to succinylated KLH using 1ethyl3(3dimethylaminopropyl)-carbodiimide. Benzyl alpha TFD (BzlαTFD) is oxidized by using galactose oxidase and conjugated to cluster peptide (Lys_5) through $NaCNBH_3$. The synthetic glycoconjugate was used as immunogen in Balb-C mice. Antibody titers were measured by using ELISA against several antigens. They recognize BzlαTFD and related TFD structures as observed by using direct and competitive ELISA. Cell ELISA method evidenced that yielded IgG and IgM antibodies bind epithelial tumor cell lines (T47D, HT29 and TA3 Ha), which are partially mediated by related TFD molecules. The present work reveals beneficial properties on the use of Lys_5 as cluster arm of BzlαTFD, with the purpose to direct the immune response to related TFD molecules expressed on epithelial tumor cells.

LP-P1.
EFFECTS OF CYCLOPHOSPHAMIDE ON TESTICULAR LIPIDS WITH VERY LONG CHAIN POLYUNSATURATED FATTY ACIDS (VLCPUFA)
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Treatment with cyclophosphamide (CP), a drug commonly used as an anticancer and also as an immunosuppressive agent, may result in oligozoospermia or azoospermia, associated with germ cell apoptosis. The objective of this study was to determine the effects of CP administration on rat testicular lipids containing VLCPUFA. These were studied at various intervals after a single i.p. dose of the drug. A decrease in the number of cells was seen in the germinal epithelium at days 5 and 15 after treatment in part of the tubules, followed by a partial recovery in most of them after 30 days. The content of testicular ceramide (Cer) showed a significant increase, reaching a peak the 5th day, and then decreased to levels slightly smaller than the initial ones. The fatty acids of the increased Cer were mostly saturates and monoenes, especially 16:0 and 18:1. The molecular species containing VLCPUFA of Cer only tended to be reduced from the beginning to the end of the interval studied. A moderate but significant increase in the amount of other VLCPUFA-containing lipids, such as cholesterol esters was observed in the 15-30 day interval. The results suggest that a single dose of CP damaged germ cells and increased Cer, the surviving cells being able to resume spermatogenesis. CP-induced lipid changes may be intensified by the administration of repeated doses of the drug.

LP-P2.
RAT, CAPRINE, EQUINE AND BOVINE ERYTHROCYTE GHOSTS EXPOSED TO T-BUTYL HYDROPEROXIDE AS A MODEL TO STUDY LIPID PEROXIDATION USING A CHEMILUMINESCENCE ASSAY
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The aim of the present study was to analyze the time-course of t-butyl hydroperoxide-induced changes in fatty acid composition and chemiluminescence intensity in rat, caprine, equine and bovine erythrocyte ghosts. A relatively high content of arachidonic acid: C20:4 n6 and docosahexaenoic acid:C22:6 n3 was characteristic of the rat erythrocyte ghosts. The fatty acid composition of native erythrocyte ghosts obtained from caprine, equine and bovine was characterized by a high content of oleic acid:C18:1n9 and a low content of the peroxidable polyunsaturated fatty acids (C20:4n6 and C22:6n3).The proportion of linoleic acid: C18:2n6 was higher in equine and bovine compared to rat and caprine. Increase in lipid peroxidation in rat erythrocyte ghosts was maximal within 12 min of incubation, t-butyl hydroperoxide concentration dependent and was paralleled by a decrease in C18:2 n6, C20:4 n6 and C22:6 n3 and an increase in chemiluminescence. Polyunsaturated fatty acids present in rat erythrocyte ghosts exhibit the highest sensitivity to oxidative damaged and their sensitivity increases as a power function of the number of double bonds per fatty acid molecule. Light emission in caprine, equine and bovine erythrocyte ghosts was very low, t-butyl hydroperoxide concentration dependent but changes in fatty acid composition were not observed. A lower unsaturation degree of fatty acids in erythrocyte ghosts of caprine, equine and bovine prevent the lipid peroxidation on those membranes when they are incubated with t-butyl hydroperoxide.

LP-P3.

AN ACTIVE GLUCOSYLCERAMIDE SYNTHASE IN EPIMASTIGOTE FORMS OF *TRYPANOSOMA CRUZI*

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Biosynthesis of glycosphingolipids involves the sequential action of glycosyltransferases and has been assumed that these enzymes are functionally organized in Golgi. However, the key step involves the transfer of glucose from UDP-glucose to ceramide catalyzed by a UDP-Glucose:glucosylceramide transferase (EC 2.4.1.80; glucosylceramide synthase (GCS) to form glucosylceramide, the precursor of most higher order glycosphingolipids. Here we report the presence of the glucosylceramide transferase involved in the biosynthesis of glycosphingolipids in epimastigote forms of *T. cruzi* CL strain, showing the characterization of other byproducts of the enzyme reaction (such as acylated glucosylceramide). In addition, in order to get some insight on the subcellular location of this enzyme, immunoelectron microscopy techniques were used. Fixed epimastiges ultrathin sections were stained with anti-human GCS 5.1 antibody (a kind gift of Drs. Marks and Pagano) followed by gold conjugated secondary antibody. Interestingly, whereas the Golgi apparatus, as expected, revealed labelling with this antisera an intense labelling was also observed in plasma membrane including extracellular aggregates all around the parasite indicating its location may be posible in lipid rafts.

LP-P4.

PHOSPHATIDYLCHOLINE PHOSPHOLIPASE A1 IS MEMBRANE ASSOCIATED IN *TRYPANOSOMA CRUZI* INFECTIVE STAGES

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We demonstrated previously a phosphatidylcholine (PC) Phospholipase A_1 (Plase A_1) in *T. cruzi* epimastigotes (Epi) with lysosomal location. We here report that Plase A_1 activity resides mainly in membranes (Mb) of amastigotes (Ama) and trypomastigotes (Trip) and it is 15-10 fold higher respectively than in Epi. Only infective stages secreted this activity. Ama and Trip were able to incorporate and degrade exogenous PC (10%, 15-30 min), but EPI was unable (less than 1%, 30 min). Sephadex G50 chromatography of Trip and Ama Mb, treated with 0.3% Triton X-100, showed only a peak close to the void volume, suggesting that this enzyme could be particulated and Mb associated. Vero cells exposed (10 min) to intact Ama or Trip, changed their lipid patterns with increase in FFA, DG and LysoPC. Mb from the infective stages induced the same lipid profile modification. Meanwhile, intact Epi or its Mb was unable to modify host cell lipid profile. In conclusion, higher Plase A_1 activity is Mb associated to *T. cruzi* infective stages supporting the view that Plase A_1 could act on the host cell, triggering particular signaling cascades. Moreover, Plase A_1 appears as the only enzyme in *T. cruzi* able to hydrolyze PC, a clear difference from PC degradation pathways in the host cells. Thus, if specific inhibitors and/or its protein sequence (*in progress*) become available, this biochemical peculiarity could be a possible chemotherapeutic target.
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LP-P5.

EFFECT OF HYPERTONICITY ON PHOSPHOLIPID METABOLISM IN RENAL EPITHELIAL CELLS

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Previous works from our laboratory demonstrated that the papilla is the zone of the kidney with the most active phospholipid (PL) metabolism. Papillary cells work under physiological stress since they are submitted to the highest renal interstitial osmolality. Hence, the active phospholipid turnover could act as a protective mechanism of cellular membrane homeostasis. In order to explore the relationship between hypertonicity and PL metabolism, we study the effect of high NaCl concentration on PL content, composition and synthesis in MDCK renal epithelial cell line. For this purpose, confluent-arrested MDCK cells were cultured under physiological (150 mM NaCl) and hypertonic (225 mM NaCl) conditions for 3, 6, 12, 24 and 48 hs. Hypertonic medium decreased cell number by 13, 44 and 78 % after 12, 24 and 48 hs, respectively, without affecting cell viability. In contrast, endogenous PL content per cell increased 68 and 157 % after 24 and 48 hs of hypertonicity. Cell membranes preserved PL profile excepting slight variations in sphingomyelin and PE percentages after 48 hs. PL synthesis was studied by ^{32}P-Pi and ^{14}C-glycerol. NaCl addition decreased PC and PI synthesis after 3 and 6 hs, but 24 and 48 hs of hypertonicity significantly increased biosynthesis of all phospholipid species. These results suggest that long exposure to hypertonicity induces cellular membrane biogenesis. Considering that cell number decreased, they may be forming new cellular compartments. The initial events leading to membrane biogenesis could involve PC and PI related-signals since both phospholipids fall after 3 hs of NaCl stimulation.

LP-P6.

BOVINE PLASMA POSSESS ABUNDANT PHOSPHOLIPID BINDING IgM MOLECULES

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Ruminants are a particular zoological group in that their erythrocytes lack phosphatidylcholine (PC), possess abundant sphingomyelin (SM) and their phosphatidylethanolamine (PE) contents is about ten times lower than in human erythrocytes. Less than 2% of PE is exposed in the cell surface. We found that bovine plasma lyses erythrocytes of other species including the closest non ruminant artiodactyl, the pig. Several lines of evidence, indicate that the relevant antigens are phospholipids. Absorption with SM/PE but not with pure SM liposomes, abrogates hemolysis. The hemolytic effect is complement dependent. Immunoblots show that SM/PE liposomes bind about the double amount of IgM than pure SM liposomes, suggesting that an unspecific binding to the liposomes is not likely to account for the differences observed with the different lipid compositions. The antibodies can be eluted from the liposomes by simple treatment with Phospholipase C, indicating the relevance of the integrity of the phospholipid molecule in the process. Similar experiments using anti bovine IgG-HRP revealed no activity. Therefore this newly described system can be considered an innate physiological mechanism. It might be particularly developed in ruminants, and not in other taxa, as a means to ward off any invasive activity from the abundant ruminal microbes that thrive in their particularly large stomachs, that characterized them.
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LP-P7.
HUMAN ERYTHROCYTE HOMEOSTASIS INVOLVES THE ACTIVATION OF A NOVEL PLC

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We have investigated the metabolic fate of 1-palmitoyl-2-[6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]caproyl]-sn-glycero-3-phosphocholine (C_6-NBD- PC) exposed to human red blood cells suspension. The method involved incubating at 37ºC the fluorescent phospholipid with freshly washed human erythrocytes for 1 h. Afterwards, the red blood cells were washed again three times to remove any unincorporated C_6-NBD-PC. The fluorescently labeled erythrocytes were added to an equal volume of plasma and incubated at 37ºC with constant stirring. Aliquots were removed at different time points and centrifuged. Three kinds of samples were thus generated: 1) total erythrocyte plus plasma suspensions, 2) plasma supernatant, and 3) red blood cell pellets. Lipids from these samples were extracted by a modification of the procedure of Bligh & Dyer and analyzed by thin layer chromatography on silica gel plates, using a double developing system, as described by Florin-Christensen et al (*J. Biol. Chem*. 267, (1992), 14783.). The results show, surprisingly, a striking formation of diacylglycerol (DG) which was entirely recovered in the plasma samples. These results demonstrate for the first time, the occurrence of a PC-PLC in human erythrocytes and suggest that plasma lipoproteins possess high affinity for the DG. These observation open the possibility that plasma membrane DG from other type of cells could be removed by interaction with plasma lipoproteins, therefore constituting a novel termination mechanism for cellular DG signals.

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LP-P8.
SPONTANEOUS ALTERATIONS OF LIPID METABOLISM IN ATHYMIC NUDE MICE

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Lipid metabolism has been scarcely studied in congenitally athymic (nude) mice. Since thymic factors have been reported to modulate cholesterol levels in rats, nude mice constitute an interesting model for the study of the impact of athymia on lipid metabolism. In the present study we characterized a number of lipidic parameters in the plasma and liver of athymic mice and compared them with those of age-matched heterozygous (control) nude mice. NIH male and female nude (nu/nu) and heterozygous (+/nu) mice were maintained on a γ-irradiate chow diet and sterilized water *ad libitum*. Nude and control mice at around 8 wk of age were fasted for 12 h and blood samples were collected from the paraorbital venous plexus to determine basal plasma cholesterol, triglycerides and glucose levels. After sacrifice livers were removed and total lipids were extracted according to Folch. Neutral lipids were separated and quantified by TLC. In some experiments, 2h before sacrifice animals were injected with 25 μCi ^{14}C-acetate. Female athymic mice had lower plasma tryglyceride levels (51.4 ± 4.5 vs 72.8 ± 13.3 gr/dl) and male athymic mice had lower plasma cholesterol levels (82.6 ± 13.0 vs 118.2 ± 10.7) as compared with their respective heterozygous counterparts. Percentage of ^{14}C-acetate incorporation into liver triglycerides was lower in female but not in male athymic animals. In addition, male nude mice showed a moderate hyperglycemia. These results constitute the initial step of a systematic study in which we plan to assess the effectiveness of thymic hormone replacement to restore lipid and endocrine parameters in athymic mice.

LP-P9.
BEHAVIOUR OF Δ^9 DESATURASE OF *T. cruzi* WITH PHOSPHATIDYLCHOLINE AS SUBSTRATE, UNDER KETOCONAZOLE EFFECT

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Previous studies from our laboratory indicated that Δ^9 desaturase of *T. cruzi* is able to use palmitic acid and phosphatidylcholine as substrates (Moyano *et al.*, SBC 2003). In this work we tested Δ^9 desaturase activity under the effect of ketoconazole using phosphatidylcholine as substrate on different subcellular fractions of *T. cruzi*. The enzymatic activity was measured using precipitate and supernatant of 105000xg obtained by differential centrifugation from *T. cruzi*. Ketoconazole (1μM) was added to the culture medium after 24 hours of growth. Reaction mixture contained phosphatidylcholine, 1, 2 -di [1-^{14}C] palmitoyl and all necessary cofactors. $AgNO_3$ TLC plates were used to separate and identify the reaction substrates and products. We found that the highest activity of Δ^9 desaturase was obtained with the precipitate fraction. It was threefold higher than the enzymatic activity from the supernatant fraction. Furthermore ketoconazole was able to reduce Δ^9 desaturase activity in approximately a 20%, in 105000xg precipitate but, it did not modify the Δ^9 desaturase activity in the supernatant fraction. Since before we found dissimilar results with palmitic acid, the present results may suggest that Δ^9 desaturase shows different behaviours depending on the substrate used. Finally we emphasize the importance that considering desaturases as a target for drugs treatment has.

LP-P10.
EFFECT OF CHRONIC EXPOSURE TO CADMIUM ON THE LIPID METABOLISM IN PROSTATE

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We have shown alterations on the histology of prostates exposed to Cadmium (Cd). The objective of this work was to determine if there were also changes in the metabolism of lipids. Wistar male rats were separated in two groups: Cd, which received 100 ppm of Cd in drinking water for 12 weeks and control (Co) which did not receive Cd. Lipids were separated by TLC. Phospholipid (PL), triglycerides (TG), total (TC), free (FC) and esterified cholesterol (EC) were determined. The activities of fatty acid synthase (FAS), glucose-6-phosphate (G6PDH), isocitrate (IDH) and malic dehydrogenases (MDH) were assayed. Total RNA was isolated with Trizol. The expression of FAS, diacylglycerol acyltransferase (DGAT) I and II, SREBP-2, peroxisome proliferator activated receptor (PPAR) γ and α, glycerol-phosphato acyltransferase (G-PAT), lipoprotein lipase (LPL) and β-actin as an internal control was studied by RT-PCR.
TG (Co:148±13,62; Cd:53,57± 1.,89 μmg/mg protein, $p<0.01$) and EC (Co:2,01±0,25; Cd: 0,62±0,04 μg/mg protein, $p<0.05$) while FC (Co:3,50±0,52; Cd: 6,06±0.,95 μg/mg protein, $p<0.05$) and PL increased (Co:6,67±0,18; Cd:28,75±5,75 μg/mg protein, $p<0.005$). The activity of the enzymes did not show variations. The expression of DGAT-I decreased and PPAR-α, SREBP-2 and GPAT increased, while DGAT-II, FAS, LPL and PPAR-γ did not show modifications. Cd induces changes in the synthesis of phospholipids and triglycerides through alterations in the expression of specific enzymes and transcription factors.

LP-P11.

EFFECTS OF DIETS WITH PROTEIN CONCENTRATE AND FLOUR OF *Amaranthus cruentus* ON GENES RELATED TO LIPIDS METABOLISM IN RAT LIVER

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Previous studies have shown the hypocholesterolemic and hypotriglyceridemic effects of the protein concentrate (PC) and flour of *Amaranthus cruentus* (F) on male Wistar rat livers. This work studied the effect of these diets on the expression of HMG-CoA and FAS genes. We used eighteen rats of 200 g of body weight. The animals were separated in three groups: one control fed with casein as protein source, a problem group A with PC and a problem group B with F as protein source. All diets had 11.9% of protein and were supplemented with 1% of cholesterol. The experience lasted 28 days. We measured total cholesterol (CT) (Zack), triglycerides (TG) (Sardesay) and FAS (Roncari) activity. RNA was extracted using TRIZOL method. RNA was transcribed to cDNA, which was used in the amplification by PCR of HMG-CoA, FAS and L19, as internal control. A decrease of CT and TG $p< 0,001$ was observed in the experimental groups when compared to control and FAS activity decreased significantly in the experimental groups. The expression of HMG-CoA was not modified and FAS decreased in the experimental groups ($p<0.05$). This let us to propose the protein concentrate as a new therapeutic strategy as hypocholesterolemic and hypotriglyceridemic agent.

LP-P12.

VITAMIN A DEFICIENCY ON MITOCHONDRIAL LIPID METABOLISM IN RAT LIVER

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We have showed that vit A deficiency alters the serum and liver lipid concentrations. Now, the mitochondrial lipid compositions and fatty acid (FA) β-oxidation are studied. Male Wistar rats at 21 d age were weaned onto either a vit A deficient diet (-A) or the same diet with 8 mg retinol/kg diet (control). They were fed for 3 months. Serum and liver Vit A levels were determined by HPLC. Acetyl CoA Carboxylase activity (ACC) was measured in the liver cytosolic fraction in presence of [^{14}C]-bicarbonate. Serum β-OH-butiric acid (β-HBA) concentrations were determined by a Sigma kit. Mitochondrial lipids were quantified after separation by TLC. Mitochondrial Carnitine Palmitoyl Transferase (CPTI) activity was measured using [^{3}H]-carnitine. Serum β-HBA was increased in -A rats. Mitochondrias of -A rats showed higher CPTI activity than controls. In adittion, there were a decrease of cholesterol, an increase of tryglicerides, and no changes in total phospholipid contents, which was due to an increase of phosphatidylcholine and a decrease of cardiolipin. The ACC activity was decreased in -A rats. Vit A deficiency increases the FA β-oxidation, possibly by decreasing the availability of the endogenous inhibitor of CPTI, malonyl-CoA, as was suggested by the decrease of ACC activity. The changes of mitochondrial lipid compositions also can play a role in the alteration of β-oxidation process.

LP-P13.

INTRACELLULAR SIGNALS MEDIATING FSH EFFECTS ON FATTY ACID COMPOSITION AND METABOLISM IN RAT SERTOLI CELLS IN CULTURE

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Follicle-stimulating hormone (FSH)-regulatory action on both fatty acid composition and metabolism was investigated in Sertoli cells from immature rats. Cells were cultured up to day 7, either under basal conditions or in the presence of r-FSH (200 mUI/ ml, 72 hs). Compared to controls, significant changes were noted in cell fatty acid profile. A decrease in 14:0, 16:1 n9 as well as in 18:2 and 24:4 n6 fatty acids was observed. An increase in 16:1 n7, 18.0, 18:1 (n9 and n7) as well as in the fatty acids of the n-6 series 22:4 and 24:5 was measured. Increases observed in the fatty acid ratios 16:1 n7/ 16:0, 20:4 n6/18:2 n6 and 24:5 n6/24:4 n6 were taken as an indirect indication of Δ9, Δ6 plus Δ5, and Δ6 desaturase -FSH stimulation. FSH up regulation of Δ9 desaturase activities was corroborated by analyzing the metabolic products formed from [1-^{14}C]16:0 after the addition of the labeled substrate to the culture medium (2 μM, 24 hs). Changes in fatty acid profile observed under FSH stimulation were reproduced by (diBu)cAMP (300 μM, 12 hs). Incubations with staurosporine (2 μM) plus FSH resulted in analytical changes that partially reproduced those changes observed with FSH alone. An increase in the ratio Σn-6 fatty acids/ Σn-3 fatty acids was observed in all experimental conditions. From these results we conclude that FSH effects on Sertoli cell fatty acid profile are partially dependent on the cAMP signal transduction pathway.

LP-P14.

CHANGES IN RAT TESTICULAR ANTIOXIDANT DEFENSE SYSTEM INDUCED BY DIETARY LIPIDS

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We studied the effect of dietary lipids on the antioxidant defense system of rat interstitial (Leydig) cells. Animals were fed on diets containing 20 % of soybean (S), olive (O), coconut (C) or grape seed oil (U) for 60 days. Testicular homogenates and suspensions of interstitial cells were prepared from each experimental group. Several biomarkers of oxidative-nitrative stress were determined under either basal conditions or after incubating the cells (5 h) with arginine (A) [50 mM] or L-NAME (N) [5 mM]. Basal or induced (Fe/ascorbate, Fe/ADP/NADPH, ACCN) lipid peroxidation (TBARS, FOX), and enzymes activities (Mn-SOD, Cu,Zn-SOD, CAT, GSH-Px, GSH-Rd, GSH-Tr) showed decreased values in the order U>S>O>C. α-Toc, ascorbate and GSH contents were higher for O and C compared to those of S and U groups. Ratio GSH/GSSG decreased in the order C>O>S>U, while FRAP assay followed the order O=C>S>U. Accumulation of nitrate+nitrite was higher in S and U groups compared to O and C. Both, increased and decreased conditions of NO biosynthesis produced complex and significant alterations in several oxidative-nitrative stress biomarkers. Cells incubated with A or N experimented major changes when they were prepared from U or S groups compared to those obtained from C or O. Taking together, results indicated that O diet had the most, and U diet the least protective effects under oxidative-nitrative stressing conditions.

LP-P15.
ACYL COA OXIDASE ACTIVITY FROM *BEAUVERIA BASSIANA*, AN ENTOMOPATHOGENIC FUNGUS
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The entomopathogenic fungus *Beauveria bassiana* has the ability to degrade a variety of hydrocarbon structures similar or identical to those of their insect host, incorporating their degradation products into different fungal lipids. After the initial oxidation step, the fatty acid product becomes the substrate of acyl CoA oxidases (ACO). *B. bassiana* was grown on glucose supplemented medium (FS_0) or hydrocarbon supplemented medium (FS_2). The ACO activity was studied by spectrophotometry, based on the determination of H_2O_2 production coupled to the oxidation of 2',7'-dichlorofluorescin diacetate in a reaction catalysed by exogenous peroxidase. The ACO activity was analyzed in the P_{20000g} fractions from FS_0 and FS_2 cultures. Activity was measured employing acyl CoA's of 18 to 24 carbons. Higher activities were always detected for FS_2 cultures in the conditions tested, i.e., using stearoyl-CoA as the substrate a 32% increment in activity was observed. Employing larger chain length acyl-CoA´s, tetracosane-grown cultures showed the highest activity with lignoceroyl CoA. This assay is quite sensitive and offers a simple approach for detection of ACO activity. The activity was linear until 20 min of incubation and up to 30 µg/µl of protein concentration. These studies will contribute to help understand the metabolic pathways involved in hydrocarbon degradation by entomopathogenic fungi, playing an important role in the biocontrol of insects.

LP-P16.
CHEMICAL TAXONOMY OF THE *TRIATOMA SORDIDA* COMPLEX : HYDROCARBON FINGERPRINTS
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Hydrocarbon fingerprints were used as a taxonomic tool to differenciate *T. sordida*, *T. garciabesi* and *T. guasayana* populations from Argentina, Bolivia, Brasil and Paraguay. Compositional and structural analyses were performed by capillary gas chromatography (CGC), and CGC coupled to mass spectrometry (MS). Intraspecific variations were analyzed by ordenation and classification multivariate techniques. As in other triatomins, the prevailing structures were mostly saturated, with straight components up to 33 carbons, whereas methyl-branched structures with one, two, and three CH3 groups inserted in terminal position (3-) or internally. *n*-Nonacosane was the major component in the complex. *T. guasayana* specimens showed homogeneous profiles regarding their geographical distribution, and easily distinguishables from the other species of the sordida complex. *T. sordida* showed complex hydrocarbon fingerprints with high intraspecific variability, significantly different patterns were detected mostly in Bolivia. Insects from neighbouring areas in Argentina and Paraguay were classified together. *T. garciabesi* from Salta (Ar) showed profiles with moderate amount of methyl-branched components, closely similar to *T. sordida* from Cochabamba area in Bolivia.

LP-P17.
THE CUTICULAR HYDROCARBON PATTERN AS A TAXONOMIC TOOL FOR STUDYING *TRIATOMA DIMIDIATA* POPULATION STRUCTURE
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The cuticular hydrocarbon fingerprint of *T. dimidiata*, a relevant Chagas disease vector in northern Latin America, was used to compare insect populations from different states from Mexico and Guatemala. The hydrocarbon composition was analyzed by capillary gas chromatography (CGC), structure identification was performed by CGC coupled to mass spectrometry (MS). The relative abundance of the hydrocarbon components was analyzed by principal component analysis and discriminant analysis. Hydrocarbon profiles showed lineal and methyl-branched chains between 21 to more than 40 carbon atoms, the prevailing structures were odd-numbered chains of 25 to 33 carbons. Quantitative rather than qualitative intraspecific differences were detected. Discriminant analysis results in 93.2% of specimens correctly classified according to their collection sites and 78.4% after reclassification by cross-validation. Four groups were easily distinguished, in coincidence to geographic vicinity; one included insects from Hidalgo, Veracruz, Chiapas and east Oaxaca states; a second group included Santa Rosa, Jutiapa and west Oaxaca, a third one corresponded to Cozumel, Yucatan and the nearby Peten jungle. A fourth group from Guatemala (Lanquin caves) was significantly different, posing concern on its taxonomic status.

LP-P18.
ANALYSIS OF TRIBOLIUM CASTANEUM VOLATILES
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Solid phase microextraction (SPME) technique was used for collecting the volatile secretions released by adult red flour beetle *Tribolium castaneum*. This major stored product pest uses prothoracic and abdominal glands to produce specific defensive secretions with repellent and irritant properties against predators, contaminating human foods. SPME samples were analyzed by capillary gas chromatography (CGC) using a non polar column, and CGC coupled to ion trap mass spectrometry (MS). Results obtained employing three different SPME fibers (a divinylbenzene/polydimethylsiloxane (DBV/PDMS) fiber, a carboxen (CAR)/PDMS fiber and a PDMS fiber were compared in different sampling conditions. Two quinones, methyl- 1,4 benzoquinone (MBQ) and ethyl- 1,4 benzoquinone (EBQ) and the unsaturated hydrocarbon 1-pentadecene were the major components of the volatile blend, small amounts of hidroquinones and 1,6-pentadecadiene were also detected. Quinone lability usually has hampered detection of the oxidized forms, the requirement of large amount of specimens, and time consuming techniques, can be efficiently replaced by SPME-CGC to detect insect volatiles.

LP-P19.
EFFECT OF FENITROTHION ON THE ACYLGLYCERIDE EXCHANGE IN CRUSTACEAN LIPOPROTEINS

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The effect of fenithrotion (FS) on the functionality of two models of crustacean lipoproteins was studied. Models used were a high density plasma lipoprotein (HDL) that is involved in the lipid - tissue exchange, and a lipovitellin (LV), the main source of embryo nutrients, which presents two forms only distinguishable in its lipid composition. The relative influence of lipid and apoprotein compositions on the FS effect in the lipid transfer, was comparatively evaluated using liposomes prepared with lipids from both lipoproteins. FS treatment of HDL modified the transfer of lipids to and from hepatopancreas, diminishing the uptake of phosphatidylcholine and triacylglicerols and increasing its capacity to release both lipids to the tissue. Likewise, FS increased the transfer of phophatidylcholine to the embryonic tissue from both LVs and from liposomes. It was assessed that apoproteins are involved in the transfer of phosphatidylcholine to tissues, though they are not involved in the changes produced by FS concerning the affinity of this lipid to lipoprotein systems.

LP-P20.
EFFFECT OF THE WATER-SOLUBLE FRACTION OF PETROLEUM ON LIPID METABOLISM OF CRUSTACEANS

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The effect of the water-soluble fraction (WSF) from crude oil on lipid classes, phosphatidylcholine (PC) molecular species, membrane fluidity and microsomal enzymes was studied in hepatopancreas (HP) and eggs of the freshwater crustacean *M. borellii*. After 7-day exposure to sublethal concentration of WSF (0.6 ppm), a triacylglicerol/phospholipid ratio increased in HP and decreased in eggs, suggesting alterations either in the mobilization of triacylglycerols to phospholipid pools or in the energy balance. WSF exposure altered PC molecular species in both tissues. From the 15 species identified, the 4 major HP molecular species (16:0/18:1, 18:1/18:2, 16:0/20:5 and 16:1/20:5) and most other PUFA-containing ones decreased. This was compensated by an increase in 16:1/18:1 (152%) and 18:1/18:1 (50%). The major egg PC molecular species decreased (16:0/18:1 and 18:1/18:2), while PUFA-containing ones increased their proportion. Nevertheless, microsomal membrane fluidity was not altered by WSF exposure. The microsomal palmitoyl-CoA ligase activity increased significantly in HP (154%) and eggs (89%). Total acylglycerides synthesis also increased in both tissues (21 and 61%, respectively) though the radioactive tracer was incorporated into different lipids. The contrasting response of both tissues to WSF contamination suggests the presence of different homeostatic mechanisms. The increase of enzymatic activities might be due to their synthesis induction or a mechanism of activation and not to changes in membrane properties.

LP-P21.
LIPID UPTAKE BY HEMOCYANIN IS ALTERED BY FENITHROTION

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We had already corroborated that fenithrotion (FS), a liposoluble insecticide, alters the structure of several lipoproteins of invertebrates. This study shows the *in vitro* effect of FS on the functionality of a spider lipoprotein (VHDL) in the uptake of different lipids: triacylgliceride, cholesterol and palmitic acid. This lipoprotein has hemocyanin (HC) as apoprotein. HC, the *in vivo* carrier of those lipids, was delipidated and treated with FS. Then, the apoprotein was incubated with each lipid and their uptake was measured by radio-HPLC. The fluidity of treated and control VHDL was determined using fluorescence anisotropy (rs) measurements. It was assessed that FS penetrates the whole lipoprotein, altering its structure and increassing rs. In a dose-dependent manner, this insecticide decreases the uptake in the three lipids assayed by 24, 29 and 29% with 10 ppm FS, and by 44, 62 and 40% when treated with 20 ppm FS, respectively. In conclusion, FS rigidizes the apoprotein, leading to a diminution of its lipid uptake capacity that, extrapolating to *in vivo* conditions, this occurrence would imply a normal function alteration.

