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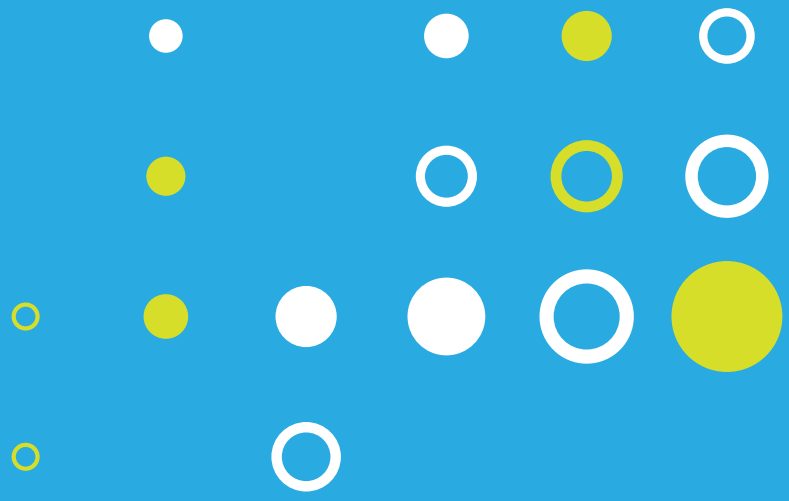

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Sociedad Argentina de
Investigaciones en Bioquímica
y Biología Molecular

B I O C E L L

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- SAIB -

43rd Annual Meeting
Argentine Society for Biochemistry and Molecular Biology

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

November 17-20, 2007

Mar del Plata, Buenos Aires
República Argentina

MEMBERS OF THE SAIB BOARD

DELEGATES OF SCIENTIFIC SECTIONS

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

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

-Microbiology-
Dr. Raúl Raya
CERELA-CONICET, Universidad Nacional de Tucumán

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

ACKNOWLEDGMENTS

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

Consejo Nacional de Investigaciones Científicas y Técnicas
(CONICET)

Agencia Nacional de Promoción Científica y Tecnológica
(ANPCyT)

European Molecular Biology Organization
(EMBO)

Instituto de Investigaciones Biológicas,
Universidad Nacional de Mar del Plata

SAIB 2007 CONGRESS OVERVIEW

Saturday, November 17th	Sunday, November 18th	Monday, November 19th	Tuesday, November 20th
	8:30-10:30 **Symposia** Room A: Plant Biochem Mol Biol Room B: Lipids	8:30-10:30 **Symposia** Room A: Microbiology Room B: Developmental Biology	8:30-10:30 **Symposium** Room A: Cell Biology
	10:30-11:00 Coffee break	10:30-11:00 Coffee break	10:30-11:00 Coffee break
	11:00-13:15 **Oral Communications** Room A: MI (C01/09) Room B: CB (C01/08) Room C: ST (C01/08)	11:00-13:15 **Oral Communications** Room A: MI (C10/18) Room B: CB (C09/16) Room C: PL (C01/09)	11:00-13:15 **Oral Communications** Room A: MI (C19/27) Room B: LI (C01/09) Room C: PL (C10/18)
	13:15-15:30 Lunch	13:15-15:30 Lunch	13:15-15:30 Lunch
14:00-18:00 Registration	15:30-16:30 **Lecture** T-Y Chang	15:30-16:30 **Presentation** Pasteur Institute of Montevideo	15:30-16:30 **Lecture** J. Pozueta Romero
18:00-18:15 **Open Ceremony**	16:30-19:00 **Posters - coffee** MI (P01/34) CB (P01/30) PL (P01/27) LI (P01/15) BT (P01/20) EN (P01/14)	16:30-19:00 **Posters - coffee** MI (P35/68) CB (P31/61) PL (P28/57) LI (P16/29) BT (P21/40) ST (P01/12)	16:30-19:00 **Posters – coffee** MI (P69/101) CB (P62/84) PL (P58/86) SB (P01/13) NS (P01/13) ST (P13/26) EN (P15/29)
18:15-19:15 **Opening Lecture** D. de Mendoza			
19:15-20:15 **Lecture** J. Bowman	19:00- 20:00 **Lecture** J. Bonifacino	19:00- 20:00 **Alberto Sols Lecture** A. Ferrer Montiel	19:00- 20:00 **EMBO Lecture** J. Errington
21:00 Cocktail	20:00-21:00 CONICET- Argentina R. Farías	20:30 SAIB Assembly	21:00 Farewell Dinner

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

PROGRAM

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

18:00-18:15 Room A
OPENING CEREMONY

18:15-19:15 Room A
OPENING LECTURE
Diego de Mendoza
Instituto de Biología Molecular y Celular de Rosario, CONICET, Universidad Nacional de Rosario
"Sensing Lipid Fluidity in Bacterial Membranes"
Chairperson: Ricardo Farías, INSIBIO-CONICET, Universidad Nacional de Tucumán

19:15-20:15 Room A
LECTURE
John Bowman
School of Biological Sciences, Monash University, Melbourne, Victoria, Australia
"Patterning genes in land plants"
Chairperson: Néstor Carrillo, IBR- CONICET, Universidad Nacional de Rosario

21:00 **COCKTAIL**

SUNDAY, November 18th, 2007

08:30-10:30 Room A
SYMPOSIUM
"Plant Biochemistry and Molecular Biology"
Chairpersons: C. Casalongué, IIB-CONICET, Universidad Nacional de Mar del Plata
R. Wolosiuk, Fundación Instituto Leloir-CONICET, Buenos Aires

08:30 - 09:00 ***Mohammad-Reza Hajirezaei***
Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany
"Fundamentals of primary metabolism: molecular and biochemical attempts to identify key steps"

09:00-09:30 ***Omar Borsani Cambón***
Universidad de la República, Montevideo, Uruguay
"Inducible endogenous siRNAs in plant stress responses"

09:30-10:00 ***Carlos L. Ballaré***
IFEVA - Escuela de Agronomía, Universidad de Buenos Aires, Argentina
"Molecular and physiological connections between light and defense signaling mechanisms"

10:00-10:30 ***María Eugenia Zanetti***
IBBM - Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Argentina
"RIP-Chip for global analysis of cell-specific gene expression in Arabidopsis"

08:30-10:30 Room B

SYMPOSIUM

"Lipids"

Chairpersons: María del R. Gonzalez Baró, INIBIOLP-CONICET, Universidad Nacional de La Plata
Marta Aveldaño, INIBIBB-CONICET, Universidad Nacional del Sur, Bahía Blanca

08:30-09:00 ***Rosalind Coleman***

Department of Nutrition and Pediatrics, University of North Carolina at Chapel Hill, USA

"Lipogenic pathways and insulin sensitivity**"**

09:00-09:30 ***Paul A. Watkins***

John Hopkins University, School of Medicine and School of Public Health, USA

"Acyl-CoA synthetases: new roles in brain cancer and GABA signaling"

09:30-10:00 ***Concetta DiRusso***

Ordway Research Institute, Center for Cardiovascular Science, Albany Medical College, USA

"Differential regulatory and metabolic effects in liver contributed by dietary fatty acids"

10:00-10:30 ***Paul N. Black***

Ordway Research Institute, Center for Metabolic Diseases, Albany Medical College, USA

"Fatty acid transport by vectorial acylation: selective trafficking of fatty acid mediated by the FATP and ACSL isoforms"

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

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

Room A

Microbiology (MI-C01 / MI-C09)

Chairpersons: Angel A. Cataldi, CICVyA, INTA Castelar
María Cecilia Mansilla, IBR-CONICET, Universidad Nacional de Rosario

11:00-11:15

MI-C01

LIGHT REGULATES VIRULENCE IN *BRUCELLA ABORTUS* BY A LOV-DOMAIN HISTIDINE KINASE PROTEIN

Paris, G.; Comerci, D.J.; Swartz, T.E.; Briggs, W.; Bogomolni, R.; Ugalde, R.A.; Goldbaum, F.A.
Fundación Instituto Leloir, IIBBA-CONICET, Bs As, Inst. Invest. Biotec., UNSAM-CONICET, San Martín.

11:15-11:30

MI-C02

DESIGN OF PEPTIDES INHIBITING THE TYPE THREE SECRETION SYSTEM FROM ENTEROPATHOGENIC *ESCHERICHIA COLI*

Cataldi; Larzabal; Vilte; Mercado; Salazar; Navarro García
Inst. Biotecnología INTA, Castelar.

11:30-11:45

MI-C03

ACYL-COA CARBOXYLASE INHIBITORS AS NEW ANTIMYCOBACTERIAL AGENTS

Kurth, D.; Gago, G.; de la Iglesia, A.; Morbidoni, H.R.; Tsai, S.-C.; Gramajo, H.
Instituto de Biología Molecular y Celular de Rosario, UNR-CONICET, Suipacha 531, Rosario, Argentina.

11:45-12:00

MI-C04

ORAL ADMINISTRATION OF A CATALASE-PRODUCING *LACTOCOCCUS LACTIS* CAN PREVENT COLON CANCER IN MICE
LeBlanc, J.G; de Moreno de LeBlanc, A; Perdigón, G.; Miyoshi, A.; Langella, P.; Azevedo, V.; Sesma, F.
Centro de Referencia para Lactobacilos, CERELA-CONICET; UFMG-ICB, Brazil; UEPD-INRA-CRJ, France.

12:00-12:15
MI-C05
GAS DISCHARGE PLASMA AS A NEW TOOL FOR BACTERIAL BIOFILM INACTIVATION
Joaquin, J.C.; Kwan, C.; Vandervoort, K.; Abramzon, N.; Brelles-Mariño, G.
Biology and Physics Departments, California State Polytechnic University, USA.

12:15-12:30
MI-C06
TDR TARGETS: PRIORITIZATION OF DRUG TARGETS FOR HUMAN NEGLECTED DISEASES
Agüero, F.[1]; Berriman, M.[2]; Buckner, F.[3]; Carmona, S.[1]; Crowther, G.[3]; Hertz-Fowler, C.[2]; Nwaka, S.[4]; Pain, A.[2]; Ralph, S.[5]; Riechers, A.[3]; Roos, D.S.[6]; Shanmugam, D.[6]; Suzuki, T.[2]; Verlinde, C.[3]; Van Voorhis, W.C.[3]
[1] Instituto de Investigaciones Biotecnológicas, UNSAM; [2]Wellcome Trust Sanger Institute, UK; [3] University of Washington, USA; [4] World Health Organization, Switzerland; [5] University of Melbourne, Australia; [6] University of Pennsylvania, USA.

12:30-12:45
MI-C07
CYTOCHROME P450 REDUCTASES IN *TRYPANOSOMA CRUZI*. TCCPR-B CONFERS INCREASED DRUG RESISTANCE
Portal, P.; Fernandez Villamil, S.; De Vas, M.; Alonso, G.; Flawiá, M.; Torres, H.; Paveto, C.
INGEBI-CONICET, Universidad de Buenos Aires.

12:45-13:00
MI-C08
ANTI-TRYPANOSOMA CRUZI HUMAN RECOMBINANT ANTIBODIES
Grippo, V.; Niborski, L.L.; Levin, M.J.
Laboratory of Molecular Biology of Chagas Disease (LaBMECh), INGEBI-CONICET.

13:00-13:15
MI-C09
IDENTIFICATION OF VIRULENCE PROTEASES FROM *BOTRYTIS CINEREA* BY CROSS-PROTEOLYSOME ANALYSIS IN FUNGI
Ten Have, A.
IIB-FCEyN, Universidad de Mar del Plata.

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

Room B

Cell Biology (CB-C01 / CB-C08)

Chairpersons: Carolina Touz, INIMEC-CONICET, Córdoba
Silvia Belmonte, IHEM-CONICET, Universidad Nacional de Cuyo, Mendoza

11:00-11:15
CB-C01
REGULATION OF CELL-MATRIX ADHESION AND CELL MIGRATION BY PTP1B
Burdisso, J.E.; Aguirre, C.E.; Mansilla, S.F.; Hernandez, M.V.; Arregui, C.O.
Instituto de Investigaciones Biotecnológicas, UNSAM-CONICET.

11:15-11:30
CB-C02
POSSIBLE SECRETORY PATHWAY OF CATHEPSIN D IN RAT EPIDIDYMIS
Carvelli, L.; Bannoud, N.; Aguilera, C.; Barrera, P.; Morales, C.R.; Sosa, M.A.*

McGill University,Canadá and IHEM-CONICET, Univ. Nac. Cuyo, Mendoza.

11:30-11:45
CB-C03
FUNCTIONAL ANALYSIS OF THE RAB1-COPII INTERACTION
Slavin, I.; Monetta, P.; Romero, N. ; Alvarez, C.
CIBICI-CONICET, Facultad Ciencias Químicas, Univ. Nac. Córdoba.

11:45-12:00
CB-C04
THE EARLY SECRETORY PATHWAY CONTRIBUTES TO THE DEVELOPMENT OF THE *COXIELLA*-REPLICATIVE NICHE
Campoy, E.M.; Zoppino, F.C.M.; Colombo, M.I.
Laboratorio de Biología Celular y Molecular, IHEM-CONICET, FCM, Univ. Nac. Cuyo, Mendoza.

12:00-12:15
CB-C05
ROLE OF BECLIN1 AND BCL-2 IN THE DEVELOPMENT OF THE *COXIELLA BURNETII* REPLICATIVE VACUOLE
Vázquez, C.L.; Colombo, M.I.
Laboratorio de Biología Celular y Molecular, IHEM-CONICET, Univ. Nac. Cuyo, Mendoza.

12:15-12:30
CB-C06
ACTIN AND RHO GTPASES REGULATE THE BIOGENESIS OF *COXIELLA BURNETII*-CONTAINING VACUOLES
Aguilera, M.; Salinas, R.; Rosales, E.; Carminati, S.; Colombo, M.; Berón, W.
IHEM-CONICET, Fac. Cs. Médicas, Univ. Nac. Cuyo, Mendoza.

12:30-12:45
CB-C07
ADAPTOR PROTEIN 2: A KEY PLAYER IN *GIARDIA* LYSOSOMAL PROTEIN TRAFFICKING
Rivero, M.C.; Vranych, C. ; Ropolo, A.S.; Touz, M.C.
Instituto Mercedes y Martín Ferreyra, INIMEC-CONICET, Córdoba.

12:45-13:00
CB-C08
LACTONES AFFECT INVASIVENESS OF *L. MEXICANA* POSSIBLY BY REDUCING EXPRESSION OF VIRULENCE FACTORS
Barrera, P.; Jimenez, V.; Carvelli, L.; Sartor, T.; Sanchez, V.; Tonn, C.; Giordano, O.*; Sosa, M.A.*
**Univ. Nac. San Luis; IHEMCONICET, Univ. Nac. Cuyo, Mendoza.*

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

Room C

Signal Transduction (ST-C01 / ST-C08)

Chairpersons: Ana Russo de Boland, Universidad Nacional del Sur, Bahía Blanca
María Teresa Téllez-Iñón, INGEBI-CONICET, Buenos Aires

11:00-11:15
ST-C01
THE MASTER TRANSCRIPTION FACTOR SPO0A REGULATES SLIDING DEVELOPMENT IN *BACILLUS SUBTILIS*
Rovetto, A.; Lombardía, E.; Coullery, R.; Grau, R.
IBR-Rosario.

11:15-11:30

ST-C02
BIOCHEMICAL CHARACTERIZATION OF DESK, THE MEMBRANE FLUIDITY SENSOR OF *BACILLUS SUBTILIS*
Martín, M.; de Mendoza, D.
IBR- CONICET, Univ. Nac. Rosario, Argentina.

11:30-11:45
ST-C03
POST-TRANSLATIONAL MODIFICATIONS MODULATE ATF7 TRANSCRIPTIONAL ACTIVITY
Diring, J.; Camuzeaux, B.; Donzeau, M.; Kedinger, C.; Chatton, B.
Institut Gilbert Laustriat, ESBS, UMR7175 CNRS-ULP, BP10413 67412 Strasbourg Illkirch, France.

11:45-12:00
ST-C04
ROLE OF ERK1/2 AND p38 MAPKS IN THE ANTIAPOPTOTIC EFFECTS OF 17β-ESTRADIOL IN MUSCLE CELLS
Ronda, A.C.; Vasconsuelo, A.; Boland, R.L.
Dpto. Biología, Bioquímica y Farmacia, Universidad Nacional del Sur; Bahía Blanca.

12:00-12:15
ST-C05
cAMP EXERTS A FINE CONTROL OF MAP KINASE PHOSPHATASE-1 LEVELS: IMPLICATIONS ON GENE TRANSCRIPTION
Brion, L; Gorostizaga, A; Suárez, G; Sequeiros García, M; Poderoso, C; Cornejo Maciel, F; Podestá, EJ; Paz, C
IIMHNO and Department of Biochemistry, School of Medicine, University of Buenos Aires.

12:15-12:30
ST-C06
ROLE OF PTPα IN 1α,25(OH)$_2$D$_3$ DEPENDENT Src ACTIVATION IN SKELETAL MUSCLE CELLS
Buitrago, C.G.; Boland, R.L.
Dpto. Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, Bahía Blanca.

12:30-12:45
ST-C07
ATP SIGNAL TRANSDUCTION PATHWAY IN MCF-7 BREAST CANCER CELLS
Scodelaro Bilbao, P.; Boland, R.; Russo de Boland A.; Santillán, G.
Dpto. Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, Bahía Blanca.

12:45-13:00
ST-C08
ALPHA2-M/LRP1 BINDING INCREASES MMP-9 SYNTHESIS BY INTRACELLULAR SIGNALING ACTIVATION IN J774 CELLS
Cáceres, L.C; Barcelona, P.F.; Sánchez, M.C.; Chiabrando, G.A.
Dpto. Bioquímica Clínica, CIBICI-CONICET, Fac. Ciencias Químicas, Univ. Nac. Córdoba.

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

15:30-16:30 Room A

LECTURE

Ta-Yuan Chang

Dartmouth Medical School, New Hampshire, USA

"Cholesterol sensing, trafficking, and esterification with relation to atherosclerosis and neurodegenerative diseases"

Chairperson: Ricardo Boland, Universidad Nacional del Sur, Bahía Blanca

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

Microbiology (MI-P01 / MI-P34)
Cell Biology (CB-P01 / CB-P30)
Plant Biochemistry and Molecular Biology (PL-C01 / PL-C27)
Lipids (LI-P01 / LI-P15)
Biotechnology (BT-P01 / BT-P20)
Enzymology (EN-P01 / EN-P14)

19:00-20:00 Room A

LECTURE

Juan Bonifacino

Cell Biology and Metabolism Branch, NICHD, National Institutes of Health, Bethesda, Maryland, USA

"Going retro: mechanisms of protein transport from endosomes to the Golgi complex"

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

20:00-21:00 Room A

Ricardo Farías

Director of CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), Argentina

"CONICET in the scientific system of Argentina. Strategies, policies and cultural institutional change 2002-2007"

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

MONDAY, November 19th, 2007

08:30-10:30 Room A

SYMPOSIUM

"Microbiology"

Chairpersons: Raúl Raya, CERELA-CONICET, Universidad Nacional de Tucumán
Antonio D. Uttaro, IBR-CONICET, Universidad Nacional de Rosario

08:30-09:00 ***Oscar P. Kuipers***

Department of Genetics, Groningen Biomolecular Sciences and Biotechnology Institute (GBB)
Rijksuniversiteit Groningen, Haren, The Netherlands

"Induction of natural competence in *Bacillus cereus*"

09:00-09:30 ***Colin Hill***

Department of Microbiology Alimentary Pharmabiotic Centre
BioSciences Institute, University College Cork, Ireland

"Shining light on how *Listeria monocytogenes* sense and respond to environmental change"

09:30-10:00 ***Eduardo Groisman***

Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, USA

"Regulatory mechanisms governing bacterial virulence"

10:00-10:30 ***Paul Michels***

University of Louvain, Belgium

"Metabolic changes during *Trypanosome* differentiation and the crucial role of glycosome turnover"

08:30-10:30 Room B

SYMPOSIUM

"Developmental Biology"

Chairpersons: Nora Calcaterra, IBR-CONICET, Universidad Nacional de Rosario
Pablo Wappner, Fundación Instituto Leloir-CONICET, Buenos Aires

08:30-09:00 ***Rolando Victor Rivera Pomar***

Centro Regional de Estudios Genómicos, Universidad Nacional de La Plata, Argentina

"Genomics and the development of *Rhodnius prolixus"*

09:00-09:30 ***José Xavier-Neto***

Hospital das Clínicas, Fac. Medicina, Universidade de São Paulo, Brasil

"Retinoic acid signaling, aldehyde dehydrogenases and the origin of chambered hearts"

09:30-10:00 ***Miguel L. Allende***

Departamento de Biología, Facultad de Ciencias, Universidad de Chile

"Development and regeneration of mechanosensory hair cells in the zebrafish lateral line system"

10:00-10:30 ***Flavio R. Zolessi***

Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay

"Neuronal differentiation in the retina: a fish and chicks story"

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

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

Room A

Microbiology (MI-C10 / MI-C18)

Chairpersons: Angeles Zorreguieta, Fundación Instituto Leloir-CONICET, Buenos Aires
Patricio J. de Urraza, Facultad Ciencias Exactas, Universidad Nacional de La Plata

11:00-11:15

MI-C10

PLURICELLULARITY AND ADHESIVE PROPERTIES OF THE ANTI-INFECTIVE PROBIOTIC BACTERIUM *BACILLUS SUBTILIS*

Rovetto, A.; Sabal, E.; Salvarrey, M.; Grau, R.
IBR-CONICET, Facultad de Bioquímica, Rosario.

11:15-11:30

MI-C11

INHIBITION OF AMIKACIN RESISTANCE USING AN RNASE P BASED STRATEGY TO SILENCE *AAC(6')-IB*

Soler Bistué, A.J.C.; Ha, H.; Zorreguieta, A; Tolmasky, M. E.
Fundación Instituto Leloir, CONICET; Dpto. Química Biológica, FCEN, UBA.

11:30-11:45

MI-C12

THE *ATTC* SITE IS A SITE SPECIFIC RECOMBINATION HOT SPOT OF DIFFERENT MOBILE ELEMENTS

Quiroga, C.; Centrón, D.
Dpto. Microbiología, Facultad de Medicina, Universidad Buenos Aires, Argentina.

11.45-12:00

MI-C13

PREDICTION AND DETECTION OF CHROMOSOMALLY ENCODED SMALL NON CODING RNAs IN *SINORHIZOBIUM MELILOTI*

Valverde, C.; Parisi, G.; Livny, J.

Programa Interacciones Biológicas, Dpto. Ciencia y Tecnología, Univ. Nac. Quilmes, Bernal.

12:00-12:15
MI-C14
EFFECT OF PBP MUTATIONS ON CELL MORPHOLOGY AND GROWTH RATE IN *STREPTOCOCCUS PNEUMONIAE*
Albarracin Orio, A. G. ; Cortes P.; Piñas G. ; Echenique, J.R.
CIBICI-CONICET, Facultad Cs. Químicas, Universidad Nacional de Córdoba.

12:15-12:30
MI-C15
ARGININE DEIMINASE PLAYS MULTIPLE REGULATORY ROLES IN THE BIOLOGY OF *GIARDIA LAMBLIA*
Vranych, C.; Rivero, R; Touz, M.C.; Rópolo, A.
Instituto M y M Ferreyra, INIMEC-CONICET, Friuli 2434, Córdoba, Argentina.

12:30-12:45
MI-C16
CARBON CATABOLITE REPRESSION OF TYPE IV PILI-DEPENDENT GLIDING
Méndez, M.; Grau, R.
IBR-Rosario.

12:45-13:00
MI-C17
CELL-TYPE SPECIFIC COMPARTMENTALIZATION OF THE *BACILLUS SUBTILIS* STRESS-RESPONSE TRANSCRIPTION FACTOR SIGMA B
Goñi, A.; Méndez, M.; Grau, R.
IBR-Rosario.

13:00-13:15
MI-C18
RELATION OF COPPER METABOLISM WITH THE *ESCHERICHIA COLI* RESPIRATORY CHAIN
Volentini, S. I.; Farias, R. N.; Rodríguez-Montelongo, L.; Rapisarda, V. A.
INSIBIO e Inst. de Química Biológica "Dr. B. Bloj", CONICET, UNT, Tucumán, Argentina.

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

Room B

Cell Biology (CB-C09 /CB-16)

Chairpersons: Laura Morelli, Fundación Instituto Leloir-CONICET, Buenos Aires
Paula Maloberti, Facultad de Medicina, Universidad de Buenos Aires

11:00-11:15
CB-C09
THE EARLY PHASE OF PROGRAMMED CELL DEATH IN CACO-2 INTESTINAL CELLS EXPOSED TO PTH
Calvo, N.; Gentili, C.; Russo de Boland, A.
Dpto. de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, Bahía Blanca.

11:15-11:30
CB-C10
ROLE OF HSP27 IN THE ANTIAPOPTOTIC EFFECTS OF 17β-ESTRADIOL IN SKELETAL MUSCLE CELLS
Vasconsuelo, A.; Milanesi, L.; Pronsato, L.; Boland, R.L.
Dpto. de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur; Bahía Blanca.

11:30-11:45
CB-C11
EFFICIENT LIPID ELIMINATION FROM SERTOLI CELLS AFTER APOPTOTIC DEATH OF SPERMATOGENIC CELLS

Ayuza Aresti, P.L.; Oresti, G.M.; Furland, N.E.; Ferraris, M.; Aveldaño, M.I.
INIBIBB-CONICET, Universidad Nacional de Sur, Bahía Blanca.

11:45-12:00
CB-C12
HYPERTONICITY INDUCES LAMIN A/C SYNTHESIS AND DISTRIBUTION IN A TONEBP/NFAT5 DEPENDENT MECHANISM
Favale, N.O. ; Sterin-Speziale, N.B.; Fernandez-Tome, M.C.
Biología Celular, FFYB-UBA, IQUIFIB-CONICET, Buenos Aires.

12:00-12:15
CB-C13
ZEBRAFISH CNBP UPSTREAM REGULATORY REGION: ISOLATION AND PROMOTER ANALYSIS
Weiner, A.M.J.; Allende, M.L.; Calcaterra, N.B.
IBR-CONICET, Univ. Nac. Rosario, Rosario,Argentina; CGC, Universidad de Chile, Santiago, Chile.

12:15-12:30
CB-C14
IDENTIFYING NATURAL ANTISENSE TRANSCRIPTS OF CHOLESTEROL TRANSPORT RELATED PROTEINS
Castillo, A.F.; Orlando, U.; Castilla, R.; Mele, P.G.; Podestá, E.J.
IIMHNO and Department of Biochemistry, School of Medicine, University of Buenos Aires.

12:30-12:45
CB-C15
TRANSCRIPTIONAL REGULATION OF INSULIN-DEGRADING ENZYME (IDE): POSSIBLE ROLE OF HYPOXIA AND NOTCH
Surace, E.I.; Leal, M.C.; Bulloj, A.; Castaño, E.M.; Morelli, L.
Laboratorio de Amiloidosis y Neurodegeneración, Fundación Instituto Leloir.

12:45-13:00
CB-C16
c-FOS, A NOVEL TARGET TO CONTROL TUMOR DEVELOPMENT IN NEUROFIBROMATOSIS TYPE 1
Silvestre, D.; Tomasini, N.; Caputto, B.
CIQUIBIC-CONICET, Dpto. Química Biológica, Fac. Cs. Químicas, Univ. Nac. Córdoba.

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

Room C

Plant Biochemistry and Molecular Biology (PL-C01 / PL-C09)

Chairpersons: Graciela Salerno, Centro de Investigaciones Biológicas, FIBA, Mar del Plata.
Ariel Goldraij, CIQUIBIC-CONICET, Universidad Nacional de Córdoba.

11:00-11:15
PL-C01
SPATIAL AND TEMPORAL CHARACTERIZATION OF THE TRANSCRIPTIONAL ACTIVITY OF miR164a PROMOTER IN PLANTS
Bassin, A., Almasia, N., Manacorda, C., Mongelli, V., Distéfano, A., Maroniche, G., Rodríguez, C., del Vas. M,, Asurmendi, S.
Instituto de Biotecnología, CICVyA, INTA-Castelar.

11:15-11:30
PL-C02
THE ARABIDOPSIS *CYTC-2* GENE IS REGULATED BY THE COOPERATIVE ACTION OF SITEII AND G-BOX MOTIFS
Welchen, E.; Viola, I.L.; Comelli, R.N.; González, D.H.
Cátedra Biología Celular y Molecular, FBCB, Univ. Nac. Litoral, Paraje "El Pozo", Santa Fe.

11:30-11:45
PL-C03
POLLEN TUBE GROWTH PROMOTION BY MrX FROM PISTILS
Wengier, D.L[1]; Mazzella, M.A.[1]; McCormick, S.[2]; Muschietti, J.P[1].
[1]INGEBI, Obligado 2490, Buenos Aires, Argentina; [2]PGEC-UC-Berkeley, 800 Buchanan St, Albany, CA, USA.

11.45-12:00
PL-C04
LEAF SIZE REGULATION BY miRNAs IN PLANTS
Mecchia, M.A.; Rodriguez-Virasoro, R.; Palatnik, J.F.
Instituto de Biología Molecular y Celular de Rosario, IBR-CONICET, Rosario, Argentina.

12:00-12:15
PL-C05
PROCESSING OF miR319 AND miR172 MicroRNA PRECURSORS IN *Arabidopsis thaliana*
Bologna, N.; Mateos, J.; Palatnik, J.
Instituto de Biología Molecular y Celular de Rosario, IBR-CONICET, Rosario, Argentina.

12:15-12:30
PL-C06
L-PROLINE CATABOLISM ALONG DEVELOPMENT OF THE HYPERSENSITIVE RESPONSE
Cecchini, N.M.[1]; Monteoliva, M.I.[1]; Nota, M.F.[1]; Blanco Herrera M.F.[2]; Holuigue Barros, M.L.[2]; Alvarez, M.E.[1]
[1]Fac. Cs. Químicas, UNC, Córdoba, Argentina. [2]Fac. Cs. Biológicas, Univ. Católica de Chile, Santiago, Chile.

12:30-12:45
PL-C07
DISSECTING IMMUNE RESPONSE AND PATHOGEN SUPPRESSION IN CITRUS-XANTHOMONAS INTERACTIONS
Enrique, R.; Siciliano, F.; Rigano, L.; Sendin, L.; Vojnov, A.; Castagnaro, A.; Marano, M.R.
IBR-Rosario; Fundación Pablo Cassará, Bs. As.; EEAOC-Tucumán.

12:45-13:00
PL-C08
CROSSTALK BETWEEN JASMONIC ACID AND ETHYLENE PATHWAYS ARE MEDIATED BY THE HAHB4 TRANSCRIPTION FACTOR
Manavella, P.A.; Dezar, C.A.; Chan, R.L.
Cátedra de Biología Celular y Molecular, FBCB, Univ. Nac. Litoral, Santa Fe.

13:00-13:15
PL-C09
TOBACCO PLANTS EXPRESSING A CYANOBACTERIAL FERREDOXIN DISPLAY INCREASED OXIDATIVE STRESS TOLERANCE
Ceccoli, R.D.; Blanco, N.E.; Carrillo, N.J.
IBR-CONICET, Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR, Rosario, Argentina.