LP-P22.
[1-^{14}C]20:4n-6 ACID IS INCORPORATED INTO THE INNER AND OUTER NUCLEAR MEMBRANE OF RAT LIVER CELL

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The aim of this work was to determine if arachidonic acid (20:4n-6) is incorporated into inner (IM) and outer (OM) nuclear membranes of rat liver cell nuclei during its trafficking from cytosol to nuclear pools. This acid regulates the expression of certain genes in the nucleus. For this reason, IM and OM were separated from isolated nuclei of rat liver cells. OM was solubilized using sodium citrate, and the resulting nucleopast was digested with DNase 1. Membranes were recovered by centrifugation in a sucrose discontinuous gradient. Then IM and OM were incubated *in vitro* with [1-^{14}C]20:4n-6, and 20:4n-6 incorporation was assessed in the lipid pools. It was noted that [1-^{14}C]20:4n-6 acid was mainly esterified in PL of nuclear IM and OM in the presence of ATP and CoA. In both membranes, the acid incorporation takes place in PtdCho > PtdEtn > PtdIns. Incorporated radioactivity increased in PtdCho and decreased in PtdIns and PtdEtn with the incubation time. In conclusion, exogenous 20:4n-6 acid is mainly esterified in PtdCho of IM and OM isolated from rat liver cell nuclei through an acyl-CoA-dependent process. The incorporation mechanisms in both membranes would be independent each other.

LP-P23.
POLYMERIC LIPOSOMES: EVALUATION OF TOXICITY AND IMMUNOADJUVANTICITY

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In order to evaluate the immunoadjuvanticity of polymeric liposomes, different doses of liposomes associated with β-lactamase (gift of Dr. ME Ermácora) protein were administrated. Balb/c mice were inoculated i.p. with DC8,9PC:DMPC polymeric, non polymeric and natural lipids formulated liposomes, to compare the humoral immune response developed by each one of this systems. As a result, it was observed that mice treated with polymeric liposomes presented an antibody titre significantly higher than if treated with non polymeric or natural lipids vesicles. Besides, the toxicity of polymeric liposomes compared with the other formulations, was evaluated using bovine red blood cells and NIH 3T3 cell line. The results of haemolisys percentage show that none of the assayed formulations presented a high level of toxicity, or at least are less toxic than some drugs approved for medical use, in the assayed conditions. When considering viability of cell culture, the polymerization did not show any association with an increase of cell death even though the mechanism of action of polymeric lipids is not known.

LP-P24.
DEVELOPMENT OF A LIPOSOME BASED VACCINE AGAINST BOVINE ROTAVIRUS INFECTION

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Rotaviruses are an important cause of diarrhea in animals and are responsible for significant production losses in some livestock species. VP7 is the major protein of the outer capsid of the rotavirus particle. Antibodies against VP7 can neutralize the virus and protect against homologous virus challenge *in vivo*. Liposome mediated vaccination is a promising alternative to achieve protection against the infection as it avoids the potential secondary effects of live vaccines. In order to evaluate the immunoadjuvanticity of cationic and polymeric liposomes, different doses of liposomes associated with plasmid DNA encoding VP7 and cellular extracts from Sf9 cells infected with recombinant baculovirus expressing VP7 were administrated. Balb/c mice were inoculated i.d. twice with EPC:DOPE:DOTAP/ plasmid DNA and with a single dose of polymeric DC8,9PC:DMPC/protein extract. Humoral immune response developed by this system was compared to the one raised by the injection of naked DNA followed by the cellular protein extract. ELISA and Western Blot assay were performed to detect and titrate specific antibody response against VP7 protein.

LP-P25.
CHARACTERIZATION OF ACYL CARRIER PROTEIN EXPRESSION IN *BACILLUS SUBTILIS*

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To form spores, *Bacillus subtilis* requires de novo fatty acid biosynthesis. In this pathway, the acyl carrier protein (ACP) carries all the acyl intermediates and presents them to each enzyme of the pathway. It is unknown if *acp* is regulated in bacteria, therefore, we started to characterize the expression of the *acpA* gene and its function during the sporulation process in *B. subtilis*. In *B. subtilis acpA* is part of a cluster of genes which code for enzymes of the fatty acid and phospholipid biosynthesis, but by *in-vitro* transcription we determined that *acpA* is transcribed from an internal promoter of that cluster. The expression of *PacpA-lacZ* fusion showed that this gene is not under the control of FapR, a global regulator of fatty acid biosynthesis, and that it was higher in *spoOA* mutant strains, being Spo0A the master regulator of the sporulation process. To characterize *acpA* expression during the sporulation, we constructed an ACP-GFP fusion and found that it localizes to the mother cell of the sporangium. Finally, gel shift assays shown that there is at least one protein of *B. subtilis* able to bind to the *acpA* promoter. These results are the first characterization of *acpA* gene regulation in bacteria and the evidences that we show suggest the existence of a mechanism that regulates the expression of *acpA* during the sporulation process.

MI-P1
REGULATION OF FOREIGN ADC GEN EXPRESSION IN *T. CRUZI*

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Previous work from our laboratory has shown that wild type *Trypanosoma cruzi* is auxotrophic for polyamines due to the absence of the ODC and ADC genes in the parasite genome.
After transfection of *T. cruzi* with a recombinant plasmid bearing the coding region of oat ADC inserted in an appropriate vector, we have obtained transgenic *T. cruzi* showing an appreciable level of expression of ADC enzymatic activity. The heterologous ADC has been characterized by identification of the products, agmatine formed during the catalysed reaction.
The expression of the forein gen was transient during the first two weeks after transfection showing decreasing levels of enzymatic activity. However, when the selective antibiotic (G418) was continuosly present in cultures of transformed parasites the enzyme activity increased again and ADC gen expression became stable. We have folowed the fate of the transforming vector by Southern hybridization analysis , PCR amplification and PFGE of DNA obtained from transgenic parasites. Our results indicated that during the period of transient expression of ADC the recombinant plasmid used for *T.cruzi*. transformation remained as episomes inside the parasites. Later on we detected two or more copies of plasmid integrated in *T.cruzi* genome with the simultaneous increase of ADC activity.

MI-P2.

THE FUNCTION OF RIBONUCLEASE G IN *ESCHERICHIA COLI* IS CONSTRAINED BY ITS AMINO AND CARBOXYL TERMINI

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RNase G is a homolog of the essential *E. coli* ribonuclease RNase E. Whereas RNase E plays a key role in the degradation of mRNA and the processing of tRNA and rRNA in *E. coli*, the biological functions of RNase G appear more limited. We report here that this difference in function is not merely a consequence of the significantly lower cellular concentration of RNase G, as overproducing wild-type RNase G at a level up to 20 times the normal cellular concentration of RNase E cannot compensate for the absence of RNase E in *E. coli*. Instead, RNase G can sustain the growth of RNase E-deficient *E. coli* cells only when it bears an unnatural extension at its amino terminus (e.g., MRKGINM) or carboxyl terminus (e.g., GHHHHHH). These extensions presumably enable RNase G to cleave critically important cellular RNAs whose efficient processing or degradation normally requires RNase E. Though able to restore growth to *E. coli* cells lacking RNase E, the finding that extending RNase G does not detectably improve tRNA processing suggests that RNase E is not essential for tRNA production and is required for cell growth because it plays an indispensable role in the maturation or decay of essential *E. coli* RNAs other than tRNA.

MI-P3.

ELECTROCHEMICAL POLARIZATION INDUCED CHANGES IN THE GROWTH OF PSEUDOMONAS FLUORESCENS (ATCC 17552) INDIVIDUAL CELLS AND BIOFILMS

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In this work the effects of surface electrochemical polarization on the growth of P. fluorescens (ATCC 17552) on gold electrodes were determined. Polarization to potentials positive and negative to the zero charge potential of gold, induced different changes in cell morphology, size at birth, duplication time, and biofilm structure. At negative potentials cells elongated and divided faster rendering long daughter cells that reached up to 3.8 μm immediately after division. Consequently, biofilms at this potentials were composed by large cells distributed in expanded microcolonies that protrude several micrometer to the solution. In contrast, under positive potentials, cells elongated slower and were shorter at division, even when the duplication time was virtually the same than in the previous case. The average length of daughter cells was around 2.5 μm. Biofilms grown under positive potentials were composed by short cells distributed in a large number of compact microcolonies, and were flatter than those grown under negative potentials. Changes were analyzed considering the possible influence of electric fields at the electrochemical interface, on membrane potential and the energetic metabolism of cells.

MI-P4.

A PROTEOMIC APPROACH FOR THE IDENTIFICATION OF NOVEL *Mycobacterium bovis* ANTIGENS

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Infection with *Mycobacterium bovis*, the causative organism of bovine tuberculosis is an important animal health problem. The disease control is carried out by intradermal test (IDT) with tuberculin and removal of positive animals. IDT is a cumbersome, *in vivo*, test. Thus, there is an important need for novel diagnostic tools for bovine tuberculosis. An interesting option is the utilization of gIFN test with whole blood.
M. bovis was cultivated and cell extract and culture supernatant were obtained., These materials were submitted to SDS-PAGE and molecular weight fractions were prepared by electroelution. The antigenicity of fractions was evaluated by gIFN assays using blood from infected cattle and those that had a high stimulation index were further separated by 2D electrophoresis to identify individual proteins. Gels were silver stained and spots examined for MALDI-TOF mass spectrometry. MS data were compared to genomic database and genes sequences were cloned into the expression vector as His-tag fusions. The following recombinant proteins were purified under native conditions: Rv1636 (14-kDa), Rv0138 (17-kDa), Rv3740 (50-kDa), Rv2524 (15-kDa) and Rv3919 (10-kDa). All of them demonstrated to be antigenic in gIFN assays. In conclusion, we obtained novel antigens from *M. bovis* using non-recombinant-native fractions and MS. This methodology may overcome certain drawbacks of recombinant libraries such as incomplete cloning or non-expression of posttransational modifications.

MI-P5.

CHARACTERIZATION OF THE REGULATORY MECHANISM OF THE VIRULENCE ASSOCIATED MCE OPERONS FROM *Mycobacterium tuberculosis*

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The analysis of *M. tuberculosis* genome revealed the existence of four homologous operons containing eight genes very similarly organized (*mce1, mce2, mce3* and *mce4*). We have recently demonstrated that mutant strains of *mce1, mce2* and *mce3* operons have a reduced ability to multiply/persist in mammalian host when inoculated in mice, strongly suggesting that these operons are involved in *M. tuberculosis* virulence.
At least two regulator genes are involved in *mce* expression in *M. tuberculosis*. Our group has found that a transcriptional regulator, called mce3R, down regulates *mce3* operon during the *in vitro* growing of *M. tuberculosis*. In this work we described the construction of a knockout *M. tuberculosis* strain in *mce3R* gene, obtained by allelic replacement. The expression profile of *mce* genes in this mutant strain was analysed by Western blotting using specific antibodies and the results were compared with those obtained for the wild type strain.

MI-P6.

IDENTIFICATION OF DISTANT CASPASE HOMOLOGUES IN THE GENOME OF *TRYPANOSOMA CRUZI*

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Metacaspases are putative caspase homologues, based on catalytic domain amino acid sequence, His-Cys catalytic diad conservation and predicted secondary structure. They were first identified by PSI-BLAST and are present in plants, fungi and protozoa. So far, five metacaspase genes have been identified in *T. brucei*. In *T. cruzi*, however, we found only sequences with homology to *TbMCA3* and *TbMCA5*. *TcMCA3* is present in approximately 16 copies per haploid genome arranged in two head-to-tail tandems, while *TcMCA5* is present as a single copy. In the vicinity of TcMCA3 ORFs we detected several retrotransposons which probably caused a great amplification of the TcMCA3 gene in *T. cruzi* and disruption in gene syntheny in comparison to *T. brucei*. Western blot experiments using antisera against the insoluble recombinant proteins showed that TcMCA3 and TcMCA5 are present in cell free extracts of different life cycle stages of *T. cruzi*. Although augmented caspase-like activity has been reported in *T. cruzi* undergoing apoptosis-like cell death, the nature of the enzyme responsible for these observations is not clear. We are producing now soluble recombinant metacaspases in order to determine whether they could be functional homologues to classical caspases.

MI-P7.

METACASPASES OF *TRYPANOSOMA CRUZI*: POSSIBLE CANDIDATES FOR PROGRAMMED CELL DEATH (PCD) MEDIATORS

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PCD is a biochemical process that plays an essential role in the development of multicellular organisms. PCD has also been reported to occur in unicellular organisms. *Trypanosoma cruzi*, the causative agent of Chagas disease, is able to undergo apoptosis-like cell death under a variety of stress conditions. In order to gain deeper insight into the mechanisms of this process we decided to study the members of a novel class of proteolytic enzymes called metacaspases (distant caspase homologues). Epimastigotes of *T. cruzi* undergo massive cell death after exposure to fresh human serum leading to DNA fragmentation and visualization of TUNEL-positive nuclei. During this process we could monitor the induction of a caspase-like activity exclusively in dying cells using the fluorescent *in vivo* caspase probe SR-VAD-FMK. We also observed, in immunofluorescence experiments, that metacaspases change their subcellular localization and co-localize with the SR-VAD-FMK signal. Moreover, preliminary experiments suggest that *T. cruzi* epimastigotes overexpressing the metacaspase 5 gene are more sensitive to serum induced cell death. Our results suggest that metacaspases are likely to be involved in the PCD of trypanosomatids, although further studies need to be carried out to confirm the mechanism of this process.

MI-P8.

EXPRESSION AND PARTIAL CHARACTERIZATION OF METALLOCARBOXYPEPTIDASES (MCPs) OF *TRYPANOSOMA CRUZI*

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The genome of *Trypanosoma cruzi*, the causative agent of Chagas Disease, encodes two MCPs of the M32 family, with 64% of identity between them: TcCP-1 and TcCP-2. These enzymes belong to a new family of peptidases whose members had been found so far exclusively in prokaryotes. This makes them possible candidates as targets for chemotherapy. Both TcCP-1 and TcCP-2 were expressed as active recombinant enzymes in *E. coli*. TcCP-1 was purified to homogeneity in three steps, namely gel filtration and two ion exchange chromatography steps. TcCP-1 acted optimally at pH 6.2 on furylacryloyl(FA)-Ala-Lys with a *Km* of 0.166 mM. Activity against N-carbobenzoxy-Ala-X (ZAX) substrates revealed a P1´ preference for basic and some neutral C-terminal residues. Western blot analysis using a polyclonal antiserum raised against recombinant TcCP-1 showed that the enzyme is expressed in all life cycle stages of *T. cruzi*. Indirect immunofluorescence staining suggested that the protein is localized in the parasite cytosol. The polyHis-tagged TcCP-2 recombinant enzyme was purified to homogeneity by IMAC (Co^{+2}). Preliminary characterization of this enzyme shows that it prefers FA-Phe-Phe at an optimum pH of 7.6-8.0. Therefore, the specificities of both MCPs are complementary.

MI-P9.

TOMATO SEED AND ROOT EXUDATES: ITS ROLE IN THE COLONIZATION BY *AZOSPIRILLUM*

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Our previous studies demonstrated that plant growth promotion and rhizosphere colonization by *Azospirillum. brasilense* strains BNM 65 and Sp7 were influenced by tomato genotype. To find the best combination of bacterial strain and tomato genotype we inoculated tomato seeds with seven *Azospirillum* spp. strains and evaluated the early stimulation in root hair production. To establish the role of seed and root exudates in the colonization of the spermosphere and rhizosphere, we determined the chemotactic response of the bacterial strains towards seeds exudates and analysed the composition of root exudates. We observed significant differences in the number of root hairs produced depending on the *Azospirillum* strain inoculated, while, no interaction between bacterial strain and tomato genotype was detected. The seven *Azospirillum* strains were positively chemo-attracted towards the seeds of cherry and fresh market tomato. The root exudates obtained were extracted with solvents of increased polarity and analysed by thin layer chromatography (TLC). Significant differences in R*f* values between genotypes were observed in the chloroform extract. The *n*-BuOH:AcOEt or H_2O extracts showed the same R*f* values but differences in their relative concentrations were evident.

MI-P10.

AZOSPIRILLUM INOCULATION INDUCES CHANGES IN THE MICROBIAL COMMUNITIES ASSOCIATED WITH TOMATO

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Community-level physiological profiles (CLPP) were used to characterize the bacterial community associated with roots and leaves of cherry and fresh market tomato inoculated or not with *Azospirillum brasilense* strain BNM65. Seeds were inoculated with BNM65 and germinated in a humid chamber for 48 h. One seed was sowed per pot and grown in a plant growth chamber. Fifty six days after sowing plants were harvested and extracts of rhizosphere, rhizoplane and phyllosphere were prepared. Extracts were inoculated in Ecoplates (Biolog™), incubated for 24, 48 and 72 h and absorbances were read at 590 nm. Data were analysed using Principal Components Analysis. There were differences in the patterns of carbon source utilization for the uninoculated rhizoplane, rhizosphere and leaf communities of both tomato genotypes. The inoculation with *A. brasilense* modified the patterns of carbon-source utilization: the rhizoplane and rhizosphere communities followed roughly the same trend, while the leaf community changed in a very different fashion. In conclusion, the inoculation with *A. brasilense* modified the species composition, their relative abundance or both in the microbial communities associated with either cherry or fresh market tomato.

MI-P11.

THE TWO POSITIVE GATA FACTORS, GLN3 AND GAT1, HAVE DIFFERENT ROLES IN THE REGULATION OF YEAST UGA4 GENE IN RESPONSE OF NUTRIENT AVAILABILITY

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The *Saccharomyces cerevisiae* targets of rapamicin, TOR1 and TOR2 are functionally and structurally conserved protein kinases that activate cell growth in response to nutrient availability, such as nitrogen and possibly carbon sources. Both TOR proteins interact with the GATA factors Gln3 and Gat1 and control their phosphorylation. Ure2 is a yaest preprionic protein that acts as an inhibitor of Gln3 and Gat1. The expression of the *UGA4* gene encoding a yeast permease, is controlled by these GATA factors among other proteins. In this study we analyse the regulation of *UGA4* by carbon and nitrogen source availability. Our results are indicating that the two positive GATA factors act on the UAS_{GATA} element present in the 5`-regulatory region of *UGA4* in different ways. While Gat1 confers a high basal expression level when low quality energy carbon source is present, Gln3 is modulating *UGA4* GABA-dependent induction. This modulation depends on the quality of carbon and nitrogen sources and it is probably mediated by both a different subcellular localization and a different activity level.

MI-P12.

INCORPORATION OF GLUCOSE INTO _ESCHERICHIA COLI_ PROTEIN

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The incubation of disrupted *Escherichia coli* with 8 µM UDP-[14C]glucose in the presence of Mn^{++} resulted in the incorporation of [14C]glucose into trichloroacetic acid insoluble reaction product (RP), non extractable by chloroform-methanol (C-M) and C-M-H_2O mixtures. The autoradiogram of the RP subjected to SDS-PAGE showed two contiguous bans of low rM, the slower moving one showing a complete dependence on Mn^{++}. Labeled ADPG did not replace UDPG. About two third of both, RP and the acceptor-transferase couple, were recovered in the pellet after centrifugation at 16,000 xg. The glucosylation reaction was reduced about five fold when *E. coli* was cultured in a medium containing glucose. Neither chase of the incorporated [14C]glucose nor change in the SDS-PAGE mobility was observed by incubation of the RP with 1.0 mM UDPG or ADPG. Serine and threonine were not involved in the glucose linkage to protein, judging from the stability of the RP to a β-elimination treatment. The incorporated glucose was not released from RP by digestion with α-amylase, amyloglucosidase or phosphorylase. As far as we know, no glucoprotein has been heretofore described in bacteria.

MI-P13.

EFFECT OF OVER-EXPRESSION OF _GUM_B AND _GUM_C GENES ON XANTHAN STRUCTURE

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The biosynthesis of the extracellular polysaccharide xanthan in *Xanthomonas campestris* pv. campestris is directed by a cluster of 12 genes, *gumB-gumM*. Several xanthan-deficient mutants of the wild-type strain 8004, which carry Tn5 insertions in this region of the chromosome, have previously been described. Insertion of a Tn5 transposon immediately upstream of *gumB* unable xanthan synthesis. This phenotype is restored by complementation with *gumB* and *gumC* to almost 30% of wild type levels, thus both genes are considered to be involved in xanthan polymerization and/or translocation. Another insertion of Tn5 in the *gumK* gene renders a mutant, strain 8396 that is only able to synthesize a polytrisaccharide composed of mannose and glucose. A liquid culture of this strain produces two polysaccharide fractions, one that is easily recovered by sticking to a revolving glass rod and another that must be recovered by centrifugation, this being probably due to its lower molecular weight. On this view, we found that over-expression of *gumB* or *gumC* alters the molecular characteristics of polytrisaccharide by modifying the polysaccharide fractions distribution, thus allowing the recovery of different proportions of them. The presence of *gumB* gene in high copy number increases the amount of the polytrisaccharide recovered by centrifugation at expenses of the other fraction, while the presence of high copy numbers of *gumC* increases the sticky polysaccharide fraction amount. We are now characterizing these polysaccharides fractions by atomic force microscopy. Our results point toward the fact that both, *gumB* and *gumC*, play a critical role on the polymerization degree and thus on the final structure of the polytrisaccharide.

MI-P14.
IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES IN THE HALOALKALIPHILIC ARCHAEON, *NATRONOCCOCUS OCCULTUS* USING RAP-PCR
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Extremophiles live in environment with conditions that are lethal for other life forms. Haloarchaea developed strategies to survive with high salt (4M NaCl) and high pH (pH10). Our research group characterized proteolytic systems in the haloalkaliphilic archaeon *N. occultus*. Besides, preliminary evidences were obtained about the occurrence of quorum sensing (q.s) in this archaeon. The use of RNA arbitrarily primed PCR (RAP-PCR) is a powerful tool to identify differencially expressed genes. Differences in gene expression can be detected using this methodology, as each sample generates its own unique cDNA fingerprint for each condition. The aim of this work was to study the differential gene expression in response to the transition from exponential to stationary phase in *N. occultus* using RAP-PCR The screening was directed to genes whose induction respond to high cellular density and that may be regulated by q.s. A preliminar analysis showed 2 transcripts that were induced in *N. occultus* cells supplemented with conditioned media suggesting that the corresponding genes may respond to "signal" present in the stationary phase. Another set of transcripts seemed to respond negatively to the transition from exponential to stationary phase. The identification and characterization of differentially expressed genes will help to understand the genetic and molecular mechanisms used in the cellular communication by haloarchaea in response to the adaptation to extreme conditions.
Supported by ANCyPT, UNMdP and CONICET.

MI-P15.
MOLECULAR DETECTION OF POLYCYCLIC AROMATIC HYDROCARBON-DEGRADING BACTERIA IN MARINE SEDIMENTS
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Marine sediments exposed to fuel spills, industrial wastes or shipping activities tend to accumulate polycyclic aromatic hydrocarbons (PAHs) due to their low aqueous solubility, low volatility and high affinity for particulate matter. Some of these compounds are highly toxic, mutagenic, teratogenic, and carcinogenic. In this work, we use PCR to detect catabolic genes involved in PAH biodegradation in marine sediments. Surficial intertidal sediment samples were collected in Puerto Madryn city, Chubut and Ushuaia city, Tierra del Fuego. Total DNA was purified from these samples using the FastDNA® SPIN kit for Soil (Q-BIOgene) and the UltraClean™ Soil DNA Isolation kit (MoBio Laboratories), applying the same bead beating step for cell lysis. In both methods, the majority of the obtained DNA was in the size range of 5 to 20 kb, and was found to be suitable for PCR amplification using bacterial 16S rRNA gene primers, suggesting the absence of PCR inhibition. The Q-BIOgene method, however, produced DNA yields 30 times higher than the MoBio method. PCR primers targeting the alpha subunit of initial PAH dioxygenases from marine bacteria were designed and applied to the purified DNA. Amplifications with these primers produced a band with the expected molecular weight in a contaminated sediment sample but not in less polluted sediments.

MI-P16.
CHARACTERIZATION OF THE TWO REGIONS COMPOSING THE *E. COLI* NADP-MALIC ENZYME
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Malic enzyme (ME) catalyses the oxidative decarboxylation of malate to yield pyruvate, CO_2 and NAD(P)H. Distinct isoforms of ME are expressed in both prokaryotic and eukaryotic organisms, where they play diverse metabolic roles. By sequence homology, two isoforms of ME have been detected in *E. coli* genome: sfcA and maeB. Both genes present high degree of homology with ME from different sources, having the product of maeB a carboxyl end extension that has not been found, until present, in any eukaryotic or Gram+ bacterial ME. The aim of the present work was the cloning and expression of the products from both regions separately: the N-terminal region that presents high degree of homology with ME and the C-terminal region of approximately 320 amino acids, which shows homology to phosphotransacetylase enzymes (EC 2.3.1.8). In this way, N- and C-terminal regions were cloned by PCR from *E. coli* K-12, ligated into the expression vector pET-32 and successfully expressed in *E. coli* BL21(DE3). The maeB deleted proteins obtained were purified and kinetically and structurally characterized. Although the product of the N-terminal region was found to retain malic enzyme activity, the properties of this deleted enzyme were different from that of the complete maeB protein. In this way, the presence of this extra region in the product of the maeB gene from Gram- bacteria may modify the enzyme in order to fulfil some particular function in these class of organisms.

MI-P17.
EFFECT OF F0.F1-ATPASE MUTANTS ON THE ACID-INDUCED AUTOLYSIS IN PNEUMOCOCCUS
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We have described that acidic stress in pneumococcus induced two different cellular processes. First, this pathogen induces an acid tolerance response (ATR), and after triggers a programmed cell death or autolysis. Recently, we also demonstrated the involvement of F0.F1-ATPase in the ATR mechanism by characterization of *atp* mutants (Cortes *et al.*, poster presentation). This enzyme facilitates the extrusion of protons from the cell cytoplasm, preventing a drop in the intracellular pH in bacteria. With the purpose to evaluate the F0.F1-ATPase effect on acid-induced autolysis, wild-type cells were incubated at pH 7,6 with 10 µM DCCD, an specific inhibitor of this enzyme that blocks its H^+-translocating activity. We found that DCCD-induced autolysis was similar to that one produced by acidic shock. To confirm the role of this enzyme in the acid-induced autolysis, two F0.F1-ATPase mutants were incubated at pH 5,6 and 37ºC for 6 hours. We observed that the *atpC* G47V strain, which showed an increased ATR, presented no lysis after 6 hours of incubation. On the contrary, the *atpC* V48L strain, which had a decreased ATR, showed an early induction of autolysis compared with the wild type strain. According to these data we conclude that functionality of the F0.F1-ATPase is required for preventing autolysis induced by acidic stress. Work is in progress to study the checkpoints by which pneumococcus, cultured under acidic conditions, pass from ATR to autolysis, or omit ATR producing directly autolysis.

MI-P18.
MOLECULAR CHARACTERIZATION OF PBP GENES IN β-LACTAMIC-RESISTANT PNEUMOCOCCAL STRAINS

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The target for β-lactamic (βL) antibiotics are enzymes involved in the cell-wall biosynthesis, known as PBPs (penicillin-binding proteins). The βL-resistant strains present altered PBPs that reduce their affinity to β-lactamics. The goal of this work was to characterize at molecular level the *pbp* gene mutations of clinical pneumococci circulating in Cordoba City. From twenty four βL-resistant strains isolated at Public Children Hospitals, we selected ten with the higher βL resistance. To identify the *pbp* mutations that confer βL resistance, these genes were amplified and PCR products and transformed to an uncapsulated βL-susceptible strain. For transformants with *pbp1a* or *pbp2b* altered genes, a two-fold increase was obtained using as reference the wild-type penicillin MIC, whereas *pbp2x* altered genes produced changes ranging from one to three-fold increase. For cefotaxime, *pbp2x* or *pbp2b* altered genes produced two- to three-fold increase in its MIC value. We could not find individual altered *pbp* genes able to confer MIC values equal to those from the original strain. To characterize these altered *pbp* genes, genetic polymorphisms were investigated by RFLP analysis. Two types for *pbp1a/pbp2b* and four types for *pbp2x* were detected. All clinical strains were genotyped by PCR-BOX, and four different box patterns were identified. We found no close relationship between *pbp* and *box* types, indicating that the βL-resistance spreading was caused by different strains, but harbouring a few *pbp* gene patterns that were transferred between them.

MI-P19.
EXPLOITING *Anaplasma marginale* GENOME

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The obligate intraerythrocytic bacterium *Anaplasma marginale* (Rickettsiales: Anaplasmataceae) is the most prevalent tick-borne livestock pathogen worldwide and is a severe constraint to animal health and production in tropic and subtropical regions of the world. Whole -genome sequencing of bacteria together with in silico analysis and different kinds of genomic approaches are powerful tools that allow prediction of genes that take part in microbial pathogenesis. Using the information that became available with the complete sequence of *A. marginale*, our study aimed at selecting approaches that led us to the identification of genes that code for putative virulence factors or immunogens. The selected methods were:- PhoA fusion system for identifying exported proteins;- PCR-based subtractive hybridization for detecting differential genes between two species;-Bioinformatics analysis. We studied eight gene products so far: five were identified using the PhoA fusion system, two were selected from the differential subtraction library and one was identified by bioinformatics analysis. We also found a polymorphic tandem repeats of 11 nucleotides located in a non-coding region of the genome. Among the selected genes, four are currently being evaluated to determine their antigenic potential and one of them is being characterized to elucidate its role as a virulence determinant. In addition, the tandem-repeat non-coding region is being validated as a diagnostic and epidemiological tool.

MI-P20.
INFLUENCE OF GLUCOSE ON THE FLOCCULATION OF *Kloeckera apiculata* IN CO-CULTURE WITH *Saccharomyces cerevisiae*

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Kloeckera apiculata mc1 isolated from wine posses a flocculent phenotype characterized by protein-carbohydrate interaction, stabilized by Ca^2. We carried out a study of the influence of glucose on the modification of the flocculent phenotype of *Kloeckera apiculata* in co-culture with *Saccharomyces cerevisiae*. The flocculation degree (DF) was determined by the method of Bonaly and Lematre and glucose by the glucose oxidase method. An increase of DF of pure culture of *Kloeckera apiculata* was observed in presence of increasing glucose concentrations. The flocculation was inhibited when the apiculate yeast was co-cultured with *Saccharomyces cerevisae*. In pure cultures of *Kloeckera apiculata*, the percentages of free cells excluded from the flocs were 92%, 61% and 53% in presence of 20, 50 y 100 g/l glucose, respectively. In mixed cultures, these percentages were higher (95%, 82% and 85%). In co-culture conditions, a higher rate of glucose consumption was observed as consequence of the greater sugar fermentative power of the elliptic yeasts. When the culture media were completely depleted of sugar, the flocculation of *Kloeckera apicuata* in pure and mixed culture was fully reverted. The results indicate that glucose is an activator of the *Kloeckera apiculata* mc1 flocculent phenotype. The carbohydrate could lead to different transduction pathways in yeast. In mixed cultures, the greater glucose consumption by *Saccharomyces cerevisae*, accelerates the flocculation reversion.

MI-P21.
A PUTATIVE POLY(ADP-RIBOSE)POLYMERASE IN *TRYPANOSOMA CRUZI*

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Poly(ADP-ribosyl)ation is a post-translational modification of nuclear proteins and has been suggested to regulate gene expression and amplification, cellular differentiation, DNA replication and repair, and maintenance of genomic stability. Using a PARP sequence from *D. discoideum* as reference we screened *T.cruzi* genome database (TIGR). A homologous sequence was identified and used to design PCR primers. The amplification product encodes a protein of 592 amino acids, with an expected molecular weight of 65 kDa and isoelectric point of 9.5. Primary structure showed high homology with members of the PARP family, mainly PARP-2 type. Presence of carboxyl-terminal catalytic, autoregulatory, DNA binding domains, and the aminoacids considered as the PARP signature (TGYMFGKG), was confirmed. Southern blot showed a single-copy gene in epimastigote genomic *T.cruzi* DNA. A single transcript of about 2 kb was observed by Northern blot of epimastigote *T.cruzi* RNA. The cloning was performed in different expression systems. *E.coli* BL21 (DE3)pLysE-pRSET-A yielded TcPARP fused to 6 histidines as inclusion bodies and proteolytic fragments. Soluble recombinant protein was obtained with *E.coli* DH5α-pMALc2 as a fusion protein to the MBP. The recombinant product was affinity purified and used to test the requirements for the activity assay. By indirect immuno-fluorescence assay we observed kinetoplastic and nuclear PARP localization in *T.cruzi* epimastigotes.

MI-P22.
PLASTIC MATERIAL BIODEGRADABILITY IN SOIL ENVIRONMENT

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The development of biodegradable plastics made from renewable biomass, emerges as a key alternative to solve severe environmental problems. The verification of new materials biodegradability, impose the implementation of methodologies, according to international test material norms. In this study, the biodegradability of plastic products previously prepared from proteins and starch isolated from beans of the North West of Argentina was analyzed in a soil microcosm based in the aerobic microbial population activity, found in soil and compost mixtures. The incubations were performed under controlled temperature and water content conditions, until the materials were apparently disintegrated totally. Periodically the degree of degradation was determined by carbon dioxide production trapped by the alkaline medium and by weight loss, complemented with scanning electronic microscopy of the material surface before and after degradation. After to months of incubation, the test materials were degraded until at least 50% of carbon content was oxidized. At the 15th days, a loss of about 30% in weigh was detected. This was reflected at the electronic micrograph which reveals a high degree of alteration on the materials surfaces. We conclude that the plastic compounds tested have a satisfactory capacity of biodegradation in a soil environment under laboratory conditions.

MI-P23.
PLASMID OF SIMILAR SIZE TO THE PYV VIRULENT PLASMID FOUND IN *YERSINIA FREDERIKSENII* AND *YERSINIA INTERMEDIA* STRAINS

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The genus *Yersinia* is composed by eleven species, of which, Y. *pestis, Y. enterocolitica*, and *Y. pseudotuberculosis* have clearly been shown to cause human infections. The remaining eight species, commonly referred as *Y. enterocolitica*-like bacteria have not been studied as extensively as those three, and not yet clearly demonstrated to cause human disease. The three recognized pathogenic species always possess a 70 Kb plasmid called pYV. Virulence is associated to this plasmid, and chromosomally encoded genes. The objective of this study was to analyze the plasmid profile of two *Y. enterocolita*-like species, *Y. frederiksenii* and *Y. intermidia* strains, previously isolated from the environment, by plasmid extraction and determine the presence of pYV by detecting plasmid encoded virulence genes (*virF, yadA* and V-antigen gene) by PCR. For DNA preparation one distinct colony from an agar plate was transferred to lysis buffer a processed for gene amplification. European pathogenic Y11, and American Y286 strains, were used as positive controls. Even though plasmids of similar pYV size were found, none of the plasmid encoded genes analyzed were detected. The obtained results indicate that virulent pYV genes are not present in these *Y. frederiksenii* and *Y. intermidia* strains plasmids.

MI-P24
ROLE OF MOLECULES INVOLVED IN PEANUT-RHIZOBIA INTERACTION UNDER ENVIRONMENTAL STRESS

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To improve the yield of peanut crops it is necessary to know the adaptative response of the nitrogen fixer micro-organisms that interact with the crops. There are bacterial components such as LPS, proteins and lipids that take part in peanut-rhizobia interaction. In this work we analysed proteins, phospholipids and fatty acids profiles of peanut-symbiotic rhizobia and also the effect of temperature and salinity on the mentioned molecules. The strains used were *Bradyrhizobium* USDA 3187 and *Rhizobium* TAL 1000, both were grown in control and stressed medium. Proteins were subjected to SDS-PAGE. Lipids were separated using TLC plates and the unsaturation degree was analysed in TLC plates impregnated with $AgNO_3$. Under stress there was a change in protein profile, bands of 0.15 and 0.24 Rf were not detected under salinity and bands that belong to 0.16 and 0.50 Rf appeared because of temperature stress. Phospholipids concentration was modified by both kinds of stress recording a 38% increase under saline stress and a 40% decrease under thermal stress. Phospholipids profile was modified mainly because of temperature with which CL increased an 80% and the fraction formed by PE + PG decrease a 37%. Hyperthermic stress reduced the unsaturation degree of fatty acids. These results suggest that lipids and proteins are implicated in the adaptative response of rhizobia that interact with peanut crops.

MI-P25.
IDENTIFICATION OF GENES REQUIRED FOR TESTOSTERONE CATABOLISM AND PRODUCTION OF A DIFFUSIBLE SIGNAL MOLECULE IN *COMAMONAS TESTOSTERONI*

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The gene loci *teor*, encoding acyl-coenzyme A [acyl-CoA] dehydrogenase homolog, *tead* encoding enoyl-CoA dehydratase homolog and *tekt* encoding a β-ketothiolase homolog which are involved in the catabolism of testosterone in *Comamonas testosteroni*, were localized on a DNA region upstream of the *teiR* transcriptional regulator recently described. To prove the essential involvement of *teor*, *tead* and *tekt* genes in the catabolism of testosterone in *C. testosteroni*, these genes were inactivated separately by the insertion of omega elements. The corresponding mutant strains were not able to grow on testosterone as well as to activate a steroid-inducible transcriptional fusion. In conclusion, the data demonstrate the necessity of a beta-oxidation cycle for testosterone degradation suggesting the activation of a testosterone metabolite to the corresponding CoA ester. The *teor* mutant phenotype complemented with a plasmid encoding *teiR* can be restored by a diffusible extracellular factor (DSF) produced by C. *testosteroni* wt or *C.testosteroni teiR* mutant grown in presence of testosterone. We propose a model for the DSF system, which represents a novel mechanism for regulating testosterone catabolism revealing a connection between the upper pathway and the TCA cycle intermediates.

MI-P26.
CHARACTERIZATION OF A GROUP OF ACYL-COA CARBOXILASES FROM *MYCOBACTERIUM TUBERCULOSIS*
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Acyl-CoA carboxylases are key enzymes for the synthesis of lipids in actinomycetes, as they provide the elongating units malonyl- and methylmalonyl-CoA. We characterized two acyl-CoA carboxylases complexes in *Streptomyces coelicolor*. They consist of an ß-subunit with the ability to carboxylate its covalently bound biotin group, a ß-subunit bearing the carboxyl transferase activity and a small ε-subunit. In *M. tuberculosis* three complete carboxylase systems could be found, each consisting of an α-(*AccA*) and a β-(*AccD*) subunit, as well as three β-subunits without an α-counterpart. There is also an ORF associated with the β-subunit *AccD5* that codes for a putative ε-subunit. Here we present the biochemical and structural characterization of a group of essential acyl-CoA carboxylases from *M. tuberculosis*. Three acyl-CoA carboxylases have been successfully reconstituted from their purified components. The three complexes consist of a specific β subunit (AccD4, AccD5, and AccD6) and share the same biotinylated α subunit (AccA3) and ε subunit (AccE5). For all complexes the addition of AccE5 dramatically increased the specific activity of the enzymes. Also, all complexes were able to carboxylate both acetyl and propionyl-CoA, although a clear preference for propionyl-CoA was evident. This is the first report of the presence of a functional e subunit in *M. tuberculosis*. These studies aim to provide a novel drug design target for tuberculosis.

MI-P27.
TRANSCRIPTIONAL REGULATION OF *macs1-fadD1* OPERON ENCODING TWO ACYL-COENZYME A SYNTHETHASES INVOLVED IN THE PHYSIOLOGICAL DIFFERENTIATION OF *S. COELICOLOR*
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fadD1 of *Streptomyces coelicolor* 3A(2) is part of the bicistronic *macs1-fadD1* operon. Both genes encode for acyl-CoA synthetases (ACS). FadD1 has a broad range of substrate specificity, although saturated long-chain fatty acids appear to be the preferred substrates. A mutation in *fadD1* alters the levels of other ACS(s) and the mutant strain also shows a severe deficiency in antibiotic production. Actinohordin production was restored by the introduction of a wild type *fadD1* allele into the cell, demonstrating a strict link between the FadD1 activity and the biosynthesis of secondary metabolites. In this work we investigate the *macs1-fadD1* transcriptional regulation. Gene expression assays clearly show that this operon is tightly regulated and that its induction only occurs when the cultures reach stationary phase, independently of the carbon source present in the media. We identified a novel regulator gene named *acsR*. This gene encodes a positive transcriptional regulator that is necessary to enhance the expression of *macs1-fadD1*. AcsR directly binds to the DNA sequence of the operon promoter region. We identified the operator sites recognized by this transcription factor. Furthermore, the regulatory role of AsR also influences the proper timing and final titers of the actinorhodin antibiotic production.

MI-P28.
STRUCTURE BASED ENGINEERING OF SUBSTRATE SPECIFICITY OF THE BETA SUBUNIT OF ACYL-COA CARBOXYLASE OF *STREPTOMYCES COELICOLOR* A3(2)
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Acetyl-CoA carboxylase (ACC) and propionyl-CoA carboxylase (PCC) catalyze the carboxylation of acetyl- and propionyl-CoA to generate malonyl- and methylmalonyl-CoA, respectively. Understanding the substrate specificity of ACC and PCC will help to develop novel structure-based inhibitors that are potential therapeutics against infectious disease. It would also facilitate bioengineering to provide novel extender units for polyketide biosynthesis. *Streptomyces coelicolor* ACC and PCC are multisubunit complexes. The core catalytic beta subunits, PccB and AccB, are 360 kDa homohexamers, catalyzing the transcarboxylation between biotin and acyl-CoAs. Apo and substrate-bound crystal structures of PccB hexamers were solved to 2.0 - 2.8 Å. The hexamer assembly forms a ring-shaped complex. The hydrophobic, highly conserved biotin-binding pocket was identified for the first time. Biotin and propionyl-CoA bind perpendicular to each other in the active site, where two oxyanion holes were identified. N1 of biotin is proposed to be the active site base. Structure-based mutagenesis at a single residue of PccB and AccB allowed interconversion of the substrate specificity of ACC and PCC. The di-domain, dimeric interaction is crucial for enzyme catalysis, stability and substrate specificity; these features are also highly conserved among biotin-dependent carboxyltransferases.

MI-P29
CELL TO CELL COMMUNICATION AND COMMUNITY FORMATIONS: ROLE OF BORATED PHEROMONES (AI-2) IN *Bacillus subtilis*
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Certain species of bacteria alter their behavior in response to intercellular signals known as autoinducers (AIs). This phenomenon, quorum sensing, allows bacteria to sense their population density through detection of the relative concentration of autoinducers in the environment. The spore-forming bacterium *B. subtilis* is able to form highly organized three dimensional structures such as biofilms and fruiting bodies. In addition, it can spread and colonize by a swarming strategy. In this work, we demonstrated that *B. subtilis luxS* gene is essential for the autoinducer-2 (AI-2) synthesis pathway, produced from S-adenosylmethionine in at least three enzymatic steps. This gene was transcripted at low levels in the wild type strain, meanwhile, an isogenic *luxS* mutant strain showed upregulation of *luxS* expression. Additionally, the hipper-induction of *luxS* by the mutant strain was abolished after the addition of the pheromone (AI-2) present in the supernatant of wild type cultures pointing out for the existence of a negative autoregulation of *luxS* expression. Additionally, we observed that the ability of the *luxS* mutant strain to form multicellular communities structures such as biofilms and fruiting bodies were significantly impaired. Our perspectives are to determinate the mechanism by which *B. subtilis* produces and senses the AI-2 and how this pheromone is used for cell to cell communication between different species.

MI-P30.
VOLATILE ORGANIC COMPOUNDS RELEASED BY THE ENTOMOPATHOGENIC FUNGI *BEAUVERIA BASSIANA*

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Hydrocarbon-growth adaptation of entomopathogenic fungi has been recently proposed as a method to reduce the time to kill its insect host. The effect of hydrocarbon utilization as the sole carbon source on the composition of fungal volatiles was investigated. Volatile organic compounds (VOC) released by the entomopathogenic fungi *Beauveria bassiana* were analyzed by solid phase microextraction (SPME) coupled to capillary gas chromatography (CGC) - mass spectrometry (MS). Fungi was grown on an incubation medium supplemented with an exogenous carbon source, either glucose or *n*-octacosane, an insect-like hydrocarbon. A 65-µm polidimethylsiloxane/divynilbenzene (PDMS/DVB) fiber was used to collect fungal volatiles. Decane accounted for almost 95% of the VOC after alkane-growth adaptation, whereas isopropyl methyl naphtalene was the major component detected in glucose-grown cultures.
Present results suggest that fungal VOC measurement is a simple and fast method to evaluate fungal adaptation to different carbon sources.

MI-P31.
PURIFICATION AND CHARACTERIZATION OF TWO CATALASES FROM THE ENTOMOPATHOGENIC FUNGUS *BEAUVERIA BASSIANA*

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Fungal catalases are a diverse group of enzymes with regard to their structure, function and cell localization. These tetrameric hemoproteins are classified in the "small-subunit" and the "large-subunit" groups. After purification to homogeneity by gel filtration and strong anion exchange chromatography, we found two different catalases in the entomopathogenic fungus *B. bassiana*. The peroxisomal enzyme, involved in ß-oxidation reactions, showed a molecular mass of 54.7 kDa for each subunity. For the cytosolic form, a longer molecular mass (84 kDa) was estimated, exhibiting a Soret peak at 406 nm in the absorption spectrum. Unlike the bifunctional catalase-peroxidases, and in coincidence with other fungal cytosolic catalases, this enzyme was not inhibited by high concentration of substrate (hydrogen peroxide), with a Km value of 35 mM, and 92% inhibition was detected after 10 min incubation with 3-amino-1,2,4-triazole. In addition, a broad range of optimal pH (6 to 11) and temperature (25 to 50ºC), with 35% activity remaining at 75ºC, were found. Present data showed the existence of two different catalases in *B. bassiana*, a peroxisomal form belonging to the small-subunit group and a monofunctional cytosolic form that belongs to the large-subunit group.

MI-P32.
GENE EXPRESSION PATTERNS IN THE EUGLENOID *EUGLENA GRACILIS*

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We spotted 1000 EST to generate cDNA microarrays to asses gene expression patterns in cultured *E. gracilis* cells after exposure to different types of environmental stress: streptomycin, darkness and chromium. After the chromium treatment, an overexpression of the putative *E. gracilis* SOD enzyme (one of the main natural antioxidant enzyme) was evident, together with an increase of mRNA levels encoding for metabolic enzymes. A decrease on the expression of the H^+-ATPase gene was observed, that might be caused by depolarization due to the metal crossing the membrane. Antibiotic treatment provoked a shift to heterotrophy, that could be followed as an increase in the mRNA encoding for glyxolytic enzymes. A decrease of diverse mRNAs encoding for bleaching indicative proteins like ribulose diP carbox., cyt.552, apoproteins and photosystem precursor was detected. Darkness induced similar results to those caused by the streptomycin treatment, although changes in expression levels were not so marked. We identified 21 and 14 ESTs following the behaviour of mRNA encoding for antioxidant enzymes and membrane proteins upon chromium treatment, respectively. In the streptomycin treatment, 30, 15, and 4 ESTs behaved as mRNAs of bleaching indicative proteins, chloroplast proteins and chlorophyll proteins, respectively. These findings will serve as a stimulus for going on increasing *E.gracilis* nuclear molecular data and trying to understand its behavior against environmental stress conditions.

MI-P33.
PHOSPHORYLCHOLINE PHOSPHATASE ACTIVITY IS ENHANCED BY A LYSR REGULATOR

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Phosphorylcholine phosphatase (PChP) of *P. aeruginosa* is a enzyme that acts coordinately with haemolytic phospholipase C to break down phosphatidylcholine into choline and Pi. Recently we have found the gene responsible for this activity, named *pchP*. By Western blot using anti PChP antibodies we could demonstrate the presence of this protein when *P. aeruginosa* grows with choline, betaine, dimethylglycine, carnitine, but not with succinate/NH_4. *pchP* gene is located at positions 5858257 to 5957208 in *P. aeruginosa* PAO1 genome. Upstream of *pchP*, a probable LysR regulator (PA5293) is located. In the present work we confirmed induction of *pchP* by choline or derivatives by Northern blot, and showed that *pchP* is transcripted monocistronically. In order to determine if probable LysR regulator located upstream of *pchP* is involved in regulation of this gene, we studied mutant 42488 from UWGC mutant collection, with a *IsphoA/hah* insertion in PA5293 gene. This mutant showed a 70% depletion of PChP activity, but nearly wild type levels were recovered when it was complemented by introduction of wild PA5293 gene in pBBR1MCS-5 vector. Western blot experiments support these observations. These results led us to propose that PA5293 gene encodes a transcriptional regulator involved in *pchP* activation.

MI-P34.

GLYCINE BETAINE TRANSMETHYLASE GENE OF *Pseudomonas aeruginosa*: SEQUENCE ANALYSIS TO OVEREXPRESS THE PROTEIN

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Glycine betaine transmethylase gene (*gbt*) had been identified in *P. aeruginosa* Fildes III. Based on DNA sequence analysis, it was located in the published genome of PAO1 as PA3082 gene. Analysis of this locus revealed two putatives overlapping ORFs in different frames, ORF1 (1.965 bp) and ORF3 (1.542 bp) with an ATG methionine and a CTG leucine initiation codon, respectively. The aim of our work is to characterize the GBT protein after its overexpression in an adequate vector. In the present work, we study which ORF encodes this protein. Different sequences, named M, T and J, in frame with ORF3 were amplified by PCR using Taq polymerase and subcloned in pET-15b expression vector. Efforts to overexpress the His-Tag fusion proteins in *E. coli* BL21(DE3) were unsuccessful. This failed protein expression was explained after DNA sequencing since a stop codon was observed as a consequence of transition mutation in ORFM and T. ORFJ presented a deletion in two nucleotides. High fidelity Pfu Turbo polymerase was used to amplified ORFT and ORF1, in order to reduce possible mistakes introduced by Taq polymerase. After sequencing, the same transition mutation was observed in both ORFs. These results suggested that Taq did not introduce the mutation, but it was present in *P. aeruginosa* Fildes III gene. Then, ORF1 would encode and allow the overexpression of a functionally GBT protein in spite of the transition mutation.

MI-P35.

CONSTITUTIVE OVEREXPRESSION OF A HETEROLOGOUS AGGLUTININ IN *RHIZOBIUM ETLI* INTERFERES N2 FIXATION IN *PHASEOLUS VULGARIS*

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The agglutinin known as RapA1 from *R. leguminosarum* bv. *trifolii* was constitutively overexpressed in a multicopy plasmid under the control of plac promoter in this same species as well as in *R. etli* or *R. tropici*. By comparison with control strains carrying the plasmid vector without *rapA1* insert we observed that although the host specificity did not change, only the *R. etli* strain overexpressing RapA1 constitutively was affected in nodulation. It produced less total nodules and at a lower rate than the wild-type with plasmid vector alone. Neither a truncated form of RapA1 lacking its C-terminal end nor the entire gene overexpressed under the control of its own promoter had any effect. Forty day-old plants -at the beginning of flowering- maintained with N-free solution were dwarf and yellow when inoculated with the constitutively overexpressing strain, as uninoculated controls. By difference, the controls inoculated with the wild-type with plasmid vector alone were green and healthy, pointing out that the constitutive overexpression of RapA1 interfered N_2 fixation.

MI-P36.

THE WATER COLUMN AS ATTENUATING FACTOR OF UV-INDUCED BACTERIAL MORTALITY IN ANTARCTICA

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The increase in UV-B radiation (280-320 nm) caused by ozone depletion affect the activity of aquatic microorganisms principally heterotrophic bacteria. The aim of this research was to study the effects of solar UV-B and UV-A (320-400 nm) on two Antarctic marine bacterial strains (*Cithophaga-Flavobacterium-Bacteroides* group-related UVps and *Arthrobacter*-UVvi) in the first meters on the water column. Several assays were carried out under different irradiance conditions on Potter Cove (South Shetland Islands, Antarctica). Quartz bottles containing mixed bacterial cultures were exposed to solar radiation at 0, 1 and 3 m deep. These experiments were carried out with or without interferential filters. Results showed that the deleterious effect of solar radiation was significant at 1 m but not at 3 m deep. When filters were present, the strains showed higher mortality values under UV-A+UV-B radiation than those observed under UV-A only. When a vertical mixing of 4 m h^{-1} was simulated, the negative effect of UVR was reduced although a significant decrease in viability was observed in the irradiated systems compared to the dark control. Results suggested that the proposed deleterious effect of UV radiation on marine bacteria would be significant mainly in the 1 m surface layer of the Potter Cove marine water column.

MI-P37.

CHARACTERIZATION OF THE GENES INVOLVED IN CITRATE FERMENTATION IN *ENTEROCOCCUS FAECALIS*

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Enterococci compose the microbial association of a variety of fermented foods such as cheese, fermented sausages and fermented vegetables. However, members of the genus Enterococcus have distinguished themselves from other lactic acid bacteria by their role in human infection, harboring a number of identified virulence factors, and for their acquired resistance to antibiotics. The purpose of the present work was to study the Citrate metabolism in *E. faecalis*. We characterized a cluster of 12 genes involved in the citrate fermentation. On the basis of their homology with previously characterized proteins we identified the citrate transporter (citH), the subunits of citrate lyase (citD, citE, citF) and the accessory genes (citC, citX and citG). Furthermore, we identified two oxalacetate decarboxylases (OAD): a soluble protein named citM and the membrane-bound biotin-dependent OAD complex (oadA, oadB, oadD). Finally, we found a transcriptional regulator (citR). Uptake experiments in *E. coli* expressing citH, shown specific requirement of Ca^{2+} for citrate uptake. Citrate lyase activity was determined in total extracts of *E. faecalis*, showing higher levels in those extracts obtained from cultures grown in presence of citrate. CitM was expressed in *E. coli*, purified as a his-tagged protein, and the kinetics parameters were determined (K_m value for oxaloacetate was 0.453 mM and V_{max} 17.7 µmol/min). Transcriptional analysis shown divergent operons citHR and oadABcitCDEFXoadDcitMG which are inducible by citrate.

MI-P38.

THE *BACILLUS SUBTILIS* YUTB GENE ENCODES A LIPOATE SYNTHETASE

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Iso- and anteiso-branched-chain fatty acids are the major components of *Bacillus subtilis* phospholipids, being essential to maintain the appropriate fluidity of the membrane. The enzyme responsible for the formation of the primers for the synthesis of these fatty acids is the branched-chain a-ketoacid dehydrogenase complex, which requires lipoic acid as coenzyme. Genome sequencing revealed that a *B. subtilis* gene, *yutB*, presents high homology with *E. coli lipA*, whose gene product has been genetically linked to the lipoic acid biosynthesis. We constructed a conditional *yutB* mutant, under the control of the xylose promoter, EL104. This strain displayed growth defects in minimal media, but growth was restored with addition of xylose, lipoic acid, or branched-chain fatty acid precursors. As determined by gas cromatography, EL104 presents increased levels of straight-chain fatty acids with the concomitant reduction of branched-chain fatty acids. Moreover, this mutant presents twice as much unsaturated fatty acids than the wild type, result coincident with the observed strong induction of the transcription of the desaturase gene. Moreover, a plasmid expressing *yutB* in the *E. coli lipA* mutant KER296 complemented its lipoic acid auxotrophy. These results suggest that *yutB* is responsible for the lipoic acid synthesis in *B. subtilis*.

MI-P39.

BIOSYNTHESIS OF MICROBIAL POLYESTER BY *AZOTOBACTER CHROOCOCCUM* 6B UTILIZING DIFFERENT ORGANIC ACIDS AND ALCOHOLS

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Microbial polyesters have attracted a great attention as biodegradable thermoplastics, their mechanical and physical properties are similar to those plastics obtained from non renovable resources. We studied the effect of different carbon source on the biosynthesis of the microbial polyester. *A. chroococcum* were grown in Burk´s medium at 30°C with aeration. Polyhydroxyalkanoates (PHAs) in the freeze-dried biomass samples were extracted with hot chloroform in a Soxhlet. The molecular mass was determined by gel permeation chromatography. Monomers were determined after methanolysis by gas chromatography. PHAs polymers produced by *A. chroococcum* from various carbon sources as substrate were only polyhydroxybutyrate with different molecular mass. When polyhydroxybutyrate homopolymer was produced from different organic acids, with even carbon number the polymer content was 40% lower than in Burk´s medium with glucose. When odd carbon number was used as the sole carbon source the chain length of the biopolymer was shorter and the molecular mass decreased from those polymers accumulated in glucose medium. When different alcohols, specially 1-butanol, were used the PHB content decreased. In the case of 1-butanol this alcohol induced cyst formation using the accumulated PHB. The physico-chemical properties of these polyesters can be regulated with the selected carbon source.

MI-P40.

SUPEROXIDE PRODUCTION WOULD BE A MICROCIN J25 ALTERNATIVE MECHANISM OF ACTION IN *E. coli*

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E. coli RNA polymerase (RNAP) is the intracellular target of microcin J25 (MccJ25). MccJ25 enters the cell after binding to a specific outer membrane receptor, FhuA. In *Salmonella* strains, MccJ25 acts on the cytoplasmic membrane affecting cell respiration. Previously, we obtained an RNAP mutant of MccJ25-hypersusceptible strain AB1133 (PA232), which showed a residual sensitivity. This result may be explained assuming the existence of an alternative mechanism of action of MccJ25, which is investigated herein. Although we did not detect any effect on cell respiration in PA232, an important inhibition was found when this strain was transformed with plasmid pGC01, carrying *fhuA*.

MccJ25 inhibits lactate and NADH dehydrogenase activities by 40% and 20 %, respectively, while cytochrome oxydase activity was increased by 25%. All measurements were made with a membrane preparation from strain AB1133. Notably, superoxide dismutase (SOD) reverted lactate dehydrogenase inhibition and growth inhibition of PA232 (pGC01) induced by MccJ25. Furthermore, we found a 25% increase in superoxide production by PA232 (pGC01) in the presence of MccJ25. We conclude that in *E. coli* AB1133 an increment of superoxide production could also be responsible for MccJ25 antibiotic activity, together with the already known RNAP inhibition.

MI-P41.

SURVIVAL OF *PSEUDOMONAS* SP. IN WHEAT RHIZOSPHERE

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We have previously isolated native *Pseudomonas* sp. from Córdoba soil. The isolates were able to promote the growth of wheat under greenhouse. The objective of the present study was to investigate the survival of *Pseudomonas* sp in nosterile soil and their biocontrol ability. The strains 10bR and 4cR are spontaneous rifampicin resistant of *Pseudomonas* sp 10b and *Pseudomonas* sp 4c respectively. The viability of the mutants and their wild types were similar in LB medium and minimal medium MML. Wheat seedlings were grown in nonsterile soil under greenhouse. After one week, each seedling was inoculated with strains 10bR or 4cR to a density of 10^8 cfu per plant. Fifteen and thirty days postinoculation, bacteria were recovered from the rhizosfere, buck soil and roots. Bacteria were counted in nutrient agar with rifampicin and cycloheximide. No indigenous soil bacteria were grown in this medium in uninoculated plants (control). The number of *Pseudomonas* sp 10bR and *Pseudomonas* sp 4cR after 15 days was 10^4-10^5 cfu/g soil in the rhizosphere and buck soil. After 30 days, the number of viable cells of the strains was maintained. These results indicate that these strains could survive in nosterile soil competing with indigenous microorganisms.

MI-P42.
SUGAR COMPOSITION OF WHEAT INOCULATED WITH *MICROBACTERIUM SP.* UNDER SALT CONDITIONS
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Microbacterium sp. is an endophyte of grasses that promotes wheat growth under saline conditions in greenhouse (Marty and Mori, 2003). Sugar content was determined on rhizosphere as well as inside the plant. Seedlings cultured under saline conditions on tubes containing Hoagland´s solution were harvested and soluble sugars were extracted with ethanolic maceration. Treated plantlets (T) were subjected to 100mM ClNa stress, while treated inoculated plantlets (Ti) were subjected to the same stress conditions but were inoculated with 10^7 ufc/ml of *Microbacterium sp*. Macerates were vacuum dried and passed through a molecular exclusion column Biogel P 4 (Bio Rad). Fractions were collected and total sugars were assessed by the antrone technique. Positive fractions were joined and evaluated by HPLC with an anionic exchange column and an amperometric detector.
Qualitative changes were observed between plantlets subjected to different treatments specially inside the plant. Trehalose was found inside treated plantlets (T) but was not present on inoculated treated plantlets (Ti) at least on the conditions assayed.
On rhizosphere there was neither qualitative nor quantitative significant differences on the sugar composition.

MI-P43.
CHARACTERIZATION OF *RHIZOBIUM TROPICI* CIAT899 *GSHB* PROMOTER
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The tripeptide glutathione is ubiquitous among most organisms studied, being the most abundant low molecular weight thiol found in most cells. Many functions have been attributed to this compound, which include participation in sulphur metabolism, maintenance of cell redox state, disulfide bond formation, detoxification, and stress protection. We have previously obtained a *R. tropici* mutant in *gsh*B gene - which codifies for glutathione synthetase, the first enzyme in glutathione biosynthesis- which is sensitive to acid, oxidative and saline stress. We have shown that transcription of *gsh*B transiently increases in response to an acid shift, in addition a putative promoter upstream from the start of transcription of this gene was identified, by sequence analysis. This putative promoter sequence was found to show similarity to that of rhizobial *rec*A promoters, which suggests that *gsh*B could be part of rhizobial general SOS response. Further analysis of this upstream sequence by using gene fusions confirmed that it displays transcriptional promoter activity that indeed responds to an acidic environment.
Supported by ANPCYT PICT 7072.

MI-P44.
CHARACTERIZATION OF A *RHIZOBIUM TROPICI* CIAT899 SIDEROPHORE MUTANT STRAIN
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Microbes are constantly challenged by different enviromental adversities, that are overcome by going through different metabolic and physiologic adaptations. The thermosensible mutant strain *R. tropici* CIAT899-19A1 was isolated from a *R. tropici* Tn5-*luxAB* induced library. The mutant strain has a lag fase of 110 h in its growth at 38°C. Sequence analysis of the region flanking the unique Tn5-*lux* revealed an ORF homologous to ORF *Y4xN* of *Rhizobium sp*. NGR234, that itself shows homology to the *E. coli iucC* gene, which is involved in siderophore biosynthesis. Siderophore production was determined by using the CAS assay. Unlike the wild type, the mutant strain does not secrete siderophore in low Fe minimal medium at 28°C. Analysis of the supernatant from cell free culture in low Fe minimal medium by using the Csáky and Arnow procedures, allowed us to classify the *R. tropici* CIAT899 siderophore as hydroxamate type. The wild type and mutant strains were unable to grow under low iron minimal medium at 38ºC. The symbiotic plasmid-cured strain derived of *R. tropici* CIAT899, does not secrete siderophores and its DNA does not PCR amplify by using primers for the ORF mutated. These results indicated that most likely the *Y4xN*-like gene is carrried on plasmid. The ORF mutated seems to be dispensable in its symbiosis with common beans.
Supported by ANPCYT PICT 7072.