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

15:30-16:30 **PASTEUR INSTITUTE OF MONTEVIDEO: AN OVERVIEW**
Chairperson: Néstor Carrillo, IBR-CONICET, Universidad Nacional de Rosario

15:30-15:50 ***Mariela Bollati***
Scientific activities at the "Pasteur Institute of Montevideo"

15:50-16:10 ***Pablo Aguilar***
"Eisosomes biogenesis and Pkh-kinases control"

16:10-16:30 ***Arlinet Kierbel***
"*Pseudomonas aeruginosa* exploits a PIP3-dependent pathway to transform apical into basolateral membrane"

16:30-19:00 **POSTERS with coffee**
Microbiology (MI-P35 / MI-P68)
Cell Biology (CB-P31 / CB-P61)
Plant Biochemistry and Molecular Biology (PL-P28 / PL-P57)
Lipids (LI-P16 / LI-P29)
Biotechnology (BT-P21 / BT-P40)
Signal Transduction (ST-P01 / ST-P12)

19:00-20:00 Room A
"Alberto Sols" LECTURE
Antonio Ferrer Montiel
Universidad Miguel Hernandez, Alicante, España
"Molecular biology of pain transduction"
Chairperson: Héctor Torres, INGEBI-CONICET, Buenos Aires

20:30 **SAIB General Assembly**

TUESDAY, November 20th, 2007

08:30-10:30 Room A
SYMPOSIUM
"Cell Biology"
Chairpersons: María Teresa Damiani, IHEM-CONICET, Universidad Nacional de Cuyo, Mendoza
Luis S. Mayorga, IHEM-CONICET, Universidad Nacional de Cuyo, Mendoza

08:30-09:00 ***José Luis Daniotti***
CIQUIBIC-CONICET, Universidad Nacional de Córdoba, Argentina
"Protein lipidation: role in membrane association, intracellular trafficking and signaling"

09:00-09:30 ***Gabriel Rabinovich***
Hospital de Clínicas "José de San Martín", Universidad de Buenos Aires, Argentina
"The sweet control: impact of differential glycosylation in the regulation of T-cell homeostasis"

09:30-10:00 ***Alejandro Aballay***
Duke University Medical Center Durham, North Carolina, USA
"Use of *C. elegans* to study conserved signaling pathways that regulate innate immunity"

10:00-10:30 ***Ariel Savina***
Institut Curie, Paris, France
"Membrane trafficking in antigen cross-presentation"

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

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

Room A

Microbiology (MI-C19 / MI-C27)

Chairpersons: José R. Echenique, CIBICI-CONICET, Universidad Nacional de Córdoba
Mónica A. Delgado, INSIBIO-CONICET, Universidad Nacional de Tucumán

11:00-11:15
MI-C19
IDENTIFICATION AND CHARACTERIZATION OF A *BRUCELLA ABORTUS* TYPE IV TRANSLOCATED PROTEIN
Marchesini, M.I.; Comerci, D.J.; Gorvel, J.P.; Ugalde, R.A.
Instituto de Investigaciones Biotecnológicas, UNSAM, Argentina and CIML, Marseille, France.

11:15-11:30
MI-C20
TRANSCRIPTION OF THE *BRUCELLA ABORTUS VIRB* OPERON IS METABOLICALLY LINKED TO HISTIDINE CATABOLISM
Sieira, R.; Comerci, D.J.; Ugalde, R.A.
Instituto de Investigaciones Biotecnológicas, UNSAM, IIB-INTECH-CONICET.

11:30-11:45
MI-C21
PROTECTIVE ACTION OF PPGPP ON MICROCIN J25 SENSITIVE STRAINS BY INCREASE OF YOJI EXPORT ACTIVITY
Pomares, M.F; Farías, R.N.; Salomón, R.A.; Vincent, P.A.
Dpto. Bioquímica de la Nutrición. INSIBIO, UNT-CONICET, Inst. Química Biológica, UNT, Tucumán.

11.45-12:00
MI-C22
IDENTIFICATION OF A NOVEL LIGASE INVOLVED IN A PROTEIN LIPOYLATION PATHWAY IN *BACILLUS SUBTILIS*
Martin, N.; de Mendoza, D.; Mansilla, M. C.
Instituto de Biología Molecular y Celular de Rosario, CONICET, Fac. Cs. Bioquímicas y Farmacéuticas, UNR.

12:00-12:15
MI-C23
AN ENZYME WITH A LENGTH PROOF-READING ACTIVITY AS A MECHANISM TO CONTROL THE SIZE OF POLYSACCHARIDES
Ciocchini, A. E.[1]; Guidolin, L. S.[1]; Casabuono, A. C.[2]; Couto, A. S.[2]; Iñón de Iannino, N.[1]; Ugalde, R. A.[1]
[1]IIB-UNSAM; [2]CIHIDECAR-CONICET, Dpto. de Química Orgánica, FCEyN, UBA, Argentina.

12:15-12:30
MI-C24
FROM SUCROSE TO MANNOSYLFRUCTOSE BIOSYNTHESIS: *AGROBACTERIUM TUMEFACIENS* NOVEL METABOLIC PATHWAY
Torres, L.L.; Salerno, G.L.
Centro de Investigaciones Biológicas, FIBA, Mar del Plata, Argentina.

12:30-12:45
MI-C25
HALOALKALIPHILIC PROTEASE FROM AN ARCHAEON: OVEREXPRESSION IN *ESCHERICHIA COLI* AND *HALOFERAX VOLCANII*
Ruiz, D.M.[1]; Maupin-Furlow, J.A.[2]; De Castro, R.E.[1]
[1]IIB, Univ. Nac. Mar del Plata, Mar del Plata, Argentina; [2]UF, USA.

12:45-13:00
MI-C26

CHARACTERISTICS OF N-TERMINAL DOMAIN OF A *PSEUDOMONAS AERUGINOSA* PAO1 RECOMBINANT POLYPHOSPHATASE
Beassoni, P.R.; Gallarato, L.A.; Garrido, M.N.
Dpto. Biología Molecular, FCEFQN, UNRC, Río Cuarto, Córdoba, Argentina.

13:00-13:15
MI-C27
IDENTIFICATION OF CATALYTIC SITE RESIDUES OF *PSEUDOMONAS AERUGINOSA* HEMOLYTIC PHOSPHOLIPASE C
Forrellad, M.A.; Zafra, M.; Lisa, A.T.
Dpto. Biología Molecular, Universidad Nacional de Río Cuarto, Córdoba.

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

Room B

Lipids (LI-C01 / LI-C09)

Chairpersons: Margarita Garcia de Bravo, INIBIOLP-CONICET, Universidad Nacional de La Plata
Gabriela Salvador, INIBIBB-CONICET, Universidad Nacional del Sur, Bahía Blanca

11:00-11:15
LI-C01
EFFECT OF CLA, LINOLEIC ACID AND ITS METHYL ESTER ON LIPID PEROXIDATION OF TRIGLYCERIDES ω-3 PUFAS
Fagali, N.S.; Catalá, A.
INIFTA-CONICET, Fac. Ciencias Exactas, UNLP, La Plata, Argentina.

11:15-11:30
LI-C02
CHARACTERIZATION OF THE MURINE CTP:PHOSPHOCHOLINE CYTIDYLYLTRANSFERASE BETA GENE PROMOTER
Marcucci, H.; Elena, C.; Banchio, C.
Área Biología, IBR-CONICET, Fac. Ciencias Bioquímicas y Farmacéuticas, Univ. Nac. Rosario.

11:30-11:45
LI-C03
PHOSPHATIDYLCHOLINE BIOSYNTHESIS AND NEURONAL DIFFERENTIATION
Banchio, C; Jackowski, S.
Área Biología, IBR-CONICET, Fac. Ciencias Bioquímicas y Farmacéuticas, Univ. Nac. Rosario.

11.45-12:00
LI-C04
PARTICIPATION OF GLYCOSPHINGOLIPID BIOSYNTHESIS IN RENAL COLLECTING DUCT CELL DIFFERENTIATION
Pescio, L.; Leocata Nieto, F.; Favale, N.; Fernandez-Tomé, M. C.; Sterin-Speziale, N.
Biología Celular, FFyB, UBA, IQUIFIB-CONICET, Buenos Aires.

12:00-12:15
LI-C05
REGULATION OF LIPID METABOLISM AND ONCOGENESIS
Duarte, A.; Maloberti, P.; Karlés, C.; Orlando, U.; Neuman, I.; Cornejo Maciel, F.; Solano, A.; Podestá, E.J.
IIMHNO and Department of Biochemistry, School of Medicine, University of Buenos Aires.

12:15-12:30
LI-C06
CADMIUM EFFECTS ON RAT TESTICULAR LIPIDS AND FATTY ACIDS
Zanetti, S.R.; Aveldaño, M.I.
INIBIBB-CONICET, Universidad Nacional del Sur, Bahía Blanca.

12:30-12:45
LI-C07
A MALONYL-COA-DEPENDENT SWITCH IN THE BACTERIAL RESPONSE TO A DYSFUNCTION OF LIPID METABOLISM
Gustavo E. Schujman; Silvia Altabe; Diego de Mendoza
IBR-CONICET, Facultad de Cs. Bioquímicas y Farmacéuticas, Univ. Nac. Rosario.

12:45-13:00
LI-C08
STRUCTURAL DETERMINANTS FOR COLD REGULATION IN METHYL-END DESATURASES OF TRYPANOSOMATIDS
Andrés Alloatti; Antonio D. Uttaro
IBR-CONICET, Dpto. Microbiología, Facultad de Cs. Bioquímicas y Farmacéuticas, Univ. Nac. Rosario.

13:00-13:15
LI-C09
FACTORS THAT MODULATE MEMBRANE FATTY ACID COMPOSITION, STRUCTURE AND PROTEIN LIPID INTERACTIONS
Brenner, R.R.; Bernasconi, A.M.; Montanaro, M.A.
Instituto de Investigaciones Bioquímicas de La Plata, INIBIOLP, Fac Cs Médicas (CONICET-UNLP).

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

Room C

Plant Biochemistry and Molecular Biology (PL-C10 / PL-C18)

Chairpersons: Fabiana Drincovich, CEFOBI-CONICET, Universidad Nacional de Rosario
Diego Gomez-Casatti, IIB-INTECH, CONICET, Universidad Nacional San Martín, Buenos Aires

11:00-11:15
PL-C10
HETEROMERIC ENZYMES: DIFFERENT PROPERTIES OF *A. thaliana* NAD-MALIC ENZYME SUBUNITS
Tronconi, M.A.[1]; Maurino, V.G.[2]; Drincovich, M.F.[1]; Andreo, C.S.[1]
[1]CEFOBI-CONICET, Fac. Cs. Bioq. y Farm. Univ. Nac. Rosario; [2]Instituto de Botánica Univ. Colonia, Alemania.

11:15-11:30
PL-C11
REGULATORY ROLE OF THE N-TERMINAL STARCH BINDING DOMAINS ON THE KINETICS OF STARCH SYNTHASE III
Valdez, H.A.; Wayllace, N.Z.; Parisi, G.; Ugalde, R.A.; Gomez-Casati, D.; Busi, M.V.
UB6-Lab. de Bioquímica y Biología Molecular de Plantas IIB-INTECH CONICET-UNSAM, Chascomus.

11:30-11:45
PL-C12
METABOLIC REGULATION AND STRUCTURE-FUNCTION RELATIONSHIP OF *A. thaliana* NADP-MALIC ENZYME ISOFORMS
Gerrard Wheeler, M.C.[1]; Arias, C.L.[1]; Maurino, V.G.[2]; Andreo, C.S.[1]; Drincovich, M.F.[1]
[1]CEFOBI. Fac. Cs. Bioq. y Farm. UNR. [2]Instituto de botánica Univ. Colonia, Alemania.

11:45-12:00
PL-C13
AUXIN AND NITRIC OXIDE TRIGGER PHOSPHATIDIC ACID ACCUMULATION VIA PHOSPHOLIPASE D IN CUCUMBER
Lanteri, M.L.; Laxalt, A.M.; Lamattina, L.
Instituto de Investigaciones Biológicas, Univ. Nac. Mar del Plata, Mar del Plata.

12:00-12:15
PL-C14
CHARACTERIZATION OF NADP-MALIC ENZYME FROM *Nicotiana tabacum:* CLONING AND BIOLOGICAL ROLE ANALYSIS
Müller, G.L.; Andreo, C.S.; Drincovich, M.F.; Lara, M.V.
CEFOBI-CONICET, Facultad de Ciencias Bioquímicas y Farmacéuticas, Univ. Nac. Rosario.

12:15-12:30
PL-C15
AUTOPHOSPHORYLATION OF RAPESEED 2-Cys PEROXYREDOXIN
Aran, M.; Caporaletti, D.; Senn, A.M.; Tellez de Iñon, M.T.; Girotti, M.R.; Llera, A.S.; Wolosiuk, R.A.
Instituto Leloir and INGEBI-CONICET, Buenos Aires, Argentina.

12:30-12:45
PL-C16
LOCALIZATION OF A PLANT LTP SUGGESTS A NOVEL ROLE OF THIS PROTEIN IN LIPID MOBILIZATION
Pagnussat, L.A.; Lombardo, M.C.; de la Canal, L.
Instituto de Investigaciones Biológicas, FCEyN, UNMdP. Funes 3250 CC 1245. 7600. Mar del Plata.

12:45-13:00
PL-C17
ATAZG IS A HIGH-AFFINITY PURINE IMPORTER WITH A POTENTIAL ROLE IN CYTOKININ UPTAKE
Maurino, V.G.[1]; Grube, E.[1]; Schumacher, B.[2]; Flügge, U-I [1]; Desimone, M.[2]
[1]Botanisches Institut, Gyrhofstr. 15, Cologne; [2]ZMBP, Auf der Morgenstelle 1, Tübingen, Germany.

13:00-13:15
PL-C18
CHARACTERIZATION OF RECOMBINANT NADP DEPENDENT MALIC ENZYME FROM C3, C4 AND C3-C4 FLAVERIA SPECIES
Saavedra, D.D.; Drincovich, M.F.; Andreo, C.S.
CEFOBI-CONICET, Facultad de Cs Bioquímicas y Farmacéuticas, Univ. Nac. Rosario.

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

15:30-16:30 Room A

LECTURE

Javier Pozueta Romero

Instituto de Agrobiotecnología, Navarra, España

"Recent advances on starch and glycogen metabolisms in plants and bacteria, respectively"

Chairperson: Alejandro Viale, IBR-CONICET, Universidad Nacional de Rosario

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

Microbiology (MI-P69/ MI-P101)
Cell Biology (CB-P62 / CB-P84)
Plant Biochemistry and Molecular Biology (PL-P58 / PL-P86)
Structural Biology (SB-P01 / SB-P13)
Neuroscience (NS-P01 / NS-P13)
Signal Transduction (ST-P13 / ST-P26)
Enzymology (EN-P15 / EN-P29)

19:00-20:00 Room A

"EMBO" LECTURE

Jeffery Errington

Institute for Cell and Molecular Biosciences, University of Newcastle, UK

"A novel protein system orchestrating the switch between growth and division in *Bacillus subtilis*"

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

21:00 **Farewell Dinner**

S-01.

FUNDAMENTALS OF PRIMARY METABOLISM: MOLECULAR AND BIOCHEMICAL ATTEMPTS TO IDENTIFY KEY STEPS

Hajirezaei MR, Mockwitz I, Kim YM, Ahkami A.
Leibniz Inst Plant Genetics & Crop Plant Research, Corrensstrasse 3, 06466 Gatersleben, Germany. E-mail: mohammad@ipk-gatersleben.de

Due to tremendous importance of primary metabolism, intensive study is carried out in our group since decades. In this presentation the three major current research topics are reviewed: (1) Hexokinases play multiple functions in the carbohydrate metabolism, contribute to energy production (glycolysis), form precursors for the synthesis of cell wall and secondary metabolites, and act as signal transducer to regulate gene expression. We isolated and characterised 10 different isoforms of hexokinases from tobacco. Transgenic plants are created with overexpression/downregulation of hexokinases to elucidate their individual functions. In addition Arabidopsis and yeast mutants lacking hexokinases are complemented to functionally analyse the different isoforms. (2) Petunia is used as a model system to study adventitious root formation. To improve the rooting behaviour, metabolic fluxes have to be manipulated via overexpression of rate limiting enzymes, inhibition of competing pathways, manipulation of the levels of signalling compounds/phytohormones, etc. (3) Creation of transgenic plants conferring tolerance against various environmental stimuli. For this purpose, the expression of heterologous enzymes is preferred to circumvent endogenous regulatory mechanisms. We generated transgenic plants expressing a bacterial flavodoxin in different compartments. Plants with plastidic targeted flavodoxin confer broad range stress tolerance. The use of flavodoxin provides new possibilities to improve the stress tolerance capacity of crops as well as to elucidate the mechanism underlying the augmented durability.

S-02.

INDUCIBLE ENDOGENOUS siRNAs IN PLANT STRESS RESPONSES

Borsani O[1], Zhu JK[2].
[1]Fac Agronomía, Univ República, Uruguay; [2]Dep Botany & Plant Sci, Univ California Riverside, USA. E-mail: oborsani@fagro.edu.uy

Aproximately 10% of *Arabidopsis thaliana* genes are in convergent overlapping gene pairs, also known as natural cis-antisense gene pairs. It has been shown that overlapping transcripts in an antisense orientation are able to form double-stranded RNAs wich are then processed into natural-antisense-transcripts small interfering RNAs (nat-siRNA). The nat-siRNA produced by the SRO5-P5CDH cis-antisense pair were the first identified nat-siRNA and they have critical role in a salt-stress regulatory network. The SRO5-P5CDH nat-siRNAs study defined a mode of siRNA biogenesis and function that may be applied to other natural cis-antisense genes. Our survey for other convergent overlapping genes potentially involved in abiotic stresses responses has yielded in the identification of several new cis-antisense gene pairs that generate nat-siRNAs. We will show that these cis-antisense gene pairs are able to generate nat-siRNA, and some of the nat-siRNA are inducible and detected only under specific abiotic-stress treatment conditions.

S-03.

MOLECULAR AND PHYSIOLOGICAL CONNECTIONS BETWEEN LIGHT AND DEFENSE SIGNALING MECHANISMS

Ballaré CL, Izaguirre MM, Mazza CA, Moreno JE.
IFEVA, CONICET and Univ Buenos Aires, Av San Martín 4453, C1417DSE Buenos Aires. E-mail: ballare@ifeva.edu.ar

Plants have evolved sophisticated mechanisms to deal with competitors and consumers. Plants use specific photoreceptors to obtain information about the proximity of neighboring plants and the intensity of future competition. The phytochromes can detect far-red (FR) radiation reflected by neighbors and elicit rapid shade-avoidance response before the plants are actually shaded by competitors. Plants can also detect mechanical damage and specific herbivore elicitors, and respond with an array of defensive mechanisms. Exposure of non-shaded plants to reflected FR can inhibit the expression of induced defenses (Izaguirre et al. PNAS 2006, 103: 7170). We have now carried out a series of experiments to elucidate the mechanistic basis of this inhibition. Arabidopsis and Nicotiana plants responded to insect herbivory by inducing a variety of direct defenses (phenolic compounds and proteinase inhibitors). This induction was the result of a clear, rapid up-regulation of several defense-related genes. Plants exposed to reflected FR failed to show this induction of defense genes and were more vulnerable to attack by a variety of insects. Analysis of gene expression data and physiological experiments suggest that the suppressing effect of FR radiation on the expression of induced defenses is caused by the down-regulation of several components of the jasmonic-acid signaling pathway.

S-04.

RIP-CHIP FOR GLOBAL ANALYSIS OF CELL-SPECIFIC GENE EXPRESSION IN ARABIDOPSIS

Zanetti ME, Mustroph A, Bailey-Serres J.
Center for Plant Cell Biology, University of California Riverside, CA 92521 USA. E-mail: meuzanetti@yahoo.com

Two significant deficiencies of routine mRNA expression profiling studies are, (1) the abundance of an individual mRNA may not reflect the level of its translation, and (2) the abundance of an mRNA in specific cell-types cannot be gleaned from organ extracts. To circumvent these challenges and augment the elucidation of developmental processes and environmental responses, we have developed a Rip-Chip ("RNA immunopurification and microarray chip hybridization") technology to isolate polyribosomes for global characterization of cell-specific gene expression in Arabidopsis thaliana (Zanetti *et al* 2005, Plant Physiol 138: 624). The basis of this technology is the use of cell-type specific promoters to drive the expression of an epitope-tagged ribosomal protein, RPL18. Rip-Chip experiments were conducted to evaluate global gene expression in specific cell-types of Arabidopsis roots (cortex, endodermis, vasculature, etc.) kept in the air or subjected to hypoxia during 2 hours. The results suggest that both qualitative and quantitative differences exist in the polysomal mRNA levels under hypoxia conditions in each root cell-type.

S-05.
LIPOGENIC PATHWAYS AND INSULIN SENSITIVITY
Nagle CA, Li LO, Ellis JM, Coleman RA.
Departments of Nutrition and Pediatrics, University of North Carolina, Chapel Hill, NC 27599. E-mail: rcoleman@unc.edu

Increased synthesis of triacylglycerol is associated with chronic metabolic disorders like obesity, cardiovascular disease, and diabetes. Acyl-CoA synthetases activate long-chain fatty acids destined for the synthesis or oxidation of complex lipids, and glycerol-sn-3-phosphate acyltransferase (GPAT) catalyzes the first step in de novo triacylglycerol synthesis. Each of these synthetic steps is catalyzed by multiple independent isoforms, each encoded by a separate gene. Further, the acyl-CoA and diacylglycerol products of acyl-CoA synthetases and the glycerol-P acyltransferases have been implicated in the development of insulin resistance. We have over-expressed and knocked out members of each family in order to determine the effect on insulin sensitivity. Gain or loss of function of the GPAT1 isoform in liver alters insulin signaling in conjunction with changes in lipid intermediates like diacylglycerol. New data will be presented on the effects of tissue-specific decreases in ACSL1.
Supported by DK56598, DK59935, and the American Heart Association (MidAtlantic Division).

S-06.
ACYL-COA SYNTHETASES: NEW ROLES IN BRAIN CANCER AND GABA SIGNALING
Watkins PA, Pei Z, Jia Z, Toomer CJ, Huang P.
Kennedy Krieger Institute and Dept of Neurology, Johns Hopkins Univ Sch Med, Baltimore, MD, USA. E-mail: watkins@kennedykrieger.org

Fatty acids must be "activated" to their CoA derivatives to participate in most downstream anabolic or catabolic pathways. This reaction is catalyzed by acyl-CoA synthetases (ACS). Investigation of these enzymes has revealed new and unexpected roles in health and disease. ACSBG1 is an ACS found only in neurons and in steroidogenic cells of the adrenal gland, testis, and ovary, and preferentially activates the 16-carbon long-chain fatty acid, palmitate. Studies of knockout mice and cells depleted of ACSBG1 by RNA interference suggest that this protein is required for normal functioning of $GABA_A$ receptors. We propose that ACSBG1 provides activated substrate for palmitoylation of the receptor γ-subunit. Another enzyme, ACSVL3, activates fatty acids containing 16-24 carbons and is a member of the very long-chain ACS family. In adult brain, ACSVL3 expression is low and is confined to neurons. However, we found very high levels of this protein in human malignant glioma cell lines and tumors. Knockdown of this enzyme by RNA interference decreased the malignant phenotype of glioma cells in culture as well as their in vivo tumorigenicity. However, the mechanism by which decreased ACS activity affects these properties has not yet been elucidated.

S-07.
DIFFERENTIAL REGULATORY AND METABOLIC EFFECTS IN LIVER CONTRIBUTED BY DIETARY FATTY ACIDS
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Nutritional fatty acids have been implicated in both disease promotion and prevention. The saturated and monounsaturated fatty acids in the diet are generally first incorporated into storage pools and have limited regulatory properties. On the other hand the omega 3 and omega 6 polyunsaturated fatty acids (PUFA) that are essential for growth, development and health maintenance have very strong regulatory effects that are transmitted primarily through signal transduction cascades or transcription. Many dietary studies to date have been conducted which compare high fat, high calorie diets with normal calorie diets containing limited fat. Less attention has been paid to evaluating the effects of the types of fat provided in a normal calorie diet, including the amount of long chain PUFA, relative to very long chain PUFA. We have examined the fates and regulatory effects of feeding mice diets that are enriched in saturated fat from lard (16:0 and 18:0), PUFA from canola oil (18:2 and 18:3), and PUFA from a fish and fungal oil mixture (20:4, 20:5 and 22:5). The results point to very profound and unique differences in the storage capacity for these fats in adipose tissue, metabolic fates in liver, and effects on gene expression in liver.

S-08.
FATTY ACID TRANSPORT BY VECTORIAL ACYLATION: SELECTIVE TRAFFICKING OF FATTY ACID MEDIATED BY THE FATP AND ACSL ISOFORMS
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The process of fatty acid transport across the plasma membrane occurs by several mechanisms that involve distinct membrane-bound and membrane-associated proteins and enzymes. Amongst these are the fatty acid transport proteins (FATP) and long-chain acyl CoA synthetases (ACSL). The yeast orthologues of FATP and ACSL form a physical complex at the plasma membrane and are required for fatty acid transport, which proceeds through a coupled process linking transport with metabolic activation and termed vectorial acylation. At present six isoforms of FATP and five isoforms of ACSL, including a number of splice variants, have been identified in mice and man. The different FATP and ACSL isoforms have distinct tissue expression profiles and along with different cellular locations suggest they function in the trafficking of fatty acids into discrete metabolic pools. More specifically, we hypothesize the different FATP and ACLS isoforms function individually and coordinately to move distinct classes of fatty acids into these different metabolic pools. The concerted activity of these proteins allows the cell to discriminate different classes of fatty acids and provides the mechanistic basis underpinning the selectivity and specificity of fatty acid transport.

S-09.

INDUCTION OF NATURAL COMPETENCE IN *BACILLUS CEREUS*

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Natural competence is the ability to take up and incorporate DNA from the environment. Competence development within the *Bacillus* family has so far been described for *Bacillus subtilis*, *B. licheniformis* and *B. amyloliquefasciens*, but not for *B. cereus* or other *Bacillus* species.We used sequences of proteins involved in competence in *B. subtilis* as query in BLAST analyses of the genomes of several *Bacillus* species. In doing so, we have identified various putative homologs of competence proteins. The *comK* gene was identified in nearly all species, but was missing in *B. clausii* and *B. halodurans*. Interestingly, several *B. cereus*, *B. antracis* and *B. thuringiensis* strains seem to contain two homologues of ComK. Recent results in our laboratory show that *B. cereus* can become natural competent. When expressing ComK proteins in *B. cereus* ATCC14579, plasmid and genomic DNA was shown to be taken up by the cells and stably maintained or integrated, respectively. DNA microarray analysis demonstrated the up-regulation of several putative competence operons in these strains. This work has several important implications. First, the combination of genome mining and characterization of competence in other species than *B. subtilis* might enable the definition of a minimal competence machinery. Secondly, it is likely to reveal differences in the regulation of competence between species. Establishing a competence regime would greatly enhance the genetic accessibility for medically and industrially relevant organisms.

S-10.

SHINING LIGHT ON HOW *LISTERIA MONOCYTOGENES* SENSE AND RESPOND TO ENVIRONMENTAL CHANGE

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Listeria monocytogenes is a rare, though often fatal, foodborne pathogen which has a lifestyle which exposes it to many different ecological niches; from food (with variations in pH, Aw, temperature, nutritional status) to the gastrointestinal tract (low pH, bile, competition from other flora, immune system) and finally, an intracellular phase (reactive oxygen species, limited iron). Despite a relatively small genome, Listeria is capable of sensing and responding to changes in its environment in a manner which allows it to overcome these challenges and become one of the most difficult organisms to control in the food chain. In order to understand the biological and physical triggers which influence Listeria, we developed a luciferase-based technology which allows us to track gene expression in realtime in situ in whole complex foods and in living animal models. The luciferase genes, originally identified in Photorhabdus, are integrated into the bacterial chromosome in stable single copy, and accurately report the expression of Listeria genes without the need for invasive assays. Using this novel technology we have analysed the expression of genes involved in stress tolerance and virulence in the mouse model.

S-11.

REGULATORY MECHANISMS GOVERNING BACTERIAL VIRULENCE

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S-12.

METABOLIC CHANGES DURING TRYPANOSOME DIFFERENTIATION AND THE CRUCIAL ROLE OF GLYCOSOME TURNOVER

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In trypanosomatids, most glycolytic enzymes are sequestered in peroxisomes designated glycosomes. The function of glycosomes and the selective advantage that led to the compartmentation of glycolysis have been a matter of year-long discussions. We hypothesized that this metabolic compartmentation might be important for increasing the adaptability of the parasite to differentiation-related changes of the environment. Previous studies on non-differentiating laboratory-adapted strains of *Trypanosoma brucei* have shown that glycolytic enzymes constitute nearly the entire glycosomal protein content in bloodstream-form cells, whereas they are quantitatively less important in cultured procyclic cells, representative of trypanosomes living in the tsetse-fly's midgut, where the organelles also contain many other enzymatic systems. Also in yeasts, the enzymatic content of peroxisomes may vary considerably and the biogenesis and degradation of these organelles are regulated according to the nutritional conditions. Old peroxisomes, no longer appropriate for altered conditions, are degraded by autophagy, while peroxisomes with a different metabolic repertoire are synthesized, enabling the organism to cope efficiently with the changed environment. We will present evidence, based on bioinformatics, morphological and metabolic studies, that in a pleiomorphic *T. brucei* strain, during its differentiation from the long-slender to the short-stumpy bloodstream form and particularly from the short-stumpy to the procyclic form, a similar enhanced turnover of glycosomes occurs, involving autophagy of the organelles.

S-13.
GENOMICS AND THE DEVELOPMENT OF *RHODNIUS PROLIXUS*
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The establishment of the insect body plan has been extensively studied in *Drosophila melanogaster* and *Tribolium castaneum* (Coleoptera). New models such as *Nasonia* (Hymenoptera) and *Oncopeltus* (Hemiptera) have provided new insights. However, a detailed comparison of regulatory networks is far from completed, as genome information is still scarse. Only four genomes have been finished: *Drosophila*, *Anopheles* and *Aedes* (Diptera) and *Apis* (Hymenoptera). Although relevant, they still correspond to holometabolous insects. The completion of the first genome draft of *Rhodnius prolixus*, a hemimetabolous insect will shed light on more complex processes of morphogenesis as the hatching individual is essentially identical to the adult. We have constructed normalized genomic libraries for gene annotation and initiated the analysis of a handful of developmentally regulated genes of the HOX class. We identified several homeobox-containing genes from *Oncopeltus* and *Rhodnius*, clone a putative Antp-ortholog and determine its genomic structure. Moreover, we analized the expression pattern and the function by systemic RNAi. We will present preliminary data the role of different segmentation genes as well as the regulatory networks acting on *Rhodnius embryogenesis*.