MI-P45.
HEPATITIS B VIRUS (HBV) QUASISPECIES EVOLUTION IN A CHRONICALLY INFECTED PATIENT DESPITE THE PRESENCE OF ANTI HBs ANTIBODIES (Ab)
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We have previously reported the unusual case of a HBV chronically infected patient despite the presence of usually neutralizing anti HBs Ab (Mathet *et al.*, *J Med Virol, 2003*).
In order to determine the HBV population dynamics through the infection course, S gene cloning was performed from PCR products obtained from serum samples collected throughout a 3-yr period (1995, 1997 and 1998; n=50).
Phylogenetic analyses ascribed all clones to genotype F.
The following findings deserve to be highlighted: 1) a higher degree of heterogeneity in the serum sample obtained at the end of the observation period, suggesting a positive selection; 2) a putative neck-bottle effect when S gene clones from 1997 were analysed; 3) a 10-fold higher substitution rate as compared with that reported for the same e^- phenotype; and 4) positional nt change at codons exhibiting mutations (i.e. from 3rd to 1st within the S gene, and from 1st to 2nd within the P gene).
Mutations detected along the HBs gene provided insights about the puzzling coexistence of both HBs Ag and anti HBs Ab, without viral clearance.

MI-P46.
EARLY INDUCTION OF EXTRACELLULAR PROTEASES IN THE HALOALKALIPHILIC ARCHAEA *NATRONOCOCCUS OCCULTUS* AND *NATRIALBA MAGADII*

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Many bacteria can communicate and have coordinate responses depending on cell density through a mechanism referred to as quorum sensing, however, this mechanism has not been demonstrated in archaea. The haloalkaliphilic archaea *N. occultus* and *Na. magadii* produce extracellular proteases in the late exponential and stationary growth phases as cell density increases. The aim of this study was to investigate the effect of stationary phase spent media on the production of the extracellular proteases of these archaea. A low density culture was centrifuged and the cells were suspended in stationary phase spent media or fresh medium. The cultures were incubated at 37°C with agitation and samples were withdrawn at various times for determination of extracellular proteolytic activity. An early induction of *N. occultus* and *Na magadii* proteolytic activities was observed in the cultures supplemented with conditioned media relative to the control cultures while the duplication times were similar. In *N. occultus*, these results were confirmed by the early accumulation of both the precursor and active forms of the protease in the cells and extracellular media by Western blotting. These results show that the production of the extracellular proteases of *N.occultus* and *Na magadii* is affected by extracellular "signals" present in stationary phase spent medium and suggest the occurrence of quorum sensing in the domain Archaea.
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MI-P47.
T-DNA TRANSFER AND PLASMID RESCUE FOR INSERTIONAL MUTAGENESIS IN *LACCARIA BICOLOR*

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The characterization of primary genetic traits controlling the mycorrhizal symbiosis development will help in understanding the ecological role of the ectomycorrhiza. The generation of null mutants for these genes has been so far hampered by the lack of transformation systems. In a previous work we reported the development of a transformation system for the ectomycorrhizal fungus *L. bicolor* by *Agrobacterium*. Herein we report the construction of a vector for a binary system in *Agrobacterium* carrying as a fungal selection marker the *hph* gene from *E. coli*, conferring resistance to hygromycin, under the control of a *Tuber. borchii* nitrate transporter promoter and *trpC* terminator from *Aspergillus niger*. The T-DNA also contains the *amp*^{r} and *ori* from plasmid pSP72 allowing marker rescue in *E. coli*. This construct has been tested for replication of the circularised T-DNA in *E. coli* and for transformation of *L. bicolor*. The presence of the *hph* and *amp*^{r} resistance genes has been demostrated by PCR. Currently, Southern blot analysis and marker rescueing from the fungal genome are under way. The transformation procedure described here is being used to generate mutants of *L. bicolor* impaired for mycorrhiza formation with Poplar.

MI-P48.
STABLE *AGROBACTERIUM* - TRANSFORMATION OF THE MYCORRHIZAL FUNGUS *LACCARIA BICOLOR*

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In nature most of the plants interact with soil fungi forming mycorrhiza. Among them ectomycorrhiza is crucial for survival and growth of forest trees. Development of EST sequencing, cDNA array and proteomics have been developed in order to understand the interactions during ectomycorrhizal functioning. The completion of the genome sequence of Poplar, the model perennial plant, has proved to be important for symbiosis research. In fact, the US Department of Energy Joint Genome Institute (JGI) is sequencing several Poplar symbionts, among them the ectomycorrhizal fungus *Laccaria bicolor*. *Laccaria* is an excellent choice as it has extensively been used in both basic and applied research. It is our aim to identify genes of interest, alter their expression and test their impact on symbiosis formation. For that purpose we need to count on an reliable an efficient transformation system. Vegetative mycelium of *L. bicolor* was transformed by *Agrobacterium*. The selection marker was the hygromycin resistance gene of *E. coli* (*hph*) under the control of the *gpd* promoter from *Agaricus bisporus* and the CaMV 35S terminator. PCR and Southern blot analyses showed that the genome of the hygromycin-resistant transformants contained the cassette. The latter proved single-copy and random integration of the transgene into the fungal genome.

MI-P49.
ANTI-*TRYPANOSOMA CRUZI* ACTIVITY OF EPIGALLOCATECHIN GALLATE

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Treatment of chagasic patients relies on two chemotherapeutic agents: Benznidazole and Nifurtimox. The severe limitations of this drugs in the Chagas' disease chemotherapy, encouraging the research of alternative more effective compounds for both, treatment and chemoprophylaxis of banked blood. We have recently reported the trypanocidal activity of some green tea catechins against infective and no infective forms of *Trypanosoma cruzi* in *in vitro* assays. Epigallocatechin gallate (EGCg) and gallocatechin gallate (GCg) were the two most active compounds with MBC_{50} values on blood trypomastigotes lower than 1 nM. EGCg was elected to study the mode of action of *in vitro* trypanocidal effect. Preliminary work demonstrated that this compound interfere with *T. cruzi* Cyt P450 reductase activity and induces an apoptotic response on cultured epimastigotes. The recently low IC_{50} values obtained on infected cultures cells and the protective effect observed on infected mice, strength the possibilities of EGCg as a valuable tool in Chagas' disease chemotherapy.

MI-P50.

IS IT POSSIBLE THE DIRECT ENERGY TRANSFER FROM THE LHII TO THE CR IN THE ABSENCE OF LHI ANTENNA COMPLEX?

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There are different results on the effectiveness of energy transfer from the peripheral light-harvesting (LH) complex 2 directly to the reaction center (RC) in mutant strains lacking the core LH1 complex. A LH1$^-$ mutant of *Rhodovulum sulfidophilum*, named rsLRI, was constructed by deleting the *puf* A and B genes, resulting in a Kanamycin resistant greenish-brown, photosynthetically positive clone.
Under photosynthetic conditions, those cells grew only at high-light intensities (500 Wm^{-2}). Below this value the efficiency of photosynthetic growth decreases and at 50 Wm^{-2} no photosynthetic growth was observed.
Light-induced FTIR difference spectra of wild type and rsLRI showed to have only marginal differences, indicating no large structural changes of the RC due to the deletion of LH1. The amount of RC in each strain was similar, according the bleaching of the absorption band at 865 nm (ΔA865, is indicative for the P→P+ transition in the reaction center).These results are interpreted as indicating that energy transfer between LH2 and RC is inefficient. It is possible that most of the energy necessary for photosynthetic growth was provided by light harvested directly by the RC.

MI-P51.

OXYGEN EFFECT ON SYNTHESIS OF PHOTOSYNTHETIC APPARATUS IN PHOTOSYNTHETIC BRADYRHIZOBIA

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The photobiological characteristics of an indigenous strain of phototosynthetic *Bradyrhizobium* strain was assessed. We present data on the effect of oxygen and light growth conditions on BChl accumulation and the purification of its light harvesting Bchl-polypeptide complex (LH1-RC complex), including the identification of the antenna polypeptides by partial amino acid sequencing of the α and ß polypeptides. *Bradyrhizobium* sp. C7T1 strain was grown on modified MAG broth at 30ºC in a 5L Bio-Flo III fermentor under different oxygen tensions and continuous dark, light or light-dark regimes. LH1 complex was purified by treating the membranes with LDAO and sucrose gradient centrifugation, and the purification of the α and ß polypeptides was performed by hydrophobic molecular sieving chromatography. BChl and carotenoids were measured after extraction with acetone:methanol. The strain C7T1 under either light-dark regimes or continuos darkness accumulate BChl. No growth was observed under anaerobic conditions in the light, and under continuous light no BChl was accumulated while canthaxanthin was accumulated during cell growth. However BChl synthesis increased under semiaerobic conditions (3% O2) reaching values up to 60% higher. This is at variance with what was observed for *Bradyrhizobium* BTAi1 and ORS278 strains. The oxygen dependence of bacterial photosynthetic activity appears to correlate with nitrogen fixation conditions occurring during stem symbiosis.

MI-P52.

THE *sae*RS OPERON OF *Staphylococcus aureus* MODULATES EXOPROTEOLYTIC ACTIVITY

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Staphylococcus aureus is a major pathogen of humans and animals which synthesizes a large number of extracellular and cell-wall associated proteins that contribute to its virulence. Several global regulatory loci regulate the production of these virulence factors, among which the most relevant are the *agr, sar* and *sae* loci. Global regulatory locus *sae* consists of a two-component signal transduction system coded by *saeR* and *saeS* genes that upregulates the synthesis of several exoproteins at the transcriptional level. *S. aureus* synthesizes several extracellular proteases. The synthesis of these proteases is positively regulated by *agr* and negatively regulated by *sarA*. The aim of the present study was to analyze the effect of the *sae* regulatory locus on exoprotease activity. Total protease activity from concentrated supernatants of cultures in late stationary phase, measured using azocasein as a substrate, showed a 3- to 4-fold increase with the *sae* mutant as compared to the wild type strain. This activity diminished about two-fold with treatment with EDTA. The *sae agr* double mutant showed a lower exoproteolytic activity than the *sae* mutant. Three bands with increased exoproteolytic activity were visualized with the *sae* mutant in sodium dodecyl sulfate polyacrylamide gels containing 0.1% gelatin. These results indicate that the *saeRS* operon, contrary to its effect as a positive regulator of several exoproteins, acts as a negative modulator of the activity of some exoproteases.

MI-P53.

PHENO AND GENOTYPING OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM HUMANS, BOVINE SUBCLINICAL MASTITIS AND FOOD SAMPLES IN ARGENTINA

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Staphylococcus aureus is an important pathogen. The aim of the present study was to characterize and compare pheno and genotypically 45 *S. aureus* strains isolated from humans, bovine mastitis and food samples in Argentina. Biotyping revealed that 50% of the human strains belonged to human ecovar and 60% of the bovine strains belonged to bovine ecovar. Resistances to various antibiotics could be observed for the human *S. aureus*, less pronounced for the bovine strains but not for the food samples strains. The strains could be further classified genotypically by *rep*-PCR and by amplification of the genes *spaA, coa, clfA, cna* (domains A and B), *cap5* and *8, agr* class I to III and *sae. rep*-PCR analyses and the different gene pattern revealed that the strains could be divided into groups matching with the origin of the isolates. According to the results of the present study, *S. aureus* strains isolated from different sources differ considerably in pheno and genotypic properties which provides evidence for a high degree of host specificity. These findings might be of importance for cases where cross infections are presumed.

MI-P54.
ADENYLATE CYCLASE ON *Bordetella pertussis* ATTACHMENT TO ALVEOLAR CELLS: ITS INTERACTION WITH FILAMENTOUS HAEMAGGLUTININ

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Bordetella pertussis is the etiological agent of whooping cough. Bacterial adherence to respiratory cells is a crucial step in colonisation. In this study we used alveolar epithelial human cells (A549) to evaluate the role of the main virulence factors on bacterial attachment. Our results showed that solely the lack of either Filamentous Hemagglutinin (FHA) or Adenylate Cyclase toxin (Cya) significantly decrease bacterial attachment rates compared to the wild type strain (80% and 40%, respectively). FHA is a well known adhesin but Cya has never been described involved in bacterial-cell interaction. Adherence assays of wild type bacteria ran in the presence or the absence of anti-FHA antibodies, anti-Cya polyclonal antibodies, or anti-Cya blocking monoclonal antibodies directed against either each or both toxic activities of Cya (adenylate cyclase and haemolitic) suggested that neither Cya has adhesive activity in this system nor any of the toxic activities of Cya are required for efficient interaction with alveolar cells. Recent studies have demonstrated Cya and FHA to be associated on the surface of *B. pertussis*. The lack of Cya might affect surface associated FHA eventually influencing *B. pertussis* attachment. Although immunoblot analysis showed the lack of Cya not to prevent FHA localisation in the outer membrane, attachment assays performed in the presence of heparin suggested that the absence of Cya indeed modifies the carbohidrate-binding site of FHA (the site involved in FHA-mediated bacterial binding to epithelial cells) in a way that it is less efficient in mediating bacterial attachment but it is not longer inhibitable by heparin. We propose the presence of Cya in the surface of *B. pertussis* to play a role in the functionality of FHA as an adhesin.

MI-P55.
DIFFERENTIAL EXPRESSION OF THE *Escherichia coli* NADH DEHYDROGENASE-2 DEPENDING ON THE MEDIUM AND THE PHASE OF GROWTH

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NADH dehydrogenase-2 (NDH-2) of *Escherichia coli* is an enzyme of the respiratory chain, coded by *ndh* gene. According to the bibliography, *ndh* is expressed only in exponential phase of growth due to the negative regulation of the global transcription factor FNR, in the absence of oxygen. In order to deepen the studies on expression, we worked with a strain that contains a plasmid with the *lacZ* gene under the *ndh* promoter. We measured the β-galactosidase activity along growth curves and we saw that the expression profile of the enzyme was coincident with the data reported previously when the cells were growing in LB (rich medium) or in MT (minimal medium). However, when the cells were growing in M9 minimal medium, the maximum level of expression was reached in early stationary phase and it was maintained in later stationary phase. These data were corroborated measuring the membrane NDH-2 activity in a wild-type strain grew in the different media and harvested either in exponential or in stationary phase of growth. In this work we demonstrated that the expression pattern of NDH-2 during the growth curves depends on the media. Further studies should be done to elucidate the participation of FNR, oxygen and metals in these processes.

MI-P56.
SPECIFIC EXTRACELLULAR REDUCTION OF CUPRIC IONS IN *Escherichia coli* DEPENDS EXCLUSIBILY ON CERTAIN REPIRATORY CHAIN COMPONENTS

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The aerobic respiratory chain (RC) of *Escherichia coli* is located in the inner membrane. We previously identified in the RC two sites of electron transfer to membrane-bound copper leading to reduction of Cu(II) to Cu(I): Site I, depending on NADH dehydrogenase-2 (NDH-2) and Site II, depending on quinones. We demonstrated also that purified NDH-2 has cupric-reductase activity. To elucidate the possible role in the extracellular Cu(II)-reduction of the members of these two Sites of the RC, we performed *in vivo* studies using *E. coli* strains that either contain or are deficient in the different components. Cells harvested in exponential phase of growth were exposed for 1 hour to 20 mM citrate-200 uM CuSO4 in the presence or in the absence of bathocuproine disulfonate. This chelator specifically binds Cu(I) and develops a colored complex. Our results demonstrated that the mutant in both NDH-2 and quinones did not generate extracellular Cu(I), while the negative mutants for quinones or NDH-2 reduce about 50% less Cu(II) than the wild-type strain. These results strongly evidence that NDH-2 and quinones are the main players involved in the cellular cupric-reduction, a relevant biological process related to the import/export system and the homeostasis of copper.

MI-P57.
GENOMIC VARIABILITY OF AN UNUSUALLY STABLE SINGLE CROSS-OVER AgMNPV RECOMBINANT

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Baculoviruses possess large circular double-stranded DNA genomes (80-180 kbp). They are infectious only to arthropods, principally lepidopterans, which makes them highly specific insecticides non-toxic to other species. They are also used as eukaryotic expression vectors. The classical method for introducing changes in a baculovirus genome relies on homologous recombination between the circular viral DNA and a transfer vector, both of which are transfected into insect host cells. An allelic replacement reaction involves two independent homologous recombination events. If both events occur a double recombinant is obtained (0.1-1% of the total virus population). Nevertheless, the single cross-over recombinant is the most common type, in which the entire plasmid has integrated into the viral genome. In these species, a second recombination event is highly probable due to the repeated homologous sequences. Single cross-over recombinants are usually resolved during a second round of amplification in cell culture. We constructed a transfer vector to introduce changes in *Anticarsia gemmatalis* nucleopolyhedrovirus (AgMNPV). After co-transfecting insect cells we isolated a single cross-over recombinant (I*Ppo*I-AgMNPV) as evidenced by DNA restriction patterns. This genome persisted after various rounds of amplification, in contrast to its expected resolution yielding the parental and the allelic replacement genomes.

MI-P58.

HIGH INTER- AND INTRA-STRAIN POLYMORPHISM IN GENES ENCODING ANTIGEN B FROM *ECHINOCOCCUS GRANULOSUS*

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Echinococcus granulosus, the causative agent of cystic hydatid disease, exists as a number of intraspecific variants that differ in biological characters, such as intermediate host specificity and infectivity to humans. One of the major antigens of the larval stage of the parasite, the heterodimeric antigen B (AgB), is widely used in diagnosis and has been involved in the evasion of host immune response. We used PCR-SSCP followed by sequencing to evaluate the intraspecific variation and expression of genes of the AgB family. Forty two genomic sequences were isolated and clustered in five genes (B1 to 5) by maximum parsimony analysis. The variants were differentially distributed among strains. Neutrality was rejected for AgB2 sequences. Intra-strain variation was also observed, mainly in sheep strain. The level of nucleotide and amino acid variation observed was higher than the reported so far for coding genes of other helminth parasites. Differential expression of AgB variants between protoscoleces of sheep and pig strains was detected by RT-PCR. These data demonstrate for the first time the genetic variability of antigen-coding genes among genetically characterised strains of *E. granulosus*.

MI-P59.

POLYAMINE METABOLISM DURING SCLEROTIAL DEVELOPMENT OF *Sclerotinia sclerotiorum*

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Polyamines (putrescine, spermidine and spermine) are polycationic compounds found in all living organisms. They have shown to be essential for growth and morphogenesis of several fungi. We have previously studied polyamine metabolism on different steps of the life-cycle of *Sclerotinia sclerotiorum* (1, 2). In this work we evaluated polyamines requirement for sclerotial development, and the effect of polyamine biosynthesis inhibitors on this process. Neither α-difluoro-methylornithine nor cyclohexylamine, inhibitors of putrescine and spermidine biosynthesis respectively, reduced the number of sclerotia produced *in vitro*. By the contrary, mycelial growth was impaired. Furthermore, the activity of key polyamine biosynthetic enzymes, as well as free spermidine and spermine levels, diminished during sclerotial maturation. These results suggest that even though vegetative growth requires suitable levels of polyamines, sclerotial development in turn seems to be less dependent on polyamine biosynthesis. Further considerations on polyamine metabolism concerning sclerotia maturation and the potential use of polyamine biosynthesis inhibitors to control sclerotial-forming fungal phytopathogens are discussed.

1-Gárriz et al. Archives of Microbiology 180: 169-175.
2-Gárriz et al., New Phytologist 161: 847-854.

MI-P60.

PARASITICIDAL EFFECTS OF NON-IMMUNOSUPPRESIVE CYCLOSPORIN A ANALOGS ON *TRYPANOSOMA CRUZI*

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In a previous report we showed that nonimmunosuppressive analogs of Cyclosporin A (CsA) H-7-94 and F-7-62 proved to be inhibitors of the protozoan parasite *Trypanosoma cruzi in vitro* and on the enzimatic activity of *Tc*CyP19 cyclophilin, the target of the CsA. This antimicrobial drug is immunossuppresive when is complexed with cyclophilins (CyPs). In this work we tested the trypanocidal activity *in vitro* and on the experimental infection in Balb/c mice, with NIM811 and MeVal4, two new nonimmunosuppressive CsA analogs, with anti-HIV activity.
NIM811 and MeVal4 CsA analogs had a potent inhibitory effects on the proliferation of *T. cruzi* epimastigotes (IC50 of 0.64 and 2.32 micromolar respectively, while CsA had an IC50 of 5.39 micromolar). NIM 811 and MeVal-4 did not show toxic effects on mammalian cells when tested up to 10 micromolar concentration. The effect on the inhibition of penetration of *T. cruzi* in Vero cells, at 25 micromolar drug concentration was 78.8% for NIM811 and 65% for MeVal4 respect to the medium control.
The *T. cruzi* experimental infection in mice resulted in a lower parasitemia and mortality in drug treated animals than controls.
We conclude these nonimmunosuppressive CsA analogs are interesting drugs to further study, in the search of novel effective drugs against Chagas´ disease.

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MI-P61.

IDENTIFICATION AND CHARACTERIZATION OF A NOVEL GLYCOGEN SYNTHASE GENE FROM *SYNECHOCYSTIS* SP. PCC 6803

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Cyanobacteria are prokaryotic organisms that perform oxygenic photosynthesis. They accumulate glycogen (main carbon storage compound) as corpuscles in the thylacoids. To date the enzymes involved in this polyglucan metabolism are not fully characterized. We have retrieved two open reading frames (ORF) from the genome sequence of the unicellular strain *Synechocystis* PCC6803 homologous to glycogen synthase (GS) genes (ORFs *sll*1393 and *sll*0945). Firstly we characterized *sll*1393 as a GS encoding sequence (*glg*A), about 46% identical to plant starch synthases. The aim of the present work was to isolate and characterized the other ORF *sll*0945 (*glg*B). After amplification of the ORF by PCR and cloning, we expressed the encoding sequence in *Escherichia coli*. Functional characterization of *glg*B was confirmed by biochemical and molecular studies of the recombinant protein and of the enzyme isolated from a Synechocystis mutant strain lacking GlgA (Δ*glg*A). Cyanobacterial GlgB (ca. 54 kDa) showed similar properties to bacterial GS. In addition, determination of glycogen content and expression analyses of *glg*A and *glg*B by RT-PCR were carried out in *Synechocystis* cells. We conclude that both GS contribute to glycogen synthesis in cells grown under different conditions.

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MI-P62.
CHARACTERIZATION OF A SUCROSE-PHOSPHATE SYNTHASE FROM A MARINE CYANOBACTERIUM
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One of the physiological responses for salt adaptation of cyanobacteria consists in the accumulation of osmoprotectants, such as glucosylglycerol (GG), trehalose and sucrose. We have been recently identified the biosynthesis of sucrose in cyanobacteria through the sequential action of sucrose-phosphate synthase (SPS) and sucrose-phosphate phosphatase (SPP). Particularly, the marine strain *Synechococcus* sp. PCC 7002 (Syc) accumulates GG and sucrose as second osmolyte. The present study describes the first isolation of a SPS encoding gene from a marine cyanobacterium, and the expression of this gene (Syc-*sps*A) in relation to the cell growth phase and to salt concentration. Syc-*sps*A encodes a 81-kDa polypeptide, which is 61% identical to *Synechocystis* sp. SPS, both bidomainal SPSs, composed by a Glucosyl-Transferase Domain (GTD) and a Phospho-Hydrolase Domain (PHD). Syc-*sps*A expression at the transcriptional level was markedly increased in cells at stationary phase and in high osmolarity conditions. As reported, starvation, growth rate modulation and osmoregulation seem to involve similar proteins, and a number of osmotically inducible genes are also growth-phase dependent. Additionally, sequence analysis of the Syc genome, revealed the existence of a second ORF encoding a putative PHD adjacent to Syc-*sps*A. This special genome arrangement, not found in other unicellular cyanobacteria, might be an evidence of possible gene duplication of a bidomainal SPS-like during evolution leading to extant SPPs.
Supported by ANPCyT, CONICET, FIBA and UNMdP.

MI-P63.
EXPRESSION OF SUCROSE-RELATED GENES IN RESPONSE TO SALT STRESS IN *Agrobacterium tumefaciens* C58
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Agrobacterium tumefaciens (*A.t.*) is an α-proteobacterium of the family Rhizobiaceae. It is an ubiquitous soil organism and the etiological agent of the crown gall disease of dicotyledonous plants. *A.t.* has been divided into three taxonomic groups based in part on the salt tolerance. Biotypes I and III are tolerant to 2% NaCl, while biotype II is not. The strain C58 belongs to biotype I. Sequence analysis of *A. t.* C58 genome revealed the existence of two sequences codifying for a Glucosyl-Transferase-Domain (GTD) and a Phospho-Hydrolase-Domain (PHD), characteristic of sucrose-biosynthesis-related proteins present in oxygenic photosynthetic organisms. The role of sucrose in salt tolerance has been reported in cyanobacteria and plants. The objective of this work was to investigate the possible role of those genes in NaCl stress in *A.t.* After cloning and expression of *At*-PHD, we showed that it codifies for a phosphohydrolase specific for the P-disaccharide. RT-PCR analyses revealed that GTD and PHD transcript levels increase with time of culturing. Moreover, their expressions were markedly higher under salt stress (0.5 M NaCl). As no significant amounts of sucrose could be detected in *A.t.* cells in similar conditions, we conclude that sucrose is not a salt osmolyte. However, the expression of those genes might be related with a salt response.
Supported by CONICET, UNMdP, FIBA.

MI-P64.
THE LEUCINE-RESPONSIVE REGULATORY PROTEIN (Lrp) IS INVOLVED IN MccJ25 RESISTANCE
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We have observed an intrinsic resistance of several *E. coli* strains to the peptide antibiotic MccJ25. We found that inactivation of one of the genes of leucine (Leu) biosynthesis increases sensitivity to MccJ25. In addition, there was a greater sensitivity to the antibiotic when the Leu concentration in the medium was increased. Intracellular amount of the global transcriptional regulator Lrp inversely depends on Leu concentration. *E. coli* RO64, an *lrp* null mutant, did not show the Leu effect on MccJ25 sensitivity. Moreover, RO64 was hypersusceptible to the antibiotic. A possible explanation of these results is that Lrp either negatively affects the import or increases the export of MccJ25. We searched for a consensus sequence for Lrp binding (TTTATTCtNaAT) in the genes involved in MccJ25 import and export. We found two potential Lrp binding sites (one of these identical to the consensus) just upstream of *yojI*, a chromosomal gene which codes for a MccJ25 pump. To prove that Lrp is a positive regulator of yojI transcription, we transformed *lrp*$^+$ and *lrp*$^-$ strains with a plasmid carrying *yojI*$^+$. Lrp$^+$ transformants displayed complete resistance to MccJ25, while the sensitivity of Lrp$^-$ strain remained unchanged. We conclude that an increased Leu concentration reduces *yojI* transcription, and therefore MccJ25 export, through a decreased intracellular level of Lrp.

MI-P65.
THE MCCJ25 STRUCTURAL GENE IS NEGATIVELY AUTOREGULATED
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Microcin J25 (MccJ25) is a peptide antibiotic produced by an enteric isolate of *Escherichia coli*. The structural gene, *mcjA*, codes for a 58-residue precursor, which is then processed by the *mcjB* and *mcjC* gene products to give the mature peptide of 21 amino acids.
With the aim of determining the existence of autoregulation of the *mcjA* gene we constructed *E. coli* strains bearing MccJ25-producing recombinant plasmids: pTUC201, pTUC202 and pTUC203, which are all derivatives of pACYC184. The cells were then transformed with plasmids pTUC342 (pBR322, McjA$^+$) o pPAV342 (pGEM, McjA$^+$), which hyperproduce the McjA precursor *in vivo*. In all cases, we observed a decrease in extracellular microcin production. This result suggested that increased levels of the precursor, or the additional microcin produced from the plasmid bearing the *mcjA* gene, exert a negative effect on the expression of the MccJ25 system. This was confirmed by using gene fusions between *mcjA* and *lacZ*. In fact, measurements of β-galactosidase levels showed that expression of the MccJ25 structural gene is autoregulated by the antibiotic: elevated amounts of intracellular microcin repress expression of *mcjA*. Further study of this regulation could allow us to optimize MccJ25 production.

MI-P66.

TolC IS REQUIRED FOR THE ACTIVITY OF A TETRACYCLINE EFFLUX PUMP (TetA)

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Tetracycline (Tc) is an antibiotic which inhibits bacterial growth through the inhibition of protein synthesis. One mechanism of resistance among pathogenic strains is mediated by Tc efflux pumps which maintain its intracellular concentration at a low level. One of these pumps is encoded by *tetA* gene of transposon Tn*10*. We observed that the *E. coli* strain MC4100 *tolC*::Tn*10* grew poorly, forming small colonies in LB medium with 15 µg/ml of Tc. The *tolC* mutation in this strain decreased the MIC for Tc by 7 fold, compared with MC4100 *thr*::Tn*10*. This reduction in the MIC was also observed both in other *tolC* mutants with a Tn*10* insertion elsewhere and MC4100 *tolC*::Tn*5* transformed with pBR322 (Tc^R). The reduction in the MIC was, in all cases, reverted by plasmid pAX629 carrying wild type *tolC* gene. We next investigated whether inhibition of the TetA efflux pump was responsible for the enhanced Tc activity on these bacterial strains. Spectrofluorometric analyses suggested an efflux inhibition. Moreover, total accumulation of Tc was increased. On the other hand, this Tc sensitivity phenotype was also seen with a Tc derivative, chlortetracycline (CTc). All *tolC* mutants failed to form colonies in LB medium with a low concentration (10 µg/ml) of CTc. Even autoclaved CTc (50 µg/ml) inhibited grow. Again, this phenotype was reverted by pAX629. Based on these results, we conclude that TolC is required in an unknown manner for the activity of the tetracycline efflux pump TetA.

MI-P67.

CHARACTERIZATION OF BINDING OF S-LAYER PROTEIN FROM *LACTOBACILLI* ON LIPOSOMES

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S-layers are isoporous structures composed of a single protein with the ability to assemble into monomolecular arrays either in suspension, at liquid-surface interfaces, on lipid films, on liposomes and on solid supports. Due to these unique features, S-layers have a broad application potential in molecular nanotechnology, nanobiotechnology and biomimetic. The aim of this work was the characterization of binding of S-layer from different lactobacilli on lipidic surfaces. For this study we used two different types of liposomes, one with DPPC, cholesterol and stearylamine and other with soybean lecithin (SL) replacing DPPC. The S-layer proteins were obtained from *L. kefir* JCM 5818, *L. brevis* JCM 1059 and *L. buchneri* ATCC 4005. Binding of S-layer protein on liposomes and particle stability were analysed. Z-potential measures showed the existence of protein-lipid interactions, which are dependent of the incubation time and the S-layer protein source, quantity and formation of self-assembly products. The binding affinity of S-layer proteins was higher on SL- than on DPPC-liposomes. Data obtained in this work agree with previously reported results for another source of S-layer protein. In the development of a future well-defined liposomal system for the attachment or encapsulation of molecules for human application, it is important to use natural components as SL and S-layer protein.

MI-P68.

***TRYPANOSOMA CRUZI* BASAL TRANSCRIPTION FACTORS IN THE POST GENOMIC ERA**

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For a long time it has been accepted that gene expression in trypanosomatids was almost exclusively post-transcriptional. However, in the last years, the description of several transcription factors in these organisms, as well as the determination of a transcription initiation region in *Leishmania* chromosomes, has led to the idea that transcription may be controled, at least to some extent, at the initiation level. In our laboratory we have cloned and characterized a TATA-binding protein from *T. cruzi* (TcTBP) This protein has shown to take part in a complex that binds the SL-RNA gene promoter, the only RNAPol II-dependent promoter described in trypanosomatids. Other protein complexes required for SL-RNA transcription, characterized in related organisms, have been found in *T. cruzi* too. In order to determine the composition and structure of the transcription initiation complexes in this parasite, we performed both homology- and motif- based searches in the available *T. cruzi* genomic sequences. We identified several putative basal transcription factors, related to RNAPol II and RNAPol III promoters. The coding sequences of these factors were PCR-amplified and cloned into Two hybrid vectors to analize the interactions between them. TcTBP showed interaction with many of the new cloned basal factors, suggesting that it takes part in different initiation complexes, as seen for TBPs from higher eukariotes.