S-14.
RETINOIC ACID SIGNALING, ALDEHYDE DEHYDROGENASES AND THE ORIGIN OF CHAMBERED HEARTS
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Retinoic acid (RA) patterns embryonic vertebrate hearts in the anterior-posterior (AP) axis, dividing them into inflow/outflow segments (e.g. atrial or ventricular chambers). RA patterning extends to teleosts, suggesting that hearts are composites of inflow/outflow segments presaged by division of precursors along embryonic axes by signaling mechanisms. We argue that chambers arose in evolution, not one by one, but simultaneously, as an ancestral peristaltic pump field that was patterned into domains later fashioned into chambers. Using RA signaling as a tool to understand the origin of chambered hearts from the peristaltic vessels of deuterostomes, we showed that, similar to vertebrates, retinaldehyde dehydrogenases mark the posterior border of the emerging invertebrate chordate pump field. This suggests scenarios in which this ancestral topology was initially exploited to establish posterior boundaries of pumping organs, but was later co-opted to AP patterning roles

S-15.
DEVELOPMENT AND REGENERATION OF MECHANOSENSORY HAIR CELLS IN THE ZEBRAFISH LATERAL LINE SYSTEM
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We are interested in exploring some of the molecular mechanisms underlying the development of the mechanosensory lateral line in the zebrafish. The lateral line sensory organs are the neuromasts, clusters of hair cells surrounded by accessory cells, distributed over the surface of the body. The neuromasts form at the end of embryogenesis through a process of highly stereotyped migration of a primordium and cell deposition. We have shown that the atonal homolog 1 gene, ath1, is essential for specification of the hair cells and that progenitor cells express the neural stem cell marker sox2. Previous studies have shown that diverse agents can destroy the lateral line hair cells, but that these can regenerate and become fully functional. We now show that regeneration of hair cells can occur through two mechanisms. The first involves a postmitotic group of precursors that can quickly replace hair cells when these are damaged. The second requires proliferation of sox2-expressing cells and occurs only when damage is extensive. Our study of this system has profited from the availability of several transgenic lines of zebrafish, which express GFP in the different cell types of neuromasts and in the migrating primordium. Therefore, we have been able to perform our analysis in vivo, in the intact zebrafish larva.
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S-16.
NEURONAL DIFFERENTIATION IN THE RETINA: A FISH AND CHICKS STORY
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The neural retina is an excellent model system for studying neuronal differentiation. In the chick embryo, the retina is big, easily dissected and free from non-neural cells, making it suitable for biochemical analyses and cell culture. The zebrafish embryo, on the other hand, is small, easily handled as a whole organism, genetically competent and optically transparent, thus offering the best vertebrate system for functional analyses *in vivo*. MARCKS is a ubiquitous, actin-modulating protein, which is particularly important for neural development, although the basis for its apparent functional specificity is unknown. We have previously described a phosphorylated MARCKS isoform that is specific for differentiating neurons in the chick embryo. The phosphorylated residue (S 25) is in a consensus sequence for cyclin-dependent kinases (Cdks). We will show here evidence indicating Cdk5 as the neuronal-specific MARCKS kinase. Treatment of cultured neural retina cells with a specific Cdk inhibitor caused a dramatic decrease in MARCKS phosphorylation, dependent on the developmental stage and the time *in vitro* of the cells. We also found that Cdk5 partially co-localizes and co-immunoprecipitates with S25p-MARCKS. We are now starting to analyze the possible roles of MARCKS in the differentiation of the zebrafish neural retina. We have found in the databases two cDNAs coding for slightly different MARCKS proteins, only one of which contains a S25-like residue. After blocking the translation of these proteins with specific morpholinos, we obtained very characteristic phenotypes including severe defects in retinal morphogenesis.

S-17.

EISOSOMES BIOGENESIS AND PKH-KINASES CONTROL

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The molecular composition of plasma membranes is constantly remodeled by endocytosis and exocytosis. Eisosomes are large cytoplasmic protein assemblies that localize to specialized domains on the yeast plasma membrane, where they mark sites of endocytosis. Here we show that eisosomes are formed *de novo* in the bud of dividing cells. Pil1, one of the two main eisosome subunits, emerges as the central regulator of this process that can determine both size and location of eisosomes. We also show that Pil1 *in vivo* is a target of the lipid signaling pathway mediated by the Pkh-kinases. Eisosomes disassemble if Pil1 is hyperphosphorylated and conversely, more Pil1 assembles into eisosomes if Pil1 is hypophosphorylated.

Taken together, our results provide a first framework defining eisosome assembly and distribution as well as the lipid mediated signaling pathway that regulates this process.

* These authors contributed equally to this work.

S-18

PSEUDOMONAS AERUGINOSA EXPLOITS A PIP3-DEPENDENT PATHWAY TO TRANSFORM APICAL INTO BASOLATERAL MEMBRANE

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Pseudomonas aeruginosa, an important human pathogen, preferentially binds and enters injured cells from the basolateral (BL) surface. We previously demonstrated that activation of phosphatidylinositol 3-kinase (PI3K) and Akt are necessary and sufficient for *P. aeruginosa* entry from the apical (AP) surface and that AP addition of phosphatidylinositol 3,4,5-trisphosphate (PIP3) is sufficient to convert AP into BL membrane. We now show that *P. aeruginosa* subverts this pathway to gain entry from the AP surface. In polarized monolayers, *P. aeruginosa* binds near cell-cell junctions without compromising them where it activates and recruits PI3K to the AP surface. Membrane protrusions enriched for PIP3 and actin accumulate at the AP surface at the site of bacterial binding. These protrusions lack AP membrane markers and are comprised of BL membrane constituents, which are trafficked there by transcytosis. The end result is that this bacterium transforms AP into BL membrane, creating a local microenvironment that facilitates its colonization and entry into the mucosal barrier.

S-19.
PROTEIN LIPIDATION: ROLE IN MEMBRANE ASSOCIATION, INTRACELLULAR TRAFFICKING AND SIGNALING

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A wide variety of proteins are modified by covalently linked fatty acids and/or prenyl groups which confer reversible association of the lipid-modified protein with membranes. Moreover, each distinct fatty acid or prenyl moiety provides particular information to assist proteins in finding their correct subcellular destination and biological function. The work in our laboratory is focused to gain new insights into the consequences of lipidation of proteins on their intracellular trafficking and subcellular distribution using as model proteins H-Ras (dually palmitoylated + farnesylated), K-Ras (farnesylated + "basic motif") and GAP-43 (dually palmitoylated). It will be described our recent results obtained by using biochemical assays and a combination of selective photobleaching techniques and time-lapse fluorescence microscopy in living epithelial cells. Our finding support and illustrate how lipid modification of proteins plays an important role in dictating precise intracellular movements within the cells going beyond that of a simple hydrophobic modification.

S-20.
THE SWEET CONTROL: IMPACT OF DIFFERENTIAL GLYCOSYLATION IN THE REGULATION OF T-CELL HOMEOSTASIS

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T cell mediated processes, including activation, differentiation and homing, are accompanied by a programmed remodeling of cell surface glycans, which themselves are the products of a repertoire of glycosyltransferases acting sequentially and dictating the glycosylation "signature" of each effector cell type. The responsibility of decoding the biological information encrypted by this glycosylation "signature" is assigned, in part, to endogenous glycan-binding proteins or lectins, whose expression and function are regulated at inflammatory sites. Galectin-1, a member of a family of highly conserved glycan-binding proteins, has emerged as a regulator of T cell homeostasis. Galectin-1 plays a pivotal role in conferring immune privilege to tumor tissues by skewing the cytokine balance toward a Th2 profile. We recently found that T_H1 and T_H-17-differentiated cells expressed the repertoire of cell surface glycans essential for galectin-1 binding, while T_H2 cells were protected from galectin-1 through differential sialylation of *N*- and *O*-glycans on cell surface glycoproteins. Consistently, galectin-1-deficient mice showed hyper-T_H1 and T_H-17 responses following antigenic challenge *in vivo* and show greatly enhanced susceptibility to autoimmune neuroinflammation. In addition, we observed that galectin-1 contributes to the homeostasis of other immune cell types (i.e. dendritic cells and macrophages) through non-apoptotic immunoregulatory mechanisms. Our findings provide a novel molecular link between differential glycosylation of immune cells and termination of the inflammatory response with potential implications in cancer, autoimmunity, and fetomaternal tolerance.

S-21.
USE OF *C. ELEGANS* TO STUDY CONSERVED SIGNALING PATHWAYS THAT REGULATE INNATE IMMUNITY

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Forward and reverse genetic studies have led to the identification of conserved *C. elegans* signaling pathways that are required for innate immunity, suggesting that the underlying mechanisms of innate immunity may be similar in evolutionary disperse organisms. Taking advantage of the *C. elegans* genetic tractability, our laboratory has studied both the mechanisms of bacterial virulence factors and conserved host immune responses. Recent work from our laboratory demonstrates that bacterial virulence factors target specific *C. elegans* immune pathways, indicating that the interactions between bacterial virulence factors and immune pathways are not as specific as previously believed. We have also found that the immune suppression caused by certain pathogens can be reversed by activating a fever-like pathway that requires heat shock transcription factor 1 (HSF-1) and both small and 90 kDa heat shock proteins. Our results also showed that the HSF-1 pathway regulates immunity independently of p38 MAPK and that it interacts with the DAF 2/DAF-16 insulin-like pathway, indicating that interacting pathways control stress response, aging, and immunity. Moreover, our latest findings indicate that pathogen recognition, through a variety of receptors, results on a neuroendocrine signaling pathway that activates downstream components of the DAF-2/DAF-16 pathway.

S-22.
MEMBRANE TRAFFICKING IN ANTIGEN CROSS-PRESENTATION

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Cross presentation is the process by which Dendritic cells (DC) phagocytose pathogens or dying cell fragments, and present proteolytic peptides derived from these antigens in association with MHC class I molecules. The reasons why DCs are the only antigen presenting cells that efficiently cross present antigens are not well understood. We have already demonstrated that the NADPH oxidase NOX2 is recruited to DC early phagosomes mediating a sustained production of low levels of reactive oxygen species and causing a maintained alkalynization of the phagosomal lumen. DCs lacking NOX2 show increased antigen degradation due to an enhanced phagosome acidification. As a result, the efficiency of *in vitro* and *in vivo* antigen cross presentation is significantly reduced in NOX2-deficient DCs. We also show that DCs derived from ashen mice, which are defective for the small GTPase Rab27a, fail to cross present antigens efficiently, due to increased phagosome acidification and antigen degradation. This defect in Rab27a-deficient DCs results from the impaired recruitment to phagosomes of the NOX2 membrane components. Therefore phagosomal alkalinization by NOX2 is controlled by Rab27a, and is required for efficient cross presentation in DCs. Interestingly; we have found that murine CD8+ spleen DCs, the unique DC subset that efficiently cross present internalized antigens *in vivo*, bear this phagosome alkalynization system. In contrast, CD8- DCs, which lack phagosomal ROS production, acidify their phagosomes causing more important antigen degradation which is unfavourable for cross presentation.

MI-C01.
LIGHT REGULATES VIRULENCE IN *BRUCELLA ABORTUS* BY A LOV-DOMAIN HISTIDINE KINASE PROTEIN

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Brucella abortus is a facultative intracellular pathogen that causes brucellosis in animals and humans. *B. abortus* contains a LOV-histidine kinase (LOV-HK) protein. LOV domains are light sensory modules that bind FMN and undergo a self-contained photocycle that is dependent on the presence of a conserved Cys residue. Upon illumination the Cys forms a covalent bond between the sulfur and C4a carbon of FMN. To investigate the *in vivo* function of *Brucella* LOV-HK a knocked-out null mutant was obtained. Cell infection assays of macrophages showed that LOV-HK mutant strain has an attenuated phenotype as compared with the wild type. A complemented strain expressing the LOV-HK gene in LOV-HK mutant strain was able to rescue the phenotype. However, a LOV-HK C69A replacement, which cannot form the covalent adduct showed the same infection profile that the LOV-HK knockout strain, indicating that formation of covalent adduct is essential for its biological activity. To determine if LOV-HK functions as photoreceptor during host-pathogen interactions, the infection experiment was performed in light vs. dark conditions. Strikingly, the number of wild type intracellular bacteria recovered from the culture kept in the dark was roughly one order of magnitude less than in the light-treated culture; moreover, no difference between dark and light conditions was detected with the LOV-HK knockout mutant.

MI-C02.
DESIGN OF PEPTIDES INHIBITING THE TYPE THREE SECRETION SYSTEM FROM ENTEROPATHOGENIC *ESCHERICHIA COLI*

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Attaching and effacing *E. coli* (AEEC) are *E. coli* strains able to colonize the intestinal mucosa of mammalians with a characteristic lesion, defined as "attaching and effacing". Enteropathogenic and Enterohaemorrhagic *E. coli* are two AEEC categories associated with human diseases. A major AEEC virulence factor is a type three secretion system (TTSS) encoded in a pathogenic island. The aim of this work is the identification and evaluation of peptides to inhibit the TTSS. Two set of peptides were synthesized: I) peptides selected because they form part of the coiled coil domain of EspA (major component of TTSS needle), EscF (a basal component of the needle) and TIR (bacterial receptor secreted by the TTSS); II) other peptides were selected using a phage display library by panning with recombinant EspA and EspB. Peptides were screened for TTSS inhibition using a Red Blood Cells Haemolysis assay. Peptides coilA, B and D were effective in inhibiting RBCs lysis. These peptides were then examined by a Fluorescence Actin Staining assay, which identify the A/E lesion. CoilA and CoilB peptides showed to be highly effective in avoiding the formation A/E lesions and in reduction of TTSS proteins secretion on Hep2 cells surface. These characteristics make the coiled-coil peptides an attractive alternative to avoid AEEC pathogenesis.

MI-C03.
ACYL-COA CARBOXYLASE INHIBITORS AS NEW ANTIMYCOBACTERIAL AGENTS

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Mycolic acids, one the crucial lipids of the sophisticated cell envelope of *Mycobacterium tuberculosis*, are essential for the survival, virulence, and antibiotic exclusion of this human pathogen. Inhibitors of mycolic acid biosynthesis, such as isoniazid and ethionamide, have long been used as one of the most efficient drugs for the treatment of tuberculosis. However, the increasing cases of multidrug-resistant tuberculosis have urged the finding of new targets. In *Mycobacterium*, the acyl-CoA carboxylases (ACCase) provide the building blocks for both *de novo* fatty acid biosynthesis and further elongation to produce mycolic acids. Previous studies suggested that AccD6 is the carboxyltransferase component of the ACCase 6 enzyme complex implicated in the biosynthesis of malonyl-CoA. We have characterized a ligand, NCI-172033, previously identified as a weak inhibitor of another ACCase from *M. tuberculosis*. The compound competitively inhibited AccD6 and had a potent bactericidal activity against several pathogenic species of *Mycobacterium*. Inhibition of both fatty acid and mycolic acid biosynthesis at minimum inhibitory concentrations was also observed. These results help to define the biological roles of key ACCases in the biosynthesis of membrane and cell envelope fatty acids, and provide a new target, as well as a new lead, for the rational development of novel antimycobacterial drugs.

MI-C04.
ORAL ADMINISTRATION OF A CATALASE-PRODUCING *LACTOCOCCUS LACTIS* CAN PREVENT COLON CANCER IN MICE

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Reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) are involved in various aspects of intestinal tumor development. Decreasing their levels can therefore be a promising approach for colon cancer prevention. The objective of this study was to evaluate the effect of a genetically modified catalase-producing *Lactococcus lactis* on the prevention of an induced colon cancer in mice. DMH-treated BALB/c mice received a catalase-producing *L. lactis* strain or the isogenic non-producing strain as control. Catalase activity and H_2O_2 levels in intestinal fluids and changes in the histology of the large intestines during tumor progression were evaluated. The catalase-producing *L. lactis* strain used in this study was able to slightly increase catalase activities (1.19 +/- 0.08 U/ml) and reduce H_2O_2 levels (3.4 +/- 1.1 µM) compared to i) animals which received the non-catalase producing strain (1.00 +/- 0.09 U/ml; 9.0 +/- 0.8 µM), and ii) those that did not receive bacterial supplementation (1.06 +/- 0.07 U/ml; 10.0 +/- 1.1 µM). Animals which received the catalase producing *L. lactis* had a significant lesser extent of colonic damage and inflammation compared to the control animals. The catalase producing *L. lactis* strain used in this study was able to prevent tumor appearance in an experimental DMH-induced colon cancer model.

MI-C05.
GAS DISCHARGE PLASMA AS A NEW TOOL FOR BACTERIAL BIOFILM INACTIVATION

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Conventional disinfection methods are often ineffective with bacterial biofilms. The use of plasmas is a novel alternative since they contain a mixture of charged particles, free radicals, and UV photons, well-known for their decontamination potential against free cells. The objective of this work was to assess the use of plasma for biofilm inactivation. Plasma was generated with an Atomflo reactor and a mixture of He and N_2. Biofilms were produced in microplates, exposed to plasma for various exposure times and processed to determine CFU/ml. For microscopy, biofilms were subjected to the same procedure and smears prepared. Cell viability was studied measuring ATP production with the BacTiter-Glo Microbial Cell Vialibity Test and by the LIVE/DEAD BacLight Bacterial Viability Test and fluorescence microscopy. Bacteria were imaged by atomic force microscopy after plasma treatment and optical emission spectroscopy used to study plasma composition. Our data show that almost 100% of the viable cells are removed after a 10-minute plasma treatment. Survivor curves show a rapid initial decline in CFU/mL followed by a slower decline. ATP determinations and microscopy show that non-culturable cells are still alive at short exposure times. These results indicate the potential of plasma for biofilm inactivation and suggest that cells go through sequential changes before complete inactivation.

MI-C06.
TDR TARGETS: PRIORITIZATION OF DRUG TARGETS FOR HUMAN NEGLECTED DISEASES

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Existing drugs against neglected tropical diseases are few and inadequate due to their limited efficacy, toxicity and/or resistance. The recent availability of the genomes of five human pathogens (*Plasmodium falciparum*, *Mycobacterium tuberculosis*, *Trypanosoma brucei*, *Leishmania major* and *Trypanosoma cruzi*) created a unique opportunity to identify potential new targets for drug discovery. To facilitate this process, we are developing an open-access database (TDR Targets). This tool allows scientists to prioritize targets by interrogating an extensive database with their own criteria. The database combines existing genomic information with both curated and automatically extracted data from existing literature and other databases relevant to each organism. A wealth of structural, genetic and inhibitor data is also available from model organisms, and the database is organized to take advantage of this data by mapping all pathogen genes to the corresponding orthologs in model prokaryotic and eukaryotic organisms. The database is available at http://tdrtargets.org.

MI-C07.
CYTOCHROME P450 REDUCTASES IN *TRYPANOSOMA CRUZI*. TCCPR-B CONFERS INCREASED DRUG RESISTANCE

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Cytochrome P450 enzymes (CYPs) are involved in reactive oxygen species detoxification cascade and in the biosynthesis of endogenous compounds. The flavoenzyme Cytochrome P450 reductase (CPR) is the electron donor for the CYP catalytic activities. Three sequences codifying for respective proteins homologous to CPRs, named *Tc*CPR-A, *Tc*CPR-B and *Tc*CPR-C, all of them showing the FMN, FAD and NADPH characteristic binding domains of reductases superfamily were found in the *Trypanosoma cruzi* genome database. Their aminoacidic sequences share 11% identity and differ mainly in the amino-terminal region. They were cloned, expressed and purified from bacterial systems. The recombinant proteins demonstrated a NADPH-dependent cytochrome c reductase activity, partially inhibited by a flavoprotein inhibitor (DPI). Native enzymes expression was demonstrated by Northern blot in CL Brener strain epimastigotes. Kinetical behaviour of the recombinant *Tc*CPRs resemble the previously reported NADPH-dependent cytochrome c reductase activity demonstrated in *T. cruzi* by biochemical approaches. Stable transfection of *Tc*CPR-B, was confirmed in *T. cruzi* epimastigotes by Southern blot. Overexpression was demonstrated by Western blot, augmented specific activity and IFI. These overexpressing parasites showed increased resistance to the anti-trypanosomal drugs Nifurtimox and Benznidazole.

MI-C08.
ANTI-*TRYPANOSOMA CRUZI* HUMAN RECOMBINANT ANTIBODIES

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To characterize the human antibody response against the parasite *Trypanosoma cruzi* (*T.cruzi*) we constructed single-chain variable fragment (scFv) libraries derived from patients with Chronic Chagas Heart Disease (cChHD). Total RNA was isolated from bone marrow, and cDNA was synthesized. Variable regions (VH, Vκ y Vλ) were amplified by PCR and cloned in the phagemid pHEN. The libraries were subsequently panned against *T.cruzi* total extract by phage display. We obtained different human recombinant antibodies (hu-rAbs) to *T.cruzi* antigens, as assessed by sequence data. Furthermore, the expressed hu-rAbs were able to recognize the parasite extract in ELISA. Interestingly, a hu-rAb set that belongs to the VH 3-30*18 family recognized a 175 kDa protein in *T.cruzi* Western blot. Comparison of *T.cruzi* recognition pattern of the different bone marrow donors allowed us to identify the origin of the hu-rAb. The molecular target of the anti-175 hu-rAb was assessed by indirect immunofluorescence (confocal microscopy) and parasite subcellular fraction Western blots. In this study, we report the construction of scFv libraries derived from cChHD patients. We have shown that phage display technology was effective to isolate the first hu-rAbs against parasite antigens. This approach may allow us to progress in the understanding of pathogenesis towards an effective immunoprofilaxis of Chagas disease.

MI-C09.
IDENTIFICATION OF VIRULENCE PROTEASES FROM *BOTRYTIS CINEREA* BY CROSS-PROTEOLYSOME ANALYSIS IN FUNGI
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Although proteases secreted by plant pathogens are envisaged to degrade structural and pathogenesis related plant proteins, it is difficult to identify proteases as virulence factor. Comparing genomes from fungi with a different phylogeny and/or lifestyle provides a new tool for identifying genes under natural selection, such as virulence protease genes. I compared the proteolysome (i.e. all known proteases) from ascomycete plant pathogen *Botrytis cinerea* (Bc) with other fungal proteolysomes in order to find virulence proteases. A genome-BlastP with the *Neurospora crassa* proteolysome (MEROPS) as a query identified 250 Bc sequences of which 195 were confirmed as putative protease. The corresponding sequences were used for a specific and saturating, manual and iterative BlastP analysis against the genomes of seven other ascomycetes and one basidiomycete. Comparison showed that Bc and related pathogen *Sclerotinia sclerotiorum* are poor in certain basic and rich in certain acid proteases, corresponding with the observed acidification during pathogenesis. Molecular evolutionary studies (e.g. Phylogeny, McDonald-Kreitman tests) are performed to determine which acid proteases are likely to act in virulence. Preliminary results suggest a horizontal gene transfer has taken place. These and other to be obtained results will be discussed in terms of evolution, pathogenesis and competition.

MI-C10.
PLURICELLULARITY AND ADHESIVE PROPERTIES OF THE ANTI-INFECTIVE PROBIOTIC BACTERIUM *BACILLUS SUBTILIS*
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In nature, microbes are able to express sophisticate and elaborate social behaviors that allow adaptation, survival and evolution. Here we report the social behavior of the undomesticated and probiotic strain *B. subtilis* RG4365. We demonstrated that the three master developmental regulators Spo0A, AbrB and SinR are crucial for the expression of social behaviors in *B. subtilis*. Biofilm development and colony morphogenesis were under the positive and negative control of Spo0A and AbrB /SinR respectively. The requirement of Spo0A during colony differentiation was not only due to its known antagonistic effects on *abrB* and *sinR* expression but also for its unique role in maintaining the structure of the aerial colony at late developmental stages. In addition, *B. subtilis* was able to adhere with high affinity and specificity to the extracellular matrix representative proteins fibronectin and collagen. This specific binding was under the negative control of AbrB and counteracted by Spo0A. *B. subtilis* was able to compete and inhibit the adherence of pathogenic *Staphylococcus aureus* to immobilized-fibronectin, a characteristic that it would be of importance for the expression of the anti-infective properties of probiotic *B. subtilis*. Finally, we present a working model summarizing how wild strains of *B. subtilis* can alternate between different communitarian styles of behaviors.

MI-C11.
INHIBITION OF AMIKACIN RESISTANCE USING AN RNASE P BASED STRATEGY TO SILENCE *AAC(6')-IB*
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Spread of *aac(6')-Ib* among pathogenic bacteria is a growing concern as it generates resistance to the clinically important aminoglycoside amikacin (Ak). A possible strategy to overcome this problem is to silence *aac(6')-Ib*. Several 17-nucleotide RNA molecules complementary to the five single stranded regions of *aac(6')-Ib* mRNA, carrying the consensus sequence for RNAseP ACCA in it's 3'-end were designed. These External Guided Sequences (EGS) were assessed in vitro for their capacity to bind to *aac(6')-Ib* mRNA and their ability to direct RNAseP digestion of the messenger. These results led to the selection of five candidates to perform in vivo experiments. In vivo expression of EGS demonstrated that EGSA2 and EGSC3 were able to reduce Ak resistance, being the latter one the most effective. In this strain the mRNA level showed a 50% reduction. Degradation of EGS is a problem to further develop this technology. To face this we designed antisense compounds with the EGSC3 sequence using several non-hydrolysable nucleic acid analogs: phosphorothioates, 2'-O-Methyl and Locked Nucleic Acids (LNA) derivatives. Their binding to the mRNA and their capacity to direct RNAseP-mediated cleavage of mRNA was studied. Our results suggest that LNA derivatives are able to induce RNAse P cleavage in vitro. The use of LNA-EGS might be a viable strategy to deal with antibiotic resistance.

MI-C12.
THE *attC* SITE IS A SITE SPECIFIC RECOMBINATION HOT SPOT OF DIFFERENT MOBILE ELEMENTS
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The *attC* sites are genetic elements involved in the site-specific recombination of gene cassettes mediated by the tyrosine recombinase integrase, and in the recombination of class C-*attC* group II (CattG2) introns. Their general structure consists of two short regions of sequence similarity at their boundaries (1R-2R and 1L-2L) separated by a stretch of imperfect internal dyad symmetry. The *attC* sites form stem-loop structures through a single strand DNA intermediate. The goal of this work was to understand the evolution of three genetic elements: *attC*s, integrases and CattG2 introns, which converge in two different site-specific recombination mechanisms. Seventy bacterial genomes were selected by their identity to the *S.ma*.I2 intron, inserted within an *attC* site of a class 1 integron. Only 11 introns (15.7%) showed an $\geq$46% identity to *S.ma*.I2 and belonged to a genome that also encoded an integrase. Sequence analysis with computational tools showed that all CattG2 introns invade the TTGTT motif and were inserted downstream of or in stem-loops structures. Three out of 5 stems were located within chromosomal integrons, which suggest that they can be identified as typical *attC* sites. Hence, genome evolution seems to converge in the use of a common target site for two different recombination systems.

MI-C13.
PREDICTION AND DETECTION OF CHROMOSOMALLY ENCODED SMALL NON CODING RNAs IN *SINORHIZOBIUM MELILOTI*
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Small non coding RNAs (sRNAs) have emerged as ubiquitous regulatory elements in bacteria and other life domains. We have started to investigate regulatory processes of gene expression mediated by sRNAs and RNA binding proteins in the model bacterium *S. meliloti* 2011, a nitrogen-fixing symbiont of legume root nodules. We have conducted a first search of putative sRNAs in the chromosomal intergenic regions (IGRs) of *S. meliloti* using sRNApredict, an algorithm that allows identifying DNA sequences within IGRs based on co-localization of genetic features associated with known sRNAs (terminators, conservation of primary and secondary structure). The initial list of 186 candidates out of 2920 IGRs was narrowed down to 76 elements upon elimination of junk repetitive regions, and it was further refined by performing predictions of putative promoters and the introduction of a scoring routine that weighs the relative orientation of promoters, terminators and their distance to neighbours ORFs, in addition to sequence and secondary structure conservation analysis. We ended with a list of 18 IGRs with high predictive scores to encode sRNAs. To date we have detected by Northern blot the presence of RNA bands showing coherent predicted sizes in 8 out of 10 tested IGRs, and interestingly, they seem to be differentially expressed in *S. meliloti* grown under different stress conditions.

MI-C14.
EFFECT OF PBP MUTATIONS ON CELL MORPHOLOGY AND GROWTH RATE IN *STREPTOCOCCUS PNEUMONIAE*
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β-Lactam (βL) resistance in *S. pneumoniae* is caused by mutations in penicillin-binding proteins (PBPs), mainly PBP1a, PBP2x, and PBP2b, which are enzymes involved in cell wall synthesis. Here, we assessed the impact of *pbp* mutations on cell shape and growth rate. In addition, we used a fluorescent derivative of vancomycin (Fl-Van) as a probe for nascent peptidoglycan synthesis to evaluate the septal and equatorial pattern by fluorescence microscopy. The *pbp1a, pbp2b, and pbp2x* genes from βL-resistant isolates were transformed into Cp1015, and the transformants were analyzed. All *pbp2b* mutants showed growth retardation, morphological alterations and simultaneous parallel septal and equatorial staining by Fl-Van, but no such differences could be detected in *pbp1a* or *pbp2x* mutants. Because no alterations were observed in the original βL- resistant strains, we constructed double and triple mutants to analyze if *pbp* genes association may compensate growth rates and cell shape alterations in the *pbp2b* mutants. The double *pbp2b/2x* or *pbp2b/1a* mutants resulted only in a partially restored morphology whereas the triple *pbp2b/2x/1a* mutant was similar in morphology, growth rate and Fl-Van staining to Cp1015. Our results suggest that acquisition of *pbp2x/pbp1a* mutations by horizontal transfer have compensatory effects on growth and shape alterations, in addition to development of βL resistance.

MI-C15.
ARGININE DEIMINASE PLAYS MULTIPLE REGULATORY ROLES IN THE BIOLOGY OF *GIARDIA LAMBLIA*
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Giardia lamblia is a food and waterborne parasite deriving from one of the earliest branches of the eukaryotic lineage. Giardia trophozoites undergo antigenic variation, a process by which the parasite switches its major surface molecules to evade the host's immune response. These variant-specific surface proteins (VSP) are membrane proteins with a variable N-terminal extracellular region, a well-conserved hydrophobic intramembranous domain, and a perfectly conserved 5 amino acid (CRGKA) C-terminal tail located in the cytoplasm. Although there is extensive data related to the characterization of VSPs, till now it is unclear how the unique structural features of VSP contribute to the biology of *Giardia*. Here, we demonstrate that arginine deiminase (gADI) binds specifically to the CRGKA cytoplasmic tail of VSPs. We found that the function of ADI in *Giardia* goes beyond energy production since it is able to deiminate protein-bound arginine and convert it to citrulline, a function restricted to higher eukaryotes. This modification is directly related to the control of the VSP switching in the process of antigenic variation and cell survival. Additionally, during differentiation, gADI seems to play a regulatory role by controlling the expression of encystation specific genes. These results define novel regulatory pathways utilized by *Giardia* for survival where gADI is a key player.

MI-C16.
CARBON CATABOLITE REPRESSION OF TYPE IV PILI-DEPENDENT GLIDING
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Clostridium perfringens is an anaerobic Gram-positive spore-forming bacterium responsible for severe histotoxic and gastrointestinal diseases in humans and animals. Genes encoding flagellar proteins and chemotaxis genes are absent in *C. perfringens*, however it exhibits Type IV pili-dependent gliding motility. Since carbon catabolite regulation (CCR) has been implicated in the control of different bacterial behaviors, we investigated the effect of glucose and other metabolized carbohydrates on gliding. We demonstrated that CCR reduces gliding of a large number of human and animal-derived pathogenic *C. perfringens* strains. Glucose produces a strong dose-dependent inhibition of gliding development without affecting vegetative growth. The inhibition of gliding in the presence of glucose was due, at least in part, to the repression of the genes pilT and pilD, whose products are essential for TFP-dependent gliding proficiency. The inhibitory effect of glucose on pilT and pilD was under the control of the key regulatory protein CcpA (catabolite control protein A). The deficiency in CcpA activity restored expression of pilT and pilD and gliding proficiency in the presence of glucose. Furthermore, we discovered a novel positive role, in the absence of CCR, of CcpA on pilT / pilD expression and gliding proficiency. We discus these finding in the context of gliding importance during infection.

MI-C17.
CELL-TYPE SPECIFIC COMPARTMENTALIZATION OF THE *BACILLUS SUBTILIS* STRESS-RESPONSE TRANSCRIPTION FACTOR SIGMA B
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B. subtilis is a soil bacterium, and hence temperature changes, starvation, and solar radiation would constitute common environmental stresses. Stress resistance in *B. subtilis* is governed by the master transcription factor sigma B (SigB). We showed in previous reports that SigB is dramatically induced after a temperature downshift from 37°C to 20°C. Loss of SigB reduces stationary-phase viability of cold-adapted cells 10 to 15-fold. Here, we show that SigB is exclusively trapped in the pre-spore compartment of *B. subtilis* during the development of the spore. The presence in the spore prepares it for future stress. Spores of sigB mutants present a significant reduction in germination rate compared to the wild type strain in alcohol stressing germination medium. Sigma B is shown to be necessary for appropriated function of germination receptors (GerBK) under stress conditions. Moreover, it was observed a delay in the outgrowth for the sigB mutant when we compared it with wild type spores under the same conditions. Importantly, we found a decrease in the viability of *sigB* mutant spores when they were exposed to extreme conditions as acid or UV-C light. These experiments are discussed in the context of the lithopanspermia theory and the novel role, discovered in this work, of sigma B as a "Trojan horse" hidden in the dormant spore.

MI-C18.
RELATION OF COPPER METABOLISM WITH THE *ESCHERICHIA COLI* RESPIRATORY CHAIN
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Respiratory chain is a main focus of free radicals production in bacterial cells. Previous results of our laboratory have shown that electron flow through the *Escherichia coli* respiratory chain promotes the reduction of cupric ions to Cu(I), which mediates damage of the respiratory system by hydroperoxides. We have also shown that respiratory NADH dehydrogenase-2 (NDH-2) in *E. coli* has Cu(II)-reductase activity. In addition, NDH-2 presence decreased the susceptibility of the respiratory chain to copper and peroxides and gives the bacteria advantages to resist extreme copper concentrations. In the present work, we carried out *in vivo* experiments to further analyze the participation of NDH-2 and other components of the respiratory chain in the copper metabolism. We determined a specific cellular reduction of Cu(II) to Cu(I) caused only by the presence of NDH-2 and quinones, when cells were exposed to sub-lethal Cu(II) concentrations. In parallel cupric ion content, unlike cuprous ion, was increased in the NDH-2 deficient mutants. Taking together our results, NDH-2 and quinones could have a role in the copper metabolism, since Cu(II)-reduction is required by the metal uptake/export systems.

MI-C19.
IDENTIFICATION AND CHARACTERIZATION OF A *BRUCELLA ABORTUS* TYPE IV TRANSLOCATED PROTEIN
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Brucella abortus is an intracellular pathogen that replicates inside mammalian phagocytic and non-phagocytic cells. A type IV secretion system (VirB) is essential to subvert lysosome fusion and to create an organelle that supports *Brucella* replication. Therefore, a possible role of VirB system is to translocate effector proteins that modulate host vesicular transport to allow the biogenesis of the endoplasmic reticulum-derived replicative organelle. Using the *Bordetella pertussis* Adenylate Cyclase reporter protein we were able to identify a *B. abortus* protein, called Bep1, that is translocated to the host cell via VirB system and to determine that substrate recognition involves an N-terminal translocation signal. Bep1 is a 17-kDa protein with a predicted signal peptide and a coiled-coil region that shows similarity to the autophagosomal protein Atg16. VirB-mediated protein translocation into phagocytic cells was confirmed by immunofluorescence confocal microscopy. The translocated protein was localized to the phagosomal membrane from 15 minutes to 10 hours post-infection. This is consistent with the biogenesis of the replicative organelle and the expression kinetics of *virB* operon. Bep1 ectopically expressed in HeLa and COS-7 cells colocalized with the endoplasmic reticulum marker calreticulin, suggesting that the protein is targeting the organelle where *Brucella* replication occurs.

MI-C20.
TRANSCRIPTION OF THE *BRUCELLA ABORTUS VirB* OPERON IS METABOLICALLY LINKED TO HISTIDINE CATABOLISM
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The *virB* operon codes for a type-IV secretion system that is essential for the pathogenesis of bacteria belonging to the genus *Brucella*. During the first hours of intracellular infection of macrophages the VirB expression is tightly regulated in *Brucella abortus*. Recently, as a part of a *virB*-transcription factor identification project, we identified a protein that binds specifically the *virB* promoter. This protein (HutC) is a transcriptional regulator involved in control of the *hut* (for histidine-utlilization) operon and it also participates in transcriptional regulation of the *virB* operon. Here we show that DNA-binding activity of HutC is negatively regulated by urocanate (an intermediate of histidine catabolism) in both *hut* and *virB* promoters. Apparent dissociation constants for HutC binding to both promoters were analyzed. In order to study the possible regulatory link between *hut* and *virB* systems, we constructed *B. abortus* deletion mutants for different *hut* genes that participate in urocanate metabolism. These mutants have a defective intracellular *virB*-expression, indicating that its regulation is connected to histidine catabolism. Using different culture media we observed that HutC participates in transcriptional regulation of the *virB* operon under a very definite culture condition that resembles the environment encountered by *Brucella* during its intracellular transit.

MI-C21.
PROTECTIVE ACTION OF ppGpp ON MICROCIN J25 SENSITIVE STRAINS BY INCREASE OF yogI EXPORT ACTIVITY

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Many reports have shown that ppGpp has a significant role in growth rate control and gene expression during stationary phase. MccJ25-sensitive strains exhibit an increased resistance in stationary phase. These results led us to suppose that the accumulation of ppGpp could have a protective action against the MccJ25. In fact, colony counts dropped five orders of magnitude for AB1133 in the presence of MccJ25, while AB1133 transformed with a plasmid that overproduces ppGpp (pALS13) remained unaffected. Using AB1133 *rpoS*::Tn*10*, we demonstrated that sigma factor is involved in the protective effect of ppGpp since sensitivity of this strain harboring pALS13 was similar to the strain producing normal levels of ppGpp. MccJ25 had no effect either on transcription activity or on oxygen consumption (MccJ25 targets) in sensitive strains harboring pALS13. The fusion activity of *yojI* (a chromosomal *E. coli* transporter involved in MccJ25 exportation) was recorded in the absence and in the presence of pALS13. The results showed an increment of 100% in the *yojI* expression in the strain harboring pALS13. Using a biossay we demonstrated that the introduction of pALS13 plasmid increment the antibiotic secretion mediate by YojI. Our results suggest that the increment in ppGpp pool promotes YojI expression, which in turn keeps the intracellular concentration of MccJ25 below a toxic level.

MI-C22.
IDENTIFICATION OF A NOVEL LIGASE INVOLVED IN A PROTEIN LIPOYLATION PATHWAY IN *BACILLUS SUBTILIS*

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Lipoic acid (LA), a covalently bound cofactor, is essential for functioning of several key enzymes involved in oxidative metabolism. The current model for protein lipoylation involves two pathways: one in which exogenous LA is transfer to apoproteins in a process mediated by LA ligase A (LplA), and an endogenous one, that involves LipB, which transfers octanoic acid to target proteins. These octanoylated domains are converted into lipoylated derivatives by lipoyl synthase. *Bacillus subtilis* has two ORFs that codify products homologous to LplAs (*yhfJ* and *yqhM*), but no one whose product is significantly similar to LipB. The aim of this work was to identify the enzyme/s involved in the endogenous lipoylation pathway. A candidate was *ywfL*, a gene whose product is slightly similar to YhfJ. We constructed an *ywfL* mutant (NM51) that was impaired to grow in minimal media. Cell growth was restored by adding acetate, succinate, and branched-chain fatty acid precursors, or LA. NM51 sporulates poorly in SM medium, but this was partially reverted by the addition of LA. This strain also showed a strong induction of the transcription of the desaturase gene, suggesting that membrane fluidity can only be increased by raising the amount of unsaturated fatty acids. These results show that YwfL is essential for the endogenous protein lipoylation pathway in *B. subtilis*, so we renamed *ywfL* as *lipL*.

MI-C23.
AN ENZYME WITH A LENGTH PROOF-READING ACTIVITY AS A MECHANISM TO CONTROL THE SIZE OF POLYSACCHARIDES

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Cyclic β-1,2-glucans are osmolite homopolysaccharides with a cyclic β-1,2-glucan backbone of 17 to 25 glucose residues present in the periplasmic space of several bacteria. Initiation, elongation and cyclization, the three distinctive reactions required for building the cyclic structure, are catalyzed by the same protein, the cyclic β-1,2-glucan synthase (Cgs). The initiation activity catalyzes the transference of the first glucose from UDP-glucose to a yet unidentified amino acid residue in the same protein. Elongation proceeds by the successive addition of glucose residues from UDP-glucose to the non-reducing end of the protein-linked β-1,2-oligosaccharide intermediate. Finally, the protein-linked intermediate is cyclized and the cyclic glucan released from the protein. These reactions do not explain, however, the mechanism by which the number of glucose residues in the cyclic structure is controlled. We now report that control of the degree of polymerization (DP) is carried out by a β-1,2-glucan phosphorylase present at the Cgs C-terminal domain. This last activity catalyzes the phosphorolysis of the β-1,2-glucosidic bond at the non-reducing end of the linear protein-linked intermediate releasing glucose 1-phosphate. The DP is thus regulated by this "length proof-reading" activity. To our knowledge, this is the first description of a control of the DP of homopolysaccharides.

MI-C24.
FROM SUCROSE TO MANNOSYLFRUCTOSE BIOSYNTHESIS: *AGROBACTERIUM TUMEFACIENS* NOVEL METABOLIC PATHWAY

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Agrobacterium tumefaciens C58 is a heterotrophic proteobacterium of the *Rhizobiaceae* family. Sequence analysis of *A. tumefaciens* C58 genome revealed the existence of two ORFs (1365 and 747) annotated at NCBI as a sucrose biosynthesis related glucosyltransferase and phosphatase. Because the ability to synthesize and accumulate sucrose has been reported only in photosynthetic organisms (plants and cyanobacteria), we found of interest to investigate the role of the protein products of *A. tumefaciens* ORFs 1365 and 747. Molecular and biochemical examinations of the recombinant proteins produced from the expression of the ORFs in *Escherichia coli* cells, revealed that they are not sucrose related proteins, instead they are novel enzymes mannosylfructose-phosphate synthase and mannosylfructose-phosphate phosphatase defining the pathway that leads to the biosynthesis of the rare disaccharide mannosylfructose. Our analyses revealed that not only is the biosynthesis of mannosylfructose mechanistically similar to that of sucrose, but the corresponding genes for the biosynthesis of both disaccharides are also phylogenetic close relatives. Importantly, a protein phylogeny analysis indicated that mannosylfructose-phosphate synthase defines a new group of mannosyltransferases.

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MI-C25.
HALOALKALIPHILIC PROTEASE FROM AN ARCHAEON: OVEREXPRESSION IN *ESCHERICHIA COLI* AND *HALOFERAX VOLCANII*

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The haloalkaliphilic archaeon *Natrialba magadii* (optimum growth in 20% NaCl, pH 12) secretes an organic solvent-tolerant protease denoted as NEP (*Natrialba magadii* Extracelular Protease) that has been biochemically characterized and the corresponding gene has been cloned and sequenced. The aim of this study was to over express the gene encoding NEP in two systems: *Escherichia coli* (bacteria) and *Haloferax volcanii* (haloarchaea). The coding region of *nep* was amplified by PCR from a genomic clone of *Nab. magadii* and the PCR product was cloned into pET24b expression vector generating pET-nep and pET-nep-His6 constructs, which were expressed in *E. coli* BL21 (DE3) Rosetta cells. *nep* and *nep-His6* were subcloned into a shuttle vector and transformed into *Hfx. volcanii*. NEP was expressed as an active enzyme in both systems, and the highest levels of extracellular protease activity were attained in *Hfx. volcanii*. Protease activity was not detected in the culture medium of *E. coli* cells suggesting that it was not secreted/processes or it was unstable. The recombinant enzyme produced in *Hfx. volcanii*, displayed similar biochemical properties and solvent-tolerance compared to the native enzyme. This research will contribute to optimize the high production of this extremozyme for basic studies and potential biotechnological applications.
This work was supported by ANPCyT, CONICET and UNMDP.

MI-C26.
CHARACTERISTICS OF N-TERMINAL DOMAIN OF A *PSEUDOMONAS AERUGINOSA* PAO1 RECOMBINANT POLYPHOSPHATASE

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In *Pseudomonas aeruginosa* PAO1 the gene PA5241 encodes a polyphosphate phosphatase (PPX) scarcely studied. To characterize the enzyme it was obtained as a recombinant protein (rPPX) through an N-terminal fusion to intein (IMPACT-CN, NEB). The PM of rPPX was lower than predicted by its DNA sequence. Mass spectrometry and MALDI-TOF analysis demonstrated that rPPX had lost a 192 residues C-terminal fragment after overexpression and purification. The truncated rPPX had optimum activity on a pH range between 8.0-8.4. Mg^{+2} was essential for its activity and the addition of 50 mM KCl increased the activity 300%. Ca^{+2}, Mn^{+2}, Co^{+2} and Cu^{+2} were less effective than Mg^{+2}. rPPX hydrolyzed different sizes of polyphosphates (PP_{25}, PP_{45}, PP_{65} and PP_{75}) exhibiting two affinity constants for each one. The catalytic efficiency data demonstrated that rPPX preferred PP_{45} over longer chain substrates. Multiple sequence analysis of different PPX exhibited sequence conservation in the N-terminal region. Comparative protein modeling of *P. aeruginosa* PPX with *E. coli* PPX (1U6Z) showed highly conserved residues which may be potential contributors to the active site or substrate binding.Our results suggest that the C-terminal domain is necessary to processive hydrolysis and a correct binding of polyphosphates higher than 50 Pi residues, whereas putative PPX active site is located in N-terminal domain.

MI-C27.
IDENTIFICATION OF CATALYTIC SITE RESIDUES OF *PSEUDOMONAS AERUGINOSA* HEMOLYTIC PHOSPHOLIPASE C

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PlcH is a virulence factor of *P. aeruginosa*. It belongs to the phosphoesterase superfamily. Sequence analysis and biochemical studies focused in phosphoesterase domain residues, allowed us to identify the residues E60, T178 and H321 as essential for PlcH activity, whereas L306, Y235 and ^{158}YAL^{160} were not. To identify the organization of PlcH catalytic site, a structural model was built based on crystal structure of acid phosphatase (AcpA) from the *F. tulariensis*. The residues E60, T178, H321, N61, H120, D357 and E358 are located in the PlcH catalytic site. All of them are identically conserved in AcpA active site. The residues D198, N274 and F275 are located far away; despite they align with the residues of AcpA catalytic site. The conserved residues were site-directed mutagenized and their relevance in the activity was analyzed. The residues N61, D357, E358, E60 and T178 are essential in PlcH activity. The residues H120 and H321 are essential for the hydrolysis of physiological substrate but not for the hydrolysis of *p*-NPPC. However, these mutants showed a low catalytic efficiency for *p*-NPPC compared with the native enzyme. As the model predicted, the residues D198, N274 and F275 are not critical in PlcH activity. These data confirm the PlcH structural model and further studies will allow us to know if PlcH catalytic mechanisms are shared with other phosphoesterase members.

CB-C01.
REGULATION OF CELL-MATRIX ADHESION AND CELL MIGRATION BY PTP1B

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Our previous work shows that ER-bound PTP1B associates with integrin complexes, and that is required for cell-matrix adhesion and spreading. Further, we and others showed that the protein tyrosine kinase Src is a substrate of PTP1B. In this work we examined the role of Src family members in the localization of PTP1B to cell-matrix adhesion sites, and the role of PTP1B in the turnover of the adhesive sites, and in cell migration. Using cell lines deficient in different members of the Src family, we found that Fyn but not Src plays an essential role in the localization of PTP1B to the cell-matrix adhesion sites. In addition, PTP1B and Fyn colocalize in small foci at the leading edge and at patches of the ventral membrane which also contain F-actin. In PTP1B knockout cells (KO cells), small focal complexes at the protruding lamella are more dynamic and incorporate less α-actinin than in cells reconstituted with wild type PTP1B (WT cells). KO cells display frequent changes in speed and pause periods, frequent changes in the direction of migration, and long and persistent trailing tails compared to the WT cells. Our results reveal a tight functional link between PTP1B and Fyn at cell-matrix adhesion sites. In addition, our data suggest a function of PTP1B in cell migration and cell-matrix turnover, which at least partly may involve the regulation of rho GTPases.
Supported by ANPCyT.

CB-C02.
POSSIBLE SECRETORY PATHWAY OF CATHEPSIN D IN RAT EPIDIDYMIS

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Mammalian epididymis participates in sperm maturation, secreting factors to the lumen. The function of this organ is maintained by androgenic hormones. Several acid hydrolases are secreted by the epididymal epithelium (e.g.procathepsin D, pcD), although their function in the lumen is unclear. In most cells and tissues, lysosomal enzymes are transported to lysosomes via mannose-6-P receptors (MPRs). Two types of MPRs have been described; the cation-independent (CI-MPR) and the cation-dependent (CD-MPR) receptors. Alternative routes to lysosomes may be mediated by sortilin (Sor) (a receptor for prosaposin pSAP). The aims were to study the possible routes for cathepsin D secretion in epididymis and its regulation by steroid hormones. Sprague-Dawley rats and RCE-1 cell line were used, and the expression and distribution of epididymal proteins were analyzed by IPP, immunoblot and immunohystochemistry (IHC). Alternatively, metabolic labeling was carried out in RCE-1 cells. It was observed that expression of both MPRs and pcD was increased in epididymis of castrated rats. Secretion of pcD was also increased under these conditions, forming complexes with pSap. An apical redistribution of cathepsin and Sor was observed. In cell cultures these changes were observed by treatment with estradiol. We concluded that pcD may be secreted via pSap-Sor or CD-MPR and controlled by estrogenic hormones.

CB-C03.
FUNCTIONAL ANALYSIS OF THE RAB1-COPII INTERACTION

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Export from the Endoplasmic Reticulum (ER) defines the first step of the secretory pathway and is mediated by the recruitment of the COPII coat complex (composed by Sar1p, sec23/24 and sec13/31). In the ER exit sites (ERES), COPII modulates sorting and concentration of cargo in COPII vesicles. Moreover, COPII assembly is up-regulated by increased levels of cargo proteins. After vesicle budding, COPII is exchanged by COPI complex, a crucial step for ER-Golgi transport. Rab1 GTPase acts as an essential component required for this transport by modulating COPI recruitment in ERES. We have previously shown that Rab1 interacts *in vitro* with Sec23. Here, we aim to explore the *in vivo* role of the Rab1-COPII interaction. We show that Rab1 also interacts *in vivo* with Sec23. Interestingly, depletion of Rab1 by expressing the Rab1 dominant negative mutant (Rab1N121I) or by iRNA, strongly suggests that COPII recruitment is Rab1 independent. However, the number of small COPII structures is markedly increased in Rab1 depleted cells, supporting our previous data showing that Rab1 modulated COPII dynamics. Furthermore, Rab1 knock down inhibits sorting of a Golgi cargo protein (GFP-GalT2) into the ERES. Taken together, our results strongly suggest that Rab1-COPII interaction is implicated in modulating COPII membrane association dynamics and cargo sorting into the ERES.

CB-C04.
THE EARLY SECRETORY PATHWAY CONTRIBUTES TO THE DEVELOPMENT OF THE *COXIELLA*-REPLICATIVE NICHE

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Coxiella burnetii is a Gram-negative obligated intracellular bacterium. After internalization, this bacterium replicates in a large parasitophorous vacuole that has features of both phagolysosomes and autophagosomal compartments. We have previously demonstrated that early after internalization *Coxiella* phagosomes interact with both the endocytic and autophagic pathways. In this report we present evidence that the *Coxiella*-replicative vacuoles (CRV) also interact with the secretory pathway. Rab1b is a small GTPase responsible for the anterograde transport between the Endoplasmic Reticulum and Golgi Apparatus. We have evidence that Rab1b is recruited to the CRV and that overexpression of GFP-Rab1b wt or the active GTPase defective mutant (Q67L) affects the development of the *Coxiella*-replicative compartment. In the present work we have examined the fusion competence of CRVs in cells overexpressing Rab1b Q67L. For this purpose *Coxiella* infected cells were incubated with rodamine-labeled heat inactivated *Staphylococcus aureus* or rhodamine-dextran, to label the phagocytic and endocytic pathways, respectively. Our results indicate that fusion between CRV and both compartments was hampered.
These results suggest that the GTPase defective mutant of Rab1b alters the normal development of the *Coxiella* vacuole by changing the fusogenic capacity of this compartment.

CB-C05.
ROLE OF BECLIN1 AND BCL-2 IN THE DEVELOPMENT OF THE *COXIELLA BURNETII* REPLICATIVE VACUOLE

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Several pathogens have developed different strategies to invade and survive into the host cell, avoiding degradation. *Coxiella burnetii* is an obligate intracellular pathogen that invades and multiplies in acidic vacuoles with lysosomal characteristics. We have previously shown that *Coxiella* interacts with the autophagic pathway as a strategy for its survival and replication. In the present work we have explored the role of Beclin1 and Bcl-2 in *C. burnetii* infected cells. Beclin1, a Bcl-2 interacting protein, is a component of the class III PI-3 kinase complex, required for autophagy. Bcl-2 functions as an anti-apoptotic protein which inhibits autophagy. HeLa cells were transfected with pFLAG-Beclin1 and then infected with *C. burnetii*. Interestingly, Beclin1 was markedly recruited to the membrane of *Coxiella*-replicative vacuoles (CRVs). Moreover, overexpression of this protein increased the size and number of CRVs, whereas Beclin1 depletion, by a short interfering RNA, caused a decrease in vacuole size. To evaluate whether Bcl-2 has an effect in *Coxiella* infection, HeLa cells were transfected with GFP-Bcl-2. Surprisingly we found that Bcl-2 was also recruited to the CRV, however, although overexpressed Bcl-2 increased vacuole number, the CRVs size was markedly reduced. These results indicate that whereas Beclin1 favors the development of CRV, Bcl-2 alters *Coxiella*-replicative niche.

CB-C06.
ACTIN AND RHO GTPASES REGULATE THE BIOGENESIS OF *COXIELLA BURNETII*-CONTAINING VACUOLES

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Q fever is a disease caused by the intracellular pathogen *C. burnetii*. This bacterium generates a large replicative compartment in the host cell, called parasitophorous vacuole (PV). We show that actin not only is recruited to the PV membrane but is also involved in its biogenesis since treatment of infected cells with actin-depolymerizing agents inhibited PV development in a reversible manner. It is known that actin dynamic is regulated by the Rho family of GTPases. To test if these GTPases play a role in PV biogenesis, after infection with C. burnetii, cells were transfected with pEGFP: Cdc42, RhoA or Rac1 wild types, constitutively active or inactive mutants. The PV was decorated by Cdc42WT and its active mutant V14. Interestingly, in cells overexpressing the V14 mutant a significant PV population of bigger size was observed. Neither co-localization nor change in size distribution of PV was observed in cell expressing the inactive mutant N17 of Cdc42. RhoA WT and its active mutant V14 were also recruited on the PV but not the inactive one. However, in this case a significant smaller size PV population was observed in cells expressing the negative mutant N17. In contrast, Rac1 WT and its mutants did not show any association with the PV. These results suggest that actin, likely modulated by Cdc42 and RhoA, is involved in PV biogenesis.

CB-C07.
ADAPTOR PROTEIN 2: A KEY PLAYER IN *GIARDIA* LYSOSOMAL PROTEIN TRAFFICKING

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In mammalian cells, the role of clathrin and adaptor protein complexes (APs) in endosomal and lysosomal protein delivery is well known. Unlike these cells, only AP1 and AP2 are present in the *Giardia* genome. We recently found that gAP1 participates in lysosomal protein trafficking from a sorting organelle to the lysosomal-like peripheral vacuoles (PVs) in *Giardia*, but the function of AP2 had not been elucidated. Based on what was described for more evolved cells, we hypothesize that gAP2 is the adaptor protein involved in vesicular trafficking from the plasma membrane to the PVs. By using a specific antibody against one of the subunits of gAP2 (μ2), we showed that it localizes in the PVs as well as close to the plasma membrane. To analyze the interaction of gAP2 with associated proteins, the YTH and CoIPP assays were performed. Production of μ2 ds RNA for protein knock-down was used to study the role of gAP2 in receptor-dependent and independent endocytic mechanisms. By using different approaches we were able to observe that gAP2 is critical in trophozoite growth and differentiation and that depletion of μ2 affects receptor-mediated endocytosis. The complete characterization of gAP2 role would contribute to increase our knowledge about this extremely simplified protein trafficking system present in this primitive human parasite.

CB-C08.
LACTONES AFFECT INVASIVENESS OF *L. MEXICANA* POSSIBLY BY REDUCING EXPRESSION OF VIRULENCE FACTORS

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Leishmania mexicana is the etiological agent of the cutaneous leishmaniasis. For decades, many natural compounds have been used against leishmaniasis, but its use has been restricted due the high citotoxicity on host cells. *Leishmania* synthesizes high-mannose containing glycoconjugates as virulence factors to invade the host cells. Gp63 is a virulence factor, which is synthesized by the action of GDP-mannose pyrophosphorylase (GDP-MP). In this study we proposed to evaluate the effect of three sesquiterpene lactones (SL); helenalin (Hln), mexicanin (Mxc) and dehydroleucodin (DhL) on the life cycle of *L. mexicana*, and the ability to invade host cells. Methods: The effect of SL was evaluated on cultured promastigotes of *L. mexicana*. The infective index was determinated by counting number of infected host cells and number of parasites per cell, after staining with hematoxylin-eosin. Expression of virulence factors was evaluated by western blot. Apoptosis was observed by TUNEL methods. Results and conclusions: The three compounds diminished proliferation of parasites, possibly by inducing apoptosis. They also reduced invasive ability to Vero cells and the expression of GDP-MP and gp63 was decreased significantly by Hln and at lesser extent by Mxc and DhL.

CB-C09.
THE EARLY PHASE OF PROGRAMMED CELL DEATH IN CACO-2 INTESTINAL CELLS EXPOSED TO PTH

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The cell has derived various mechanisms to precisely control the balance between survival and apoptotic signaling. Parathyroid hormone (PTH) function as a major mediator of bone remodeling and as an essential regulator of calcium homeostasis. In a previous work we found that PTH promotes the apoptosis of human Caco-2 intestinal cells. In the current study, we demonstrate, for the first time, that stimulation of Caco-2 cells with PTH (10^{-8} M) results in the dephosphorylation and translocation of pro-apoptotic protein Bad from the cytosol to mitochondria and release of cytochrome c. The hormone also triggers mitochondria distribution to the perinuclear region, morphological features consistent with apoptosis. PTH increases the enzymatic activity of caspase-3 (48 h) that is also evidenced from the appearance of its cleaved fragments in western blot experiments. Moreover, active caspase-3 is present in nucleus after PTH treatment. In addition, PARP, a caspase-3 substrate, is degraded by 48 h of PTH treatment. These results suggest that, in Caco-2 cells, the induction of apoptosis in response to PTH is mediated by translocation of mitochondria to the perinuclear region, dephosphorylation and movement of Bad to the mitochondria and subsequent release of cytochrome c, which result in activation of downstream caspase-3.

CB-C10.
ROLE OF HSP27 IN THE ANTIAPOPTOTIC EFFECTS OF 17β-ESTRADIOL IN SKELETAL MUSCLE CELLS
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We have demonstrated that 17β-estradiol (E2) at physiological doses inhibits apoptosis in skeletal muscle cells (C2C12 murine cell line) (Vasconsuelo et al., SAIB 2007). This protective effect involves estrogen receptors β and α and fast activation of the PI3K/Akt/Bad pathway. In the present study, we established that the antiapoptotic action of E2 requires the participation of heat shock protein 27 (HSP27). RT-PCR and immunocytochemistry assays showed that 17β-estradiol at longer treatment times upregulates expression of HSP27 in C2C12 cells whereas inhibition, by the hormone, of caspase-3 cleavage was evidenced. Moreover, in presence of quercetin, the inhibitory effect of E2 on caspase-3 cleavage was diminished. Co-immunoprecipitation assays demonstrated the interaction of HSP27 with caspase-3 in non-apoptotic cells. These results reveal a novel aspect of the mechanism of HSP27-mediated 17β-estradiol antiapoptotic activity, not reported before for any estrogen target cell, namely modulation of caspase-3 activity.

CB-C11.
EFFICIENT LIPID ELIMINATION FROM SERTOLI CELLS AFTER APOPTOTIC DEATH OF SPERMATOGENIC CELLS
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Testicular exposure to X-rays and to temperatures higher than 38ºC induces apoptosis of spermatogenic cells, compromising fertility. The former mostly affects mitotically dividing spermatogonia and the latter non-dividing spermatocytes and spermatids, both sparing Sertoli cells. In this study we surveyed the *in vivo* effects on rat testicular lipids and their fatty acids several weeks after having locally irradiated (6.5 Gy) or heated (15 min, 43ºC) the testis. In both cases Sertoli cells behaved as competent phagocytes, efficiently processing and disposing of materials formerly composing germ cells, including cholesterol and phospholipids. Germ-cell related 22:5n-6-rich glycerophospholipids and species of sphingomyelin and ceramide with 28:4n-6 and 30:5n-6 were cleared from the testis in a few weeks. In the process, neutral glycerides and cholesterol esters (CE) temporarily increased, accumulating more 22:5n-6 than other fatty acids. The buildup of CE reached a maximum and eventually decreased. The described changes including complete clearing of CE occurred earlier after hyperthermia than after irradiation, a slow but significant re-population of the testis with germ cells ensuing. The results suggest that only when Sertoli cells have processed and got rid of all materials derived from germ cell corpses they are ready to resume support of a new round of spermatogenesis.

CB-C12.
HYPERTONICITY INDUCES LAMIN A/C SYNTHESIS AND DISTRIBUTION IN A TONEBP/NFAT5 DEPENDENT MECHANISM
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A-type lamins (lamin A/C) are the most widely studied nucleoskeletal proteins. It has also been shown that lamin A/C is a structural component of nuclear speckles, nucleoplasmic structures enriched in pre-mRNA, splicing and transcription factors, and plays a key role in differentiation process. It has been reported that changes in environmental tonicity induces renal epithelial cell differentiation and that the tonicity-responsive enhancer binding protein (TonEBP/NFAT) regulates gene expression induced by osmotic stress. In the present work we examined how hypertonic medium affects the concentration and nuclear distribution of the lamin A/C in MDCK cells. We also evaluated the relationship between the lamin A/C and TonEBP/NFAT5. Data herein demonstrate that hypertonicity induces lamin A/C increase and nuclear redistribution to nucleoplasmic speckles. Microscopy shows the codistribution of TonEBP/NFAT5 and lamin A/C in nucleoplasmic speckles and immunoprecipitation assays demonstrate the interaction of Lamin A (but not C) with TonEBP/NFAT5. Silencing of TonEBP/NFAT5 causes the redistribution of lamin A/C from speckles to periphery followed by the reduction in lamin A/C levels, thus reflecting that hypertonicity induces lamin A/C specked pattern by a TonEBP-dependent mechanism. We propose that lamin A/C-speckles sequesters TonEBP/NFAT5 thus favoring differentiation process activity.