MI-P69.

***GIARDIA LAMBLIA* TBP AND BRF BASAL FACTORS**

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The TATA Binding protein (TBP) is an initiation factor that participates in the transcription together with the three eucaryotic RNA polymerases. Other initiation factors are also necessary for the polymerases to bind promoter sequences, among them is the TFIIB-related BRF, which acts in the ARN pol III dependent transcription. Exhaustive searches in the available genomic sequences of *G. lamblia* allowed us the identification of genes corresponding to orthologs of the eucaryotic factors TBP and BRF (glTBP and glBRF). Northern blot experiments showed that both transcripts are present in the parasites, with a higher expression on trofozoites than during the induction of encystment. Electrophoretic mobility shift assays performed with the recombinant proteins and different probes showed that both factors can bind double stranded DNA without an apparent sequence specificity. GlBRF heparin-resistant DNA-binding could be associated with a criptic DNA-binding site described at the C-terminus region of yeast BRF. Two hybrid experiments demonstrated that these factors interact with each other. Using this metodology, we also defined which domains are involved in the binding. DNA-protein and protein-protein interactions involving glTBP and glBRF represent the first experimental data that contributes to the understanding of the divergent mechanisms of transcription initiation regulation in *Giardia lamblia*.

MI-P70.
HIGH FREQUENCY OF MORPHOTYPICAL DIVERSIFICATION IN *PSEUDOMONAS AERUGINOSA* mutS POPULATIONS IS RELATED TO QUORUM SENSING

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Pseudomonas aeruginosa is an extraordinarily versatile species involved in severe and often fatal infection in Cystic Fibrosis, AIDS or severe burn wounds patients. Its pathogenicity is based on the production and secretion of extracellular virulence factors whose expression is tightly regulated by quorum-sensing (QS) systems that are hierarchically controlled by global regulators. We recently described that, in contrast to the parental strain, when a hypermutator *P. aeruginosa* mutant was grown for a short period in LB medium, it reproducibly generated two morphotypes in a high frequency, mS1 and mS2 (Microbiology, 150:1327-1338, 2004). Notably, mS2 displayed differences in virulence traits, including antibiotic resistance, altered motility behavior, hyperpigmentation, reduced protease activity and non-cytotoxic invasive phenotype, suggesting that they stem from mutations in major regulators related to QS. In this work we strengthen this hypothesis using a *Caenorhabditis elegans-P.aeruginosa* pathogenesis model. The mS1 and mS2 variants were tested for virulent/nonvirulent phenotypes in comparison with *gacA* and *lasR* mutants. As described for the global regulator GacA and for the QS regulator LasR, mS2 failed to kill *C.elegans* in slow and paralitic assays. For exoprotease and elastaseB production, mS2 was similar to *gacA* and *lasR* mutants, although its hyperpigmentation and swarming motility were equivalent to *lasR*. Our results indicate that mS2 variant is affected in QS regulators and suggest that in *P. aeruginosa*, hipermutability could constitute an adaptive strategy by modulating virulence.

MI-P71.
FREQUENCY OF SMALL COLONY VARIANT PHASE-VARIATION IS INCREASED IN *Pseudomonas aeruginosa* mutS MUTANTS

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Pseudomonas aeruginosa is a ubiquitous and remarkably versatile bacterium emerging as one of the most important causes of opportunistic infections in humans. Particularly in Cystic Fibrosis (CF), *P. aeruginosa* develops in biofilms which are thought to be responsible for persistent lung infections conferring protective advantage against antibiotic therapy, oxidative stress, and immune system attack. Moreover, CF lung seems to select for adaptive variants such as autoaggregative small-colonies (SCV) recognized by their increased ability to form biofilms and whose occurrence is regulated by phase-variation mechanism. Recently, we observed that a higher frequency of SCV emerged from biofilms formed by a hypermutator *P. aeruginosa* mutS mutant compared with non-mutator wild-type biofilms. Additionally, we observe that, in batch and solid cultures, these SCV can revert to the non aggregative wild-type morphotypes indicating a variation between two phases. In order to reinforce the view that hypermutability favors adaptive strategies, we test if the mutation frequency affects the velocity of SCV reversion. SCV obtained from hypermutator biofilms were transformed with a plasmid containing the *P. aeruginosa mutS* gene and their reversion rate were compared with not complemented hypermutator SCV clones. Clearly, we observe that after six days, 100% of hypermutator SCV reverted, while complemented SCV only reverted 13%. Our results prompt the hypothesis that hypermutability favors phase-variation strategies and constitutes a major factor contributing adaptability of *P. aeruginosa* in the CF lung.

MI-P72.
GOLD RESISTANCE IN *Salmonella typhimurium*

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We have screened the *Salmonella* genome for loci encoding *Salmonella*-specific metal-responsive MerR homologues. Among the genes detected, we have identified one that we termed *godS*. GodS controls the expression of two transcriptional units, *godTS* and *godB*, in response to gold salts. These genes code for a P-type ATPase with homology to metal transporters, the regulator, and a metal-binding protein, respectively. We investigated the role of this regulon in the resistance to gold and other heavy metals salts. We found that a mutant strain deleted in *godS* was more susceptible only to gold salts than the wild type. Mutants in either *godT* or *godB* or in both genes showed an intermediate tolerance, suggesting that other GodS-controlled genes are required for full tolerance. We analyzed a series of putative cation efflux systems in order to identify additional genes involved in gold tolerance. Mutants in the copper efflux systems *cusBAC* and *copA* were more sensitive to gold than the wild type. However, the expression of these loci was not modulated by gold suggesting that these systems are not controlled by the God regulon. In sum, to our knowledge this is the first gold-specific detoxification system horizontally acquired into the *Salmonella* genome, that allow this pathogenic bacterium to monitor and to resist environments with high concentration of gold salts.

MI-P73.
GodS, A TRANSCRIPTIONAL REGULATOR THAT RESPONDS TO GOLD

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The MerR family is a group of transcriptional regulators that respond specifically to a variety of environmental stimuli, such as oxidative stress, heavy metals or antibiotics. We have identified a gene, *godS*, coding for a MerR-like protein located in a *Salmonella* specific region. *godS* forms an operon with *godT* that encodes a metal transporter and is located upstream of *godB*, whose product has homology to copper chaperones. We demonstrated by ß-galactosidase activity and western blot assays that GodS regulates not only the expression of *godT* and *godB* but also its own synthesis. The autoinduction reported here was not previously observed in other MerR like regulators from gram negative bacteria. Transcriptional activity analysis in the presence of mono, di or trivalent metal ions showed that this regulon is exclusively induced by gold salts. Moreover, this induction is abrogated by the deletion of *godS*, and fully restored upon expression of the regulator from a plasmid. We observed that GodS specifically binds to the promoter regions of *godTS* and *godB in vitro* even in the absence of gold. However, the presence of $AuCl_3$ in the culture medium increased the level of GodS associated to these promoters. To our knowledge, this is the first evidence of transcriptional autoregulation for this kind of metalloregulatory proteins.

MI-P74.
INTERACTIONS OF MutL WITH SINGLE- AND DOUBLE-STRANDED DNAs ARE MODULATED BY ADENINE NUCLEOTIDE COFACTORS

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MutL has been previously implicated in the initiation and excision steps of methyl-directed mismatch repair. To clarify the interactions among nucleotide and single- and double-stranded DNA binding sites on the protein, we studied complex formation by fluorescence, crosslinking and gel shift methods. Conditions where nucleotide-binding site occupancy is empty has a modest effect on ssDNA affinity. Upon AMPPNP binding, the protein undergoes conformational changes that result in the highest stabilization of the complex as judged by gel shift. MutL binding to dsDNA is distinct. A stable complex is detected when the nucleotide cofactor is absent whereas addition of AMPPNP reduces the dsDNA binding. Conversely, formation of the complexes was equally achieved for gapped and partial DNA molecules in the presence and absence of nucleotide cofactors. ATP hydrolysis studies revealed that the degree of ATPase stimulation is sensitive to the nature of the DNA, with dsDNA molecule yielding only a 2.5-fold stimulation compared to 8-fold stimulation for the corresponding ssDNA molecule. These findings imply a number of potential conformational states in the protein and suggest that one particular species of MutL is dominant at a particular time and serves a specific function.

MI-P75.
A B-CELL MITOGEN OF *Brucella abortus* IS A PROLINE-RACEMASE

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Brucellosis is characterized by an initial acute phase followed by a subversion of the host innate and adaptive immune responses that allow a chronic infection. The isolation and characterization of pathogen molecules essential for the interactions with the host immune defense are keyed issues for the understanding of the pathology and the development of rational strategies for vaccination, immunotherapy and drug design. Recently a proline-racemase (RAC) was reported in *Trypanosoma cruzi* to act as B-cell polyclonal activator. It has been demonstrated that mitogens are associated with immunomodulation and immunoevasion by many pathogenic microorganisms. In this study we have cloned, sequenced and expressed a putative proline-racemase (*rac1*) of *B. abortus*. Enzymatic activity of the rRAC1 showed that it is capable of racemizing only L-Proline. Stimulation of murine naive spleen-purified B-cells with 50ug/ml of rRAC1 induced a 77-fold increase in B-cell proliferation. Interestingly, as other known virulence fac-tors, *rac1* expression is induced under starving conditions. Experi-mental infection of Balb/c mice showed that *B. abortus rac1* mu-tant was less virulent than its parental strain, and exerted splenic disorders characterized by total loss of architecture, hemorrhagic and apoptosis. These results suggest that RAC1 is a B-cell polyclo-nal activator that strongly contributes to *Brucella pathobiology*.

MI-P76.
IDENTIFICATION AND CHARACTERIZATION OF A TRANSCRIPTIONAL REGULATOR OF THE *BRUCELLA ABORTUS virB* OPERON

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In the last years several studies demonstrated that the *Brucella* virulence is directly related to a type-IV secretion system encoded by the *virB* operon. In *B. abortus* 2308 this system is essential for virulence and intracellular multiplication. In the present study we analyzed the structure of *virB* promoter (P_{virB}). The P_{virB} transcription start site was identified using primer extension experiments. *lacZ* transcriptional fusions allowed us to identify the minimal region necessary for promoter activity of P_{virB}. Using gel shift assays, affinity cromatrography and tandem mass spectrometry we isolated and identified a transcriptional regulator of the *virB* operon. This protein, homologue to Integration Host Factor (IHF), belongs to a family of bacterial histone-like proteins. The P_{virB} IHF-binding site was identified using DNaseI footprinting experiments. Single IHF-P_{virB} complexes were visualized by Atomic force microscopy, showing that IHF interacts with P_{virB} inducing a DNA bending angle of 50.36°. P_{virB}-*lacZ* fusions showed that in *B. abortus* IHF participates in the regulation of P_{virB} activity during the intracellular infection of J774 macrophages. A mutant strain with a 20 bp IHF-binding site replacement failed to turn on the *virB* operon during the initial stages of J774 infection and displayed severe intracellular multiplication defects. These data indicate that IHF plays a key role during intracellular *virB* operon expression.

MI-P77.
***Brucella abortus* SYNTHESIZES PHOSPHATIDYL-CHOLINE BY USE OF THE PHOSPHATIDYLCHOLINE SYNTHASE PATHWAY**

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Phosphatidylcholine (PC) is one of the major structural constituents of the eukaryotic membranes and the principal source of lipid second messenger involved in signal transduction pathways. PC is also found in an increasing number of prokaryotes, mainly in sym-bionts, pathogens and photosynthetic bacteria. Besides the current-ly known methylation pathway for PC formation, a novel pathway was described in the legume symbiont *Sinorhizobium meliloti* in which choline is directly condensed with CDP-DAG by the action the enzyme phosphatidylcholine synthase (Pcs). The *Brucella* cell envelope has unique characteristics such as a low endotoxic LPS, several porins and OMPs covalently bound to the peptidoglycan layer and the presence of PC as one of the main phospholipids. Until now, the metabolic pathway for PC formation in *Brucella* and the enzymes involved are completely unknown, although *pmta* and *pcs* genes were detected in the three *Brucella* sequenced geno-mes. We cloned the *pmta* and *pcs* genes from *B. abortus* S2308 and characterized mutants defective in PC synthesis. Our results indicate that Pcs is the only pathway for PC formation in *Brucella*. Although the ability of the mutant to invade and sustain intracell-ular replication was not affected, experimental infections indicate a reduction of virulence. A possible role for PC as a modulator of the inflammatory response generated during infection is discussed.

MI-P78.

Brucella abortus CHOLOYL GLYCINE HYDROLASE MUTANT HAS AN ALTERED CELL ENVELOPE AND IS DEFECTIVE IN CELLULAR INTERNALIZATION

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Choloyl glycine hydrolase (CGH) is a conjugated bile salt hydrolase that catalyses hydrolysis of the amide bond between glycine and cholic acid. Bile salt hydrolases are expressed by gastrointestinal bacteria and they presumably decrease the toxicity of host conjugated bile salts. *Brucella* CGH is highly conserved in the three species whose genomes have been sequenced and has a putative secretion signal. In order to understand the role of CGH in *Brucella* virulence, we constructed a *B. abortus* S2308 strain with an unmarked deletion of the gene (Δ*cgh*). As expected, Δ*cgh* was more sensitive to bile salts than the parental strain. In contrast, Δ*cgh* showed increased resistance to polymyxin B. Western Blot analyses of Outer Membrane Proteins (Omps) showed a reduced expression of Omp2b in Δ*cgh*. To compensate for this reduction, expression of Omp31b, a paralogue of Omp31 (which is absent in *B. abortus*), was only detected in Δ*cgh*. Two-dimensional electrophoretic profiles of membrane proteins also revealed differences between both strains. *In vivo* experiments showed that, although wild type and mutant strains reached similar CFU values at 48 hs post-infection, Δ*cgh* displayed reduced invasiveness in murine macrophages and HeLa cells. However, the mutant strain was virulent in Balb/C mice inoculated intraperitoneally. Taken together, these results suggest that *B. abortus* CGH may play a role in membrane integrity and in host-bacteria interactions.

MI-P79.

TRANSCRIPTIONAL REGULATION OF CYCLIC β-1,2-GLUCAN BIOSYNTHESIS IN *BRUCELLA ABORTUS*

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Cyclic β-1,2-glucans are important components of gram negative bacterial envelope. In *Brucella spp*. the biosynthesis of cyclic β-1,2-glucan proceeds through a membrane-bound cyclic glucan synthase (Cgs) and secretion into the perisplasmic space through a membrane-bound cyclic glucan transporter (Cgt). It was observed that *cgt* mutant accumulates in the membrane higher amounts of Cgs protein than in the wild type. Accordingly, *cgt* mutant incorporates *in vitro* significant higher amount of [^{14}C]-glucose into cyclic β-1,2-glucan and TCA insoluble glucoprotein. A *cgs*::*lacZ* transcriptional fusion was constructed and analysed in three different backgrounds, wild type 2308, *cgs* and c*gt* mutants. Compare to wild type significant increment of β-galactosidase activity was observed in the *cgt* and *cgs* mutant backgrounds, thus suggesting that cyclic β-1,2-glucan is involved in the transcriptional regulation of *cgs*. Moreover complementation of *cgs* and *cgt* mutants with plasmids containing *cgs* or *cgt* inactive genes obtained by site directed mutagenesis in the active site and the ATP binding site respectively, did not abolished this effect. These results strongly suggest that the absence of cyclic glucan in the periplasm and not the absence of proteins Cgt or Cgs in the membrane may be the signal that regulates transcription of *cgs* gene.

MI-P80.

NATURAL INSERTIONAL INACTIVATION OF AN OUTER MEMBRANE PROTEIN GENE PROMOTES CARBAPENEM RESISTANCE IN *Acinetobacter baumannii*

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We previously demostrated that the loss of an outer membrane protein of 29 kDa, CarP, is associated to carbapenem resistance in the Gram-negative non-fermentative pathogen *A. baumannii* (Limansky *et al*., 2002, *J.Clin.Microbiol*. 40, 4776-78). We cloned and characterized the genomic *carP* locus of this bacterium. The *carP* gene constitutes a single transcriptional unit in the *A. baumannii* genome and codes for a novel polypeptide of 247 amino acid residues with a characteristic signal peptide. Structural predictions indicate a ß-barrel topology in the bacterial outer membrane with 10 ß-strands spanning regions. Database searches indicated the presence of *carP* homologs only in species belonging to the family *Moraxellaceae* among the γ-*Proteobacteria*. PCR analysis of a comprehensive collection of *A. baumannii* isolates indicated that the disruption of *carP* by insertion sequence (IS) elements constituted one of the mechanisms responsible for the absence of this protein in carbapenem-resistant strains. Two distinct IS were identified within the *carP* gene: one of them, ISAba825, represents a novel mobile element. The other, ISAba125, is a new member of the widely-distributed IS30 family. A second mechanism of inactivation of the *carP* gene is present in *A. baumannii* which does not involve the disruption of the structural gene.

MI-P81.

COOPERACIÓN FUNCIONAL ENTRE CHAPERONES MAYORITARIOS DE *Escherichia coli* K-12

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It has been proposed that trigger factor (TF) and the DnaK system perform redundant functions in the folding of newly-synthesized polypeptides in *E. coli*. Accordingly, the functioning of the DnaK system would be highly compromised by mutations in the *tig* gene or genes encoding components of the DnaK system such as *dnaJ*. To test this hypothesis, we constructed double mutants of *E. coli* K-12 lacking DnaJ and TF (*ΔtigΔdnaJ*, GE4103), and characterized them both genetically and biochemically. Contrasting the situation found in simple *Δtig* or *ΔdnaJ* mutants, GE4103 showed much lower duplication rates at permissive temperatures, and an upper limit of growth temperatures of 37ºC. DnaK and GroEL, the major heat-shock proteins of *E. coli*, were largely overproduced in GE4103, to levels much higher than the wild-type strain or the single mutants. This indicates a deficient functioning of DnaK as a negative regulator of the heat-shock response. Moreover, two major proteins, identified by MALDI-TOF as the outer membrane protein OmpW and the general regulator of the stationary phase Dps, were absent in GE4103. These results indicate that their expression or secretion depends on the cooperation between TF and the DnaK system.

MI-P82.
COOPERATION OF CYTOPLASMATIC CHAPERONES IN THE SECRETION OF A NOVEL OUTER MEMBRANE PROTEIN OF *Acinetobacter baumannii*

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We cloned and characterized a gene, *carP*, coding for a new outer membrane protein (OMP) of the Gram-negative bacterial pathogen *Acinetobacter baumannii*, which is associated to carbapenem resistance. Expression of the *carP* gene in *E. coli* resulted in the localization of the corresponding protein, CarP, in the bacterial outer membrane. This indicates that this protein contains all the information to direct its proper secretion. The chaperones involved in the export of this protein were studied by genetic procedures. The presence of CarA in the outer membranes was visualized by western blots using specific antibodies. The use of *E. coli* mutants in the cytoplasmatic chaperones trigger factor (TF) and DnaK presented alterations in the secretion pattern. Still, secretion was independent of DnaK co-chaperones, DnaJ and CbpA, suggesting the involvement of other factor in this proccess. Moreover, CarP secretion was severely impaired in double *secA* tig mutants of *E. coli*, implying a cooperation between the two systems. The overall data indicate that the secretion of CarP to the outer membrane of Gram-negative bacteria is a complex process that requires the cooperation of cytoplasmatic chaperones and the Sec system. DnaK and TF may bind selectively to precursors of OMPs and maintain their transport competence until the Sec system becomes available.

MI-P83.
MOLECULAR CHAPERONES INVOLVED IN THE SECRETION OF METALLO-ß-LACTAMASES IN GRAM-NEGATIVE BACTERIA

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The proper functioning of extracytoplasmatic proteins requires their export to their proper compartment. In *Escherichia coli*, periplasmatic proteins are synthesized in the cytoplasm as precursors and directed to the general secretory pathway (Sec). Whether and how cytoplasmatic chaperones participate in their secretion is still obscure. We studied the cooperation of these chaperones with the Sec pathway in *E. coli* by genetic procedures using as a model system the metallo-ß-lactamase GOB from *Chryseobacterium meningosepticum*. We developed a functional procedure to analyze the proper expression and secretion of GOB in *E. coli* chaperone mutants, consisting in determining ß-lactam resistance by a plate dilution assay. We demonstrated that SecA and SecY play fundamental roles in proper GOB secretion. Cytoplasmatic chaperones including DnaK, DnaJ and Trigger Factor (TF) were also necessary for this process. The use of double mutants provided further evidence for a cooperation between TF and SecA in GOB secretion.
These overall data further highlight the existence of complex interactions between cytoplasmic chaperones and the Sec machinery in the secretion of pathogenicity factors such as metallo-ß-lactamases in Gram-negative bacteria.

MI-P84.
MICROCIN J25 (MccJ25) ACTIVITY IN BLOOD-DERIVED MATRICES

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MccJ25 is a plasmid-encoded antibiotic consisting of 21 amino acid residues. MccJ25 has extreme resistance to heat, denaturing agents and proteases. The latter property led us to predict that it could survive *in vivo* proteolytic activity. To test this hypothesis, MccJ25 antimicrobial activity after incubation in different biomatrices and LB medium (as a control) was compared. No significant difference was found between the activity of biomatrix-treated MccJ25 and that of the control after 24-h incubation at 37ºC. MccJ25 (0.1 µM) exerted potent antimicrobial activity on *Salmonella newport* when co-incubated with blood and blood fractions, since viable cell counts drop from 10^6 CFU/ml to 10^4 CFU/ml after 2 h incubation at 37ºC. When the same concentration of MccJ25 incubated in LB with an inoculum of 10^6 cells, the mean final viable count was 10^3 cells. For comparison, the antimicrobial activity of 1 µM MccJ25, which gave complete killing of *S. newport* in biomatrices, was greater than that of ampicillin and rifampicin at the same concentration. Incubation with biomatrices alone caused no significant reduction in *S. newport* viability. We conclude that MccJ25 retains antimicrobial efficacy in complex biomatrices, including whole blood, plasma and serum. The present results are useful to establish conditions for an animal infection model in which to study *in vivo* the therapeutic action of MccJ25.

MI-P85.
CHARACTERISATION OF A DSF-HYPER PRODUCING MUTANTS OF XANTHOMONAS AXONOPODIS PV. CITRI (*XAC*)

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Xac causes bacterial chancrous a serious disease of citrus. In the related bacterium *Xanthomonas campestris* pv. *campestris* (*Xcc*), the *rpfF* gene is involved in production of a diffusible extracellular factor (DSF) that positively regulates synthesis of virulence-associated functions like extracellular polysaccharide (EPS) and extracellular enzymes. In addition, in L medium, strains of Xcc carrying mutations in genes within the *rpf* cluster grew as matrix-enclosed aggregates. DSF perception and signal transduction have been suggested to involve the two-component system comprising RpfC and RpfG. Mutation of *rpfC*, which encodes the sensor component, leads to over-production of DSF and reduced production in virulence factors. The addition of DSF can phenotypically restore *rpfF* but not *rpfC* mutants to wild type. We demonstrated here that a deletion and kanamicin resistance cassette insertion in the *rpfC* homolog of *Xac*, as in *Xcc*, leads to over-production of DSF. *rpfC Xac* mutant has shown a decreased of cyclic ß-1,2 glucan and the extracellular enzyme endoglucanase productions. Supplementation of L medium with DSF (from the *rpfC Xac* mutant) before bacterial inoculation allowed for normal dispersed growth of *rpfF Xcc* mutant but not in *rpfC* of both *Xac* and *Xcc*. The results suggest that *rpfC* may be involved in controlling virulence factors of *Xac* mediated by DSF.

MI-P86.
CHARACTERIZATION OF A TYPE I SECRETION SYSTEM IN *AGROBACTERIUM TUMEFACIENS*
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Type I secretion systems enable a wide variety of Gram negative species to export proteins to the extracellular media. The objective of this work is to explore the role of type I secretion systems in *Agrobacterium tumefaciens*. The type I PrsD/PrsE secretion system of *Rhizobium leguminosarum* was extensively characterized. We previously reported that a chromosomal region of the *A. tumefaciens* genome restored the secretion of the extracellular glycanases PlyA and PlyB and four additional proteins in both the *prsD* and the *prsE* mutants of *Rhizobium*. The complementing locus encodes for putative ABC and MFP components of type I systems. This locus and adjacent regions conferred the ability to *Rhizobium* to secrete large amounts of a 33-kDa protein and two additional proteins. Comparison of the N-terminal aminoacid sequence of the 33 kDa protein with the annotated *Agrobacterium* genome revealed that the coding gene (that we named *expA*) is located upstream the putative secretion locus; the deduced protein sequence showed no significant homology with characterized proteins and have no conserved domains. However, ExpA contains a nonapeptide that differs in one aminoacid from the repetitive consensus sequence of RTX (repetitive toxins) toxins. Plate assays using different closely related species and phylogenetically unrelated species as indicator strains showed that the presence of ExpA correlates with a bacteriocin activity. ExpA would be negatively regulated in *Agrobacterium*.

MI-P87.
FUNCTIONAL CHARACTERIZATION OF THE *tolC* GENE OF *BRUCELLA SUIS* IN HETEROLOGOUS HOST
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Outer membrane proteins of the TolC family have been implicated in several functions including efflux of toxic compounds and protein secretion. In the alpha-2 group of proteobacteria, like in *Rhizobium* spp. and *Brucella* spp. the role of this protein family remains unknown. Only one member of the TolC family was identified by Blast search in the *Brucella suis* genome. The $tolC^b$ gene was cloned and expressed in heterologous bacteria. The hemolysin A secretion defect of an *E. coli tolC* mutant was weakly complemented by $tolC^b$. We also intend to rescue the drug hypersensitive phenotype of the *E. coli tolC* mutant. No complementation of Nalidixic acid susceptibility of the *tolC* mutant was observed. A *Rhizobium leguminosarum* mutant in a gene of the *tolC* family (*nodT*) was previously isolated. However, no clear phenotype was associated with mutation in *nodT*. Interestingly, we found that the *nodT* mutant was unable to grow in minimal medium and growth in a rich medium was consistently low compared with the wild type. In addition, the extracellular protein profile of the *nodT* mutant was altered. The *tolCb* gene was able to restore growth on rich medium, slightly complemented growth on minimal medium and considerably restored the extracellular protein profile of the *nodT* mutant. These observations suggest a more crucial physiological role for proteins of the TolC family in bacteria of the *Rhizobiaceae* group.

PL-P1.
INTERACTION OF THE PLANT HOMEODOMAIN PROTEIN ATH1 WITH DNA
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ATH1 is a light-regulated *Arabidopsis* gene that encodes a member of the Bell family of plant TALE homeodomain (HD) proteins. The HD is a conserved compact domain that directly contacts DNA. In this study, the ATH1 binding sequence, TGACAGGT, was determined by selecting binding sites from random-sequence oligonucleotides through several rounds of binding and amplification (SELEX). *In vitro* DNA binding assays with oligonucleotides containing variations of the consensus sequence were performed with ATH1 and STM (a class I Knox HD protein). These assays indicated that both HDs recognize similar sequences with different affinity and that changes within the GAC core deeply affect their interaction with DNA. A more detailed picture of the binding of these proteins to its target site was obtained by footprinting and missing nucleoside experiments using hydroxyl radical cleavage of DNA. Exchange of residues present at position 54 of the HDs of ATH1 and STM, Val and Lys, respectively, indicated that this residue is a determinant of binding affinity. Changes at positions 5 and 6 of the TGACAGGT sequence significantly affect the interaction when position 54 is Val, while position 7 is very important for all proteins. The results indicate that ATH1 and STM bind similar sequences with different affinity and that subtle differences exist in their interaction with DNA. Residue 54 would be in part responsible for the different properties of both HDs.

PL-P2.
GUARD CELL-SPECIFIC ANTISENSE INHIBITION OF *ARABIDOPSIS* MAP KINASE 3 REDUCES STOMATAL SENSITIVITY TO ABA AND H_2O_2
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In order to explore possible physiological roles of *Arabidopsis thaliana* MAP kinase 3 (AtMPK3), we examined the phenoptypes arisen from inhibiting its expression in guard cells. Such an inhibition was possible by transformation of Arabidopsis with a fragment of the coding region of the AtMPK3 gene in antisense orientation driven by the guard cell-specific promoter kst1.
Results: three independent clones displayed reduced sensitivity to 20 µM ABA in inhibition of stomatal opening compared to controls, but responded normally to the hormone in promotion of closure experiments. Therefore, we investigated the possibility that the known ABA-evoked pH increase in guard cells can compensate for the absence of AtMPK3 in stomatal closure. To test this, 1 mM sodium butyrate was used to prevent pH increases inside guard cells. Under this treatment, AtMPK3 antisense plants showed a slightly reduced response to ABA compared to controls, suggesting that the pH increase acts in an ABA-signaling pathway parallel to that of MAP kinase 3.
The response to H2O2 was different from that to ABA in that antisense plants showed reduced sensitivity to 100 µM H_2O_2 in both inhibition of stomatal opening and promotion of closure.

PL-M3

A MATURASE K-LIKE TRANSCRIPT IS REGULATED BY AUXINS IN *ARABIDOPSIS THALIANA* SEEDLINGS

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Auxins are hormones that mediate a variety of developmental processes in plants. The precise molecular mechanisms involved in the hormone actions remains uncertain. Recently, it has been described that nitric oxide (NO) in plants is involved in some well-defined auxin-promoted physiological responses. The objetive of this work was to identify changes in the transcriptoma profile of *A. thaliana* treated with NO and to study if they were involved in auxin signaling pathway. Thereby we developed a differential display (DD) assay on seedlings treated for 12 and 24h with the NO donor. Seven differentially expressed cDNAs were detected from the DD analysis in NO-treated seedlings. We selected one of them (*At04*) *to use it as a probe for a* Northern blot analysis of NO-and 2.4-D-trated seedlings. Surprisingly *At04* was up-regulated in 2.4-D-treated seedlings, but remained unmodified by NO. The nucleotide sequence revealed that *At04* shares high homology with a chloroplastic maturase K cDNA. The maturases are proteins involved in the regulation of splicing of certain mRNAs. Further analysis will be presented in order to integrate know auxin- and NO-signaling pathways with the role of maturases in the control of plant gene expression.

**These authors contributed equally to the present work.*

PL-P4.

PHOTOSYNTHESIS AND OXIDATIVE STRESS TOLERANCE OF TOBACCO TRANSGENIC PLANTS OVEREXPRESSING FERREDOXIN-NADP(H) REDUCTASE

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Ferredoxin-NADP(H) reductases (FNR) catalyse the reversible electron transfer between two molecules of obligatory one-electron carriers as ferredoxin and flavodoxin (Fd) and a single molecule of NADP(H). Antisense tobacco plants with reduced amounts of this enzime display diminished photosynthetic activity (Hajirezaei *et al*., Plant J. (2002) 29:281) and enhanced photo-oxidative damage (Palatnik *et al*., Plant J. (2003) 35:332). In this work we describe the preparation of transgenic tobacco plants overexpressing pea FNR and the evaluation of their photosynthetic activity and oxidative stress tolerance. Altough FNR levels raised up to 500% in these transgenic lines, only a moderate increase in CO_2 asimilation was observed (30%) under saturating light and CO_2. These plants were subjected to oxidative stress conditions by exposure to the superoxide radical propagator metyl viologen or combinations of high irradiance and low temperature. Measurements of chlorophyll degradation, membrane damage and chlorophyll?a fluorescence shows that the overexpressing transgenic plants are more tolerant to the oxidative stress conditions assayed.