CB-C13.
ZEBRAFISH CNBP UPSTREAM REGULATORY REGION: ISOLATION AND PROMOTER ANALYSIS
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Striking conservation in various organisms suggests that Cellular Nucleic acid Binding Protein (CNBP) plays a fundamental biological role across different species. During chick and mouse embryogenesis, it was reported that CNBP is required for forebrain formation. By performing knockdown experiments in zebrafish, we have demonstrated an essential role for CNBP in mediating neural crest expansion by controlling proliferation and cell survival during rostral head development. Based on these results, we explained the craniofacial anomalies observed in zebrafish as well as those ones reported for mice and chicken and, moreover, confirmed that CNBP plays an essential and conserved role during vertebrate head development. In the present study we identified and analyzed zebrafish *cnbp* promoter to elucidate the regulatory mechanism behind the temporally and spatially restricted and highly conserved gene expression pattern observed in vertebrate embryos. Different genomic DNA fragments 5' upstream of zebrafish *cnbp* gene were cloned into the Tol2 transposon vector containing the EGFP reporter. The characterization of *cnbp* minimal promoter was detected by examining the ability of 5'-upstream fragments microinjected in one-cell embryos to drive EGFP expression in live embryos resembling *cnbp* gene expression pattern.

CB-C14.
IDENTIFYING NATURAL ANTISENSE TRANSCRIPTS OF CHOLESTEROL TRANSPORT RELATED PROTEINS
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Natural Antisense Transcripts (NATs) are endogenous RNAs complementary to sense mRNAs. Several NATs regulate the expression of their sense counterparts at many levels of gene expression (transcription, maturation, transport, stability and translation). Since a fine regulation of gene expression and protein synthesis is needed for steroid synthesis, NATs could play a role in the control of the process. The aim of this study was to evaluate the existence of NATs for three proteins involved in cholesterol transport into the mitochondria, the rate-limiting step of steroid synthesis: Steroidogenic acute regulatory protein (StAR), acyl-CoA thioesterase (Acot2) and acyl-CoA synthetase 4 (ACS4). Total RNA of MA10 mouse Leydig cells was examined for NATs using a Rapid Amplification of cDNA Ends method and nested PCR. Several NATs were identified and sequenced. Their existence was verified by RT-PCR and Ribonuclease Protection Assay. We sought to evaluate the hormonal regulation of StAR NAT. A temporal hormone dependent increase in the expression of StAR antisense transcript was observed in MA10 cells incubated with 8Br-cAMP or hCG by semiquantitative RT-PCR. Here we demonstrate the presence of NATs of StAR, Acot2 and ACS4 and the hormonal regulation of StAR NAT. These findings will help to understand the role of NATs in molecular mechanisms of gene expression and protein synthesis.

CB-C15.
TRANSCRIPTIONAL REGULATION OF INSULIN-DEGRADING ENZYME (IDE): POSSIBLE ROLE OF HYPOXIA AND NOTCH
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Amyloid beta (Aβ) is accumulated in the brains of Alzheimer's disease (AD) patients and its steady-state level is regulated by the expression and activity of IDE. Here, we determined the effect of presenilin-1 (PS1; the catalytic subunit of γ-secretase involved in Aβ generation) and hypoxia (a known risk factor for sporadic AD) in the modulation of IDE expression. First, rat primary astrocytic cultures or CHO cells were placed in hypoxia (0.1% O_2) and IDE mRNA levels measured by RT-PCR. We observed a significant increase in IDE mRNA levels in both cell types in hypoxia compared to normoxia (23%). Interestingly, CHO cells stably expressing PS1 exhibited decreased IDE mRNA levels in hypoxia while a PS1 dominant-negative mutant partially rescues this phenotype. This suggests the existence of a γ-secretase-dependent transcriptional inhibitory mechanism. HEY proteins are known Notch- and HIF-1-α- mediated transcriptional repressors. Transfection of HEY into wild-type CHO cells significantly reduced IDE transcription (50%). Co-transfection of Notch intracellular domain and HIF-1-α completely abrogated IDE mRNA levels. Overall, we show that IDE expression is modulated in hypoxia in a gamma-secretase manner. Also, we describe, for the first time, an IDE transcriptional inhibitory mechanism mediated by HEY which may be relevant in the pathogenesis of sporadic AD.

CB-C16.
C-FOS, A NOVEL TARGET TO CONTROL TUMOR DEVELOPMENT IN NEUROFIBROMATOSIS TYPE 1
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As a member of the family of AP-1 transcription factors, c-Fos forms heterodimers with c-Jun, thus regulating the expression of genes involved in mitosis and differentiation. We found that in addition to its AP-1 activity, c-Fos associates to the endoplasmic reticulum (ER) and activates the synthesis of phospholipids for the genesis of membrane required for cell proliferation and growth of human brain tumors. Herein, this phenomena was examined in human peripheral nerve sheath tumors from patients suffering from the Neurofibromatosis Type I (NF1) disease. In these tumors, high levels of c-Fos were observed co-localizing with ER markers which correlate with high rates of cell proliferation and activated phospholipid synthesis levels. Similar results were observed in NPcis mice, an animal model for the NF1 human disease in which it was verified that these effects of c-Fos are independent of its AP-1 nuclear activity. At present, we are generating NPcis mice on a fos-/- background, so as to confirm the direct participation of c-Fos on the neoplastic process by activating phospholipid synthesis. These results point to c-Fos as a new and therapeutically important target for controlling tumor growth in Nf1.

ST-C01.
THE MASTER TRANSCRIPTION FACTOR SPO0A REGULATES SLIDING DEVELOPMENT IN *BACILLUS SUBTILIS*
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Surface-associated motility such as swarming, sliding, twitching, gliding are of capital importance to understand the behavior not only of pathogenic but also of beneficial bacteria with plant-growth promoting activities and human friendly (probiotic) properties. Here, we demonstrate that sliding motility (a sort of surface-associated motility independent of flagella) of *B. subtilis* undomesticated cells was under the positive control of Spo0A and to a lesser extent, under the positive control of AbrB and SinR. Single spo0A minus cells were completely unable to slide, while single abrB and sinR mutants were partially defective in motility. However sliding phenotype was completely abolished in a double abrB-sinR mutant strain. Therefore, Spo0A was essential and AbrB and/or SinR were required for sliding motility. Interestingly, sliding motility was also under the strict control of Spo0A in the undomesticated Marburg-derived strain NCIB3610. This phenotype was clearly demonstrated by the inability of a NCIB 3610-derived hag mutant strain to slide in the absence of Spo0A production. Spo0A regulates production of the biosurfactan surfactin that apart from its antimicrobial and antiviral properties was essential for sliding motility as we show analysing the sliding behavior of surfactin-deficient cells in the absence and presence of synthetic biosurfactan.

ST-C02.
BIOCHEMICAL CHARACTERIZATION OF DESK, THE MEMBRANE FLUIDITY SENSOR OF *BACILLUS SUBTILIS*
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The Des pathway of *Bacillus subtillis*, entirely unveiled in our laboratory, is composed of the Δ5-acyl lipid desaturase that introduces double bounds into saturated membrane phospholipids and the two component system DesK/DesR which stringently controls *des* expression. The induction of the pathway is brought about by the ability of the bifunctional histidine kinase/phosphatase DesK to assume different signalling states in response to changes in membrane lipid fluidity. This is accomplished *in vivo* by regulating the ratio of kinase to phosphatase activities of DesK over its response regulator, DesR.
To gain insight into the mechanism by which the DesK sensor protein adjusts its signaling state in response to membrane fluidity changes, we wished to biochemically characterize DesK integrated into proteoliposomes. To this end, selected detergents or lipids of a comprehensive variety have been systematically evaluated with respect to their impact on the *in vitro* expression of DesK and their efficiency to keep synthesized DesK in solution when produced by means of a cell-free expression system. We found conditions in which DesK can be expressed in a soluble and functional form at high yields. Besides, DesK proteoliposomes were characterized in relation to its autokinase, phosphotransfer and phosphatase activities in order to identify which is the activity regulated by membrane lipids.

ST-C03.
POST-TRANSLATIONAL MODIFICATIONS MODULATE ATF7 TRANSCRIPTIONAL ACTIVITY
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ATF7 is a ubiquitously expressed leucine-zipper containing transcription factor, characterized by its ability to bind ATF/CRE sequences of different cellular and viral promoters. Its heterodimerization with Jun, Fos or related b-Zip factors leads to transcription regulation of target genes, via a direct interaction with TFIID general transcription cofactors TAF12 and TAF4. To gain further insight into the molecular mechanisms of ATF7-mediated activation, we have investigated the contribution of post-translational modifications. We found that ATF7 is SUMOylated, which delays its nuclear translocation and inhibits its activity by impairing its interaction with TAF12. We have also shown that ATF7 is phosphorylated after stimulation of p38 MAP kinase pathway. Ongoing experiments are aimed at elucidating the functional role of this modification in ATF7 transcriptional activity.
A novel alternatively spliced ATF7 isoform has recently been characterized. This protein (ATF7-4) shares with full length ATF7 the N-terminal activation domain but lacks the C-terminal b-Zip domain and therefore does not bind to DNA. Our observations suggest that ATF7-4 may act as a dominant negative, as it inhibits ATF7 transcriptional activity on reporter genes. A functional characterization of ATF7-4 will allow us to elucidate the molecular mechanisms involved in regulating ATF7 dependent transcription.

ST-C04.
ROLE OF ERK1/2 AND p38 MAPKS IN THE ANTIAPOPTOTIC EFFECTS OF 17β-ESTRADIOL IN MUSCLE CELLS
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Estrogens exert antiapoptotic effects in various cell types, e.g. vascular endothelial, smooth muscle and breast cancer cells. It has been reported that ERK1/2 is involved in the antiapoptotic action of 17β-estradiol (E2). We have recently shown that E2 abrogates apoptosis in C2C12 murine skeletal muscle cells. Rapid stimulation of ERK1/2 and p38 MAPK by E2 was also observed in the same cell line. In the present work, we studied the function of MAPKs in the protective effects of 17β-estradiol in C2C12 muscle cells. Immunocytochemical assays demonstrated that ERK1/2 activated by E2 localizes mainly in mitochondria. Incubation of the cells with 17β-estradiol (10 nM, 40 min) prior to treatment with H_2O_2 reversed the changes induced by this apoptotic agent on the phosphorylation of Akt and Bad, cytochrome c and Smac/Diablo release, caspase 3 and PARP cleavage, and morphological changes in the cells. Of relevance, the protective effect of E2 was abolished in presence of U0126 (ERK1/2 inhibitor) and SB203580 (p38 inhibitor), thereby involving ERK1/2 and p38 MAPKs as part of the mechanism that underlies the protective action of 17β-estradiol in muscle cells.

ST-C05.
cAMP EXERTS A FINE CONTROL OF MAP KINASE PHOSPHATASE-1 LEVELS: IMPLICATIONS ON GENE TRANSCRIPTION
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MAP kinase phosphatase-1 (MKP-1) controls nuclear MAP kinase activity with important consequences for gene transcription. In adrenal and Leydig cells, trophic hormones trigger ERK1/2 activation, a key step in Steroidogenic Acute Regulatory (StAR) protein induction and steroidogenesis. In addition, we have reported a hormone/cAMP-dependent transcriptional increase of MKP-1 in those cells. In this study we analyzed the post translational regulation of MKP-1 and its functional role on gene transcription. Western blot analysis showed that 8Br-cAMP stimulation (0-3 h) up regulates MKP-1 in MA-10 Leydig cells transiently overexpressing the protein in a magnitude higher than that observed in mock transfected cells, an effect that was reduced by blocking ERK1/2 activation. Since proteasome inhibitors also produced MKP-1 accumulation, our study suggests that PKA/ERK1/2mediated phosphorylation of the enzyme impairs its proteasomal degradation. We also tested the role of MKP-1 on cAMP-stimulated StAR promoter activity using a reporter (luciferase) system. 8Br-cAMP stimulation (6 h) enhanced promoter activity (Control=0.36±0.03 vs 8Br=0.99±0.10, $P<0.01$) and MKP-1 overexpression reduced the effect (8Br/MKP1=0.46±0.09, $P<0.01$ vs 8Br). We conclude that cAMP exerts a fine control of MKP-1 levels that sets a temporal limit to the regulation of MAP kinase-induced gene transcription.

ST-C06.

ROLE OF PTPα IN 1α,25(OH)$_2$D$_3$ DEPENDENT Src ACTIVATION IN SKELETAL MUSCLE CELLS

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C-Src is a member of a tyrosine kinase family involved in ERK 1/2 and p38 MAPK stimulation in several tissues. Dephosphorylation of c-Src in Tyr 527 residues leads to its activation, so c-Src has been shown to be a substrate for protein tyrosine phosphatase alpha (PTPα), which can be in turn activated by PKC. We have previously reported that stimulation of ERK 1/2 and p38 MAPK by 1α,25-dihydroxy-vitamin D$_3$ [1α,25(OH)$_2$D$_3$] in the skeletal muscle cell line C2C12 is mediated by c-Src. It was also observed that the steroid hormone regulates c-Src in a PKC-dependent manner. Now, we have studied the role of PTPα in c-Src activation by1α,25(OH)$_2$D$_3$ in these cells. Our results demonstrate that 1α,25(OH)$_2$D$_3$ promotes Tyr 789 phosphorylation of PTPα, which is necessary to activate c-Src by tyrosine dephosphorylation. An acute augment in PTPα phosphatase activity is detected after 60 min of hormone stimulation. Moreover, confocal immunocytochemistry showed co-localization of PTPα and c-Src promoted by 1α,25(OH)$_2$D$_3$ that is blocked with the specific PKC inhibitor Ro 318220. Finally, we corroborated the association of PTPα and c-Src by co-immunoprecipitation assays after hormone and TPA treatments. We conclude that 1α,25(OH)$_2$D$_3$ activates PKC which increases c-Src kinase activity via stimulation of PTPα, events upstream to activation of MAPKs.

ST-C07.

ATP SIGNAL TRANSDUCTION PATHWAY IN MCF-7 BREAST CANCER CELLS

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We previously showed that ATP increases in MCF-7 breast cancer cells [Ca^{2+}]i and the phosphorylation of MAP kinases ERK1/2, p38 and JNK1 via $P2Y_2$ receptor activation. MAPK stimulation by ATP was dependent on intracellular Ca^{2+} store release but independent of mechanical stressactivated Ca^{2+} (SAC) influx. In this work we investigated the participation of PKC and Src family kinases in ATP-induced MAPK phosphorylation in MCF-7 cells. The use of 5 μM Ro 318220, a PKC inhibitor, or 75 μM PP2, a Src family kinases inhibitor, showed that PKC but not Src participate in the phosphorylation of MAPKs by ATP. The induction of c-fos expression and the phosphorylation of c-jun, jun D and ATF-1 transcription factors in response to ATP was also studied. Western blot analysis showed maximum levels of c-fos induction and of c-jun and jun D phosphorylation after 30 min treatment with ATP, while maximum phosphorylation of ATF-1 occurred after 15 min. Lack of inhibition of these changes by 10 μM Gd^{3+} or La^{3+} ruled out the contribution of ATP-dependent SAC influx to the regulation of the transcription factors.
Pharmacological inhibition of MAP kinases demonstrated that c-fos induction and phosphorylation of c-jun and jun D, but not of ATF-1, partially depend on p38 and ERK1/2 activation. These results provide information on the mechanism underlying the proliferative action of ATP on breast cancer cells.

ST-C08.

ALPHA2-M/LRP1 BINDING INCREASES MMP-9 SYNTHESIS BY INTRACELLULAR SIGNALING ACTIVATION IN J774 CELLS

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Macrophages play a key role in atherosclerosis involving an increased production of extracellular matrix metalloproteinase-9 (MMP-9). LRP1 is a LDL receptor gene family member constituted by a 515-kDa extracelullar α chain and 85-kDa transmembrane and intracelullar β chain. LRP1 α chain contains multiple ligand recognition sites and β chain harbors motifs for endocytosis and intracellular signalling events. α2-macroglobulin-protease complexes (α2M*) is recognized by LRP1 and its binding is inhibited by RAP. Previously, we have demonstrated that α2M*/LRP1 interaction promotes cell proliferation mediated by intracelullar Ca2+ mobilization, MAPK-ERK1/2 phosphorylation and PKC activation in J774 cells. In this work we investigated whether the α2M*/LRP1-activated intracellular signaling events modify the MMP-9 synthesis in J774 macrophage-derived cell line. By Western blot we observed that α2M* 20 nM promoted MAPK-ERK1/2 phosphorilation, which was inhibited by PD98059, RAP, BAPTA and Calphostin C. By RT-PCR we showed that α2M* induced MMP-9 mRNA expression. By zymography we demonstrated that α2M* increased activity of MMP-9 in conditioned medium of J774 cells incubated with α2M*. The α2M-induced MMP-9 synthesis was abolished by the presence of PD98059, RAP, BAPTA and Calphostin C. Thus, the α2M*/LRP1 interaction increases MMP-9 synthesis mediated by intracellular signaling activation.

PL-C01.

SPATIAL AND TEMPORAL CHARACTERIZATION OF THE TRANSCRIPTIONAL ACTIVITY OF miR164a PROMOTER IN PLANTS

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MicroRNAs (miRNAs) are 21 nucleotide RNAs that regulate gene expression. miR164 regulates a family of NAC-domain transcription factors required for plant development. miR164 is potentially transcribed from three loci, miR164a, b and c and it is accumulated in different tissues, indicating that miRNA accumulation is spatially regulated. This could be due to an increased transcription level or to an increase on post-transcriptional maturation of such miRNA. To establish if miR164a is transcriptionally regulated, we PCR a 2500 pb fragment located immediately upstream of the *Arabidopsis* miRNA164a gene and subcloned it upstream of the uidA gene. Several stress responding boxes were bioinformatically identified on this region. The promoter was found active in onion, potato, sunflower and *Nicotiana benthamiana* by transient expression. In contrast, it was inactive in insect (Sf9) and mammal (BHK) cells. Next, we obtained several transgenic *Arabidopsis* lines, and analyzed GUS activity derived from the miR164a promoter. The data showed a specific pattern of transcriptional activity, particularly in vascular tissues, among others. The level of transcription peaks at five weeks and then disappears. In conclusion, we showed that the transcription driven by miR164a promoter is spatial and temporarily regulated. We intend to assess next if hormone or virus induce or repress the promoter.

PL-C02.
THE ARABIDOPSIS *CYTC-2* GENE IS REGULATED BY THE COOPERATIVE ACTION OF SITEII AND G-BOX MOTIFS

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The Arabidopsis *cytc-2* (At4g10040) gene has been shown to be up-regulated in response to carbohydrates, cicloheximide and under stress conditions. Progressive deletions and base substitution analysis of the cytc-2 promoter region revealed a cooperative action between site II elements, G-box and ACGT motifs. While site II motifs regulate the magnitude of the response to different treatments and the basal expression in reproductive tissues, the main expression characteristics of the *cytc-2* gene are dependent on an essential fragment (-189 to -139) containing a G-box and an ACGT motif. These motifs regulate the response to different effectors, such as light, carbohydrates, hormones, UV and environmental stress. By means of yeast one-hybrid screening we identified a group of transcription factors from the bZip and bHLH families (AtABF4, AtGBF1, AtGBF3, AtGBFlike, AtHLH080) that are able to bind to the cytc-2 G-box motif. Electrophoretic mobility shift assays using nuclear extracts indicated that the integrity of the region from -149 to -139 is required for efficient binding of proteins to the G-box motif, suggesting the existence of cooperative interactions. We conclude that site II motifs and the G-box are involved in determining specific aspects of the *cytc-2* expression patterns through the interaction with several transcription factors.

PL-C03.
POLLEN TUBE GROWTH PROMOTION BY MrX FROM PISTILS

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Higher plants depend on pollen tube growth for the delivery of male gametes to the embryo sac. Upon arrival to the stigma, pollen grain hydrates and produces a structure that grows through the female tissue directionally to the ovules. This growth is regulated positively and negatively by numerous factors such as proteins in the extracellular matrix, low molecular weight compounds, nutrients, ions, etc; this suggests an active communication between female and male tissues. In this context, we purified and characterized MrX, a small molecular weight compound from pistils that specifically dephosphorylates the receptor kinase LePRK2 in tomato pollen plasma membrane. To determine the physiological effects of MrX on pollen grains, in vitro pollen tube growth experiments were done in the presence of a purified fraction of MrX. Tube length was determined on 1- and 3-hour-germinated pollen grains. Results show that pollen tube length increased in a dose-dependent manner. Indeed, after 3 hour of germination, concentrations as low as 0.0003 Abs280nm/microliter induced a statistically significant increase in pollen tube length when compared to pollen grains incubated with a non-active fraction. Our observations suggest that communication between female and male tissues through MrX and LePRK2, results in growth stimulation from the onset of pollen tube development.

PL-C04.
LEAF SIZE REGULATION BY miRNAs IN PLANTS

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MicroRNAs (miRNAs) have been recognized as a novel mechanism to regulate gene expression in multicellular organisms. They are small RNAs of ~21 nt in length that recognize partially complementary sequences in target mRNAs and guide them to cleavage or translational arrest. They have been implicated in many key processes such as development and stress responses.
In *Arabidopsis thaliana* the miR396 family includes two genes. These miRNAs regulate seven GRF transcription factors. The interaction between miR396 and the target site has near perfect complementarity, with a bulged base at position 7.
We analyzed A. thaliana plants that overexpress the miR396b precursor. We also generated transgenic lines with a GRF version that is resistant to miR396 regulation (rGRF). From the morphological and molecular assays we concluded that the miR396/GRF system controls cell size and proliferation in leaves. To study the mechanism of miR396 action, we made translational fusions of GRF2 to the reporter gene GUS. We compared the wt version of GRF, rGRF and another version with perfect match to the miRNA. We found that miR396 regulates GRFs by two mechanisms, mRNA cleavage and translational repression.

PL-C05.
PROCESSING OF miR319 AND miR172 MicroRNA PRECURSORS IN *Arabidopsis thaliana*

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MicroRNAs (miRNAs) are small RNAs of ~21 nt that recognize partially complementary sites in target mRNAs and guide them to cleavage or translational arrest. They are transcribed as larger precursors that contain fold-back structures that are processed by RNAse III complexes. The precursor contains spatial clues that determine the position of the miRNA along its sequence. However, at a difference with animals, plant miRNA precursors are heterogeneous in size and shape, and the processing mechanisms still remain largely unknown.
We have focused on miR172 and miR319 that regulate AP2 and TCP transcription factors respectively. Overexpression of miR172 precursor causes an early transition from vegetative to reproductive phase, while miR319 causes an increased cell proliferation and changes in leaf morphogenesis. Analysis of the processing intermediates of these miRNAs revealed that the miR319 precursor requires four sequential cuts to release the mature miRNA, instead of the two cuts observed in miR172. To analyze the structural requirements for miRNA processing, we have performed site directed mutagenesis on the precursors to change their spatial structure. We are currently analysing the processing intermediates of the different miRNA precursors in *Arabidopsis* plants.

PL-C06.
L-PROLINE CATABOLISM ALONG DEVELOPMENT OF THE HYPERSENSITIVE RESPONSE

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Although plants accumulate L-proline (Pro) in response to several abiotic stresses, external Pro supply results toxic and this toxicity is apparently mediated by accumulation of reactive oxygen species (ROS). Pro catabolism takes place in mitochondria involving two consecutive steps catalyzed by ProDH and P5CDH. We have observed that Pro catabolism is altered in *Arabidopsis thaliana* tissues developing the Hypersensitive Response (HR) triggered by avirulent races of *Pseudomonas syringae*. Induction of *ProDH* and repression of *P5CDH* occur in these tissues. We here evaluated if *ProDH* and *P5CDH* genes are regulated by SA/JA/ROS and analyzed the requirement of ProDH for HR development. We found that *SRO5*, a salt- and ROS-sensitive gene which down regulates *P5CDH* trough a *SRO5-P5CDH* natural siRNA, was activated in HR. SA and JA stimulate similar *ProDH*, *P5CDH* and *SRO5* transcriptional changes than those induced by the avirulent pathogen. To analyze if ProDH affects ROS generation, siRNA*ProDH* lines were constructed. ROS induced by the avirulent pathogen or by external Pro treatment were diminished in these transgenic plants. Our results suggest that ProDH-mediated Pro degradation may affect HR development by modulating ROS generation.

PL-C07.
DISSECTING IMMUNE RESPONSE AND PATHOGEN SUPPRESSION IN CITRUS-XANTHOMONAS INTERACTIONS

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Xanthomonas axonopodis pv. citri (Xac) is responsible for the canker disease affecting citrus plants throughout the world. Many studies have implicated extracellular polysaccharides (EPS, i.e. xanthan) secreted by different species of *Xanthomonas* in pathogenicity and fitness. Recently, we have shown that xanthan plays an important role in the formation of biofilms and in bacterial survival on the leaf leading to canker development. However, other roles played by xanthan in disease development should not be ruled out. We have shown that xanthan from other *Xanthomonas* species is able to mediate suppression of plant defense response. We postulate that, after invasion of host intercellular spaces, sequestration of the Ca^{++} ions by xanthan prevents activation of defenses. Callose deposition and an oxidative burst are triggered by xanthan defective mutant on lemon leaves, suggesting that xanthan plays a key role in establishing compatibility by suppressing PAMP recognition. We have established a *Citrus limon Xac* assay and have used it to identify PAMP-mediated differential gene expression during *Xac*-citrus interaction. In order to study the gene function of these isolated genes we developed a RNAi transient assay to induced post transcriptional gene silencing in lemon seedling. These results will be discussed.

PL-C08.
CROSSTALK BETWEEN JASMONIC ACID AND ETHYLENE PATHWAYS ARE MEDIATED BY THE HAHB4 TRANSCRIPTION FACTOR

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Plants continuously suffer injuries, both mechanical or as an effect of insects attack. The caused damage led them to generate a fast and strong response that includes the expression of specific genes. Two hormones, jasmonic acid (JA) and ethylene (ET), sometimes acting cooperatively, are also involved in this response. In this work we show that HAHB4 plays a central role in the insects-induced defence response. This sunflower transcription factor is up-regulated when the plants suffer a damage caused by herbivores as indicated by qRT-PCR and transiently transformed leaves with a construct where GUS is fused to the HAHB4 promoter. This gene is also up-regulated by JA and ET following a feedback mechanism. Once enhanced, it positively regulates genes involved in JA synthesis generating a defence response. Target genes levels were quantified in treated and non-treated plants by qRT-PCR indicating that both, a local and a systemic response, are waked up in the presence of HAHB4. Enzymatic measurements show that the accumulation of HAHB4 leads to the formation of green leaf volatiles and trypsin protease inhibitors which trigger a strong defence. In addition, transgenic plants overexpressing this gene show a remarkable tolerance to insect's attack.
In conclusion, HAHB4 mediates the defence against the insect's attack through a coordination of the JA and ET action in this response.

PL-C09.
TOBACCO PLANTS EXPRESSING A CYANOBACTERIAL FERREDOXIN DISPLAY INCREASED OXIDATIVE STRESS TOLERANCE

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Ferredoxins (Fd) are iron-sulfur proteins involved in many different one-electron transfer pathways in plants, animals and microorganisms. In chloroplasts and cyanobacteria, Fd distributes the reducing equivalents generated in the photosynthetic electron transport chain to various essential oxidoreductive pathways. Fd levels decrease under adverse environmental conditions, affecting severely plant physiology. Moreover, antisense Fd plants display drastic alterations in growth and development. The aim of this study is to evaluate the effect of the expression of a bacterial version of this protein which is free from endogenous regulation on the tolerance to oxidative stress conditions. We generated transgenic tobacco plants expressing *Anabaena* PCC7120 Fd in plastids under the control of the cauliflower mosaic virus 35S promoter by *Agrobacterium tumefaciens*-mediated leaf disc transformation. Immunoblots were used to confirm the expression of the alien Fd. No phenotypic differences were observed between wild-type and transgenic plants cultured in a growth chamber. We evaluated oxidative stress tolerance using the redox-cycling herbicide methyl-viologen (MV) that propagates reactive oxygen species. The transgenic plants exhibit enhanced tolerance to oxidative damage. This suggests that expression of the cyanobacterial Fd might contribute to improve stress tolerance.

PL-C10
HETEROMERIC ENZYMES: DIFFERENT PROPERTIES OF *A. thaliana* NAD-MALIC ENZYME SUBUNITS

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Malic enzyme (ME) is classified into three different types: EC 1.1.1.38, 39 and 40. The class EC 1.1.1.39 is exclusive of plant mitochondria, and it seems to assemble as an heteromeric oligomer. In the present work, the two genes (*nad-me1 and 2*) encoding *A. thaliana* mitochondrial ME were studied. The recombinant proteins were separately expressed and characterized. NAD-ME1 and NAD-ME2 showed NAD-ME activity alone, with well-distinct kinetic and regulatory properties. While NAD-ME1 could not be detected by native PAGE revealed by activity, NAD-ME2 showed a dimeric active band. Native PAGE coupled to SDS-PAGE of *A. thaliana* mitochondrial extracts, indicate that NAD-ME could assemble as a dimer of non-identical subunits *in vivo*. Further results confirming this conclusion were obtained by reconstitution of the dimer *in vitro*. The characterization of loss-of function mutants plants for both nad-me genes indicated that each NAD-ME alone exhibits enzymatic activity in vivo, as observed for the recombinant enzymes. In addition, the single and double *nad-me* mutants showed no visible phenotype under standard conditions. These results indicate that, although *A. thaliana* NAD-ME assembles as a heterodimer, each subunit can catalyze ME reaction with regulatory and kinetic distinct properties. The results obtained are discussed in terms of the role that this enzyme can fulfill in this C3 plant.

PL-C11.
REGULATORY ROLE OF THE N-TERMINAL STARCH BINDING DOMAINS ON THE KINETICS OF STARCH SYNTHASE III

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Starch-synthase III (SSIII), one of the SS isoforms involved in plant starch metabolism was reported to have a regulatory role on the synthesis of transient starch. SSIII from A. thaliana has an N-terminal transit peptide for chloroplast localization which is followed by three repeated starch-binding domains, SBD, (SSIII residues 22 to 591) and a C-terminal domain (residues 592 to 1025) similar to bacterial glycogen synthase. To elucidate the enzymatic properties of SSIII and the function of the N- domain, we constructed recombinant full length and truncated isoforms of SSIII, lacking one, two or the three SBDs and recombinant SBDs with three (D123), two (D23) or one (D3) SBD domains. Results revealed that the presence of the SBDs confers particular properties to each isoform, increasing the apparent affinity and the Vmax for the oligosaccharide substrate. Under glycogen saturating conditions, the presence of SBDs increases progressively the apparent affinity and Vmax for ADPGlc. Studies on the N-domain confirm our previous results indicating that this region is a carbohydrate binding module and contributes to the binding of different polysaccharides. The results presented here suggest that the N- and C-terminal regions of SSIII have different functions: The N-domain is involved in the binding of the polysaccharide and the C-domain contributes to the molecular activity of the enzyme.

PL-C12.
METABOLIC REGULATION AND STRUCTURE-FUNCTION RELATIONSHIP OF *A. thaliana* NADP-MALIC ENZYME ISOFORMS

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The *A. thaliana* genome contains four genes encoding NADP-malic enzymes (*ME1-4*), which are different in their expression pattern and in subcellular location of their products. Although the four proteins share a remarkably high degree of identity, recombinant ME1-4 show well-distinct kinetic properties, both in forward -malate oxidative decarboxylation- and reverse pyruvate reductive carboxylation- reactions. When analyzing the activity of each isoform in the presence of possible metabolic effectors, ME2 resulted the most highly regulated. Furthermore, several metabolites modulate both reactions, exhibiting reciprocal effects activation or inhibition- in some cases. Thus, the results obtained indicated that forward and reverse reactions catalyzed by ME1-4 may be relevant *in vivo*, at least in some particular tissues or metabolic situations. In order to identify residues or segments of the primary structure that could be responsible in the differential properties, ME2 mutants and deletions and several quimeras were constructed and analysed. The results obtained show that Arg115 is involved in fumarate activation of ME2, while the amino-terminal regions is critical for aspartate and CoA activation, as well as for the reverse reaction. In conclusion, these studies show that minimal changes in the primary structure are responsible for the different kinetic behavior of each ME isoform.

PL-C13.
AUXIN AND NITRIC OXIDE TRIGGER PHOSPHATIDIC ACID ACCUMULATION VIA PHOSPHOLIPASE D IN CUCUMBER

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We have previously demonstrated that nitric oxide (NO), cGMP, MAP kinases, Ca^{2+} and Ca^{2+}-dependent protein kinases are messengers in the auxin-induced adventitious root (AR) formation in cucumber (*Cucumis sativus*). The goal of this work was to study phospholipid (PL) signaling during auxin treatments and to evaluate the requirement of NO on this. Cucumber explants were labeled with $^{32}P_i$ and treated with auxin or NO, in the presence or absence of the specific NO-scavenger cPTIO. PLs were analyzed by thin layer chromatography. Here we report that the PL signaling molecules phosphatidic acid (PA), phosphatidylinositol phosphate and bisphosphate are rapidly (after 1 min) and transiently (within 30 min) accumulated during auxin treatment. The application of cPTIO abolished this auxin effect, indicating that auxin depends on NO in inducing PL accumulation. Accordingly, the application of NO mimicked the effect of auxin. Furthermore, we demonstrate that auxin and NO trigger PA formation via phospholipase D (PLD) activity. We show that 10 min treatment with auxin or NO is sufficient to induce AR formation, and that PLD activity is required on this. Finally, AR formation increased 150 % by exogenously applied PA. Altogether, our data suggest that PLD-derived PA is an early signaling event in the AR formation process induced by auxin and NO.

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PL-C14.
CHARACTERIZATION OF NADP-MALIC ENZYME FROM *Nicotiana tabacum:* CLONING AND BIOLOGICAL ROLE ANALYSIS

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NADP-Malic Enzyme (NADP-ME) catalyzes the oxidative decarboxylation of L-malate producing pyruvate, CO2 and NADPH. In plants the enzyme is related to carbon fixation in C4 and Crassulacean Acid Metabolism (CAM) plants. In C3, C4 and CAM plants non-photosynthetic isoforms have also been described. The aim of this study was to characterize the different isoforms of NADP-ME from *Nicotiana tabacum*. Three different enzymes were identified. All of them are expressed in vegetative as well as in reproductive tissues but with different expression patterns. Root is the tissue that displays the highest activity. Only one isoform is targeted to plastids (DQ923119) while the others are cytosolic (DQ923118, EH663836). Real time RT-PCR studies and activity assays showed that NADP-MEs from tobacco respond to different biotic and abiotic stress stimulus. Enzymes encoded by DQ923118 and DQ923119 were recombinant expressed in *Escherichia coli* and their kinetic parameters and response to different effectors were analyzed. Studies carried out with crude extracts and with the recombinant proteins indicated that the cytosolic and plastidic isoforms aggregate as tetramers of subunits of 65.4 and 63.3 kDa, respectively.
Finally, the physiological role of each isoform is discussed in terms of the occurrence, kinetic properties and response to stress.

PL-C15.
AUTOPHOSPHORYLATION OF RAPESEED 2-Cys PEROXYREDOXIN

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2-Cys peroxiredoxins (2-Cys Prx) are widely distributed thiol-containing peroxidases that have been implicated in various cellular processes. We have used functional and structural approaches to demonstrate that rapeseed 2-Cys Prx is a direct target for nucleotides. The concerted action of a nucleoside triphosphate and Mg^{2+} impairs reversibly the peroxidase activity, being purine derivatives more efficient than pyrimidine counterparts. In particular, structural and site-directed mutagenesis studies are consistent with a mechanism where ATP interacts noncovalently with a region that contains the conserved Cys175. Most importantly, ATP triggers the autophosphorylation of 2-Cys Prx upon reduction with thiol-bearing compounds or phosphines followed by oxidation with hydroperoxides, quinones, tetrathionate, selenate or diamide. Mass spectrometry analysis reveals that 2-Cys Prx incorporates the phosphoryl moiety into the Cys175 residue yielding the sulfinic-phosphoryl [Prx-(Cys175)-$SO_2PO_3^{2-}$] and the sulfonic-phosphoryl [Prx-(Cys175)-$SO_3PO_3^{2-}$] anhydrides. Hence, the functional coupling between ATP and 2-Cys Prx brings novel insights not only to the removal of toxic reactive oxygen species but also to mechanisms that link the status of cell energy to the oxidation of reactive cysteine residues.

PL-C16.
LOCALIZATION OF A PLANT LTP SUGGESTS A NOVEL ROLE OF THIS PROTEIN IN LIPID MOBILIZATION

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Lipid transfer proteins (LTPs) are low molecular-mass proteins extensively studied in plants. The mayor characteristic of this family is its ability to transfer acyl lipids between artificial membranes in vitro. The extracellular localization of LTPs suggested its role in cutin assembly or defense, but to date, its biological function remains elusive. Our lab has characterized a sunflower LTP (Ha-AP10), present in seed apoplast. Recent evidences indicate that a fraction of Ha-AP10 is associated to the microsomal fraction. To determine the subcellular localization of this protein, we performed fluorimmunolocalization studies on germinating "Paraplast"-embedded sunflower seeds.This assay revealed that during germination Ha-AP10 has both apoplastic and intracellular localization. It also showed that the intracellular fraction of the LTP was bound to internal vesicles similar to oil bodies. This presumption was confirmed by Western blot analysis of the oil bodies' fraction using anti-Ha-AP10 antibodies as well as coimmunolocalization experiments using anti-oleosins (oil body membrane-specific proteins) antibodies. Carbon storage in the form of triacylglycerides (TAG) is responsible to fuel post-germinative growth in oilseeds but little is known about the transport of fatty acids from oil bodies to glyoxysomes, where beta-oxidation and glyoxylate cycle take place. The presence of an LTP in oil body membranes suggests a novel role of this protein in fatty acid mobilization from the oil bodies to the glyoxysome.

PL-C17.
AtAzg IS A HIGH-AFFINITY PURINE IMPORTER WITH A POTENTIAL ROLE IN CYTOKININ UPTAKE

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A high-affinity purine transporter of Aspergillus has been recently identified as a member of a novel protein family with members in prokaryotes, fungi and plants. Two orthologous proteins are encoded in the Arabidopsis thaliana genome. In this work, AtAzg1 (Arabidopsis thaliana Aza-guanine resistant) was functionally expressed in a yeast mutant deficient in adenine uptake to study transport features. AtAzg1 mediated H+-gradient dependent high-affinity transport of adenine with a broad substrate specificity including adenine, hypoxanthine, guanine, cytokinins and toxic purine analogs. In contrast, other structurally related compounds, such as pyrimidines, nucleosides, caffeine and degradation products of purines were not transported. Transient expression of AtAzg1-GFP fusion proteins in cultured Arabidopsis cells and in onion epidermal cells revealed that AtAzg1 is localized to the plasma membrane indicating a function as cell importer. Azg1 knock-out mutants presented a conditional phenotype resistant to purine analogs and toxic concentrations of trans-zeatin. On the contrary, Arabidopsis overexpressing lines showed hypersensitivity to purine analogs and tZ and presented uptake rates for adenine and tZ several times higher than the wild-type. These results indicate that AtAzg1 functions as a purine importer and suggest a possible involvement in cytokinin transport *in vivo*.

PL-C18.
CHARACTERIZATION OF RECOMBINANT NADP DEPENDENT MALIC ENZYME FROM C3, C4 AND C3-C4 *FLAVERIA* SPECIES

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NADP-malic enzyme (NADP-ME) is a widely distributed enzyme involved in different metabolic pathways. The photosynthetic isoforms of this enzyme has evolved from non-photosynthetic counterparts. In order to analyze the origin of the C4-NADP-ME specific isoform, cDNAs encoding this enzyme were isolated in the genus *Flaveria*. This genus is well suited for studying the evolution of photosynthesis because it contains a continuous range of species between C3 and C4. In the present work, four cDNAs corresponding to plastidic isoforms from C3-C4 *F. floridana,* C4like *F. palmeri* and C4 *F. bidentis* and *F. trinervia* species were isolated by RACE method and sequenced. Although these sequences share a high degree of identity, the phylogenetic tree constructed with plant NADP-MEs show that *Flaveria* isoforms group into two separate branches, corresponding to photosynthetic and non-photosynthetic isoforms. The complete codifying sequences of *Flaveria* NADP-ME were subcloned into the pET 32 expression vector and successfully expressed in *E. coli*. Recombinant proteins were purified and kinetically and structurally characterized. The results obtained indicate that, in spite of the high degree of similarity, these proteins display differences in optimum pH, km values and regulation, which may be important for their specific physiological function *in vivo* and related to the evolution towards C4-NADP-ME.

LI-C02.
CHARACTERIZATION OF THE MURINE CTP:PHOSPHOCHOLINE CYTIDYLYLTRANSFERASE BETA GENE PROMOTER

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CTP:phosphocholine cytidylyltransferase (CCT) is a key regulatory enzyme in phosphatidylcholine (PC) biosynthesis by the Kennedy pathway. In mammals, there are two genes that encode enzymes that catalyze this reaction: Pcyt1α for CCTα and Pcyt1β for CCTβ isoforms. In mice, two isoforms named CCTβ 2 and CCTβ 3 can be generated from the Pcyt1β gene. We characterized two promoters that drive the expression of each CCTβ isoforms (CCTβ 2 and CCTβ 3). The promoters were isolated from mouse DNA and its activity delineated by luciferase reporter assay and gel-shift analysis in Neuro2A (mouse neuroblastoma), TM4 (Sertoli) and C3H10T1/2 (mouse embryo fibroblast) cells. We also mapped by primer extension the transcription start sites of both promoters.
The physiological role of CCTβ is not clear, however, previous reports have shown that the expression of CCTβ 2 enhance during neurite outgrowth and CCTβ 2 specific knockout mice revealed an essential role for this isoform in ovary maturation and in the maintenance of sperm production. To identify the transcription factors that bind to the CCTβ 2 promoter and regulate its expression we performed EMSA and DNasaI footprinting.
Our results show that the expression of the CCTβ isoforms is driven by two alternatives promoters and we propose AP1 as a transcription factor that could regulate the expression of CCTβ 2 in brain.

LI-C01.
EFFECT OF CLA, LINOLEIC ACID AND ITS METHYL ESTER ON LIPID PEROXIDATION OF TRIGLYCERIDES ω-3 PUFAS

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Conjugated linoleic acid (CLA) is a generic term used to describe a group of positional and geometric isomers of linoleic acid (LA). In CLA, two double bonds are conjugated, whereas they are methylene-interrupted in LA. Some authors showed that CLA had antioxidative activity and proposed this as a possible explanation for the anticarcinogenic and antiatherogenic effect. However, other investigations have found that CLA was a prooxidant. The effect of CLA isomers (c9,t11 and t10,c12), LA and its methyl ester (LAME) on lipid peroxidation of triglycerides rich in C20:5ω3 and C22:6ω3 were analyzed by photoemission techniques. The reaction initiated by tert-butyl hydroperoxide (t-BHP) was measured at 37ºC using six different concentrations of the studied compounds. t-BHP produced an increase of chemiluminescence with a maximum at 5 min after its addition. CLAs had inhibition on photoemission being more effective the t10,c12-CLA than the c9,t11-CLA isomer. LA and LAME did not have effect on lipid peroxidation of Tg ω-3 PUFAs. The free radical scavenging properties of CLAs, LA and LAME were also assayed against the stable 2,2-diphenyl-1-picryhydrazyl radical (DPPH•). The reaction kinetics showed that t10,c12-CLA is more effective than c9,t11-CLA, whereas LA and LAME did not exhibit free radical scavenging properties. CLAs and LA act differently in the presence of free radicals.

LI-C03.
PHOSPHATIDYLCHOLINE BIOSYNTHESIS AND NEURONAL DIFFERENTIATION

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Neuronal differentiation is a complex process characterized by a halt in proliferation and extension of neurites from the cell body. Changes in gene expression mediate the metabolic redirection leading to neurite formation and function. Acceleration of membrane phospholipid synthesis is associated with neurite elongation and phosphatidylcholine (PtdCho) is the major membrane phospholipid in mammalian cells. Our results indicate that membrane biogenesis is driven by the elevated expression of a subset of lipid biosynthetic genes following retinoic acid (RA)-induced differentiation of Neuro2A cells. The transcription of genes encoding key enzymes in the CDP-choline pathway of phospholipids biosynthesis is stimulated, including the Chka gene for choline kinase (CK) and the Pcyt1α/β gene for the CTP:phosphocholine cytidylyltransferase (CCT). The promoter regions for both genes indicated common DNA sequences which are proposed to be binding sites for transcription factors (TFs) that coordinately activate expression in a RA-dependent manner. Alteration of CK or CCT expression overexpression regulated PtdCho synthesis and neurite extension following RA treatment. These results indicate that PtdCho synthesis is necessary for RA-dependent differentiation of Neuro2A cells.

LI-C04.
PARTICIPATION OF GLYCOSPHINGOLIPID BIOSYNTHESIS IN RENAL COLLECTING DUCT CELL DIFFERENTIATION

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MDCK is a renal collecting duct cellular line that conserves the capacity to differentiate in particular conditions of cell culture. To differentiate, they acquire a polarized phenotype characterized by the presence of apical and basolateral membrane domains with different protein and lipid composition. Glycosphingolipids (GSL) predominate in the apical membrane. It has been previously reported that extracellular hypertonicity induces cell differentiation, but the mechanism involved is not well understood. In this study, we have examined the role of GSL biosynthesis in collecting duct cell differentiation. Confluent MDCK cells were submitted to 300 mM NaCl for 24 and 48h. Glycosphingolipid metabolism was determined by using ^{14}C-galactose as radioactive precursor and cell differentiation was followed by using fluorescent anti gp135, a marker of apical membrane, and visualized by confocal fluorescent microscopy. The results demonstrate that hypertonicity induced an early increase in GSL biosynthesis and the formation of apical membrane domain reflected by the apical accumulation of gp135. The pretreatment with PDMP, an inhibitor of Glucosylceramide synthase, evoked a 70% decrease in GSL biosynthesis and induced the disappearing of gp135 accumulation in apical membrane domain. From these results we conclude that GSL biosynthesis play a central role in MDCK cells differentiation.

LI-C05.
REGULATION OF LIPID METABOLISM AND ONCOGENESIS

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The translocator protein (TSPO), a mitochondrial acyl-CoA thioesterase (Acot2) and an arachidonic acid (AA) preferring acyl-CoA synthetase (ACS4) are important for cholesterol transport and regulation of intramitochondrial AA levels in steroidogenic tissues. Some studies demonstrate the role of TSPO on the development of different types of cancer. The overexpression of TSPO correlates with the development of the aggressive phenotype of breast cancers, affecting cholesterol transport and cellular proliferation. It is known that ACS4 is overexpressed in hepatocarcinoma and colon cancer. In this work we studied the role of this enzyme, taking as model MCF7, a non aggressive and MDA-MB-231 a high aggressive breast cancer cell lines. The ACS4 levels expression correlate with the high cellular aggressiveness phenotype, as was previously described for TSPO levels in this cell lines. The overexpression of ACS4 produces a more aggressive cell phenotype, measured by proliferation, migration and cellular invasion. Moreover, we demonstrated that the intramitochondrial AA levels are elevated in high aggressive cell lines. The overexpression of ACS4 in several types of tumors would support ACS4 as a potentially early tumor marker. This is in agreement with recently results where ACS4 is one protein that is increased during the transition from normal to preneoplastic mammary tissue.

LI-C06.
CADMIUM EFFECTS ON RAT TESTICULAR LIPIDS AND FATTY ACIDS

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The molecular mechanisms by which cadmium, a ubiquitous environmental contaminant, damages major organs in mammals are largely unknown. At high doses ($\geq$ 5 mg/kg) $CdCl_2$ induces testicular damage in rodents. Necrosis is evident in 2 days, followed by interstitial edema, hemorrhage, and germ cell degeneration. Here we surveyed the effects on rat testis of repeated sc doses (1mg/kg) of $CdCl_2$ (one every 4 days) administered for a month. The effects on lipids were studied at the end of this month and 2 weeks later, most suggesting irreversibility. In treated animals the testicular weight decreased to one-third and the testicular lipid phosphorus to one-fifth that of controls. All fatty acids decreased, but 22:5n-6 was the most affected, its percentage falling 4-fold in the decreased glycerophospholipids (GPL) (20% to 5% of the acyl chains). Neutral glycerides including triacyl and diacylglycerols decreased, but less than GPL. Cholesterol esters accumulated, in part storing temporarily fatty acids originally composing germ cell GPL. Sphingomyelin decreased, and a concomitant accumulation of ceramide (Cer) resulted. Unexpectedly, this Cer remained unchanged 2 weeks after finishing treatment, suggesting a Cd-induced inhibition of Cer metabolism or transport. This possibility is worth investigating among the factors determining Cd toxicity in the testis and possibly also in other organs.

LI-C07.
A MALONYL-COA-DEPENDENT SWITCH IN THE BACTERIAL RESPONSE TO A DYSFUNCTION OF LIPID METABOLISM

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Bacteria stringently regulate the synthesis of their membrane phospholipids but the responsible regulatory mechanisms are incompletely understood. Bacillus subtilis FabF, the target of the fungal micotoxin cerulenin, catalyzes the condensation of malonyl-ACP with acyl-ACP and is an important determinant of the product distribution of the fatty acid biosynthetic pathway. Here we show that B. subtilis strains containing the fabF1 allele, which codes for the cerulenin-insensitive protein FabF[I108F], overexpressed several genes involved in fatty acid and phospholipid biosynthesis (the fap regulon) and had significantly elevated levels of malonyl-CoA. These results pinpointed FabF[I108F] as the responsible for the increased malonyl-CoA production, which in turn acts as an inducer of the fap regulon by impairing the binding of the FapR repressor to its DNA targets. Analysis of the ACP pool produced by the fatty acid synthase of fabF1 cells showed the accumulation of short- and medium-chain acyl-ACPs. These results support the concept that the acyl-ACP chain-length acceptance of the FabF condensing enzyme is tightly coupled to the transcriptional regulation of membrane lipid synthesis in Gram-positive bacteria. In addition, these data provide a biochemical and physiological framework for understanding antibiotic resistance within these types of condensing enzymes.

LI-C08.

STRUCTURAL DETERMINANTS FOR COLD REGULATION IN METHYL-END DESATURASES OF TRYPANOSOMATIDS

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We have previously characterized trypanosomatid desaturases involved in the conversion of oleoyl- to linoleoyl-moieties of phospholipids in the endoplasmic reticulum membrane of the parasites. *Trypanosoma brucei* and *Leishmania major* oleoyl desaturases share 56% of identity and 75% of similarity, and comparable percentage of conversion (*in vivo*) of 16:1 and 18:1 fatty acids into 16:2 and 18:2 respectively, when expressed in yeasts at 30ºC. Interestingly, *T. brucei* desaturase showed an increased substrate conversion at lower temperatures, more significant for 16:1, whereas *L. major* enzyme has the opposite effect. Analysis of primary structure for both enzymes revealed a conserved theoretical membrane topology and consensus sequences for the three clusters of histidines, presumed to be part of the active site. However, both desaturases show some significant differences in certain regions, for example in the N-terminus or the catalytic domain located between the first and second hydrophobic domains. Construction of truncated and chimeric desaturases between the orthologous genes allowed us to localize a region involved in thermal regulation near the second histidine cluster. This region is a 20 aminoacid domain that has differences in charge and in the volume of some aminoacidic residues. We propose that this domain is also involved in the substrate selectivity of the desaturases.

LI-C09.

FACTORS THAT MODULATE MEMBRANE FATTY ACID COMPOSITION, STRUCTURE AND PROTEIN LIPID INTERACTIONS

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Dietary component hormones, nuclear receptors like LXR, PPAR, RXR and many exogenous agonists modulate endoplasmic reticulum fatty acid desaturases, that control unsaturated fatty acid biosynthesis and therefore the lipidic composition and packing of membranes and the interaction with proteins. These factors and effects are currently outlined, measured and discussed. Through the translocon, intrinsic proteins synthesized in the rough endoplasmic reticulum are introduced through hydrophobic interaction with the acyl chains of the phospholipids to the microsomal membrane. Due to the presence of many molecular species of phospholipids in the membranes, with saturated chains bound to sn-1 and unsaturated acids to sn-2 positions preferentially, the existence of different domains is visualized. The domains are of raft-like and non-raft structure. The raft-like domain contains cholesterol and sphingomyelin and cholesterol interaction with saturated acyl chains is a favourable factor whereas unsaturated acids and cholesterol are incompatible. The effect of unsaturated acyl chains in the microsomes, fluidity, and dynamics correlative to alterations of intrinsic enzyme properties is discussed. The case of desaturases and the structure of their four hydrophobic aminoacid chains spanning the ER bilayer is also shown and discussed.

MI-P01.
GROWTH AND TOLERANCE OF FUNGAL *RHIZOCTONIA* SPECIES TO TOXIC XENOBIOTIC CHLORINATED PHENOLS

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Chlorinated phenols (CP) are toxic compounds, which are used as fungicides and herbicides. Due to its toxicity, CP-laden soils demand an immediate decontamination. Bioremediation is an alternative to conventional clean-up. The success of this approach depends on finding microorganisms able to degrade and support unusual-high concentration of CP. Many filamentous fungi were isolated from contaminated soils and its tolerance to CP was tested, looking for a potential degradation strain. In this work, *Rhizoctonia* isolates were studied for their ability to growth and survive to toxic concentrations of CP. *R. solani* (AG-3) and binucleated *Rhizoctonia* (BNR) were grown on PDA plus 10 ppm of the following compounds: phenol (P), trichlorophenol (TCP), tetrachlorophenol (TECP) and pentachlorophenol (PCP). The presence of P in the culture medium did not affect the growth of both fungal isolates. When TECP and TCP were used, AG-3 and BNR grew slowly compared to controls. Using an arbitrary scale from 1= no growth, to 5= growth equal to control, both isolates show a scale of 4. In contrast, with PCP the two isolates grew with a scale of 2. Also, macroscopic changes were observed in the mycelium of AG-3: concentric rings appeared in presence of CP. And both isolates showed a darker mycelium in presence of PCP. Future efforts will focus on the potential of these fungal isolates to degrade CP.

MI-P02.
CROSS-DOMAIN ANTAGONIC INTERACTIONS IN A SALTERN POND

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Salinibacter rubber is a halophilic bacterium that represents 10-30% of the microorganisms in hipersaline communities. The regulation of its population size consists in one of the research aims of this work. Pure cultures of *S. ruber* were exposed to a water sample from a high salinity pond of a saltern system, as well as to the same water sample previously enriched with 0.3% yeast extract. After two weeks, a clear inhibition in bacterial growth was observed. DGGE analysis performed with these water samples showed an increased content of *Halorubrum sp*. Furthermore, in top agar plates assays performed between this enriched water and *S. ruber* as sensible microorganism, the formation of growth inhibition zones was detected. This could indicate the production and releasing to the media of an antimicrobial compound. Several microorganisms were isolated from this water and the responsible of this effect was identified as *Halorubrum fugingense* (98% homology). To investigate the conditions at which these interactions were produced, cultures of *S. ruber* and two species of *Halorubrum* were grown at different NaCl concentrations. Top agar plates assays where performed and the formation of bigger inhibition zones was observed when *S. ruber* was grown in SW with 10% and 15% NaCl. These results suggest a relation between the sensibility to the antimicrobial compound and the ionic strength of the water.

MI-P03.
EFFECT OF CU(II) AND CR(VI) ON OXIDATIVE STRESS IN *ACTINOMYCETE* STRAINS ISOLATED FROM POLLUTED AREA

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Heavy metals can induce the formation of reactive oxygen species in the cell due to its redox properties. These forms of oxygen damage the cellular constituents causing oxidative stress. However, these molecules are detoxified by superoxide dismutases (SOD) and catalases (CAT). The aim of this work was to study the Cu(II) or Cr(VI) effect on the SOD and CAT activities in two actinomycete strains. *Amycolatopsis sp*. ABO and *Streptomyces sp*. MC1, isolated from contaminated sediments by heavy metals. The microorganisms were incubated in Minimal Medium-glucose supplemented with 10 and 20 ppm Cu(II)/Cr(VI). SOD activity was detected at 24, 48 and 72 h of incubation for native polyacrylamide gel electrophoresis and activity staining. The catalase activity was measured spectrophotometricaly by absorption decreasing at 240nm. Cells incubated in the absence of heavy metals were used as control. Cu(II) and Cr(VI) concentrations enhanced the total SOD and CAT activities from both strains and they increased with incubation time. While *Amycolatopsis sp*. ABO presented two bands, *Streptomyces sp*. MC1 showed a single band with SOD activity. One of the band from *Amycolatopsis sp.* ABO and the band from *Streptomyces sp*. MC1 were sensitive to H_2O_2; thus, they correspond to FeZnSOD. The second band from *Amycolatopsis sp*. ABO were not inhibited neither with H_2O_2 nor N_aN_3, corresponding to MnSOD.

MI-P04.
PARTIAL CHARACTERIZATION OF POLYGALACTURONASE FROM *FUSARIUM GRAMINEARUM*

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Properties of ecosystems are influential in the reduction of plant host cell-wall integrity by pathogen virulence factors. Phytopathogenic fungi are able to secrete lytic enzymes as virulence factors. Among them, pectinases are one of the most important biocatalysts. *F. graminearum* is the causal agent of the Fusarium head-blight (FHB), a destructive disease of wheat. *F. graminearum* isolates from different agroecological regions were cultivated in a medium with 2.5 g/l glucose, 1.25 g/l citrus pectin, 1.25 g/l oat bran and salts, for 2 weeks. An isolate from Marcos Juarez, Argentina, showed the highest levels of polygalacturonase (PGase) activity, at 48 h of culture. PGase was highly active from pH 3 to 5.5, and between 40 and 50ºC, being the optimum pH and temperature 4.5 and 50ºC, respectively. The enzyme was stable from pH 3.5 to 6.5, at 40ºC. Different metal ions (10 mM) were tested on PGase activity, increasing inhibition was observed as follows: $Mg^{2+} < Mn^{2+} < Co^{2+} < Zn^{2+} < Cu^{2+} < Ca^{2+} < Ba^{2+} < Hg^{2+}$. Ion-exchange chromatography was carried out by FPLC with a HiTrap SP anion-exchange column. PGase activity was eluted in a single sharp peak and overlapped with the protein peak. Despite the fact that biological function of PGases are controversial related to plant-pathogen interaction, it is considered as an important parameter in these kinds of interactions.

MI-P05.
CYANOBACTERIAL PHOTOSYNTHETIC ACTIVITY CAN BE ELECTROCHEMICALLY DETECTED ON STAINLESS STEEL

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Aiming to get insight on microbial corrosion mechanisms of stainless steel (SS) in seawater, we investigated the influence of photosynthetic microorganisms (PMO) on the electrochemical behavior of this material. Coupons of UNS30403 SS were exposed to pure cultures of *Synechococcus sp*. (PCC 7002) in the logarithmic phase of growth. Cultures were maintained at 30ºC ± 1ºC under a 16/8 h light/dark photoperiod. Chlorophyll content, photosynthetic activity and pH were periodically determined. The open circuit potential (OCP) of samples was continuously registered, while cathodic polarization curves were performed at each sampling time to evaluate changes in the concentration of dissolved oxygen. Results indicate that the interface of SS is highly sensitive to chemical changes introduced by the photosynthetic process. OCP rises were consistently observed during light hours in spite of the alkalinization produced by photosynthesis. A possible explanation is the depolarization of the cathodic reaction by the increment of dissolved oxygen.
OCP ennoblement is the main cause of failures of SS by microbiologically induced corrosion. Results reported here provide new evidences about the participation of photosynthetic microorganisms in the process. Besides this, the interface sensitivity clearly shows the possibility for the development of whole cell PMO-based biosensors of environmental use.

MI-P06.
IMPORTANCE OF GLUTATHIONE IN GROWTH OF PEANUT RHIZOBIA UNDER CADMIUM EXPOSURE

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Glutathione (GSH) is one of the best known non-enzymatic defense systems. We demonstrated that GSH plays a crucial role in the defense of *Bradyrhizobium* sp. SEMIA 6144 against different environmental stresses. For that, a GSH-deficient mutant (*B*. sp 6144-S7Z) was obtained by disruption of *gsh*A gene which encodes the enzyme γ–glutamylcysteine synthetase. Taking into account that cadmium (Cd) is a toxic heavy metal that induces oxidative stress in microorganisms, our objective was to investigate the effect of this metal on the growth and survival of *B*. sp recommended as inoculants to peanut as well as the role of GSH in the strategy of tolerance to Cd. The wild-type and GSH-deficient mutant of *B*. sp. SEMIA 6144 and *B*. sp. C-145 strains were grown at different concentrations of Cd (0-15 µM) in the YEM medium. *B*. sp. SEMIA 6144 grew up to 15 µM Cd without difference in the GSH content respect to control meanwhile the mutant strain was unable to grow at 15 µM Cd. *B*. sp. C-145 showed a 75% of decrease of growth with an increase in the GSH content at 15 µM Cd. This result could suggest that the GSH content was not enough to avoid the deleterious effect of Cd on the growth of *B*. sp. C-145. In conclusion, our findings show that the strains used as field peanut inoculants differ in the tolerance to Cd being *B*. sp. SEMIA 6144 tolerant and *B*. sp. C-145 sensitive.