PL-P5.

PARTICIPATION OF *XANTHOMONAS AXONOPODIS* PV. CITRI HRP CLUSTER IN CITRUS CANKER AND IN NON-HOST PLANTS RESPONSE

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Xanthomonas axonopodis pv. *citri (Xac)* causes citrus canker, a serious disease of the citrus genus that results in important losses in citriculture regions. Type III secretion system, indispensable for the trafficking of proteins to the plant cell in pathogenicity and avirulence models, is encoded by *hrp* (hypersensitive response and pathogenicity) cluster. We have characterized the participation of *Xac hrp* cluster in the interaction with host and non-host plants. We isolated a region of the *hrp* cluster of *Xac* and we constructed mutants in the operons *hrpB* and *hrpD* and the *hrpF* gene, using used a suicide vector transferred by biparental mating. For disease symptoms assays bacterial suspensions were infiltrated into leaves of host plant orange (*Citrus sinensis*) and into leaves of non-host plant cotton. Bacteria mutant strains failed to produce canker in citrus plants or hypersensitive response in cotton plants. We also characterized the interaction of the phytopathogen with different non-host plants. The results show that hypersensitive response is produced in leaves of cotton, bean, tobacco, tomato, pepper and *Nicotiana benthamiana* and that genes present in operons *hrpB* and *hrpD* and the *hrpF* gene are required for pathogenicity in hosts and hypersensitive response induction in non-host plants.

PL-P6.

POSSIBLE ROLES OF THE GLYCOSYLATION OF *SOLANUM TUBEROSUM* ASPARTIC PROTEINASES IN PLANT DEFENSE MECHANISM

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Plant aspartic proteinases (AP) (EC 3.4.23) contain one or more consensus N-glycosylation sites. However the importance of those for the activity, specify and function of AP is not well understood. Two AP from potato tubers (*St*AP1) and leaves (*St*AP3) have been purified and characterized in our laboratory. Both enzymes are glycosylated; however, the glycosylation type is different.
In this work we investigated the effect of the glycosylation of *St*AP on the proteolytic, antimicrobial activity, binding capacity (using FITC conjugate to protein) and ability to induce membrane permeabilization (using Sytox Green probe) in conidia of *F. solani*. Deglycosylated *St*AP (*St*APdg) did not show differences with native *St*APs in its ability of produce growth inhibition, binding capacity to conidia of *F. solani* and proteolytic activity at different pH. However *St*AP1dg and *St*AP3dg showed lower capacity (up to 30%) to induce membrane permeabilization and subsequently cell death than *St*APs. In the presence of pepstatin, specific inhibitor of APs, those results are reverted.
These results suggest that glycosylation is important in the *St*APs antimicrobial activity, because it would increase the capacity to induce membrane permeabilization.

PL-P7.

A GAF-DOMAIN MUTANTION OF PHYTOCHROME A IMPAIRS CHROMOPHORE INCORPORATION AND LIGHT RESPONSES IN *ARABIDOPSIS*

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Phytochromes A (phyA) is a photoreceptor that modulate plant growth and development in response to far-red light (FR) compared to darkness. We isolated an *Arabidopsis* mutant impaired in the response to continuous FR but not in the response to transient FR. Genetic complementation tests indicated a mutation in the *PHYA* gene. Molecular characterization of the *PHYA* gene revealed a single nucleotide mutation that changed a conserved arginine residue to lysine in the GAF motif of the N-terminal domain. The heterologously expressed PHYA-303 N-terminal domain showed poor *in vitro* assembly with the cyanobacterial chromophore PCB when compared to wild-type PHYA N-terminal domain. Feeding the seedlings with biliverdin, a precursor of the natural chromophore, partially rescued the *phyA-303* mutant phenotype. This suggests that the *phyA-303* mutation affects chromophore incorporation not only *in vitro* but also *in vivo*.

PL-P8.

OXIDATIVE STRESS AND MITOCHONDRIAL DYSFUNCTION IN FROST-EXPOSED VALENCIA ORANGE FRUIT

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Low temperatures cause severe damage in Valencia orange fruit (*Citrus sinensis* L. Osbeck). We have reported before an increased fermentative metabolism in the endocarp of frost-damaged fruits. In this work, levels of antioxidant enzymes and mitochondrial functionality were analyzed in affected tissue. Total protein content on a fresh weight basis showed a sharp decrease (37%) in frozen fruits (both in soluble and mitochondrial fractions). Induction of APX, CAT and POD was observed in native gels by activity staining, while a Mn-SOD slightly decreased. MDH activity in, and reduction of exogenous cytocrome *c* by, isolated mitochondria indicated a lower organelle integrity. Respiration rate of whole fruit was significatively reduced by 30%, whereas the flavedo showed a more pronounced descent (53%). Biochemical and internal quality parameters were also affected: volatile compounds, L-malate and titratable acidity increased, while juice content and maturity index showed a marked decrease in frozen fruits compared to a control. In conclusion, results suggest that cold imposes oxidative stress in oranges, associated to less active and functional mitochondria. Oxidative stress could be used as a marker to detect frost-induced damage in citrus fruit.

PL-P9.

VICINAL DITHIOL-BINDING AGENT, PHENYLARSINE OXIDE, INHIBITS THE α-AMYLASE SECRETION IN BARLEY ALEURONE

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We demonstrated previously that PLC-PI is involved in GA response, since GA increases the polyphosphoinositides synthesis and turnover and IP_3/IPs levels. These responses and α-amylase secretion were inhibited by U73122, a PLC inhibitor (Villasuso *et al.,* 2003; Villasuso & Machado-Domenech, 2004). Due to the canonical role of PtdIns-4 kinase in the synthesis of $PtdInsP_2$, the effect of PtdIns-4 kinase inhibitor PAO on α-amilase secretion was studied. In the present study, we found that the vicinal dithiol-binding agent, PAO inhibited the GA-stimulated α-amylase secretion in a dose-dependent fashion. However, simultaneous addition of a vicinal dithiol compound, 2,3-dimercaptopropanol (BAL), with PAO abolished this inhibition. Attenuation of hydrolase secretion by PAO was coincident with the decrease of phosphoinositide levels in membrane fractions; thus PAO evoked changes in the membrane-associated PtdIns-4 kinase activity. By contrary, treatment of layers with PAO did not affect the amylase expression, suggesting that it specifically blocks the secretion. PAO was able to affect the secretion stimulated by mas7 (a peptide able to activate G protein) and it was also correlated with the inhibition of PtdIns-4 kinase activity. In conclusion, the effect of PAO on α-amylase secretion suggest that PtdIns-4 kinase is essential for sustaining the aleurone capacity to secrete hydrolases during GA stimulation.

PL-P10.

DECONDENSATION OF *ARABIDOPSIS* CENTROMERES CAUSED BY EARLY *PSEUDOMONAS* INFECTION

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Methylation of cytosine residues is a key process for regulation of chromatin structure and gene expression in several species. We have previously demonstrated that the *Arabidopsis* genome display a 70% reduction of 5-methyl cytosine (5-mC) content at 24 hours post-infection with *Pseudomonas*. The main targets of this modification are CpG and/or asymmetric cytosines located in *Arabidopsis* centromeric regions. Although transcriptional activity of pericentric coding sequences is not substantially altered, we here demonstrate that 5-mC loss causes main changes in the chromatin organization of intephasic nuclei from infected cells. DAPI staining combined with inmunolabelling of 5-mC with monoclonal antibodies showed that reduction of 5-mC residues at centromeres precedes decondensation of these heterochromatic regions. Both responses were only observed at the inoculation sites of infected tissues but not at distal sites of challenged leaves. Neither molecular markers of cell cycle progression nor BrdU incorporation was detected at bacterial-treated tissues, indicating that an active demethylation mechanism might operate. Whether this event is part of *Pseudomonas* pathogenicity strategies or it is involved in *Arabidopsis* defense responses remains to be elucidated.

PL-P11.
ARABIDOPSIS LARGE-SCALE GENE EXPRESSION PROFILING AT EARLY STEPS OF INFECTION BY ERYSIPHE

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Erysiphe cichoracearum UCSD is a virulent fungal isolate that causes disease on *Arabidopsis thaliana* Col-0 plants. A key early step for establishment of plant disease is the development of fungal haustorium. We here characterize the transcriptome of susceptible plants at this stage of infection and evaluate the contributions of salicylic acid (SA) and jasmonic acid (JA) to this interaction. cDNA microarrays (AFGC; 11500 ESTs; 4 biological replicates) were hybridized with labeled cDNAs derived from healthy and infected leaf tissues of Col-0 plants and *npr-1* or *jar-1* mutants impaired in SA and JA signaling, respectively. Stringent statistic analysis (*Significance Analysis of Microarrays* and ANOVA) allowed us to identify 173 genes affected with similar pattern by infection in all three plants. These genes might be putative markers of plant susceptibility. On the other hand, differential gene expression among wild type and mutant plants was studied. For that purpose, a standardized differences matrix was performed to further develop *Principal Component Analysis (PCA)*. The second and third principal components define a bidimentional space were genes were clustered in four groups by *Cluster Analysis*. Genes sensitive to SA and JA were therefore identified. Expression profiles of a discrete number of genes selected by both kind of analysis (susceptibility, JA- SA dependence) were confirmed by Northern blots.

PL-P12.
A COMPARATIVE STUDY OF PLASMA MEMBRANE AND TONOPLAST AQUAPORINS SHOWS DIFFERENT REGULATORY MECHANISMS

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Water balance in plants is a topic under continuous study due to its physiological relevance. In previous works we demonstrated the presence of a transcellular water pathway sensible to mercurial compounds in *Beta vulgaris* storage roots and functional evidences of the existence of aquaporins in plasma membrane (PIPs) and tonoplast (TIPs) of these root cells. To further investigate the contribution of the water permeability of these two main membranes in water transport, we perform two approaches: molecular detection of aquaporins (western blot) and experiments to discriminate differential regulation of the two subfamilies of water channel (volume changes of vesicles under osmotic shock followed by light scattering). The obtained results showed: 1) high water permeability values in both membrane fractions; 2) the presence of PIP1 and PIP2 in PM fractions and TIP3:1 in TP fraction, 3) different inhibitory effects by HgCl2 and 4) a complete blockage by acidic pH of PIPs contrasting with only a partial blockage of TIPs.
We postulate that this different behaviour of aquaporins in both membranes allow a fine tuning of water redistribution in the cell to cell pathway.

PL-P13.
AMARANTH STORAGE PROTEINS MOBILIZATION DURING GERMINATION

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An outstanding feature of seed storage proteins is their large size and oligomeric structure, like shown by globulins and prolamins. Low molecular-weight proteins, classified as albumins due to their solubility, have been found in certain species to act as storage proteins. In Amaranth, we have observed albumin mobilization during early seed imbibition.
The objective of the present work was to study protein fractions localization in the seed and their participation during germination. Protein bodies and protein fractions were obtained from *Amaranthus hypochondriacus* seeds at different stages of imbibition. These proteins were characterized by extraction solubility, electrophoretic and chromatographic bahavior. Protease activity was also analysed in these fractions.
Results demonstrated that proteins previously characterized as albumins were located outside the protein bodies whereas globulins and glutelins were inside them. Protein degradation took place in both compartments where protease activity at pH 5 was detected. A low molecular weight globulin fraction not yet described was found inside the protein body and degraded during imbibition. The albumin degradation outside the PB may constitute a more accessible source of nitrogen whereas inide the protein body the non aggregated, small globulins would be more easily mobilized than the oligomeric ones.

PL-P14.
SURFACE PROPERTIES OF AMARANTH GLOBULINS

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Globulin-P and 11S-globulin are major amaranth storage proteins. They are extracted with different solvents, a saline neutral buffer for 11S-globulin and water for globulin-P. Both globulins are oligomers with similar subunit composition. Globulin-P presents a great trend to polymerization and is integrated by molecular units (MU) and aggregates (A). To study the different behavior of these globulins, in this work we analyze their surface characteristics and try to find structural differences. Globulin-P and 11S-globulin were isolated and purified by isoelectric precipitation and gel filtration chromatography. Their solubility in different solvents was determined by the Lowry method and the soluble fractions composition was analyzed by gel filtration chromatography. The molecular surface charge was analyzed by ionic-exchange chromatography and surface hydrophobicity was evaluated using the fluorescent probe ANS. Both globulins showed different solubility properties. Their chromatographic behavior was similar suggesting that there were no charge differences among them. On the other hand, (A) presented different surface hydrophobic properties from (MU) and 11S-globulin. (A) showed a higher number of hydrophobic sites per molecule and a lower hydrophobic association constant (Ka) than the other two molecules. These results indicated that (A) may have distinctive structural characteristics than (MU), whereas MU and 11S-globulin structure would resemble. On this basis we may propose that the amaranth storage globulins present structural differences related to their aggregation state which might confer them different protease accessibility during mobilization.

PL-P15.
CADMIUM TOXICITY IS REDUCED BY NITRIC OXIDE IN SUNFLOWER LEAVES

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Cadmium (Cd) is a heavy metal present in soils, toxic for humans, animals and plants at very low concentrations. Nitric oxide is a bioactive free radical involved in many physiological responses. Cadmium toxicity and NO protection were evaluated in relation to some antioxidant parameters, as soluble and enzymatic antioxidants, in sunflower leaves, after 10 days of treatment with 0.5 mM Cd_2Cl, in plants pretreated for 7 days with 100 µM SNP (as NO donor). Plant growth (DW) was significantly reduced by Cd and was recovered in plants pretreated with NO. Chlorophyll content decreased to 48% and TBARS increased 33% over the controls, but NO returned their contents to 88% and 100% of the C. Ascorbate peroxidase (APOX) and superoxide dismutase (SOD) activities increased significantly with Cd, while CAT and GPOX activities decreased. Glutathione (GSH) content was lower and ascorbic acid (AsA) content was higher than controls in Cd-treated plants. Nitric oxide pretreatment alone did not modify the studied parameters respect to the control, except for APOX activity and AsA content, which increased even in the absence of the metal. SOD activity was reduced to C levels, CAT and GPOX activities were returned to values close to the controls, while GSH decline was reverted by NO pretreatment. In the present work, we present evidence of the protection exerted by NO against the oxidative stress and toxicity induced by Cd on sunflower leaves.

PL-P16.
***Helianthus annuus* L. ANTIOXIDANT RESPONSES AGAINST CADMIUM OR COPPER TOXICITY**

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During the last years, it has been observed a significant increase of metal soil contamination. Thus, the aim of this work was the study of cadmium and copper toxicity in sunflower plants exposed to 0.5 mM $CdCl_2$ or $CuCl_2$ during 16 days. Metal effect on sunflower growth was evaluated by the length of roots and shoots and the relative water content (RWC). Both metals produced an inhibition of plant growth, but RWC was only affected by cadmium. The raise in TBARS content evidenced the oxidative damage produced by these metals. Sunflower plants responded to stress increasing ascorbate peroxidase activity at day 7 and 10 in shoots and at day 16 in roots. On the other hand, cadmium and copper diminished guaiacol peroxidase activity in shoots at day 10 and 16 respectively. However, in roots, it was observed an increase of the enzyme activity. Superoxide dismutase showed a very variable behavior, depending on the metal used, the plant tissue and /or the day of treatment. Glutathione content decreased at day 10 and then increased at day 16. Polyamine and proline, reported as antioxidant metabolites, were also measured. Although putrescine increased in shoots at day 7 with both metals, it decreased the following days. In roots, it increased from day 7 of treatment. Proline content showed a raise both in shoots and roots. It is clear that sunflower is responding against the metal stress by increasing some antioxidant defenses. However, growth inhibition and membrane lipid peroxidation could not be avoided.

PL-P17.
BIOCHEMICAL ASPECTS OF THE INTERACTION BETWEEN *AZOSPIRILLUM BRASILENSE* AND RICE SEEDLINGS. INVOLVEMENT OF CA++ DEPENDENT PROTEIN KINASE (CDPK) IN THE MECHANISM OF GROWTH PROMOTION

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The main goal of this work is to study the signaling pathway involved in the *Azospirillum*-rice plant interaction which results in plant growth promotion. It is widely accepted that the free cytosolic calcium concentration is a convergent point of diverse signaling pathways in plants. This study was focused on the involvement of calcium-dependent protein phosphorylation in the interaction plant/bacteria. We found that a calcium dependent protein kinase (CDPK) activity is stimulated in response to *Azospirillum brasilense* inoculation. The activation is particularly evident in crude extract prepared from leaves and roots (grain discarded) after 48 h inoculation. At the physiological level the rise in CDPK activity is accompanied by an increase in the number and length of lateral and secondary roots. On the other hand, in non inoculated plants, these physiological changes are blocked by the presence of W7, a calmodulin antagonist inhibitor of CDPK activity. Altogether these results seems to indicate that CDPK activity is involved in the development of roots and that bacteria could also promote plant growth through a pathway that involves activation and/or enhancement of CDPK expression.

PL-P18.
ENHANCED PLANT TOLERANCE TO IRON STARVATION BY FUNCTIONAL SUBSTITUTION OF CHLOROPLAST FERREDOXIN WITH A BACTERIAL FLAVODOXIN

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Iron is essential for respiration, photosynthesis and general plant metabolism. Its deficiency imposes severe limitations to plant development, mainly in calcareous soils in which it is not readily available. Cyanobacteria and certain unicellular phototrophic eukaryotes elicit adaptive mechanisms to overcome nutritional limitations, e.g., under Fe deprivation they induce the expression of flavodoxin (Fld), a flavoprotein that exerts a partial functional substitution of the metalloprotein ferredoxin (Fd) in relevant processes such as photosynthesis. As vascular plants lack Fld we decided to assess the behavior of iron starved transgenic tobacco lines expressing Fld of *Anabaena* PCC7119. Plants accumulating the transgenic product in chloroplasts turned out to be more tolerant to iron shortage than the wildtype siblings showing a higher growth rate and development. Photosynthetic activity was higher in the transformants even though the Fd levels were also low in these plants, suggesting a functional substitution of the ferrosulfoprotein by Fld.

PL-P19.

HAHB-4 (*HELIANTHUS ANNUUS* HOMEOBOX) EXPRESSION LEVELS IN ARABIDOPSIS TRANSGENIC PLANTS ARE CORRELATED WITH DROUGHT TOLERANCE

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Hd-Zip proteins constitute a family of transcription factors found only in plants. *Hahb-4* is a previously described member of sunflower subfamily I. Transgenic Arabidopsis plants that overexpress *Hahb-4* under the control of the 35S CaMV promoter were obtained. These plants showed a marked tolerance, improved development, a healthier aspect and higher survival rates that wild-type ones when they were subjected to severe water stress conditions.
In this work we have made a comparison between the phenotypes generated by several constructions bearing *Hahb-4* under the control of different promoters. The 35S CaMV promoter was compared with *Hahb-4* or RubisCO SSU ones. Stronger stress tolerance have been observed with the SSU promoter than with the 35S CaMV while *Hahb-4* own promoter region has shown a similar behaviour than the first one. Neither of them affect seed production. SSU in some of the transgenic lines expressing *Hahb-4* at high levels, produced an enlargement of the life cycle at the same time of a better adaptation to stress conditions. We propose the construction of quimeric promoters to obtain optimal results.

PL-P20.

NADP-MALIC ENZYME FAMILY FROM *ARABIDOPSIS THALIANA*

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The *A. thaliana* genome contains four genes encoding putative NADP-malic enzymes (NADP-ME). One of the genes encodes a putative plastidic protein, while the other three do not possess any organellar targeting sequence. In order to characterize the AtNADP-ME gene family, the full length cDNAs of each isoform were isolated by RT-PCR. The amplified products were subcloned into pET-32 expression vector. The recombinant proteins were purified and used for kinetic and structural characterizations, as well as for the production of polyclonal antibodies. The expression pattern of NADP-ME in *A. thaliana* was analyzed during the whole life cycle. For this purpose, promoter regions of all four members of the NADP-ME family were amplified by PCR and cloned into the binary vector pGPTV-BAR, which carries the GUS gene. The plasmids containing the chimeric *MEA*::*GUS* genes were introduced into *A. thaliana* by *A. tumefaciens* mediated transformation and the transgenic lines obtained were analyzed for GUS activity. On the other hand, knock-out lines carrying T-DNA insertion in the different *A. thaliana* NADP-ME genes were isolated. Loss-of-function mutants not exhibit major phenotypic differences respect to wild type at non-stressed conditions. However, comparative enzymatic activity assays in several tissues indicated specific patterns of expression of the different isoforms, as obtained by GUS activity. In summary, we present the characterization of a complete NADP-ME gene family, which is the basis to unreveal the biological function of each NADP-ME isoform.

PL-P21.

***ARABIDOPSIS THALIANA* EXPRESSING HIGH LEVELS OF MAIZE NADP MALIC ENZYME DISPLAYS MODIFIED PHOTOSYNTHETIC PARAMETERS**

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Arabidopsis thaliana (Columbia-0 ecotype) was transformed with a vector containing the maize chloroplastic NADP-malic enzyme (NADP-ME) cDNA under the control of 35SCaMV promoter. This enzyme catalyzes the oxidative decarboxylation of malate, generating pyruvate, CO_2 and NADPH. Homozygous plants for the transgene were selected and the level of ME expression was examined in several lines. The line with the highest expression level showed a 30-fold increase in NADP-ME activity. Western blots, as well as non denaturing electrophoresis stained for ME activity, confirmed the presence of 62 kDa ME in this plant. Plants grown under normal light intensity showed no major morphological differences compared to the wild type line (WT). However, chlorophyll content as well as photosynthetic performance indicators like CO_2 assimilation, Fv/Fm and ΦPSII were significantly lower for transgenic lines. These results suggest that the presence of a highly active NADP-ME in chloroplasts of *A. thaliana*, could have important effects on the reductive power balance of this organelle, diminishing the efficiency of the overall photosynthetic process. In this way, the mechanisms that protect the photosynthetic machinery, normally triggered by a raise in NADPH level, are exceeded in the transgenic line leading to photoinhibition. The transgenic plants obtained in the present work will be use as a tool to better understand the regulation of plastid redox metabolism.

PL-P22.

OXIDATIVE STRESS AND *A. thaliana Frataxin homolog* (*AtFH*)

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Frataxin is a highly conserved protein from bacteria to mammals that has been proposed to participate in iron-sulfur cluster assembly, mitochondrial iron homeostasis and defense against oxidative stress. We reported the cloning of *AtFH*, a plant gene with significant homology to other members of the frataxin family. Transcription analysis showed that *AtFH* is ubiquitously expressed with higher levels in flowers. Complementation of a *S. cerevisiae* mutant (Δ*yfh*) proved that AtFH is a functional protein, because it restored normal rates of respiration, growth, and sensitivity to H_2O_2. Infiltration of *Arabidopsis* leaves with H_2O_2 showed that *AtFH* is induced compared with control leaves. In *AtFH* null mutants mRNA levels of *Peroxiredoxin, Calreticulin* and *GAPDH* were induced and we also observed an increased production of superoxide in leaves by histochemical detection. Considered together, our results support the involvement of AtFH in mitochondrial respiration and survival during oxidative stress in plants.

PL-P23.

FUNCTIONAL STUDIES OF PLANT MITOCHONDRIAL CYTOCHROME *C* OXIDASE GENE PROMOTERS

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Cytochrome *c* oxidase (COX), the terminal respiratory chain complex of mitochondria, is composed of 3 subunits encoded in the mitochondrial genome and several subunits encoded in the nuclear genome. In plants, there are 4 polypeptides encoded in the nuclear genome associated with the enzymatic core: COX5b, COX5c, COX6a and COX6b. We have previously obtained evidence of a coordinated control of gene expression for some of these subunits. In this work, we tried to gain insight into the structure and function of *Arabidopsis COX5c* and *COX6b* promoters by the analysis of transgenic plants that contain promoter/gus fusions. Promoter sequences from two different *COX5c* genes produced significant GUS expression in meristems, vascular tissues and pollen. Reduced expression was also observed in the root cortex, cotyledon blades, flower organs, silique veins and developing seeds. In lines that contained *COX6b1* promoter sequences, GUS activity was detected in leaf veins and hydathodes, and in sepal veins and blades, ovary, pollen and anther filaments. In *COX6b2* lines, GUS expression was significantly lower than in *COX6b1* lines, and the stained tissues included leaf veins and hydathodes and pollen. The results indicate that the promoters under study are preferentially active in tissues with higher proliferative activity and produce similar, though not identical, expression patterns.

PL-P24.

EXPRESSION ANALYSIS OF *COX19* GENES IN *ARABIDOPSIS THALIANA*

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We have studied the expression of an Arabidopsis novel nuclear gene, Atcox19, that shares sequence similarity to cox19, a gene encoding a protein required for expression of cytochrome c oxidase in *Saccharomyces cerevisiae*. COX19p is present in the cytoplasm and mitochondria of yeast. This protein probably functions post-translationally during the assembly of the enzyme and is probably involved in metal ion transport. In Arabidopsis, there are two genes for cox19 with high sequence homology. We have detected the presence of two different transcripts originated by alternative splicing from one of the genes. To study the expression mechanisms of this gene we have made northern blot and RT-PCR analysis of the corresponding transcripts. Incubation of plants in solutions containing metal ions (Cu^{2+}, Fe^{2+}, Zn^{2+}) or compounds related with the generation of reactive oxygen species (salicylic acid, sodium nitroprusside, H_2O_2), produced an increase in transcript levels. Induction was also observed in the presence of the cytokinin 6-benzylaminopurine and sucrose. Competitive RT-PCR showed that the shorter transcript is considerably more abundant under most conditions. Our results, as well as the presence of four conserved cysteine residues in AtCOX19p, suggest that the products of Atcox19 may function in metal transport to mitochondria and cytochrome c oxidase assembly in plants.

PL-P25.

NITRIC OXIDE REGULATES CELL CYCLE PROGRESSION DURING LATERAL ROOT DEVELOPMENT

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Lateral root (LR) formation consists of two mayor steps: cell cycle reactivation in the pericycle and establishment of a new meristem. Nitric oxide (NO) is a bioactive molecule that functions in numerous processes in plants. Recently we have reported that the NO depletion results in a drastic reduction of LR primordia in tomato (*Lycopersicon esculentum*). Moreover, NO induces cell cycle genes like cyclins and cyclin dependent kinases (CDK) in roots. However, the lack of synchrony of the initiation events makes difficult to follow the development of LRs and attribute a more precise role to NO in cell cycle regulation. In this work we developed a system that allows the synchronization of the pericycle cells. The system was based on seeds incubated in the presence of the NO scavenger CPTIO for 48 h to prevent LR formation and then treat the seedlings with the auxin naphtyl acetic acid (NAA) or the NO donor sodium nitroprusside (SNP) to induce pericycle activation. Semiquantitative RT-PCR analysis showed that NO stimulates the progression of G1-S transition by the induction of D-type cyclin. In addition, the transcript of the CDK inhibitory protein KPR2 was downregulated by NO. Supporting these evidence, roots treated with SNP showed higher levels of DNA compared to control ones. All together, these results suggest a novel role for NO in the regulation of cell cycle progression in plants.

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PL-P26.

EXPRESSION OF BOTH GENE OF REVERSIBLY GLYCOSYLATED POLYPEPTIDE OF RICE PLANTS

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Reversibly glycosylated polypeptides (RGPs) are involved in plant polysaccharide synthesis such as in hemicellulose and starch synthesis [1]. Previously, we reported the presence of an RGP protein in rice (*Oryza sativa*) seeds and *in vitro* plants which posse activity upon incubation with UDP-Glc, UDP-Xyl, UDP-Gal. When OsRGP reaction was carried out in the presence of UDP-[^{14}C]Glc as sugar donor and then 1 mM UDP was added in a chase-out experiment, we observed that OsRGP reaction seems to be reversible. Here, we studied the expression of the RGP protein in ungerminated seeds, early-germinated seeds (two days of culture), or *in vitro* plants (5, 8 and 10 days of culture) with the aim to analyze protein regulation. Also, we investigate whether the protein is regulated by hormone involved in plant growing. We found a clear activity and protein induction after AIA+BAP treatment. Different plant developmental states shown different electrophoretic mobility of the 38-kDa subunit. In order to investigate if a post traducctional modification or different isoforms are present, we perform northern blot analysis using two specific probes corresponding to both genes described in rice (RGP1 and RGP2). We conclude that OsRGPs polypeptides are developmentally and also hormonally regulated.

PL-P27.

A DOMINANT NEGATIVE MUTATION IN A TIR-NBS-LRR GENE INDUCES CONSTITUTIVE SHADE AVOIDANCE DEVELOPMENTAL PATTERNS IN *ARABIDOPSIS*

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Plants can detect the presence of neighbour through the reduction in the ratio of red (R) to far-red (FR) light caused by the selected transmission and/or reflection of light in green tissues. In response to this signal, plants accelerated stem growth and place the leaves at a more erect position, a developmental strategy known as shade avoidance response. Here we describe the novel constitutive *csa1* mutant, which displays elongated stems and erect leaves under high R/FR ratios, and did not respond to light signal that simulate the presence of neighbours. All these phenotypes are also characteristics of the phytochromes B mutant (phyB mutant). However, phyB levels were mot affected in *csa1* seedlings, indicating the mutant affects signalling downstream of this photoreceptor.
The *csa1* has a T-DNA insertion within the second exon of a Toll/ Interleukin 1receptor-nucleotide binding site-leucine-rich-repeat (TIR-NBS-LRR) type gene, which led to the production of a truncated mRNA. Transgenic plants expressing the truncated TIR-NBS-LRR gene recapitulated the mutant phonotype, indicating that *csa1* is a dominant negative mutation. In plants TIR domains containing proteins had only been implicated in the perceptions of pathogens. Our results indicate that response to pathogens and neighbours share evolutionary related core-signalling components.

PL-P28.

REGULATORY PHOSPHORYLATION OF PEP CARBOXYLASE IN DEVELOPING CASTOR OIL SEEDS

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Two PEPCase isoforms (PEPC1 & PEPC2) were recently purified and characterized from endosperm of developing castor oil seeds (COS) (Blonde & Plaxton 2003 J Biol Chem 278:11867). The association of a common 107 kDa catalytic subunit (p107) with an unrelated 64 kDa polypeptide (p64) leads to marked physical and kinetic differences between the PEPC1 p107 homotetramer and novel PEPC2 p107/p64 heterooctamer. Here we describe the production of anti-phospho-site specific IgG to the conserved *N*-terminal phosphorylation site (Ser5) of p107. This IgG was used to establish that p107 phosphorylation is: (i) maximal in full cotyledon (stage VII) developing COS, (ii) more pronounced in PEPC1 than in PEPC2, (iii) reversed following PEPC1 incubation with bovine PP2A, and (iv) not involved in a possible interconversion of PEPC1 and PEPC2. Enhanced p107 phosphorylation during COS development was correlated with an 8-fold increase in PEPC's I_{50}(malate). Ca^{2+}-independent PEPC protein kinase activity was detected throughout COS development. One day following decapitation of shoots containing intact developing COS, COS PEPC kinase activity was unaffected, whereas the p107 of stage VII COS was entirely dephosphorylated. Thus, p107 phosphorylation appears to be at least partially dependent upon the 'fine control' activation of COS PEPC kinase due to sucrose import from source tissue.