MI-P07.
CHARACTERIZATION OF AROMATIC HYDROCARBON-DEGRADING BACTERIA FROM MARINE SEDIMENTS OF PATAGONIA

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Aromatic hydrocarbons are widespread pollutants in the marine environment that can represent a threat to marine life. The aim of this research was to characterize aromatic hydrocarbon degrading bacteria from marine sediments of the Patagonian coast. Using mineral media supplemented with phenanthrene and naphthalene, we isolated three halotolerant strains (PC57, PC515 and PC412), analyzed temperature and salinity growth ranges and evaluated their ability to degrade hydrocarbons. Partial 16S rRNA gene analysis showed that these strains are closely related to *Bacillus subtilis* QW10-11 (99.9%), *Psychrobacter glacincola* ANT9253 (100%) and *Cobetia marina* DSM4741 (100%), respectively. Using a culture-independent approach, we amplified aromatic ring-hydroxylating dioxygenase genes from sediment DNA and the PCR products were cloned and sequenced. Deduced amino acid sequences of two of the analyzed clones were most closely related to the diterpenoid dioxygenase from *Pseudomonas abietaniphila* BKME-9 (58% identity). Two other clones exhibited between 43 and 44% sequence identity with biphenyl dioxygenase from *Rhodococcus erythropolis* TA421, and six clones showed low identity values (33 to 36%) with PAH ring-hydroxylating dioxygenase from *Mycobacterium vanbaalenii* PYR-1. These results show the presence of diverse aromatic degrading bacteria in this coastal marine environment.

MI-P08.
INCIDENCE OF ARTIFICIAL UV-B RADIATION ON BACTERIAL COMMUNITY COMPOSITION IN TWO ANDEAN WETLANDS

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Laguna (L.) Negra and L. Verde are oligotrophic Andean wetlands, similar in altitude and environmental conditions, but different in salinity level. In this study we compared artificial UV-B radiation (UVR) incidence in bacterioplankton community of these two lakes. We have carried out expositions of both wetlands water to artificial UVR in the laboratory during 24 hrs. Samples were extracted at different times for determination of cultivable bacteria, by plating in two kinds of broth with different salinities and identified by rDNA 16S sequence. Total community composition was studied by DGGE profiles with subsequent sequencing of the bands. DNA damage was determinated with CPDs accumulation. We found 32 DGGE bands in L. Negra and 13 in L. Verde (13 and 2 corresponding to UVR resistance bacteria, respectively). In these experimental conditions, 32 strains were isolated from L. Negra and 12 from L. Verde. DNA damage was increased in L. Negra total DNA and L.Verde total DNA from 0 to 1700 CPDs MB-1 and 1500 CPD MB-1, respectively. Bacteroidetes represented an important fraction of bacterial diversity and the community was enriched in this organism by UVR stress in both lakes. These results suggest that abundance of bacteroidetes in highly irradiated environments might be connected with their UV resistances rather than with their ability to survive in halophile environments.

MI-P09.
DENITRIFIER COMMUNITY COMPOSITION ALONG NITRATE GRADIENT IN SUQUIA RIVER BED-SEDIMENT
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To understand the composition and structure of denitrifying communities along nitrate gradient in the Suquía River, the molecular diversity of *narG* gene from sediments obtained at seven stations was examined. The *narG* gene encoding the membrane bound nitrate reductase was selected as a functional marker for the nitrate-reducing community. The composition was analyzed using RFLP of *narG*, together with cloning and sequencing. In addition, a fingerprint of the total bacterial community was assessed by RISA analysis.
The results show different *narG* RFLP patterns in each community site. Two libraries of the DNA samples of the most distant sites were constructed. The clones were subjected to RFLP analysis and grouped. Nine and 20 different sequences from each library were obtained with all *narG* clones exhibiting between 62-90 % identity at the amino acid level to those of cultivated denitrifiers and other environmental clones in the database. *NarG* sequences clustered into three major groups supported by phylogenetic analyses and, all sequences amplified from environmental DNA clustered in the Gram-negative group. The results indicate that some populations were indeed distinct, although further sequencing would be required to fully characterize the highly diverse denitrifying communities at all sites.
Supported by CONICET, FONCyT and SECyT-UNC.

MI-P10.
BROAD SPECTRUM OF POTATO ASPARTIC PROTEASES (STAPS) CYTOTOXIC ACTIVITY AGAINST POTATO PATHOGENS
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Plant aspartic proteinases (EC 3.4.23) (APs) have been involved in the plant defense response. We have previously reported the purification of two potato APs (StAP1 and StAP3) that are induced by abiotic and biotic stresses and have antimicrobial activity towards the fungus *F. solani* and the oomycete *P. infestans*. The aim of this work was to characterize those antimicrobial activities and to determine the antimicrobial activity of StAPs towards others potato pathogens: the actinomycete *Streptomyces scabies* and the bacterium *Erwinia carotovora*. The results indicate that both StAPs are able to reduce the growth of *S. scabies* and *E. carotovora* cultures in a dose-dependent manner. Besides, StAPs have a potent cytotoxic activity and the lethal doses necessary to reduce the viability by 50% (IC50) are: 2.5 µM StAP1 and 7.75 µM StAP3 for *F. solani*, 5 nM StAP1 and 370 nM StAP3 for *P. infestans*, 1.5 µM StAP1 and 1.2 µM StAP3 for *S. scabies*, 3.7 µM StAP1 and 3.75 µM StAP3 for *E. carotovora*. Assays with FITC-labelled StAPs show that proteins interact directly with the surface of pathogens. Additionally, StAPs produce in all microorganisms assayed, the generation of ROS and cellular death. The results obtained here demonstrate the broad spectrum of StAP1 and StAP3 cytotoxic activity in vitro against four different potato pathogens, suggesting their potential role in plant potato protection.

MI-P11.
A NOVEL RIVET SYSTEM SUITABLE FOR THE STUDY OF PLANT-BACTERIA INTERACTIONS
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In cases where bacterial gene expression specifically occurs in biological environments of difficult access, RIVET (Recombination-based *In Vivo* Expression Technology) can be a suitable approach to search for markers that are differentially expressed only under those particular conditions. We developed a new RIVET variant based on the appearance of a gentamycin (Gm) resistance upon expression of genes of interest. The system was designed to be suitable for the study of plant-bacteria interactions where other selection markers such as sacB may be useless due to the presence of sucrose in several plant compartments. The expression of the gamma-delta-Tn-resolvase, TnpR, results in: a) the excision of an *nptII* (Nm resistance) cassette flanked by res sites, and b) the simultaneous appearance of a transcriptional fusion *aacCI-gfp* leading to a Gm resistant-green fluorescent phenotype. Using the N2-fixing legume-symbiont *Sinorhizobium meliloti* we tested the new RIVET tool with two promoters. While the expression of *tnpR* from the constitutive *p-nptII* promoter resulted in the excision of the *nptII* cassette, expression of *tnpR* from the symbiotic *p-nifH* promoter generated Nm(s)-Gm(r) clones only when rhizobia where recovered from 3-week old (N2-fixing) alfalfa nodules. The validated system will now be used to try unraveling new rhizobial markers expressed early in infection thread formation.

MI-P12.
CHARACTERIZATION OF AN *SINORHIZOBIUM MELILOTI* ACID SENSITIVE MUTANT
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Soil acidity may reduce the productivity of alfalfa plants mainly derived from the marked acid-sensitivity of the prokaryote symbiont *Sinorhizobium meliloti* which cannot grow at pH below 5.5. The identification of genetic determinants of tolerance in *S. meliloti* has been considered as an important target of research. The molecular mechanisms of acid tolerance has shown to be complex and need additional fundamental research to understand how rhizobia protect themselves from the extracellular hydrogen ions, and how they infect the plant symbiont. To advance on the genetics of acid tolerance in *S. meliloti* we generated a collection of *Tn*5B20 mutants and we screen the collection for acid sensitive phenotypes. Three mutants out of 2,000 clones failed to grow on minimal medium at pH 5.5 but not at neutral pH. Genetic analysis of one of them, namely mutant LPU630, showed a transposon insertion within an ORF with sequence similarity to a putative ABC transporter with an unusual central domain located immediately upstream of a putative transcriptional regulatory protein. We will now investigate the impact of the new marker on the ability of rhizobia to tolerate other abiotic stresses, and on the capacity to do symbiosis under moderate acid media.

MI-P13.
EFFECT OF SOLAR UVR ON VIABILITY AND OXIDATIVE STATUS OF TWO ANTARCTIC MARINE BACTERIA

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The ozone hole causes an increase in the UVB radiation, determining an increasing damage on aquatic microorganisms. The aim of this work was to study the effects of solar UVB and UVA radiation on the viability and some parameters of oxidative status of two Antarctic marine bacteria: *Arthrobacter* UVvi and *Bizionia* UVps. Studies were made under different irradiance conditions near Jubany station, Antarctica. Quartz bottles containing bacterial cultures were exposed to solar radiation. Four treatments were performed using interferential filters: Dark, PAR, PAR+UVA and PAR+UVA+UVB. Incident UVR, viability, catalase, superoxide dismutase and glutathione S-transferase activities as well as protein and lipids oxidation were measured. Both strains showed a significant mortality ($p<0.05$) under PAR+UVA and PAR+UVA+UVB. Glutathione S-transferase activities, protein and lipids oxidation did not differ ($p>0.05$) between treatments. Catalase activity decreased in 98% (UVps) and 72% (UVvi) under PAR+UVA+UVB treatment. UVps showed a similar decrease under both, PAR and PAR+UVA. Only UVps showed a significant increase (300%) in SOD activity under UV treatments ($p<0.05$). Results showed that activity of these Antarctic bacteria is deeply affected by the exposure to solar UVR and suggested that the higher sensitivity showed by UVps to UVR could be related to a significant reduction in catalase activity.

MI-P14.
BIOREMEDIATION OF ANTARCTIC SOILS: STRATEGIES TOWARDS THE SUCCESS OF BIOAUGMENTATION

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Bioaugmentation of chronically contaminated soils to increase biodegradation is usually unsuccessful. This failure has been attributed to predation and competition by the microbiota present in the target soil. Also the size of the inoculum seems to be something to consider when biomagnification is applied. In a field trial (soil plots) we observed by T-RFLP analysis that only one of the major populations of a bacterial consortia (M10), inoculated at the same density as that of the indigenous culturable bacteria per gram of soil, seemed to survive only a few days. This population was also present in soil microbiota and responded quickly to nutrient addition. Thus, we studied in microcosms the success in the settlement of M10 in biostimulated (N+P) sterile and non-sterile hydrocarbon-contaminated soil, bioaugmented at densities of 10^6 CFU/g and 10^9 CFU/g. Biostimulation alone favored the growth of both, non-degrading and degrading bacteria. In comparison, adding M10 at 10^6 CFU/g showed no differences by day 15. Finally, biomagnification with 10^9 CFU/g led to an increment in total and degrading bacterial counts by day 10, dropping then to similar counts than the other systems by day 30. The results showed that the size of inoculum should be higher than that of the autochthonous microbiota to overcome predation and competition and improve the efficiency of the process.

MI-P15.
IDENTIFICATION OF LIPOPEPTIDES INVOLVED IN BIOLOGICAL CONTROL PRODUCED BY NATIVE *BACILLUS* STRAINS

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Some *Bacillus* species are able to produce a diverse set of antibiotics compounds against phytopathogenic fungi. Many of them belong to the family of cyclic lipopeptide synthesized by large peptide synthetases. These amphiphilic cyclic biosurfactants were suggested to play an important role in biological activities and they have many advantages over traditional pesticides: low toxicity, high biodegradability, and environmentally friendly characteristics. From a bacterial collection, isolated form Córdoba soils, we have selected four isolates belonging to *Bacillus* genus capable to inhibit the growth of several *Fusarium* and *Sclerotinia* species *in vitro*. At least one gene encoding peptide synthetases was detected by a PCR assay in all isolates. The antifungal compounds were isolated by acid precipitation of cell-free supernatants, purified by RP-HPLC and then tested for antagonistic activity against *S. sclerotiorum*. MALDI-TOF mass spectra analysis of two major active fractions of *Bacillus* sp. A6 showed molecular ion peaks similar to iturin A (m/z 1056.6 Da as $[M+H^+]$). *Bacillus* sp. $AR_2 3$ showed two mainly peaks at m/z 1044.7 and 1058.7 Da as $[M+Na^+]$ corresponding to surfactin isoforms Leu/Ile-7 C_{14} and Leu/Ile-7 C_{16}, respectively. These results indicate that isolates A6 and $AR_2 3$ produce antifungal lipopeptides related to the iturin and surfactin families respectively.

MI-P16.
DEHYDROGENASES/REDUCTASES INVOLVED IN SALT TOLERANCE IN *OCHROBACTRUM*

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In plants and microorganisms, salt stress regulates the expression of a large number of genes. In such conditions, bacteria must be able to both respond immediately to osmotic shock and sustain growth under these conditions. *Ochrobactrum* sp. 11a employs adaptive responses that evolved for survival in diverse and often stressful environments conditions such as heavy metals, antibiotics and high levels of salt. In the present study, by *Tn*5 mutagenesis we have identified two genes involved in salt tolerance in *Ochrobactrum* sp. 11a. The nucleotide sequence adjacent to the *Tn*5 insertion in each mutant showed high levels of identity to acyl CoA dehydrogenase and short chain dehydrogenase/reductase (SDR) respectively. The effects of another osmotic and heavy metals stress were analyzed and different effects were observed in both mutants. The SDR and acyl CoA dehydrogenase shared a 98% and 100% of identity with the orthologs of *Ochrobactrum anthropi* ATCC 49188, respectively. SDR are important contributors to the catabolic capacity of bacteria in natural environments such as soil and wastewaters and the acyl CoA dehydrogenases are involved in changes of membrane fluidity in response to hyperosmotic stress. Modulating the composition of cell envelope and reinforcing the metabolism might play an important role in combating environmental stress in free-living bacteria.

MI-P17.
GLYCOSYL TRANSFERASES INVOLVED IN THE LPS-CORE BIOSYNTHESIS FROM BACTERIAL PATHOGENS AND SYMBIONTS

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LPS mutants of several rhizobia were shown to be altered in their symbiotic association with their host-legumes, as it is the case for *lpcC* mutants of *R. leguminosarum* lacking the mannosyl transferase LpcC, which are affected in the LPS-core and unable to form functional nodules on *Pisum sativum*. *S. meliloti* mutants altered in the LPS-core were found to be delayed in nodulation and poorly competitive for nodule occupancy on *Medicago sativa*. Complementation studies demonstrated that LpsB from *S. meliloti* is able to complement the *lpcC* mutation of *R. leguminosarum*. However, *lpcC* from *R. leguminosarum* was not able to restore the symbiotic deficiency of the *S. meliloti lpsB* mutant. LpsB and LpcC orthologs are widely distributed among either, pathogens or symbionts bacteria such as *A. tumefaciens*, *B. japonicum*, *M. loti*, *B. melitensis*, *B. henselae*, etc. In order to evaluate the biochemical and physiological significance of such conservation we performed site-directed mutagenesis and heterologous complementation. Preliminary results, showed that *lpcC* from *B. japonicum* complemented the *A. tumefaciens lpcC* mutation restoring the wild type LPS phenotype while, *rfaC* from *M. loti* R7A, and *lpsB* from *B. henselae* did not. On the other hand, *lpcC* from *B. japonicum*, *lpcC* from *A. tumefaciens*, *lpsB* from *B. henselae*, and *rfaC* from *M. loti* were unable to complement the *S. meliloti lpsB* mutant.

MI-P18.
ISOLATION AND CHARACTERIZATION OF PLANT GROWTH-PROMOTING *PSEUDOMONAS* FROM THE RHIZOSPHERE OF MAIZE

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The aim of present study was to isolate PGPR *Pseudomonas* spp. from *Zea mays* grown in field of Cordoba region. Bacterial strains were isolated from bulk soil and rhizosphere and endorhizosphere of maize plants at different growth stages (flowering plants, in December, and physiological maturity, in March). Gram stain, biochemical tests and genetic analyses were performed. Isolates belonging to *Pseudomonas* genus were screened by their ability to solubilize phosphate, to produce siderophores, indole acetic acid (IAA), extracellular enzymes and to inhibit phytopathogens fungi. Approximately, 240 isolates were obtained from maize root and soil in King B and Gould´s S1 medium. Gram negative, oxidase and catalase positive, motile and producer of pigment strains were confirmed by PCR as belonging to fluorescent *Pseudomonas* group. The highest percent of PGPR characteristic was observed among *Pseudomonas* populations from March. On the other hands, of strains from December, 39% were able to solubilize phosphate, 78%, 22% and 67% produce proteases, cellulases and siderophores respectively. Some isolates were able to produce IAA and others could inhibit the growth of *Rhizoctonia solani in vitro*. Conclusion: we obtained several *Pseudomonas* spp. from maize root with PGPR trait in vitro. In the future, promotion of plant growth by these strains should be analyzed in greenhouse conditions.

MI-P19.
HIERARCHICAL CONTROL OF PHA BIOSYNTHESIS IN AN ANTARCTIC *PSEUDOMONAS* STRAIN

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Pseudomonas sp. 14-3 is an Antarctic strain isolated in our laboratory that accumulates high amounts of polyhydroxyalkanoates (PHA) in form of polyhydroxybutyrate (PHB) a polymer composed by short chain length monomers. Recently, PHB genes have been characterized. This strain accumulates only PHB when grown on a medium supplemented with octanoate and no other medium chain length polymer is detected. However, analysis of the polymer production in a *phaC* mutant strain, unable to accumulate PHB, showed PHA accumulation. In this work we looked for PHA genes in the mutant strain, quantified its PHA production and analyzed the phylogenetic relationships of these genes. Determination of the composition of polymer showed that the wild type and the complemented mutant strain produced only PHB indicating the functionality of classI polymerase (PhaC). By contrast, the mutant strain produced PHA composed by C8 monomers (14.4 ± 1.2 wt %) suggesting that a classII polymerase is involved and a probable hierarchic control of PHA synthesis. PCR amplification and sequencing allowed identify a classII polymerase (*phaC2*) and phylogenetic analysis showed its association with other *Pseudomonas* species. These results in line with other literature data could suggest an evolution of PHA biosynthesis in *Pseudomonas* by gene displacement that probably is associated with a complex net of regulatory mechanisms.

MI-P20.
ABSORPTION OF UV RADIATION BY METABOLITES OF NAPHTHALENE DEGRADATION IN *PSEUDOMONAS FLUORESCENS*

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In sunlight exposed environments, solar UV radiation might affect biodegradation of hydrocarbons spilled in water. As an approach to this subject, a naphthalene-degrading *Pseudomonas fluorescens* strain was isolated from a slick at Tigre River (Bs As) and its UV response during naphthalene utilization was studied. Increased UV sensitivity and accumulation of UV absorbing compounds in the medium were observed. The identification of these compounds was the objective of this study. A protocol including ethyl acetate extraction, chromatography on molecular sieves and reverse phase HPLC was applied to spent medium after growth with naphthalene. Pyoverdine was detected, in addition to compounds absorbing at $\lambda<350$ nm, which seem to be catechol derivatives since similar peaks were observed after catechol incubation in sterile medium. Strong absorption of spent medium at 375 nm was not produced by catechol since absorbing material remained in aqueous phase after ethyl acetate extraction. Acidification with HCl and extraction with diethyl ether revealed accumulation of muconic semialdehyde during culture growth explaining the absorbance observed at 375nm.
During naphthalene degradation, siderophores and metabolites could act as either photo-protectors or photo-sensitizers. This fact should be considered in design and interpretation of assays involving irradiation of growing cultures.

MI-P21.
MICROBIAL DIVERSITY IN PRISTINE, DEFORESTED AND AGRICULTURAL SOILS FROM THE YUNGAS IN ARGENTINA

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Although the Yungas ecoregion is well known for its enormous diversity of flora and fauna, no studies looked at the soil microbial diversity. We analyzed soil samples from native forest, mountain and pedemountain forests, a field recently deforested and cultivated with soybean, and fields with sugarcane (40 or 100 years) or soybean (20 years) monoculture. PCR-DGGE analysis of 16S rRNA genes was used to study whether differences exist among bacterial communities inhabiting these soils. Characteristic DGGE fingerprints were obtained for the soil microbial community from each sampling site. Cluster analysis based on DGGE banding patterns indicated that soil bacterial communities from sugarcane fields were similar to each other but different from that characterizing the pristine adjacent soil. Coincidentally, DGGE profiles of bacterial communities of the recently deforested soil were similar to those of a soybean field with 26 years of agriculture and significantly different from those of the adjacent native forest. Microbial communities from forest mountain soils clustered separately from all the others communities and also showed the highest bacterial diversity as revealed by the number of bands in their DGGE profiles. The structure of bacterial communities was different among the soils analyzed and these differences depended on geographical location and their agricultural use.

MI-P22.
POSTHARVEST OXIDATIVE TREATMENT AGAINST THE CITRIC FRUITS PATHOGEN *PENICILLIUM DIGITATUM*

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Infections by *Penicillium digitatum* and *italicum* generate the green and blue mold on citric fruits. In order to avoid the attack of these pathogens, fruits are intensely treated with synthetic fungicides, leading to the proliferation of resistant strains and to the international markets rejection of fresh fruits due to the increment of toxic residues. The aim of this work was to generate some alternative treatments to eliminate or to minimize the use of fungicides. Oxidative treatments with peroxides and heavy metal salts were applied on spores of *P. digitatum*. The results indicate that these spores lost viability when they were treated with $CuSO_4$ and H_2O_2 after a brief incubation with NaClO. Thus, a lethal standard protocol (LSP) for the pathogen was elaborated, using 10 ppm NaClO, 6 mM $CuSO_4$ and 50 mM H_2O_2. The spores treated with the LSP and analyzed by electron microscopy showed drastic alterations in cellular structures, although the damage was not observed at the wall level. In addition, a decrease in the infectivity of the spores was observed after this treatment. The oxidative procedure presented here, could be an important tool that may be applied in packinghouses, replacing the fungicides in the postharvest manipulation of fruits and protecting the human health and the environment.

MI-P23.
ANTIBIOTIC ACTIVITY OF A *BACILLUS AMYLOLIQUEFACIENS* SUPERNATANT AGAINST *XANTHOMONAS* SP

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Using a screening method to find new antibiotics, a bacterial strain producing an antagonistic compound against *Xanthomonas* sp. was isolated from a water sample. Genus and speciation determination were carried out by 16S rDNA and *gyrB* sequencing and subsequent analysis of the sequences using BLAST. The strain was identified as *Bacillus amyloliquefaciens*. The antibiotic produced by this strain showed activity against different field isolates of *Xanthomonas axonopodis* pv. *citri* (citrus canker agent) and *Xanthomonas campestris* pv. *campestris* (black rot agent). This activity was measured by the agar-disk diffusion assay. The antimicrobial substance diffused through a dialysis membrane with a 12 kDa cut off and maximum activity was observed at stationary growth phase. Release of this substance was enhanced in a minimal medium compared with a rich medium. The antibiotic was resistant to all proteases tested (trypsin, chemotrypsin and proteinase K). Thermal sensitivity was also tested by exposing the culture supernatant to temperatures of 121ºC for 30 minutes resulting in a partial antimicrobial activity decrease. The compound showed stability when was exposed to extreme pHs (3-11). Identification and characterization of this compound produced by *B. amyloliquefaciens* may represent a novel antibiotic with potential application in biological control of pathogenic microorganisms.

MI-P24.
INFLUENCE OF BRADYRHIZOBIA LOCATION AND MOVEMENT ON BIOLOGICAL NITROGEN FIXATION

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We previously reported that the lack of response to peanut inoculation in soils of Argentina may be due to the presence of a competitive indigenous population of rhizobia. In the present study, ERIC-PCR analysis of wild-type and native strains showed different electrophoretic patterns produced with ERIC primers. The wild type strain C-145 (TAL-1371) and indigenous isolates each exhibited a unique profile, indicating that peanut nodulating rhizobia are highly diverse. Based on these results, we did field experiments in which the strain C-145 resistant to gentamicin at 80 $\mu g\ ml^{-1}$ (C-145 Gm^r) was inoculated in different ways so that distribution of strains was altered. This mutant provides a useful tool to evaluate nodule occupancy in soil environments since we confirmed that native strains are sensitive to gentamicin. Significant differences were observed in biological nitrogen fixation, such as nodule number, symbiotic effectiveness, nitrogenase activity and plant dry biomass for in-furrow inoculation compared to seed inoculation or control treatments. Nodule occupancy of the C-145 Gm^r strain was very high for in-furrow inoculation, indicating a positional advantage or movement into soil of in-furrow inoculated rhizobia for nodulation in peanut.

This work was supported by ANPCyT, CONICET and SECYT-UNRC.

MI-P25.
SELECTION AND EVALUATION OF NATIVE PEANUT NODULATING ISOLATES FOR THEIR USE AS POTENTIAL INOCULANTS

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Rhizobia are soil bacteria able to attach to legumes roots and induce the formation of nodules where they fix atmospheric nitrogen to the plants. The soil inoculation with nitrogen fixing bacteria is a risk-free and cost-effective alternative to the chemical fertilization. Rhizobial inoculants should contain a high number of efficient and competitive strains. The objectives of this work were (i) the identification of effective and competitive native peanut nodulating strains, (ii) the evaluation of the optimum culture medium for the production and commercialization of peanut inoculants. From 220 native isolates obtained from nodules of peanut plants (Vincent, 1970) growing in different localities of Córdoba (Argentina), we selected two (named J-81 and J-237) considering their high effectiveness (measured as shoot dry weight) and competitiveness (Somasegaran and Hoben, 1994) when compared with the reference strains *Bradyrhizobium sp* SEMIA 6144 and C-145. Their growth and viability were evaluated in different culture media. For both, the number of viable cell reached in a balanced medium was 1010 cfu/ml while in YEM, YLG, YG and LV this value was 109cfu/ml. Our results demonstrated the better symbiotic behavior of these isolates in microcosm assays.

Supported by SECyT-UNRC, CONICET, ANPCyT.

MI-P26.
***OITHONA NANA* FROM THE ARGENTINE SEA: TOWARDS AN ACCURATE TAXONOMIC IDENTIFICATION**

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Recent studies have demonstrated the numerical and ecological importance of cyclopoid copepods, particularly the genus *Oithona*, in coastal marine ecosystems. There are still, however, many uncertainties concerning their taxonomy, the cornerstone of any ecological study. Because of the small size of these copepods, the application of conventional microscopy methods became insufficient, and in this case a molecular approach may provide an useful complement. Bearing this in mind, we proposed to advance into the correct identification of *Oithona nana* in the Argentine Sea, by molecular analysis. The first step was to sequence the 28S ribosomal subunit from females of the species obtained from the type locality: Gulf of Naples, Italy. Samples were taken with a 200μm mesh size and preserved in 95% ethanol. From these samples, one specimen female was placed and squashed in a PCR tube. The 28S ribosomal was amplified using universal primers P63 and LR6 and the product was cloned and sequenced. The identity of the nucleotide fragment was tested using BLASTn and then the corresponding dendrogram was generated. This result constitutes the first step towards an accurate species identification of *O. nana* in the Argentine Sea. Our next goal will be to compare this sequence with the native one to test their homology.

MI-P27.
DISPERSION OF ANTIBIOTIC RESISTANT BACTERIA BY FLAMINGOES IN EXTREME HIGH ALTITUDE WETLANDS

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In Argentinean Andes, at high altitude oligotrophic lakes, present extreme environmental characteristic: high salinity, UV radiation, heavy metal and flamingoes presence that are known to migrate between these lakes. The aim of this work was to determine if bacterial ATB resistances was an event related with the extreme condition of environment and the possible dispersion of those bacteria by flamingoes migration. We studied ATB resistance profiles in bacterial community from bacterioplancton and flamingoes faeces by genomics methods (DGGE) in two wetlands: L. Negra and Aparejos (Argentina Catamarca), at 4400 masl with low As content. We included an external control, L. Chaxas (Chilean Atacama) at 2000 masl, which means lower UV exposition and higher As content. Most of the water and feces OTUs represented by γ-proteobacteria (*Stenotrophomonas, Aeromonas*) in L Negra and Aparejos. β-proteobacteria (*Comamonas, Duganella*) was also identified exclusively in L. Aparejos. The presence of Firmicutes (*Bacillus, Clostridium*) was indicated in the three studied environments. However many bacteria were only found in faeces those are the cases of typical enteric bacteria like *E. coli* and *Rahnella*. The colistine resistant bacteria were the most abundant. Mostly bands identified in water were also found in faeces, denoting that the birds enteric biota is in close interaction of water lake diversity.

MI-P28.
FLAGELLAR CAP PROTEIN IS INVOLVED IN THE EARLY STAGES OF *XANTHOMONAS* BIOFILM FORMATION

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Xanthomonas campestris pv. *campestris* (Xcc) is the causal agent of black rot, which affects crucifers such as *Brassica campestris* and *Arabidopsis thaliana*, and *Xanthomonas axonopodis pv. citri* (Xac) is the causative agent of citrus canker. Previous works in our laboratory demonstrated that these bacteria were able to produce a functional biofilm, a community of bacteria embedded in an extracellular matrix attached to a surface. This structure is believed to provide protection to bacteria from environmental aggressiveness. By using confocal laser scanning microscopy, we analyzed the biofilm formation of both Xcc *fliD* and Xac *fliD* mutants, involved in flagella structure, comparing with wild types Xcc and Xac, respectively. The flagellar cap protein FliD is localized in the tip of the flagella structure. As demonstrated in other bacteria, such as *Pseudomonas aeruginosa* and *Vibrio chloerae*, the flagella showed to be important in early attachment processes and our results showed that this protein could be implicated in adhesion of bacteria to the surface of the chambers but not in the maturation of the biofilm. These mutants were able to form organized structures however these were diminished in surface attachment.

MI-P29.
INTRACELLULAR MOBILIZATION OF POLYHYDROXYBUTYRATE. EFFECT ON CELLULAR AGGREGATION IN *AZOSPIRILLUM BRASILENSE*
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Bacteria of the genus Azospirillum are able to accumulate polyhydroxybutyrate (PHB). The objective was to study the mobilization of PHB and the cellular aggregation in different systems. *Azospirillum brasilense* Sp7 was incubated in different culture media with minerals salts, fructose, KNO_3 and NH_4Cl. Yeast extract were from 0 to 1,0 mg/ml. The accumulated PHB was measured by the formation of the crotonic acid and by epifluorescence microscopy. Aggregation was determined by the relationship between the optic density of the culture static 20 minutes and homogenized. The accumulation of PHB was 2.4 times higher when it was cultivated with KNO_3 instead of NH_4Cl. The yeast extract affected the accumulation of PHB. With 1 mg/ml in the culture medium the intracellular PHB was mobilized. This effect was not observed at lower concentrations. When yeast extract concentration was 0 or 0,4 mg/ml the aggregation increases along the incubation time, while at 0,7 or 1,0 mg/ml the aggregation diminishes. The accumulation of PHB was bigger agitated than static. However cellular aggregation was similar in both cases. These results would indicate the complexity of the processes that are involved in the accumulation of PHB and the cellular aggregation. The presence of yeast extract (high content in amino acids, nucleic acids, vitamins, etc), is probably related to the cellular morphogenesis along the time.

MI-P30.
PROLIFERATIVE FEATURES AND FIRST EVIDENCE OF ORNITHINE DECARBOXYLASE IN *PHYTOMONAS* JMA
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The Phytomonas genus comprises trypanosomatid flagellates infecting a wide variety of plants. Despite *Phytomonas spp.* produce important economic losses and are phylogenetically close to human pathogen parasites, their life cycle and biochemical features are still poorly known.
In this work we have studied the *in vitro* growth kinetic in *Phytomonas* Jma promastigotes cultured in different media (LIT, BHT and SDM79). Cultures were started at $1x10^6$ cells/ml and were counted periodically. In all cases the doubling time in log phase was ~20h and the maximal cell densities were ~$9x10^7$, in all media. *Phytomonas* growth was not affected by the inhibitors of polyamine biosynthesis DFMO or CHA separately, but was markedly reduced when both inhibitors were combined, reducing doubling time and maximal densities to $2x10^7$cells/ml. As an initial step for the characterization of the polyamine metabolism in *Phytomonas* Jma, we measured ornithine decarboxylase (ODC) activity; it was dependent on the growth phase, being increased at log phase (from 20 to100 pmol/h/mg prot) and was inhibited by DFMO as much as *Crithidia fasciculata* ODC, a related trypanosomatid used as control. As these are preliminary results, further investigation on polyamine metabolism is currently underway in our lab.