PL-P29.

CDPK EXPRESSION AND ACTIVITY DURING SPROUTING AND LEAF DEVELOPMENT IN POTATO PLANTS

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The sessile form of plants emphasizes the requirement of efficient adaptation to environmental conditions. Calcium is a second messenger that plays a main role in plant physiology and Calcium-dependent calmodulin-independent protein kinases (CDPKs) are key intermediates in calcium signaling, that couple changes in Ca^{2+} levels to a specific response. Tuberization in potato plants (*Solanum tuberosum*) is triggered in response to changing environmental conditions. StCDPK1, an active CDPK isoform (59 kDa), is transiently induced in swelling stolons while StCDPK3 is expressed in early non swelling stolons. Semiquantitative RT-PCR and Northern blot assays were used to monitor CDPK expression in dormant tubers, sprouts, emerging and fully expanded leaves and the PCR fragments were subcloned and sequenced. Though no significant expression or CDPK activity was detected in the dormant tuber, StCDPK1 expression was enhanced in sprouting tubers and in emerging leaves but disappeared in fully expanded leaves. In contrast, StCDPK2 and StCDPK4 were expressed in green tissues confirming previous Western blot data that suggested that different CDPK isoforms are present in potato leaves.

PL-P30.

MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF T-DNA INSERTIONAL MUTANTS IN THREE γ CARBONIC ANHYDRASE SUBUNITS OF ARABIDOPSIS MITOCHONDRIAL COMPLEX I

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The respiratory chain of plant mitochondria has unique features. Electron transport is especially branched due to the presence of rotenone insensitive NAD(P)H dehydrogenases and a cyanide insensitive terminal oxidase. Moreover, the respiratory protein complexes in plant mitochondria contain additional subunits that allow them to catalyse secondary o modified functions. Recently, we have described a novel group of structurally related complex I subunits in *Arabidopsis* that show high structural similarity to γ carbonic anhydrases (γCAs). In addition, it has been reported that γCAs are only present in mitochondria of plants and green algae suggesting these proteins are important for mitochondrial complex I function of photosynthetic eukaryotes.
In this work we show that *γca1*, *γca2* and *γca3* Arabidopsis knock-out lines differentially impair complex I assembly. In addition, all three γCAs occur in several isoforms presumably by post translational modifications. Involvement of these proteins in plant respiration will be discussed.

PL-P31.
PHOSPHORYLATION OF THE MOVEMENT PROTEIN TGBp1 OF POTATO VIRUS X BY A CK2-LIKE PROTEIN KINASE

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Potato virus X (PVX) is a member of the potexvirus whose RNA genome codes for the viral replicase, three movement proteins (MPs: TGBp1, TGBp2 and TGBp3) and the viral capsid protein (CP). The principal role of MPs is to assist in the spreading of viral progeny from cell to cell and over long distances. Growing evidences suggests that phosphorylation events can regulate MP functions. We have demonstrated that PVX TGBp1 is phosphorylated on serine and threonine residues by a kinase present in both PVX-infected and non-infected *Nicotiana tabacum*. Here we show that this kinase activity displays characteristics of casein kinase CK2: it is inhibited by heparin, activated by polylysine, and is able to use both ATP and GTP as a phosphoryl donors. We also demonstrate that TGBp1 is phosphorylated by a human CK2 α subunit and a partially purified *N. tabacum* CK2. In addittion, *in situ* phosphorylation assays show that native PVX TGBp1 is phosphorylated in plant extracts of PVX-infected plants by a CK2-like cellular kinase. Based on comparative analyses of potexvirus TGBp1 we have developed two different PVX mutant on threonine residues. Preliminary infection experiments show that the mutants are not able to produce systemic infection. These results show that TGBp1 is phosphorylated by a host kinase closely related to CK2.

PL-P32.
DISSECTING THE EARLY MOLECULAR EVENTS IN POPLAR LEAVES UPON INFECTION WITH RUST

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Melampsora larici-populina, the basidiomycete responsible for poplar rust, causes the most important damages in european poplar plantations. Nowadays, no more resistant cultivars are available since new virulent strains of *M. larici-populina* are developping regularly. To characterize the response of *Populus trichocarpa x deltoides* cv. Beaupré to virulent (98AG31) and avirulent (93ID6) strains of *M. larici-populina* and identify genes that may play a role in resistance, expression profiling of plants have been carried out. The fold-change and significance (t-test) helped us to derive groups of genes coordinately regulated during both compatible (disease) and incompatible (resistance) interactions. A marked change in gene expression was observed at multiple levels: (a) a general activation of defense and stress-response genes during incompatible interaction which peaked 48h post-infection, and (b) the early down-regulation of genes encoding primary metabolism, photosynthesis and photorespiration enzymes in both interactions. This set of pathogenesis-modulated genes is an important resource for understanding the genetic interactions underlying defense mechanisms, signaling and responses.
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PL-P33.
CHARACTERIZATION OF WATER PERMEABILITY OF *Beta vulgaris* ROOT PROTOPLASTS

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Previous works showed that water transport studies performed in a purified fraction of root plasma membrane vesicles of *Beta vulgaris* are an excellent approach for characterising water channel properties. Nevertheless, in this model the cytoplasmic machinery of the cell is disrupted. In order to study water transport at a cellular level, swelling assays must be performed in protoplasts. To achieve this strategy, *Beta vulgaris* root protoplasts were isolated from the root parenchyma and exposed to a hypoosmotic shock employing two different methodologies: one in which protoplasts were held with a glass pipette during the osmotic shock (microchamber technique) and other where protoplasts were transferred from iso to hypoosmotic solution (step changes). In both cases, volume changes after osmotic shock were followed by light microscopy to calculate osmotic water permeability coefficient (P*f*). P*f* was 25 ± 4 $\mu m.s^{-1}$ (n=18) and 24 ± 8 $\mu m.s^{-1}$ (n=22) respectively, showing no differences between both methodologies. Permeability was not altered in the presence or absence of 5 mM K^+ in the external solution, but was dramatically reduced by 10 mM external Ca^{+2}. In accordance with our previous results in isolated membranes, calcium reduces water permeability also at a cellular level, proving to be an important regulatory mechanism of water transport in *Beta vulgaris* root cells.

PL-P34.
THE STIMULATION OF THE CHLOROPLAST FERREDOXIN-THIOREDOXIN ROUTE BY A CYANOBACTERIAL FLAVODOXIN CONFERRED OXIDATIVE STRESS TOLERANCE IN PLANTS

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Flavodoxins (Fld) are FMN- containing electron carriers present in bacteria, diatomea and a few planctonic algae, but not in plants. In cyanobacteria, flavodoxin can replace ferredoxin (Fd) in many reactions. Overexpression of Fld I in *E. coli* leads to augmented tolerance toward various sources of oxidative stress. These results prompted us to evaluate whether introduction of flavodoxin in tobacco plants could result in a similar increase of stress resistance. Tobacco plants expressing *Anabaena* flavodoxin in chloroplasts developed tolerance to multiple sources of stress, including redox-cycling herbicides, ultraviolet radiation, extreme temperatures, high irradiation, drought and pathogen-induced necrosis. Tolerance correlated with flavodoxin levels in chloroplasts, whereas cytosolic expression of the flavoprotein was without effect. Oxidant build-up and oxidative inactivation of thioredoxin-dependent plastidic enzymes was ameliorated in the stressed tolerant plants, and flavodoxin-driven reduction of thioredoxin was demonstrated *in vitro*. Because the Fd amounts declined under oxidative stress while Fld levels were not affected, the tolerance thus depends on functional replacement of Fd by Fld in the Fd-thioredoxin dependant pathways of the host.

PL-P35.

GENETIC DIVERSITY OF ASPIDOSPERMA QUEBRACHO-BLANCO SCHLTDL, REVEALED BY RAPD ANALYSIS

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Aspidosperma quebracho-blanco Schltdl is a native species, present in naturals forest of the Provincia Chaqueña. RAPDs markers were used to study genetic diversity between and within diferent populations in San Luis province. Twenty arbitrary primers 10-mer (Kit A Operon Technologies) were used to test 80 samples from different locations to determine which primers generate clear bands. A total of 134 different RAPDs bands were generated by 9 of these primers. Genetic diversity was estimated by the Shannon-Weaver index and thus, a high genetic variability was detected within each population. Jaccard's similarity coefficient was calculated using NTSYS-PC software. A cluster analisys based on the similarity matrix was performed using unweighted pair group method with arithmetical averages (UPGMA). The molecular dendrogram suggests that samples can be grouped into clusters which correlates with the geographical populations. This study represents a first report in molecular analysis in *Aspidosperma quebracho-blanco* in our country and it could be useful in conservation of forest genetic resources.

PL-P36.

POTATO ASPARTIC PROTEASES (*St*APs) ANTIMICROBIAL SPECIFICITY

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Plant aspartic proteinases (EC 3.4.23) (APs) have been implicated in the plant defence response; however the role of these proteins in this mechanism is not clear. We have previously reported the purification of two potato APs (*St*APs1 and 3) that are induced by both abiotic and biotic stresses. Both enzymes have antimicrobial activity towards two potato pathogens. On the other hand, we have isolated from potato leaves a cDNA encoding an aspartic proteinase (Gen Bank accession number AY672651). *St*Asp has an internal domain (PSI) with a high homology to saposin like-proteins (protein family associated to antitumoral and antimicrobial activity related to their membrane leakage activity).The PSI presence in mature StAPs could explain the bifunctional activity (proteolytic and antimicrobial) previously reported for *St*APs.
In order to study the *St*APs antimicrobial activity specificity to compare it with SAPLIP proteins, we have performed *in vitro* assays incubating different amounts of StAP1 with liquid cultures of *Escherichia coli*, *Bacillus cereus* and *Staphylococcus aureus*. Results obtained show that *St*AP1 was able to reduce the growth of these bacterial cultures in a doses dependent form as well as inhibit the cyst of *P. infestans* and conidia of *F. solani* germination. Differences in the minimum *St*AP1 amounts to decreased bacterial growth were observed between bacterial cultures. This is the first evidence about antibacterial activity of plant AP; these results and the presence of PSI domain in *St*Aps suggest a functional homology of these enzymes with SAPLIPs proteins.

PL-P37.

CHARACTERIZATION OF A NOVEL NON-PLASTIDIC NADP-MALIC ENZYME FROM MAIZE ROOT

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NADP-malic enzyme (NADP-ME 1.1.1.40) catalyses the oxidative decarboxylation of L-malate to yield pyruvate, CO_2 and NADPH. In maize, two plastidic isoforms have been characterized, whereas a genomic clone that codifies for a putative NADP-ME, specifically expressed in root epidermis, was cloned. In this work, the cDNA for this third isoform was obtained by RT-PCR using 1-2 day-old roots, expressed in *E.coli*, and the product obtained purified to homogeneity. The kinetic characterization of the recombinant NADP-ME (53 UI/mg, Km_{malate} 1.4 mM, Km_{NADP} 19 µM) were significantly different from the previous characterized maize NADP-MEs. From various metabolites analysed, fumarate presented a strong activation effect, while fructose1,6-biP inhibited the enzyme. The analysis of the protein by native PAGE suggests that the enzyme exists in a tetrameric form *in vitro*. Western blot analysis using antibodies raised against this recombinant NADP-ME detected a 65 kDa-protein in maize root extracts. *In vitro* import studies to chloroplasts using the complete cDNA isolated suggested that this NADP-ME protein is not sorted to this organelle, while prediction programmes detect a putative mitochondrial transit peptide in this sequence. In summary, in the present work a novel maize malic enzyme with properties resembling animal NADP-MEs (e.g. fumarate activation and subcellular localization) was characterized.

PL-P38.

EARLY EVENTS IN THE DEFENSE RESPONSE OF STRAWBERRY TO *COLLETOTRICHUM SPP*

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Plants exhibit a wide array of defense strategies against potential pathogens. They possess preformed biochemical defenses and physical barriers that are presented to intruders to hinder the invasion. Additionally, fast inducible defenses may be activated by a pathogen attack. The oxidative burst, a rapid and transient generation of reactive oxygen species (ROS), is one of the earliest events of the plant defense response. We have investigated the occurrence and localization of oxidative burst in compatible and incompatible interactions of *Colletotrichum spp* with *Fragaria x ananassa cv. Pájaro*. In the incompatible interaction, leaves spray-inoculated with a conidial suspension, showed positive DAB (3,3'-diaminobenzinide) staining in small clusters of cells 2 hs after infection. These microbursts were not observed in the compatible interaction. When inoculation were carried out at the primary leaf, microbursts appeared in secondary leaves 4 hs after the infection, indicating the generation of a diffusible signal able to induce systemic microbursts. We have also studied the temporal expression of genes involved in the regulation of the cellular redox status and others, such as the glutathione-S-transferase, Ca^{++} signaling and PR proteins, and evaluate their participation in the oxidative burst and the defense response observed.

PL-P39.
IDENTIFICATION OF A NITRIC OXIDE-REGULATED DIFFERENTIALLY EXPRESSED mRNA DURING THE LATERAL ROOT DEVELOPMENT IN TOMATO

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Nitric Oxide (NO) is a free radical involved in several physiological processes in plants, many of which are related with responses mediated by phytohormones. For instance, NO participates in the signaling pathway triggered by Auxins that induces the development of lateral roots and inhibits the elongation of the primary root in tomato. In this study, the technique of Differential RNA Display (DD) was used for the identification of NO-induced differentially expressed genes in tomato roots. The profile of DD showed changes in gene expression of seedlings treated with the NO donor sodium nitroprussiate (SNP). One cDNA clone (*Le16*) corresponding to differentially expressed transcripts was sequenced since it displayed low amounts of RNA in NO treatments in both DD and Northern Blot analysis. The nucleotide sequence of *Le16* was 100% homologue with the 3'-UTR of a gene that encodes for a protein that binds to an Ethylene Reponse Element (EREBP). A DNA binding domain AP2 was found from the deduced aminoacid sequence of EREBP which is also present in many plant transcriptional regulators. These findings suggest that *Le16* would act as a transcriptional regulator of genes that encode for proteins whose expression links NO and ethylene signaling cascades during the lateral root formation.
**These authors contributed equally to the present work.*

PL-P40.
FUNCTIONAL STUDY OF POLLEN-SPECIFIC AQUAPORINS IN *Arabidopsis thaliana*

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The first step for an efficient pollination and fertilization is the hydration of pollen grains, suggesting that the movement of water through the pollen grain is a physiologically regulated process that does not depend only on osmolarity and water potential differences. Aquaporins may be involved in this regulation, increasing the water movement through the pollen grain membrane. Using available information about *Arabidopsis thaliana´*s loci (www.arabidopsis.org) which shows high homology with aquaporin genes and also analyzing available transcription data of tissue-specific arrays (http://affymetrix.arabidopsis.info), we selected four pollen-specific loci (At3g47440, At4g01470, At5g37810 and At3g04090) homologous to aquaporin genes. We measured volume changes in *Xenopus leavis* oocytes injected with the four different aquaporin cRNAs. We will assess the importance of these four aquaporin genes in the proposed pathways, isolating mutant homozygote plants for all these genes and then cross them to obtain double, triple and quadruple mutants. We will analyse their *in vitro* germination pollen ability and also the fertility of these plants evaluating the number and viability of obtained seeds. We will test for the localization of aquaporin proteins in pollen grains by transforming plants with a construct in which the LAT52 promoter is driving expression of the aquaporin proteins tagged with a C-terminal GFP fusion.

PL-P41.
PHYSIOLOGICAL PRODUCTION OF NITRIC OXIDE DURING GERMINATION OF SORGHUM SEEDS

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Nitric oxide (NO) content and its enzymatic sources of generation were evaluated during the germination of sorghum seeds. Germination of embryonic axes, defined as the development of a root 1-2 mm long, started after 24 h of imbibition. A distinctive EPR signal for the adduct MGD-Fe-NO was detected in the homogenates from axes isolated from seeds imbibed in the presence of 12 mM nitrate in a time-dependent manner. Also a close association between MGD-Fe-NO adduct content in the homogenates and nitrate supplementation to the incubation medium was observed. The activities of the NADH-dependent nitrate reductase and nitric oxide synthase were measured in axes, by the detection of NO by EPR under conditions of maximal supplementation of substrates. A significant increase in both enzymatic activities was determined between 24 to 30 h of imbibition. Total content in sorghum axes of β-carotene and α-tocopherol increased significantly as the germination occurred. The data reported here are the first observations employing EPR to assess for NR and NOS activities simultaneously in an active growing tissue. Moreover, since the increase in NO content preceded the initiation of phase II of development and the sharp increase in oxygen consumption, a potential role for NO as a signal molecule should be considered.
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PL-P42.
REGULATION OF THE EXPRESSION OF GENES IN PLANTS BY FOTORRECEPTORES

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Light is one of the most important factors in plant development of plants. Plants have involved systems that allow them to sense the light quality and intensity. Phytochromes, specific photoreceptors, are in charge of sensing the luminous signal, which is change in the gene expression followed by.
We study the signal transduction chains of the plant photoreceptors and the transcription factors that act at the end of the signaling cascades. With this purpose, the full-length promoter Lhcb1*2 and several DNA fragments of it were fused to a gusA coding region (GUS), and transferred to *Arabidopsis thaliana* plants via *Agrobacterium tumefaciens*. Transgenics plants were treated under different light conditions and the GUS activity was considered a measure of the Lhcb1*2 gene expression.
We found that a 35 bp fragment of the tobacco Lhcb1*2 promoter was involved in the *phyA* mediated high-irradiance response (HIR). The 35 bp fragment was used in one-hybrid experiments to isolate the transcription factor involved in the HIR response. Reverse genetic was used to address the "*in vivo*" role of the cloned transcription factor.

PL-P43.

STCDPK1 TRANSGENIC POTATO PLANTS RESPOND ANOMALLY TO GIBBERELLINS (GAs)

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Potato tuberization is a complex organogenic process in which an underground stolon develops into a storage organ. During stolon to tuber transition different calcium dependent protein kinases (StCDPKs) are differentially expressed. StCDPK1 expression is enhanced in developing stolons and is induced by sucrose suggesting that it could be involved in the signalling cascade triggered upon tuberization. Transgenic potato plants that displayed different StCDPK1 expression (0,1X to 4X) than the wild type plants were produced.
Stem cuttings of the different transgenic lines were grown in tuber inducing conditions (MS with 8% sucrose) with the addition of GAs (0,05 and 0, 5uM) or of the GAs inhibitor, CCC (0,05 -0,5 mg/ml). B7 plants with reduced StCDPK1 expression didn't respond to GAs (0,05 amd 0, 5) while the other transgenic lines showed an anomalous dose-response pattern. In addition, B7 plants developed tubers in MS 8% earlier than the control plants.

PL-P44.

BACTERIAL CYCLIC β1-2) GLUCAN (CβG) ACTS IN SYSTEMIC SUPPRESSION OF PLANT DEFENSE RESPONSES

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To cause disease, a successful pathogen must counter or evade pre-formed defenses and suppress or fail to elicit induced defences. Here we identify the CβG from the phytopathogen *Xanthomonas campestris* pv. *campestris* as a systemic suppressor of plant immune responses. To investigate the proposed role of the *Xcc* CβG in suppression of plant defense, *N. benthamiana* leaves were pre-infiltrated with purified CβG before inoculation 24h later with *Xcc* strains. Interestingly, leaves pre-infiltrated with CβG showed disease symptoms in response to *Xcc* strain 8523, unable to produce CβG, whereas pre-infiltrated with water did not. Northern blot experiments showed that CβG also suppressed *PR1*(pathogenicity related) gene expression in a local and systemic fashion. Radio-labelled CβG(^{14}C) was generated by incubation with membrane preparation from *Xcc* 8004 (wt) with UDP-[U-^{14}C]-D-glucose. Leaves from four weeks old plants were infiltrated and the radioactivity in both, these leaves and distant leaves, was assessed at different times after infiltration. The radioactivity per unit area decreased with time in infiltrated leaves, whereas distant leaves showed a significant increase in radioactivity 6 hours after inoculation. These findings suggest that the systemic effects of CβG on suppression of plant defence could be due to the systemic spread of the molecule itself.

PL-P45.

IS ANY RESIDUE ON *ANABAENA* FLAVODOXIN SURFACE CRITICAL FOR THE INTERACTION AND ELECTRON TRANSFER WITH FERREDOXIN NADP-REDUCTASE?

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During the photosynthesis process, Ferredoxin (Fd) transfers electrons from Photosystem I to Ferredoxin-NADP$^+$ reductase (FNR). When some algae and cyanobacteria are grown under iron-deficient conditions, Fd is efficiently replaced in this function by Flavodoxin (Fld). Although it is generally accepted that both Fd and Fld interact with the same region of FNR, there is no evidence to suggest how the formation of the Fld:FNR complex occurs. In this study we have try to identify critical residues on the Fld surface for complex formation and electron transfer to FNR. Having into account theoretical three-dimensional models proposed for the Fld:FNR interaction the T12, E16, W57, E67, D126 and D144 side-chains of *Anabaena* Fld have been replaced to study their implication in complex formation and electron transfer with FNR. Kinetic parameters and redox potentials have been established. Our results provide a substantial evidence that each studied residue contributes with a limited effect on the stability and functionality of the FNR:Fld complex. However, the data indicate that there is no single residue on the Fld surface critical in its interaction with FNR.

PL-P46.

ROLE OF FERREDOXIN ON THE MECHANISM OF ANTIOXIDANT DEFENCE USING TRANSGENIC PLANTS

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Ferredoxins (Fd) are a group of low molecular-weight proteins which have Fe-S centres. Their major function is electronic transfer. The Fds of the 2Fe-2S class act as a soluble electronic transporters between Photosystem I and ferredoxin-NADPH reductase (FNR) during the light phase of photosynthesis, to generate NADPH needed for CO2 fixation. A novel cDNA encoding tobacco ferredoxin (*Nicotiana tabacum* cv Petit Havana) was isolated by PCR (fdn-1, accesion number: AY552781). When compared to another sequence of plant ferredoxin, fdn-1 was identified as the leaf-specific ferredoxin isoform I. This sequence was cloned in *antisense* orientation into a pCAMBIA vector under the control of the constitutive promotor CAMV 35S, in order to generate transgenic tobacco plants with decreased Fd levels. These plants showed variegated levels of chlorophyll in the same leaf, that resemble photoinhibition characteristics observed in FNR *antisense* plant. As compared to the wild-type siblings, sense and antisense plants show similar growth rates. In addition, transgenic plants overexpressing pea Fd display higher sensitivity to the redox-cycling herbicide methyl viologen.

PL-P47.

CLONING AND CHARACTERIZATION OF NADP DEPENDENT MALIC ENZYME FROM C_3 AND C_4 *FLAVERIA SPECIES*

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C_4 plants have evolved independently from C_3 ancestral species many times during the evolution of angiosperms. NADP-malic enzyme (NADP-ME) is a widely distributed enzyme involved in different metabolic pathways. The photosynthetic isoform of this enzyme is found in the bundle sheath chloroplasts of certain C_4 plants and has evolved from non-photosynthetic isoforms. In order to analyze the origin of the C_4-specific isoform of NADP-ME within the dicots, we isolated cDNAs encoding this enzyme in the genus *Flaveria*. This genus is well suited for studying the evolution of photosynthesis because it contains a more or less continuous range of species between C_3 and C_4, including C_3-C_4 and C_4-like species. In the present work, a suitable method to isolate RNA from *Flaveria* tissue was developed. Then, the full length cDNAs of ChlME1 (codifying a NADP-ME plastidic isoform from the C_4 *F. bidentis*) and modA (codifying a NADP-ME plastidic isoform from the C_3 *F. pringlei*) were isolated by RT-PCR. The amplified products were cloned into pGEM-T, sequenced and subcloned into the pET 32 expression vector. The recombinant proteins were expressed in *E. coli*, purified and used for kinetic and structural characterizations. The results obtained indicate that, although both genes show a high degree of homology, the proteins display several kinetic and structural differences, which may be important for their specific physiological function *in vivo*.

PL-P48.

NITRIC OXIDE REGULATES IRON DEFICIENCY-INDUCED GENE EXPRESSION IN TOMATO

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Iron deficiency is among the most common nutritional disorders in plants. To cope with low iron supply, plants increase the solubility and uptake of iron by inducing physiological and developmental processes including iron reduction, soil acidification, Fe(II) transport and root-hair proliferation. We have already shown that the bioactive free radical nitric oxide (NO) plays an important role in iron availability, avoiding iron-deficiency-induced chlorosis in leaves. Also, NO production in tomato roots is strongly augmented by iron deficiency. In this work, we test the expression of several genes related to iron transport and homeostasis in roots and leaves of hydroponic-grown tomato plants. We show that LeFRO1 [an Fe(III)-chelate reductase] and LeIRT1 [an Fe(II) transporter] are differentially regulated in iron-deficient tomato plants treated with 0.1 mM of the NO donor nitrosoglutathione. Also, activities of Fe(III)-chelate reductase and acidification of external media were increased by NO treatment in iron-deficient roots. NO also induced the accumulation of the Fer transcript which controls physiological responses to iron deficiency in roots. A tomato mutant impaired in the FER gene that fails to activate the root responses to iron deficiency, did not respond to NO. These results suggest that NO may control root physiological responses to iron-deficiency through the expression of the FER gene.

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PL-P49.

INTERACTION BETWEEN PHYTOCHROMES IN THE REGULATION OF FLOWERING TIME

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Plants regulate the onset of flowering very precisely, producing fruits during the favorable season, maximizing reproductive output. Endogenous and environmental factors are important to regulate this developmental process. Several pathways have been defined genetically that regulate the time to flowering. However, another pathway that acts independently was described recently, the light-quality pathway. We are investigating the role of PFT1 (PHYTOCHROME AND FLOWERING TIME 1) in the light quality pathway. PFT1 regulates flowering time downstream phyB in response to changes in light quality. Using Far-Red light enriched environments we found that other photoreceptor phytochromes other than phyA or phyB are also involved in this process and decided to study the interactions with PFT1. Given that *pft1* is in the Columbia ecotype we had to isolate new alleles of *phyD* and *phyE*, to complete all the set of mutants in the Columbia genetic background. Single mutants did not display any visible flowering phenotype under normal growth conditions, confirming what is known for the Landsberg *erecta* ecotype. However, an acceleration of flowering was observed when these mutations were introduced in a *phyB* background. Furthermore, we found that the *pft1* mutation has a differential effect in *phyB, phyE* and *phyD* mutants, suggesting that signaling pathways are diverging downstream these photoreceptors. These results indicate that light quality regulates flowering through a pathway that is neither linear nor common to all phytochrome photoreceptors.

PL-P50.

***Xanthomonas campestris* pv *campestris (Xcc)-Nicotiana benthamiana (Nb)* INTERACTION**

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Xcc is the causal agent of black rot disease of crucifers. This strain produces a series of extracellular enzymes, cyclic glucan, and an exopolysaccharide (EPS), xanthan, which are collectively essential for pathogenicity. The chemical structure of xanthan has been shown to consists in a main linear cellulose chain, β-glucose-1,4 linked and side chains integrated by the trisaccharide mannose-β-1,4-glucuronic acid-β-1,2-mannose-α-1,3 linked to every two glucoses of the cellulose backbone. Xanthan biosynthesis consist of the polymerization of pentasaccharide repeating units which are assembled by the sequential addition of glucose-1-phosphate, glucose, mannose, glucuronic acid, and mannose on a polyprenol phosphate carrier. The genes involved in this process, from the respective nucleotide donors, constitute a totally sequenced 16 Kb cluster, called *gum B-M*, containing 12 genes. A Tn5 insertion mutant upstream in gene *gumK*, which codifies for the glucuronyl transferase, produces a truncated repeating unit, containing only 2 glucoses and mannose, the first 3 sugars of the repeating unit, which are polymerized to produce a "polytrisaccharide". This polymer is a mannosylated cellulose and turned out to be not virulent on *Nb*. Another not virulent mutant, the 8397, containing a Tn5 insertion in *gumB* gene, was unable to produce xanthan. Partial xanthan synthesis was obtained by introducing in this strain *gumBC* genes that also recovered the ability to cause damage on *Nb* leaves. This and other evidences are indicating that xanthan is an important and specific virulence factor.

PL-P51.

COMPETITION BETWEEN PHENYLPROPANOID AND ANTRHAQUINONE PATHWAYS IN *RUBIA TINCTORUM* CELL SUSPENSIONS

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High intracellular proline levels deregulate proline synthesis in plants which leads to an overexpressed pentose phosphate pathway which results in an increasing carbon flux through the shikimate pathway. The stimulation of shikimate pathway produced an increase in phenylpropanoid related secondary metabolites (phenolics) in plants. Chorismate, which is the end product of the shikimate pathway, becomes the branch point for the synthesis of phenylpropanoid and anthraquinones (AQs) in *Rubia tinctorum* secondary metabolism. We tested the effect of proline addition in plant suspension cultures of *R. tinctorum* in order to study the competition between the two above mentioned secondary metabolic pathways. Suspension cultures were treated with proline at different concentrations 5 and 25 mM. High levels of proline (5 and 25 mM) showed a significant decrease on AQs accumulation (35% an 50%) and an increase in phenolics concentration. The addition of the phenylalanine ammonia liase inhibitor, 2-aminoindan-2-phosphonic acid, at 40 and 100 µM resulted in an increase of AQs concentration in suspension cultures of 18 and 40% respectively. These results show that is possible to increase AQs production at expense of the phenylpropanoid pathway in *R. tinctorum* suspension cultures.

PL-P52.

ASR1, A TOMATO PROTEIN ASSOCIATED WITH STRESS, PROTECTS YEAST FROM OSMOTIC STRESS

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Asr1, a tomato gene induced by stress, belongs to a family composed by at least four members. The biochemical function of these genes remains unknown although two alternative and not mutually exclusive possible functions have been postulated: 1, the promotion of direct protection from water loss based on ASR features such as a high degree of hydrophylicity, and 2, as a transcription factor based on nuclear localization and DNA-binding activity.

To test the possible mechanism of ASR1 in protecting cells from water loss, this protein was expressed in the heterologous expression system *Saccharomyces cerevisiae* under the control of a galactose-inducible promoter. In a mutant yeast strain deficient in the osmotic stress response MAP kinase Hog1, the synthesis of heterologous ASR1 enables growth under osmotic stress conditions such as 0.5 M NaCl, 1.2 M KCl and 1.2 M sorbitol. Interestingly, this effect was less evident using a wild type strain or a MAP kinase kinase-deficient strain. These results suggest that ASR1 complements yeast Hog1 deficiency either through a direct dehydration-protective effect or by inducing downstream components of the Hog pathway.

ST-P1.

A *TRYPANOSOMA CRUZI* MEMBRANE ASSOCIATED CYCLIC NUCLEOTIDE PHOSPHODIESTERASE

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In *Trypanosoma cruzi*, cAMP has been shown to downregulate cell proliferation. High intracellular cAMP levels inhibit DNA, RNA and protein synthesis. Of the 11 families of PDEs thus far identified, PDE4, PDE7 and PDE8 are specific for cAMP; besides, the PDE4 family often account for most of the cAMP-hydrolyzing activity of a cell. Previous reports from our laboratory described a calcium-stimulated adenylyl cyclase and a flagellum associated phosphodiesterase. Using PDE catalytic domain to screen *T.cruzi* genome project database (TIGR) a sequence was identified and named PDE 703. This sequence encodes a 925 amino acids protein, with an expected molecular weight of 103.27 kDa. Primary structure showed high homology with other PDEs, mainly PDE4 type. Furthermore, presence of characteristic domains of the phosphodiesterases, FIVE and PDEaseI, were confirmed. Southern blot showed a pattern coincident with a single-copy gene and Northern blot of epimastigote *T.cruzi* RNA revealed a single transcript larger than 2.4 kb. *E.coli* recombinant protein was expressed using pET22b+ vector yielding a high catalytic activity. In addition, PDE 703 cloned in pADNs vector complemented a heat-shock-sensitive yeast mutant deficient in phosphodiesterase genes. Finally, the recombinant enzyme expressed in yeast was used to biochemical characterization and its sensitivity to different inhibitors was tested.

ST-P2.

T-TYPE CALCIUM CHANNEL SPLICE ISOFORM VARIANT ASSOCIATION WITH ANNEXIN III IN NEURONAL TUMORS

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In humans, alternate splicing of exons 25 and 26 in the III-IV linker region can give rise to multiple $Ca_v3.1$ T-type calcium channel variants ($Ca_v3.1a$, $Ca_v3.1b$ or $Ca_v3.1bc$). Our results reveal that in the normal adult brain, $Ca_v3.1a$ transcripts predominate while $Ca_v3.1b$ is mostly fetal-specific. RT-PCR results on glioma and glioma cell lines showed that $Ca_v3.1$ expression in tumor cells resemble fetal brain expression pattern as $Ca_v3.1bc$ is predominantly expressed. The $Ca_v3.1ac$ variant was found in three glioma biopsies, one glioma cell line and retinoblastoma cells, but not in normal brain or fetal astrocytes. Transient expression of this variant reveals that $Ca_v3.1ac$ displays similar current-voltage and steady-state inactivation properties compared to $Ca_v3.1b$, but a slower recovery from inactivation. A proteomic approach coupled with mass spectrometry fingerprinting resulted in the identification of the calcium binding protein annexin III as a selective binding partner for the $Ca_v3.1ac$ channel III-IV linker region *in vitro*. Considering the well documented role of annexins in cell proliferation, and the notion that T-type channel inhibitors antagonize growth of retinoblastoma cells, we propose that calcium entry via T-type channels may result in the activation of an annexin III signaling pathway linked to tumor pathogenesis.

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ST-P3.

EXTRACELLULAR INOSINE MODULATES ERK ½ AND P38 PHOSPHORYLATION IN CULTURED SERTOLI CELLS: POSSIBLE PARTICIPATION IN TNF-α MODULATION OF ERK ½

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Extracellular ATP and adenosine modulation of MAPKs is well described in different cells, but extracellular inosine action it is not known. Previous reports showed that H_2O_2 and TNF-α increases extracellular inosine concentration in cultured Sertoli cells. This nucleoside protects Sertoli cells against H_2O_2 induced damage and participates in TNF-α induced nitric oxide production on these cells. Considering that MAPKs are key mediators of cellular response to a great variety of stimulus, in this work it was investigated the effect of extracellular inosine in the phosphorylation of ERK ½ and p38 MAPKs in cultured Sertoli cells. Also it was investigated if this nucleoside could be involved in TNF-α modulation of ERK ½ phosphorylation. Extracellular inosine increased the phosphorylation of ERK ½ and p38, as well as a selective A1 adenosine receptor agonist. Selective A1 adenosine receptors antagonists inhibited extracellular inosine effects on ERK ½ and p38. These antagonists also inhibited TNF-α increase in the phosphorylation of ERK ½. Additionally, TNF-α rapidly augmented extracellular inosine concentration in cultured Sertoli cells. These results shown that extracellular inosine modulates ERK ½ and p38 in Sertoli cells, possible trought A1 adenosine receptor activation. Besides, this nucleoside participates in TNF-α modulation of ERK ½.

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ST-P4.

G-PROTEINS PARTICIPATE IN THE CHITOSAN-INDUCED ANTHRAQUINONE PRODUCTION IN *Rubia tinctorum* L.

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Plants have acquired economical importance due to the synthesis of secondary metabolites, but the levels produced *in vitro* are generally too low for commercial applications. Elicitation with chitosan (200 mg/l) significantly stimulated (≡ 100%) anthraquinone (Aq) synthesis in *R. tinctorum* cell cultures. In previous studies we have shown that chitosan effects involve the activation of the phospholipase C (PLC)/protein kinase C (PKC) pathway, phosphoinositide 3'-OH-kinase (PI3K), the mitogenic activated protein kinase (MAPK) cascade and the participacion of Ca^{2+}. Here we demonstrate that stimulation by the elicitor of Aq production could be blocked with 1 mM suramin, 1 mM GDPβS or with an anti-Gαq/11 antibody (20 μg/ml). In addition, chitosan elicitation could be mimicked by 10μM mastoparan, 10mM AlF^{-4} or with 300 μM GTPγS. These evidences strongly suggest that a G-protein, with immunological characteristics of Gαq/11 protein, is the nexus between chitosan extracellular signal and the intracellular messengers systems mentioned above.

ST-P5.

17β-ESTRADIOL BINDING PROTEINS IN THE MUSCLE C2C12 CELL LINE

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In the last years increasing evidence for non-genomic estrogen short-term effects in different cells types has been accumulated and led to hypothesize the existence of cell-surface resident estrogen receptor (ER) forms triggering membrane events. The present study was aimed to analyze the presence and localization of ER binding proteins in C2C12 cells (a murine skeletal muscle cell line). In competition binding assays of subcellular fractions employing [^{3}H]17β-estradiol and 17β-estradiol, we have detected specific binding sites for the tritiated steroid in mitochondrial and microsomal fractions. Immunochemical studies employing specific monoclonal antibodies against different domains of the classical ER (ERα) detected the 67 kDa immunoreactive band expected for the ERα and, additionally, low molecular weight bands present in mitochondria and microsomes. These reactive bands were able to bind the steroid hormone in Ligand blot assays. Also these studies showed a clear surface labeling of the macromolecular complex E_2-BSA-FITC in non-permeabilized living cells. The characterization of these estrogen binding proteins with a non-classical localization and the signaling cascades activated thereby by the steroid hormone may provide information about the non-genomic mechanism of action of 17β-estradiol.

ST-P6.

DIFFERENT POLYADENYLATION SITES ARE USED IN THE EXPRESSION OF THE *TPK2* GENE IN THE FUNGUS *CANDIDA ALBICANS*

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Catalytic subunits of PKA encoded by *TPK1* and *TPK2* genes have been shown to be essential for filamentous growth of the dimorphic fungus *Candida albicans*. We have demonstrated that these isoforms were differentially expressed during vegetative growth and during the dimorphic transition yeast-mycelia. Interestingly, two transcripts of 1.8 kb and 1.4 kb of the *TPK2* gene were observed during vegetative growth and during germ-tube formation, being the former the most abundant form. Analysis of the sequence of 3´ untranslated region of the *TPK2* gene revealed three different consensus polyadenylation signals downstream of the transcription initiation site at + 1270, + 1342 and + 1582 position. Sequencing the 3´ untranslated region of the *TPK2* mRNA by 3´RACE technique revealed three bands of approximately 700, 500 and 400 pb.

These results indicate that different polyA sites are used, possibly as part of a mechanism of transcriptional control.

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ST-P7.

MORPHOGENETIC BEHAVIOUR OF A *CANDIDA ALBICANS* MUTANT STRAIN LACKING ONE OF THE ALLELES THAT CODIFY FOR THE REGULATORY SUBUNIT OF PKA

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Previous work from our laboratory indicates that both isoforms of the catalytic subunit of PKA (tpk1p and tpk2p) play a positive rol in the dimorphic transition of *C. albicans*. We have also demonstrated that *tpk2 bcy1*, a homozygous strain lacking the gene coding for the regulatory subunit (R), in spite of its deregulated catalytic activity, displays an agerminative phenotype. In this work we analysed the germinative behaviour of a *C. albicans* strain lacking one of the *BCY1* alleles (*tpk2 BCY1/bcy1* strain). We observed that although this strain is able to form true hyphae, pseudohyphal morphology is predominant. Comparison of the levels of R in this mutant with those of the original *tpk2* strain let us establish a direct correlation among the amount of R, the number of alleles present in the cell and the germinative behaviour. Visualization of the BCY1-GFP fused protein in *tpk2* and *tpk2 BCY1/bcy1* strains support our previous hypothesis on the nuclear localization of R, which in turn, would tether the catalytic subunit to the nucleus. Western blots using anti R and anti GFP antibodies showed that in the tpk2 strain both alleles, the normal and the GFP fused one, are expressed.

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ST-P8.

INHIBITORY EFFECT OF QUERCETIN ON THE INTRACELLULAR SIGNALING AND CELL CYCLE IN INSULIN-STIMULATED CELL LINE

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Quercetin is the mayor flavonoid present in human diet which contain anti-inflammatory and anti-carcinogenic properties by inhibiting several intracellular signaling pathways. Although the main action of Insulin is to control the glucose level, also can regulate the cellular growth and differentiation mediated by its receptor (IR). This receptor is a tyrosine kinase receptor that down-stream activate signaling pathways such as PI3K/Akt and Ras/Raf. The Insulin Resistance Syndrome (IRS) is characterized by the inability of glucose control and plasma hyperinsulin mainly developed during Type II Diabetes and stress, which has been associated with atherosclerosis and cancer development. In the present work we evaluate the inhibitory effect of Quercetin on intracellular signaling and cell cycle in insulin-stimulated CHOK1 cell line . By western blotting we measure phosphorylated ERK-1/2 (ERK-p) in the presence of Quercetin 100 mM (preincubated for 30 min) and diverse doses of Insulin (1.10^{-4} to 1.0 U/ml) for several time of incubation (15 to 240 min.). We demonstrate that Quercetin increase the Insulin activity on ERK-p at short time of incubation (up to 90 min) but exert an inhibitory effect on ERK-p after 90 min of insulin incubation. In addition, by FACS analysis we demonstrate that Quercetin can inhibit the proliferation effect of insulin on CHOK1 cell line. In conclusion, in this work we demonstrate that Quercetin modify the insulin activity mainly on intracellular signaling and cellular proliferation.

ST-P9.

CONSERVATION OF THE CONSTITUTIVE ACTIVITY MECHANISM IN TRYPANOSOMATIDS SRPKs

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The regulation of the genetic expression in trypanosomatids is exerted mainly on the post-transcriptional level. Trypanosomatids are organisms that not only perform *trans*- but also *cis*-splicing. The *trans*- and *cis*-spliceosome contain conserved elements, that includes snRNPs (small nuclear RiboNucleoProteins) and non-snRNPs. From these last, SR proteins are the main components of this structure and together with their specific kinases constitute the "SR Network". We have identified and characterized for the first time components of this network in *Trypanosoma cruzi* and *Trypanosoma brucei*. The serine/arginine-rich protein (TcSR) and the specific SR kinases, TcSRPK and TbSRPK. These kinases belong to one, SRPK, of the four families characterized in diferent organisms, extending them to all the eukariotic linage. Recently the structural mechanism of the constitutive activity of Sky1p, homolog protein of *S. cerevisiae*, has been characterized. In this work we show the modeling of the 3D-structure of the trypanosomatids kinases together with the characterization of the generated mutant proteins, demonstrating the conservation of such mechanism at this evolutionary level.

ST-P10.

CARBAMYLCHOLINE INDUCES CALCIUM SIGNALLING IN *TRYPANOSOMA CRUZI* EPIMASTIGOTES VIA IP_3 AND NA^+/H^+ EXCHANGER ACTIVATION

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In a previous study we reported that stimulation of *T. cruzi* epimastigotes with carbamylcholine (Cch) resulted in Ins(1,4,5)P_3 increase level and Ca^{2+} mobilisation from intracellular stores; Ins(1,4,5)P_3 variation was abolished by PMA, an activator of protein kinase C. Now, we report that PMA significantly increased the Ca^{2+} mobilisation induced by Cch, whereas H7, an inhibitor of the kinase, produced a significant decrease of Ca^{2+} signalling. The PMA effect was inhibited when epimastigotes were preincubated with H7. The addition of the Na^+/H^+ ionophore monensin evoked Ca^{2+}_i increase from acidic intracellular stores in the presence and absence of extracellular sodium. In a Na^+ free-medium, the Ca^{2+} mobilisation induced by Cch decreased, while the Ins(1,4,5)P_3 increase remained unchanged. This Ca^{2+} signalling progressively increased when pH of the medium changed from 7.0 to 7.8. The presence of EIPA, an inhibitor of the Na^+/H^+ exchanger, inhibited the alkalinization of the acidic compartment and Ca^{2+} signalling; this effect was reverted by monensin. In other hand, the Ca^{2+} signalling evoked by Cch was also inhibited by caffeine, an inhibitor of IP3Rc.

The results suggest that the Ca^{2+}_i increase evoked by carbamylcholine depends on IP3 levels and Na^+/H^+ exchanger activation.

ST-P11.
FUNCTIONAL ANALYSIS OF THE CYTOPLASMIC DOMAINS OF POLLEN RECEPTOR KINASES LePRK1 AND LePRK2 FROM TOMATO

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We previously characterized two pollen-specific receptor kinases, LePRK1 and LePRK2, from Lycopersicon esculentum (tomato) (Muschietti *et al*., The Plant Cell 1998, 319-330). They are localized in the plasma membrane of the pollen tube and contain an active kinase domain potentially involved in transducing an extracellular signal to the pollen tube through phosphorylation-dephosphorylation reactions. LePRK2 is phosphorylated in pollen membranes, and specifically dephosphorylated by tomato or tobacco style extracts. We showed that LePRK1 and LePRK2 can be co-immunoprecipitated from pollen or when expressed together in yeast cellular membranes (Wengier *et al*., 2003, PNAS 2003, 6860-6865). In order to characterize this interaction, three types of cytoplasmic deletion constructs were made for both LePRK1 and LePRK2 and expressed in yeast. All of them lacked the extracellular domain and have different portions of the cytoplasmic domain. We then co-expressed in yeast each of the deletion constructs of one of the receptors with the corresponding full-length of the other receptor. We showed that all the constructs are properly expressed in the yeast membrane fractions. Results obtained by co-immunoprecipitation assays suggest that the carboxyl-terminal domain of LePRK2 might be necessary for their interaction. These deletions would be useful for further characterization of the LePRK signaling pathway in tomato pollen.

ST-P12.
PARTIAL PURIFICATION OF A 115 kDa PROTEIN TYROSINE PHOSPHATASE FROM RAT ADRENAL CORTEX

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We have previously demonstrated that ACTH promotes protein tyrosine dephosphorylation through the PKA-dependent activation of protein tyrosine phosphatases (PTPs). By in gel tyrosine phosphatase assay we detected an increase in the activity of three enzymes by hormone action in rat adrenal zona fasciculata (ZF). One of them exhibits an apparent molecular weight of 115 kDa approximately (PTP115) and can be activated by *in vitro* PKA phosphorylation. In this report we describe the procedure followed in order to partially purify and identify this PTP. Cytosolic proteins obtained from rat ZF were subjected to ammonium sulfate precipitation, determining by in gel PTP assay that PTP115 mainly precipitates with ammonium sulfate 30%. When this fraction was analyzed by DEAE-cellulose chromatography, PTP115 elutes with 250-400 mM of NaCl. Proteins from this eluate were fractionated by SDS-PAGE in order to isolate PTP115 by elution from the gel. The analysis by in gel bioactivity assay of the eluted material demonstrated that the protein under purification process retains its activity after this step. Even when the identity of PTP115 is still unknown, a commercially available antibody against PTP-PEST (80 kDa) recognizes a band migrating at 115 kDa in adrenocortical cells, suggesting that PTP115 could be related to this known PTP.

ST-P13.
REGULATION OF ACS4 EXPRESSION IN ZONA GLOMERULOSA OF ADRENAL GLAND

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In steroidogenic tissues arachidonic acid (AA) plays an obligatory role in the regulation of steroid synthesis. In adrenal and in Leydig cells the hormonal regulation of free AA concentration is mediated by the concerted action of two enzymes: an acyl-CoA thioesterase (MTE-I) and an acyl-CoA synthetase (ACS4). We have demonstrated in our laboratory that ACS4 is essential for StAR (Steroidogenic Acute Regulatory protein) induction and steroidogenesis. In this work we studied the regulation of ACS4 in rat glomerulosa zone, a steroidogenic tissue that is subjected to different stimulous that increase several intracellular pathways. Given the fact that in Y1 adrenocortical cells ACS4 rapidly increases its expression by ACTH, we also studied if the protein has the same behaviour in rat fasciculata cells. For this purpose, rats were injected subcutaneously following dexamethasone treatment with 18 UI of ACTH per kg and killed at different times. Adrenal glands were removed, and zona fasciculata (ZF) and zona glomerulosa (ZG) were obtained. Samples were analyzed by Western blot using antibodies against ACS4 and MTE-I. In zona glomerulosa, ACTH administration produced an increase in ACS4 expression of 109±13% at 30 minutes ($p<0.05$). In zona fasciculata, the increase was of 65±11% ($p<0.05$) at 30 min. No variation in MTE-I was detected. At these times we also observed an increase in steroid production. These results suggest an early induction of ACS4 by ACTH in glomerulosa zone, and confirms the role of this new pathway in the control of intracellular levels of AA in the regulation of hormone induced steroidogenesis.

ST-P14.
SUCROSE ENHANCES THE EXPRESSION OF A CDPK IN EXCISED LEAVES OF WHEAT

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In a previous work we have shown the involvement of the second messenger calcium and Ser/Thr protein kinases (PK) in the process that leads to the induction of fructan synthesis by sucrose in wheat (*Triticum aestivum*). Based on these results, we cloned from wheat leaves treated with sucrose, a complete cDNA with an ORF, 1828 nt long from the ATG to the stop codon, encoding a 58.4-kDa predicted protein homologous to plant calcium-dependent and calmodulin-independent PK (CDPK) (gi: 47522359, TaCDPK1). The deduced amino acid sequence showed all the CDPK characteristic domains and putative consensus motifs for N-myristoylation and phosphorylation. We demonstrated by RT-PCR and real-time PCR that TaCDPK1 expression is induced by sucrose. Besides, specific inhibitors of calcium channels, Ser/Thr PK and protein phosphatases (PP), which were shown to be effective on suppress sucrose induction of fructan synthesis, also blocked the sucrose effect on the accumulation of TaCDPK1 transcripts. Also, the inhibitors decreased the basal gene expression. These results suggest that the putative components of the sucrose signaling pathway -calcium, PK and PP2A- are required for upregulation of TaCDPK1 by sucrose. Further studies are necessary to understand whether TaCDPK1 is a specific target of sucrose regulation or it is a component of the sugar signalling cascade.
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ST-P15.
NONCATALYTIC DOMAINS OUTSIDE THE ACTIVE CLEFT AND REGULATION OF THE PHOSPHORYLATING ACTIVITY OF PROTEIN KINASE A

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There are great differences among species in the affinity between regulatory (R) and catalytic (C) subunits of cAMP dependent protein kinase. These differences can not be explained by the present structural data. In order to analyse how the R subunit interacts with C beyond its autophosphorylation site we studied the effects of several peptides on the phosphorylating activity of *Mucor rouxii* and bovine heart C subunits. The peptide sequences (18 aa) correspond to the autophosphorylation site and the hydrophobic residue-rich region toward the amino-termini of *M. rouxii* and *S. cerevisiae* R subunits. The non-phosphorylable peptides (Ala instead of Ser) have a dual effect on kemptide phosphorylation: not only do they inhibit C activity, probably by competition for the active site, but also activate. The phosphorylable peptides (P) have higher k_M and higher v_{MAX} on bovine heart C, while v_{MAX} is higher and k_M is lower on *M. rouxii* when compared to kemptide. All parameters change in the same degree for both series, with exception of v_{MAX} on bovine heart C: it is twice higher for PMrouxii than for PScerevisiae. Peptides containing only the autophosphorylaton site (8aa) have a v_{MAX} similar to kemptide for both series of peptide and both enzymes. On the other hand, preliminary results show that a peptide (10 aa) that corresponds only to the hydrophobic residue-rich region of *M. rouxii* produces activation on *M. rouxii*, when added simultaneously with kemptide, while no effect is observed on bovine heart C. These results can be interpreted as noncatalytic domains outside the active cleft regulate the phosphorylating activity of C by the interaction with peptides derived from R sequence.

ST-P16.
STUDY OF THE INTERACTION BETWEEN THE R AND C SUBUNITS OF THE PROTEIN KINASE A IN THE FUNGUS *MUCOR CIRCINELLOIDES*

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Protein kinase A is a tetramer composed of a dimer of regulatory subunits (R) and two monomeric catalytic subunits (C). In the holoenzyme C interacts with the pseudosubstrate site in R. This pseudosubstrate site and additional contact sites in R render an affinity in the nanomolar range. The affinities between R and C subunits of PKA are quite different depending on the specie, finding the highest values in *M.rouxii* and *M.circinelloides*.
Our hypothesis is that in the amino-termini region of R subunits exist elements that interact with C subunits that are responsible for the different affinity. The wild type R subunit and two mutant R1 that lacks the N-terminal and R2 that lacks an acidic residues region were cloned and expressed in a *S.cerevisiae* strain tpk2$^-$ tpk3$^-$ bcy1$^-$ (133). Western-blot analysis and measurement of cAMP binding activity showed that the different R subunits can be expressed and are functional in yeast. Determinations of kinase activity in crude extracts have been made in the strain expressing wt R, R1 and R2. The total PKA activity is increased in the three expressing strains as compared with the control one 133. The PKA activity ratio determined measuring the activity with and without cAMP was 1 in the 133 strain and 0.17-0.25 in the strains that expressed the different R. *In vivo* analysis of a phenotype associated with cAMP-PKA signaling pathway , glycogen accumulation, showed that the expressing R strains have a phenotype which is associated with a inactivation of PKA . These results indicates that The wt R, R1 and R2 subunits of *M.circinelloides* expressed in *S.cerevisiae* are functional and interact with tpk1.

ST-P17.
1,25(OH)2-VITAMIN D3 STIMULATES P38 MAP KINASE IN RAT INTESTINAL CELLS

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The stress-activated protein kinase p38 MAPK has been shown to be expressed in intestinal tissues and to play a role in regulating a variety of intestinal processes, including epithelial restitution, cytoskeletal organization and ion transport. In the present study, we have demonstrated that the hormone 1,25(OH)2-vitamin D3 [1,25(OH)2D3] induces the phosphorylation and activation of p38 MAPK in rat intestinal cells. This effect was dose and time-dependent, with maximal stimulation at 2 min (+3 fold) and 1 nM. Calcium chelation with EGTA or BAPTA-AM, inhibition of the Src-family tyrosine kinase with PP1 or PKA inhibition with Rp-cAMP, suppressed hormone-dependent p38 phosphorylation. The physiological significance of 1,25(OH)2D3-dependent activation of MAP kinases was addressed by monitoring c-fos expression in intestinal cells. Incubation of the cells with the hormone was followed by rapid induction of c-fos expression (+1 fold, 5 min) which was blocked by the p38 inhibitor SB203580 and partially suppressed by the ERK1/2 inhibitor, PD 98059. Taken together, our results suggest that 1,25(OH)2D3 activates p38 MAPK, involving Calcium, c-Src and PKA as upstream regulators and that p38 has a central rol in the hormone-induction of the oncoprotein c-fos in rat intestinal cells.

ST-P18.
CALCITRIOL SIGNALING THROUGH MAP KINASES IN SKELETAL MUSCLE CELLS

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In skeletal muscle, calcitriol (1α,25-dihydroxy-vitamin D3), the hormonally active form of vitamin D, regulates intracellular calcium levels and contraction, and promotes muscle cell proliferation and differentiation. The mitogen activated proteins kinases (MAPKs): ERKs, p38 kinase and JNK (c-jun NH2-terminal kinase), have been shown to be involved in cellular growth and differentiation. In this work, using as experimental model the skeletal muscle cell line C2C12, we demonstrate that calcitriol activates p38 kinase and JNK. Both kinases were rapidly phosphorylated and activated with different temporal profiles, maximal phosphorylation of the p38 and JNK was reached at physiological doses of calcitriol (1 nM). Of relevance, the hormone induced in the C2C12 line the stimulation of MAPKAP-kinase 2 and subsequent phosphorylation of HSP27 in a p38 kinase activation-dependent manner. As the MAPKAP-kinase 2/HSP27 pathway modulates F-actin polimerization promoting thereby cellular proliferation, additional studies are required to elucidate the signalling events involved in the final stage of regulation of muscle cell proliferation by calcitriol.

ST-P19.
PTH ACTIVATES c-SRC KINASE VIA G PROTEINS IN RAT ENTEROCYTES

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PTH interacts in target tissues with a G protein-coupled receptor but the mechanism by which G proteins activate tyrosine kinases is not completely understood. In this work we demonstrated that PTH rapidly increases the activity of the tyrosine kinase c-Src in rat enterocytes. The response is biphasic, the early phase is transient, peaking at 30 sec. (+120%) while the second phase is maximal at 5 min. (+250%) of treatment with PTH. To evaluate whether G-proteins are required for PTH-induced c-Src activation, the enterocytes were treated with PTH (10^{-8} M, 30 sec-5 min) and c-Src was co-immunoprecipitated with anti-cSrc and resolved by Western blotting with anti-Gβ, anti-Gαs or anti-Gαi antibodies. In basal condition the three subunits associate with c-Src and this association increases two-three fold, in cells treated with PTH. In addition, the hormone activates c-Src in enterocytes through changes in the tyrosine phosphorylation of the enzyme. We demonstrate that the first event in the activation of c-Src is the dephosphorylation of Y527 followed by a second event of activation with phosphorylation at Y416. Futhermore, preincubation with anti-Gβ antibody inhibited PTH-mediated increase in c-Src Y416 phosphorylation and c-Src-dependent activation of the MAP kinases ERK1 and ERK2. These results show that PTH activates c-Src in rat enterocytes through conformational changes via G proteins and modulation of tyrosine phosphorylation and that PTH receptor activation is followed by ERK stimulation through Gβγ-c-Src.

ST-P20.
REGULATION OF ALTERNATIVE SPLICING BY THE EXTRACELLULAR MATRIX

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Alternative splicing generates distinct proteins from a single gene. Fibronectin (FN) plays a key role in cell adhesive and migratory behaviour and provides a paradigmatic model of alternative splicing. Communication between mammary epithelium and stroma and their interaction with the extracellular matrix is required for proper function of the mammary gland. In this context, we study the effect of extracellular signals on the regulation of FN alternative splicing.
We found that soluble factors secreted by mesenchymal cells stimulate the inclusion of FN EDI and IIICS exons in epithelial cells through a PI3K-dependent pathway. Inhibition of the JNK pathway potentiates this effect, suggesting a negative role for JNK in the the inclusion of these two exons. Treatment of epithelial cells with a basement membrane-like extracellular matrix stimulates EDI and IIICS skipping. Moreover, this effect is entirely dependent on the activation of JNK, confirming the negative role for this pathway in the inclusion of EDI and IIICS, as proposed above These results strengthen our understanding of how extracellular stimuli are converted into changes in splicing patterns.

ST-P21.
HISTONE H1 PHOSPHORYLATION DURING *TRYPANOSOMA CRUZI* CELL CYCLE

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Histone H1, also known as linker histone, affects many features of chromatin structure and function. It stabilizes high order structure of the chromatin and has an effect on nucleosome position and spacing. Phosphorylation seems to regulate histone H1 functions in most eukaryotes and it increases as cell progress in the cell cycle. The phosphorylations are catalyzed by cyclin-dependent protein kinases (CDKs).
Recently, it was found that *Trypanosoma cruzi* histone H1 is differently phosphorylated in proliferating vs. non-proliferating forms of the parasite (Marques-Porto et al., 2002). cdc2-related protein kinases TzCRK1 and 3 able to phosphorylate mammalian histone H1 were characterized in *T. cruzi*. In this report we described the phosphorylation of endogenous purified *T. cruzi* H1 and the recombinant protein by cyclin- related TzCRKs.
Antiserum raised against TzCRK1 and 3 were employed to immunoprecipitate parasites extracts from epimastigotes forms of Y and CLBrenner strains. The *T.cruzi* histone H1 is phosphorylated by both immunoprecipitated kinases. The level of phosphorylation is higher with the CRK3 kinase than with CRK1. This data suggests that these enzymes could be involved in the phosphorylation *in vivo* of the parasite histone, and perhaps in the regulation of binding of the histone to the DNA.

ST-P22.
PROTEOMIC AND GENOMIC APPROACH TO *Trypanosoma cruzi* CYCLIN PROTEINS

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Cell cycle is a highly regulated process that controls the replication and growth population in eukaryotic cells. CDKs (cyclin-dependent kinases) are the masterpiece that controls the system in association with cyclins, CDKs protein inhibitors, and through degradation of proteins.
In *Trypanosoma cruzi* we have identified two CDK-related proteins named TzCRK1 and TzCRK3, without identified cyclins that regulates its activity. Using the yeast two- hybrid system we characterized three cyclins TzCYC2, 4 and 5 (yeast PHO family). By bioinformatics methods we found in the *T.cruzi* genome database (TIGR) TzCYCs 6, 7, 8 and 9 homologous to *T. brucei* cyclins. Sequence analyzes of TzCYC2 shows a short degradation peptide signal (PEST) in the C-terminal region of the protein. TzCYC6 presents a Destruction Box Domain (DB) in the N-terminal domain, involved in the degradation by ubiquitination. Expression of the TzCYC6-9 was confirmed searching in cDNA database of *T. cruzi* (TIGR). TzCYC6 regulates in *T. brucei* the transition G2/M while TbCYC2 the passage S/G2.
T. cruzi extracts immunoprecipitated with a polyclonal specific antibody obtained against the purified (His)6-TzCYC2, and revealed in Western blot with the same antibody shows the TzCyc2 protein of 23 kDa. This indicates the presence of the cyclin 2 in the epimastigotes forms of *T. cruzi*.

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DNA-BASED CHARACTERIZATION OF SPECIES AND POPULATIONS OF CHAGA'S DISEASE VECTORS

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DNA-based methods are important tools to discriminate insect species and populations. These are important in studies related to control of vectors diseases. We have studied rDNA sequences to characterize species of triatomines. A striking characteristic of insects internal transcribed spacers 1 (ITS-1) is a well-conserved species-specific length. We have studied the lenghth of ITS-1 from *T. infestans, P. megistus* (Triatomini tribe) and *R. prolixus* (Rhodniini tribe) all of them peridomestic, as well as from a non vector, *T. eratyrusiformis.* As expected, each one of the species possesses an ITS-1 of specific length: between 560 and 710 bp for members of the Triatomini, and 110 bp length for *R. prolixus*. Using the RAPD-PCR technique, we obtained diagnostic profiles (SDP) for each species and diagnostic bands (SDB) unique for a given species. Primers OPC-14, OPH-07. OPK-06 and OPK-18 were able to amplify SDBs for *T. infestans* and *R. prolixus.* The same approach was followed to identify genetic variability among populations within species. OPH-18 and OPH-19 showed differential specific population profiles (PDP) in most of *T. infestans* populations from Argentina. In addition, these primers generated diagnostic bands (PDB) present only in populations from three geographically locations from two NW provinces of Argentina, Salta and Santiago del Estero. OPH-18 amplified a 0.38 Kb PDB band that allowed us to discriminate Salta populations from all the other analyzed.