MI-P31.
GENERATION OF NEW TOOLS TO STUDY THE ROLE OF AURORA KINASES IN CHROMATIN DYNAMICS IN *TRYPANOSOMA CRUZI*
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Aurora kinases (AUKs) have been implicated in regulating several cellular processes such as chromosome segregation and cell division. Three isoforms of these proteins, aurora A, B, and C, have been identified in mammals. Consistent with their localizations, AUK A regulates spindle assembly, AUK B controls chromosome segregation and cytokinesis initiation and AUK C was found in testis and certain tumor cell lines. Previously, we reported the finding of three AUK homologues (TcAUK1, 2 and 3) in *Trypanosoma cruzi*. The protein products were obtained by expression in *Escherichia coli* but we failed to measure the enzymatic activity by using H2AS Histone mix from Sigma and Myelin Basic Protein as substrates. Taking into account the differences between the *T. cruzi* histones and their orthologs from other eukaryotes, we decided to perform the cloning and expression of the H3, H3 variant and H4 *T. cruzi* histones in bacterial systems. In addition, to investigate the AUK1 sub-cellular localization, we are using affinity purified recombinant protein to generate specific antiserum in mouse. Finally, to obtain new knowledge on the function of AUK1 in vivo we transfect epimastigote cells with the pTREX expression vector carrying the coding sequence of this enzyme. These evidences not only point out a conserved pathway along the evolution but also open new insights in drug therapy against Chagas' disease.

MI-P32.
A NATURAL ANTIVIRAL TETRANORTRITERPENOID WITH IMMUNOMODULATORY PROPERTIES
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The 1-cinnamoyl-3,11-dihydroxymeliacarpin (CDM), isolated from purified leaf extracts of *Melia azedarach* L., exhibits anti-HSV-1 activity, inhibits HSV-1-induced NF-kB translocation and increases TNF-alpha production in an infected macrophage cell line (J774A.1). Also, in the presence of CDM, murine peritoneal macrophages stimulated with HSV-2 increment TNF-alpha secretion. To extend our understanding about CDM properties we investigated its effect on NF-kB activation and cytokine secretion in J774A.1 cells and peritoneal macrophages induced with herpes viral and no-viral stimuli. Results showed that HSV-2 was not able to multiply in peritoneal macrophages, neither induced persistent NF-kB translocation nor IkB-alpha degradation, as determined by immunofluorescence and Western blot assays, respectively. On the contrary, HSV-1 induced IkB-alpha degradation in infected J774A.1, which was prevented by CDM. Besides, the production of TNF-alpha in LPS-stimulated J774A.1 cells was strongly enhanced by CDM, as well as in peritoneal macrophages, as determined by a biological assay. Our data suggest that CDM would prevent NF-kB translocation induced by virus multiplication, as a consequence of its antiviral activity. Furthermore, CDM could enhance TNF-alpha production regardless the stimuli involved. Taken together, this study shows that CDM exhibits immunomodulatory properties.

MI-P33.
INHIBITION OF VSV-INDUCED NF-KB TRANSLOCATION BY CDM, A NATURAL BIOACTIVE COMPOUND

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The tetranortriterpenoid 1-cinnamoyl-3,11-dihydroxymeliacarpin (CDM), isolated from purified leaf extracts of *Melia azedarach* L., displays antiviral activity and nuclear factor kB (NF-kB) modulating properties. CDM is able to inhibit vesicular stomatitis virus (VSV) multiplication in a human lung epithelial cell line (A549) by pre or post-treatment. To increase our understanding about CDM action, we investigated its effect on the NF-kB activation induced with VSV and TNF-alpha in A549 cells. Preincubation or simultaneous addition of CDM neither restrained TNF-alpha-induced translocation of NF-kB nor degradation of IkB-alpha, as determined by immunofluorescence and Western blot assays, respectively. VSV induced translocation of NF-kB and degradation of IkB-alpha at 6 h post-infection. When added 2 h before infection, CDM completely blocked VSV-induced NF-kB activation; however, this effect was not observed in CDM-post-treated infected cells. We conclude that CDM would not interfere with TNF-alpha-induced NF-kB activation in this epithelial cell line, but would prevent VSV-induced NF-kB translocation. Since CDM induces cytoplasmic alkalinization in A549 treated cultures, the mechanism involved in NF-kB inhibition would be by impeding VSV uncoating in pre-treated cells.

MI-P34.
IMMUNOMODULATING ACTIVITY OF A SYNTHETIC BRASSINOSTEROID ANALOG

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HSV-1 induces an ocular chronic inflammatory syndrome named Herpetic Stromal Keratitis (HSK), that can lead to blindness. The synthetic brassinosteroid 2, or compound 2, is a potent antiviral *in vitro* and reduces the incidence of murine HSK, although it does not exert an antiviral effect *in vivo*. In order to explain the immunomodulating action of the compound that would account for the improvement of murine HSK, we investigated the role of compound 2 in the modulation of the response of epithelial and immune cells to viral and non-viral stimuli. Immunofluorescence assays showed that compound 2 blocked HSV-1-induced nuclear translocation of NF-kB in epithelial HCLE and NHC cells, as well as significantly reduced the secretion of TNF-α in NHC, as determined by ELISA. IL-6 production was enhanced by compound 2 after HSV-1 infection in both cell types, while IL-8 levels remained unchanged. Despite NF-kB translocation was not inhibited in LPS-stimulated macrophages after treatment with compound 2 *in vitro*, the production of TNF-α and IL-6 was significantly reduced, as determined by a biological assay and ELISA, respectively. Compound 2 would be playing a modulating effect as an inductor or inhibitor, depending on the cell type and stimuli involved. The improvement of murine HSK could be a balance between stimulating and suppressive effects of compound 2 *in vivo*.

MI-P35.
***BRUCELLA ABORTUS* STRAIN S19 AS AN EXPRESSION VECTOR FOR *BABESIA BOVIS* RHOPTRY-ASSOCIATED (RAP1)**

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Brucella abortus strain 19 (live vaccine) induces a strong humoral and cellular immune response and therefore, it is an attractive vector for the delivery of heterologous antigens. The objective of the present study was to study the cellular immune response of recombinant strains of *B. abortus* S19 expressing the rhoptry associated protein (RAP1) of *Babesia bovis*, as a model for heterologous expression of antigens from veterinary pathogens. In a previous study, we have reported that recombinant strains of *B. abortus* S19 expressing RAP1 as a fusion protein either with the first aminoacids of β-galactosidase (S19pBB-RAP1) or *B. abortus* OMP19 (S19pBB19-RAP1) developed specific humoral immune response to RAP1, being IgG2a the predominant antibody isotype in mice immunized with these strains. In this work, we showed that a specific cellular immune response to recombinant RAP1 was elicited in vitro by lymphocytes from mice immunized with both strains. Furthermore, studies with mice indicated that expression of RAP1 did not alter either the attenuation characteristics of strain S19 or its vaccine efficacy against *B. abortus* 2308 challenge. Therefore, we concluded that *B. abortus* S19 expressing RAP1 is immunostimulatory and may provide the basis for combined heterologous vaccines for babesiosis and brucellosis.

MI-P36.
STUDY OF JUNÍN ARENAVIRUS GLYCOPROTEINS ASSOCIATION TO CHOLESTEROL ENRICHED MEMBRANE DOMAINS

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Arenavirus morphogenesis and budding occurs at cellular plasma membrane; however, the nature of membrane assembly sites remains poorly understood. In this study we examined the effect of several cholesterol-lowering agents on Junín virus (JUNV) multiplication in Vero cells. We found that cholesterol cell depletion reduced JUNV infectivity and glycoproteins (GPs) localization on the cell surface, while nucleoprotein (NP) and GPs expression remains constant inside. Analysis of NP presence in supernatant of cells depleted of cholesterol suggests that a reduced amount of virions are budding from infected cells. Treatment with triton X-100 followed for gradient fractionation demonstrated that JUNV GPs associate with cholesterol enriched membranes microdomains. Membrane raft localization of JUNV GPs was impaired at warm detergent extraction or when cholesterol was removed by methyl-β-cyclodextrin compound. Specific cross-linking studies with anti-GP antibodies demonstrated to improve detergent insolubility at 37ºC by rafts stabilization. On the other hand our findings showed that NP was differentially localized in soluble fractions. These results demonstrate the association of JUNV GPs to cholesterol enriched membranes and suggest a role of rafts microdomains in the process of viral assembly and budding.

MI-P37.
PRESENCE OF AN ACTIVE GALACTOSYLCERAMIDE SYNTHASE IN *TRYPANOSOMA CRUZI*
Parente JE, Landoni M, Duschak V, Couto AS.
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Glycosphingolipids form a highly polymorphic class of lipids. In mammalian cells they occur in a number of cellular membranes but are mainly expressed on the cell surface, where they are thought to play a role in cell signaling and recognition. The carbohydrate structures in most glycolipid series are based on the (Galβ1-4Glcβ1) of lactosylceramide. Glucosylceramide therefore is the precursor for most glycolipid species. The other major monohexosylceramide, GalactosylCeramide, serves as a precursor for only a few simple glycolipids, sulfatide, galabiosylceramide, and the ganglioside sialo-Galceramide.
Although the biochemical pathways of glycosphingolipid biosynthesis are relatively well understood in mammalian cells, little is known about them in parasites. Recently, we have shown the presence of an active glucosyl ceramide synthase in epimastigote forms of *T. cruzi*. In the present work, another enzyme of these pathways was detected for the first time and purified: a galatosyl ceramide synthase. CGalT activity was measured in lysates obtained from epimastigote forms of *T. cruzi*. Fractions, pretreated with saponine, were incubated at 37ºC in the presence of 2 mM UDP-Gal and NBD- ceramide. Lipids were extracted and separated by TLC.
A putative CGalT was purified using a ConA-Sepharose column. On going studies are being carried out to determine the sequence of the purified protein.

MI-P38.
CALMODULIN ANTAGONIST INHIBIT *ECHINOCOCCUS GRANULOSUS*: IMPLICATIONS FOR DRUG DESING
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Shape change is required for the invasion the same protozoans and these transitions morphology are driven by calcium-dependent signaling pathways. The presence of specific calcium storage compartments, calcium transport and different calcium-binding proteins are vital signal systems for these eukaryotes. On the other hand, broad spectrum antiparasitic agents altered Ca^{2+}-dependent metabolism processes in the cells. In *Echinococcus granulosus*, the Ca^{2+} deposits, IP6 and proteins (EgCaBP) are very abundant in the calcareous corpuscles and in the cystic membranes but physiologic function is ignored. We found by RT-PCR that *Echinococcus* larvae contain calmodulin. Additionally, agents that affect both intracellular (BAPTA) and extracellular calcium (EGTA, verapamil) homeostasis reduced the vitality of protoscoleces in a dose-dependent manner. Scanning electron and confocal microscopy were determined. The ID_{50} concentration were determined for CaM antagonists (W7), phenothiazines (TFP) and protein-kinase inhibitors and was demonstrated the deleterious effect on laminated layer of intact cysts. Protoscolex mortality was 100% after CaM antagonist incubation for 18 h. Further study of this process may identify novel mechanisms involved in the development of the cestode and may contribute to the design of novel strategies to control echinococcosis transmission.

MI-P39.
MOLECULAR IDENTIFICATION OF CUTANEOUS HUMAN PAPILLOMAVIRUSES ASSOCIATED WITH SKIN CANCER
Chouhy D, De Lorenzi A, Squeff M, Gorosito M, Cesarios G, Sanchez A, Bergero A, Fernandez Bussy R, Gardiol D, Giri A.
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Human papillomaviruses are heterogeneous DNA viruses that include more than 100 different types, 75% of them with cutaneous epithelia tropism. HPV contribution to the development of skin neoplasias is poorly characterized. Also, their epidemiology is poorly understood due to technical difficulties in viral detection that affects the sensitivity, specificity and typing. Argentine is a high-risk skin cancer region; however there are neither studies about the incidence of cutaneous HPV in our population, nor the cooperation of HPV infections to skin cancer. Our goal was the development of a colorimetric PCR-based method to evaluate HPV circulating types in our country, its frequency and their possible skin cancer association. Therefore, we optimized a "Hanging droplet" PCR technique with colorimetric detection for HPV detection. HPV types were identified by direct sequencing of PCR products. We analyzed 32 swabs samples of different skin areas (lesions, UV exposed and no exposed zones) from 7 patients. The frequency of HPV DNA was 62% (20/32), with a predominant presence in lesions and UV exposed skin. Several cutaneous HPV types were found and 4 new viral types were identified from HPV-positive samples. Results have shown the methodology developed is sensitive and specific for HPV DNA detection of a wide range of cutaneous HPVs circulating in our region.

MI-P40.
ANTIBODIES AGAINST *TRYPANOSOMA CRUZI* RIBOSOMAL P PROTEINS INDUCE APOPTOSIS ON HL-1 CARDIAC CELLS
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Objectives: The aim of this work was to analyze the long-term stimulation effect induced by antibodies (Ab) directed to the C-terminal regions of *Trypanosoma cruzi* ribosomal P2β protein, defined by epitope R13, on cardiac cells. *Methods*: Cardiac HL-1 cells were treated with A) mAb anti-R13, named 17.2; B) an irrelevant mAb, named 40.14; and C) IgG from patients with chronic Chagas Heart Disease (cChHD). Cell apoptosis was evaluated with annexin-V-FITC and Propidium Iodide (PI) staining by flow cytometry, terminal deoxynucleotidyl transferase-mediated UTP end labeling assay (TUNEL) and Bax/BclXl mRNA levels by Quantitative Real-Time PCR. *Results*: mAb 17.2 induced an increase of phosphatidylserine translocation to the outer layer of HL-1 cells together with DNA fragmentation and an augmentation in Bax/BclXl ratio. mAb 40.14 did not. The late apoptosis induced by mAb 17.2 was abolished after preincubation with β-adrenergic receptor (β-AR) antagonist, propranolol. IgG fractions with an exclusive β-AR stimulating activity induced apoptosis, but IgG fractions with both β-AR and muscarinic receptor stimulating effects only induced apoptosis when cells were incubated with atropine. *Conclusions*: These results support the hypothesis that Ab against the C-terminal end of TcP2β may contribute to the cardiac damage observed in patients with cChHD through long lasting stimulation of β1-AR.

MI-P41.

IMMUNOAFFINITY PURIFICATION AND CHARACTERIZATION OF BACULOVIRUS-EXPRESSED MEASLES VIRUS NUCLEOPROTEIN

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Introduction: Measles virus (MV), a member of the Morbillivirus genus of the paramyxovirus family, is an enveloped virus containing a single-stranded negative RNA genome. The nucleocapsid of the virus is composed of the RNA surrounded by the nucleoprotein (N). The N is the most abundant and immunogenic protein of the virus, and the presence of antibodies to this major antigen can be taken as marker of sero-conversion in both vaccinated and naturally infected individuals. *Objectives*: Expression, purification and characterization of recombinant MV nucleoprotein. The purified N protein will be used to evaluate parameters of humoral and cellular immunity in vaccinated and naturally infected individuals. *Materials and methods*: A recombinant baculovirus expressing the nucleoprotein of measles virus was generated. The expressed protein was purified by immunoaffinity chromatography using polyclonal antibodies to MV immobilized on Affi-Gel HZ. The purified nucleoprotein was characterized by EIAs, SDS-PAGE and Western Blot techniques. *Conclusions*: The baculovirus-expressed measles virus nucleoprotein was purified by immunoaffinity chromatography, and its purity demonstrated by SDS-PAGE. Recombinant viral proteins expressed in eukaryotic systems and purified by immunoaffinity chromatography could be used both in diagnosis and evaluation of immunity.

MI-P42.

CLONING AND EXPRESSION OF JUNÍN VIRUS, CANDID#1 STRAIN, PROTEINS AND REVERSE GENETICS SYSTEM DESIGN

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Arenaviridae comprises 22 recognized virus species with a bipartite ssRNA genome and an ambisense coding strategy. The virions are enveloped and include non-equimolar amounts of each genomic RNA species, designated L (ca. 7200 nt) and S (ca. 3500 nt), coding for four ORFs (N, GPC, L and Z). After expression, GPC protein is processed into three mature peptides (G1. G2 and a signal peptide). Junín virus is the ethiological agent of Argentine hemorrhagic fever (AHF), an acute disease with high mortality rate. In the '80s, the Candid#1 strain of Junín virus (CD1) was developed as a live attenuated vaccine for AHF. Molecular characterization of CD1, and of its more virulent direct ancestors, XJ-13 and XJ#44, permits a systematic approach to study the basis of Junín virus virulence. We sequenced both genomic RNAs of CD1 and XJ#44 and compared them to XJ-13. Furthermore we analyzed the secondary structure predicted for the complete set of proteins coded by the viral genome. Our results revealed 15 amino acid changes among CD1 proteins and the wild-type ones. We are developing a reverse genetic system in order to test the attenuation hypothesis. We cloned and expressed the four Junín virus ORFs in different systems (bacterial and eukaryote), and generate a set of minigenomes containing Junin virus non-coding sequences and marker genes.

MI-P43.

PURIFICATION, CHARACTERIZATION AND APPLICATION OF RECOMBINANT HEMAGGLUTININ DERIVED FROM MEASLES VIRUS

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Introduction: Measles virus, a member of the Paramyxoviridae, is capable of causing acute and persistent infections. The acute infection is followed by life-long immunity in which neutralizing antibodies against the viral haemagglutinin play an important role. The HA protein is a type II surface glycoprotein, and is involved in the attachment to the host cell. *Objectives*: Purification and evaluation of recombinant HA protein expressed in baculovirus system and production of polyclonal antibodies. Evaluation of neutralizing antibodies in infected and vaccinated individuals using the recombinant protein. *Methods*: A truncated version of Measles HA protein derived from Edmonston strain was expressed using baculovirus system. The expressed HA was purified with Ni-Nta agarose and evaluated by EIA, SDS-PAGE and Western blot. The recombinant HA was used in a capture EIA assay to evaluate neutralizing antibodies. *Conclusions*: The purified recombinant HA protein was characterized by EIA, SDS-PAGE and Western blot analysis. Results with these methods demonstrated that the protein has been successfully purified from culture supernatants and, as judged by EIA with a conformation similar to the native HA of the MV. Furthermore, application of the recombinant HA in a capture EIA demonstrated the presence of neutralizing IgG antibodies in sera of naturally infected and vaccinated individuals.

MI-P44.

***GIARDIA LAMBLIA*: PI-3K ACTIVITY AND ITS INVOLVEMENT IN THE ENCYSTEMENT**

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Giardia lamblia is a parasitic protozoan and major cause of diarrhoeal disease. Disease transmission is dependent on the ability of the parasite to differentiate back and forth between two states: trophozoite and cyst. The current understanding of the intracellular signaling mechanisms that regulate the parasite replication and differentiation is limited. In this work we demonstrate the phosphatidylinositol pathway in *G. lamblia* trophozoites by determination of lipid kinase activities: PI-3K, PI-4K, PI4P-5K and PAK. An increase of PI3-K activity in encysting trophozoites and a decrease in the protein level of CPW2 (cyst wall protein), a differentiation marker was also observed when ecysting trophozoites were incubated with specific inhibitors of PI3-K. The enzymatic activities were assayed by phosphorylation of endogenous and exogenous substrates with [Y^{32}-P] ATP in the trophozoites membrane under the both conditions. Western blot was done using an antibody against the protein CPW2. The results show the involvement of PI3-K signalling pathway in the encystation of the *G. lamblia*.

MI-P45.
TRYPANOSOMA CRUZI: PHOSPHATIDYLINOSITOL-3-KINASE SIGNALING PATHWAY IN RESPONSE TO HYPEROSMOTIC STRESS
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Phosphatidylinositol kinases play a central role in different signaling pathways. The product of PI 3-kinase, PI3P, is implicated in control of several cellular processes as differentiation between others. The aim of this work was to determinate the involvement of PI3K activity in the response of *Trypanosoma cruzi* epimastigote forms to hyperosmolarity. Bioinformatic and phylogenetic analysis showed that this parasite have two putatives PI3Ks: TcVps34p, homologue of class III PI3Ks, and TcPI3K-I, homologue of class I PI3Ks. A PI3K protein of 110 kDa was identified in epimastigote membranes and its activity was assayed using lipid kinase assay with ^{32}-PγATP and developed in TLC-borate system. Treatment of epimastigotes with wortmannin and LY294002 decreased kinase activity in 30-40%. In order to analyze PI3K behaviour under hyperosmotic stress conditions, epimastigotes were incubated with manitol 1M or NaCl 0,5M. In both cases, the activity was increased and such effects were suppressed in the presence of inhibitors. The results show that *T. cruzi* expresses a protein with PI3K activity which is a component in the signaling pathways activated by hyperosmotic stress in epimastigotes.

MI-P46.
ADENYLATE KINASE 4 (TzAdK4) IN _TRYPANOSOMA CRUZI_
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Adenylate kinases are phosphotransferases, which catalyze the interconversion of adenosine nucleotides. These enzymes maintain the cellular ATP homeostasis and duplicate ATP´s energetic potential. While eukaryotic cells typically contain three adenylate kinase isoforms, kinetoplastid parasites present at least three additional isoenzymes. The unusual large amount of adenilate kinase variants present in these organisms could be explained by their differential intracellular positioning or their differential expression patterns along the life cycle. Each variant presents particular structural characteristics which could account for their localization. In *Trypanosoma cruzi* apart from TzAdK1, TzADK4 is another isoform with a particular long N-terminal domain. This enzyme is homologous to TbADKE from *Trypanosoma brucei,* which has been shown to be targeted to the flagellar axoneme. In this work we study the localization of TzAdK4, expression pattern along the life cycle and the association to cellular structures by means of Western-Blot and the expression of tagged proteins in *Trypanosoma cruzi*.

MI-P47.
STUDY OF THE SUBCELLULAR LOCALIZATION OF TRYPANOSOMA CRUZI ARGININE KINASE
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Arginine kinase belongs to the guanidine kinase enzyme family and catalyzes the reversible synthesis of phosphoarginine, a molecule involved in cell energy homeostasis. It was postulated that phosphagen kinases participate in an enzymatic “phosphotransfer network” that communicates spatially separated intracellular ATP consumption and production processes. In order to understand the physiological role of arginine kinase in *Trypanosoma cruzi*, the causative agent of Chagas´ Disease, we investigated the presence of isoforms in the genome of the parasite by data mining and the subcellular localization by digitonin extraction and inmunofluorescence approaches. Although two putative isoforms were annotated in the genome, only one is expressed during the parasite life cycle. In epimastigotes arginine kinase is mainly localized in vacuolar structures that co-localize at specific points with the mitochondrial reticulum, in addition to a soluble enzyme in the bulk cytosol. The determination of arginine kinase subcellular localization will be a critical feature to better understand the enzymatic phosphotransfer network in this parasite.

MI-P48.
MOLECULAR DETERMINANTS OF PANCREATIC AND CARDIAC DISEASE IN COXSACKIEVIRUS B1 INFECTION
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Coxsakievirus B (CVB) are etiological agents of pancreatitis and myocarditis, being this last condition linked to dilated cardiomyopathy. To investigate the role of different parts of the viral genome in the pathogenicity of CVB1 (CVB serotype 1), we inoculated a virus derived from an infectious cDNA clone (gently provided by Dr. Nomoto, University of Tokyo)(CVB1N) into weanling C3H/HeJ mice. At 10 days postinfection, animals were killed and their tissues processed for virological and histological studies. CVB1N did not induce pathology in pancreas and heart. By passaging CVB1N in SCID mice, a variant (CVB1Nm) was obtained that induced a severe pancreatitis and myocarditis. The RNA of CVB1Nm was amplified by RT-PCR, cloned, sequenced and used to construct recombinant viruses, which were assessed for their capacity to generate pancreatitis and myocarditis. Results showed that the P1 region is critical for pathogenicity in the murine pancreas but affects pathogenicity in the heart to a lesser extent, implying that the molecular determinants for disease of these two organs are not exactly the same. Although sequencing studies showed relatively few differences (23 mutations) between the parental nonpathogenic CVB1N and the pathogenic CVB1Nm, many are located in critical regions as suggested by computer modelling of viral surface.

MI-P49.
IDENTIFICATION OF THE HOMOLOGUE WEE1 GENE IN TRYPANOSOMATIDS

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In eukaryotic cells activation of the complex cyclin B- cdc2 kinase is a pivotal step in mitotic initiation of cell cycle. Phosphorylation of cdc2 in the Tyr15 inhibitory site by the Wee1 kinase suppresses the cdc2 activity during interphase. In several organisms homologues of Wee have been identified. In Trypanosomatids, the presence of kinases that modify CRKs (Cyclin- Related Kinases) have not been reported. Querying the Trypanosoma genome database (GeneDB), we have identified a homologue of the Wee1 family. *T cruzi* Wee sequence has 1761 bp, which encodes a protein of 586 aa with a predicted molecular weight of 65 kDa. In *T brucei*, the sequence identified contains 1812 bp and a protein of 603 aa (66.5 kDa). The *T. cruzi* and *T. brucei* wee homologues are 52% identical to one another, and only 20-30% identical to *A. thaliana*, *S. pombe* and human homologues. The protein kinase domain exhibits the greatest similarity at the amino acid level. The Wee1 *T. brucei* homologue was cloned into the expression vector pDEST17 with an N-terminal His tag. Polyclonal antibodies raised in mouse against His-TbWee1 detected the recombinant Wee protein and a protein of the expected weight in *T. cruzi* and *T. brucei* extracts. Reverse genetics is being carried out to identify the function that this kinase has in the cell cycle of these eukaryotic cells.

MI-P50.
IDENTIFICATION AND CHARACTERIZATION OF AN HPAR14 HOMOLOG IN *TRYPANOSOMA CRUZI*

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A second member of the parvulin family of peptidyl-prolyl cis/trans isomerases was identified in *Trypanosoma cruzi*. A database search was performed to screen for additional parvulin homologous genes in the GeneDB trypanosomatid database. The encoding region of the gene consists of 375 bp and encodes a protein of 124 amino acids. The TcPar14 gene was expressed and purified as described for TcPIN1 (Erben *et al*., 2007). The PPIase-activity of TcPar14 was investigated using the protease-free method. Using the substrate Suc-Ala-Arg-Pro-Phe-NH-Np, a specificity constant kcat/KM of 194 /mM/s was found for TcPar14. This is about 50- fold higher than the respective value of kcat/KM for human Par14. A comparison of the relative specificity constants for various substrates shows a strong preference for a substrate with the basic arginine residue preceding proline. The analysis of the sequence of TcPar14 showed the existence of the sequence motifs typical of the parvulin catalytic core. Like most hPar14 homolog parvulins, TcPar14 has an N-terminal extension but in contrast to hPin1, a WW domain for protein/protein interactions motif could not be identified.

MI-P51.
BIOCHEMICAL CHARACTERIZATION AND SUB-CELLULAR LOCALIZATION OF AN ALDO-CETO REDUCTASE FROM *TRYPANOSOMA CRUZI*

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A novel aldo-keto reductase (AKR) gene from *T. cruzi*, TcAKR, was cloned and expressed in *Escherichia coli*. The recombinant TcAKR showed NADPH dependent reductase activity with two commonly used AKR substrates, 4-nitrobenzaldehyde (4-NBA) and 2-hydroxyacetone (2-DHA), with Km values of 0.67 mM and 30 mM, respectively and 0.026 mM for NADPH. The pH optimum for both substrates was 6.5. No activity was detected with NADH as a co-factor. The native TcAKR was also partially purified from epimastigotes of *T. cruzi* by affinity chromatography on Cibacron Blue-Sepharose and showed a specific activity for 4-NBA of 0.27 U/mg protein. The sub-cellular localization of TcAKR in epimastigote and trypomastigote stages of *T. cruzi* was investigated by electronic microscopy using an anti-recombinant TcAKR serum. Immunogold staining showed a widespread localization throughout the parasite. Otherwise, the release profile of TcAKR from intact epimastigotes by titration with digitonin was similar to the cytosolic marker pyruvate kinase. Altogether, these results suggest TcAKR is mainly localized in cytosol.

MI-P52.
CHARACTERIZATION OF *IN VITRO* INVASION OF *SHIGELLA SPP.* IPABCDA NEGATIVE MUTANTS

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It is known that genes encoded in the ipaBCDA operon of *Shigella* spp are necessary to invade and escape from infected enterocytes. In previous works we described clinical isolates of *Shigella* that invade Hep-2 cells monolayers even thought the absence of amplification of the genes of the ipaBCDA operon by PCR assays. The aims of this work are analyze ipaBCDA mutants for Ipa proteins production by immunoblotting and in vitro invasion by Confocal Microscopy. Four isolates of ipaBCDA negative mutants were analyzed by dot-blot and Western-blot with anti-IpaB monoclonal antibodies. In these assays whole cell extract of bacteria with or without incubation with Hep-2 cells were used. Three of these mutants were tagged with GFP reporter plasmids and used for invasion assays in Hep-2 cells. For this assay WT *Shigella* ipaBCDA positive isolate were labeled with carboxyfluorescein. Hep-2 infected cells with WT and mutants of *Shigella* were analyzed by Confocal Microscopy to locate intracellular bacteria. One of the ipa$^-$ mutants was located in the cytoplasm of the Hep-2 infected cells, as well as WT. Dot and Western-blot analysis of the mutant confirm the absence of IpaB protein in detectable limits of all four ipaBCDA negative mutants. According with these observations it is possible that *Shigella* ipa$^-$ mutant posses a different *in vitro* invasion mechanism than WT bacteria.

MI-P53.
LINKS BETWEEN QUORUM SENSING GENETIC MARKERS AND PHENOTYPIC TRAITS IN CLINICAL *ACINETOBACTER* STRAINS

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Acinetobacter isolates previously characterized by DNA-DNA hybridization from hospitalized patients (Leiden University Medical Center, Holland) showed quorum sensing (QS) signals when cultured in minimal or complex medium. In the former, 76% of the isolates displayed at least one QS signal as detected with *Agrobacterium* QS reporter strains. A number of these strains were analyzed with respect to the presence of *luxI* and *luxR* type sequences, using primers corresponding to the *luxI* putative QS signal synthase from *A. baumannii* ATCC 17978 and the QS signal receptor *luxR* from *A. baylyi* ADP-1. The isolates tested were *A. baumannii-A. calcoaceticus* complex (4), genogroup 3 (1), 13TU(1), *A. haemolyticus* (1) and *A. lwoffi*(1). For each strain, the QS signal profile with *A. tumefaciens* NT1/pZLR4 reporter, extracellular lipase, protease, biofilm formation and antibiotic resistance were studied using overnight cultures obtained in minimal medium with lactic acid as carbon source or complex medium (LB plus 1% glucose). Although QS signals were present in all the strains tested, PCR amplification of luxI type sequences were only found in the A. baumannii isolates. These results suggest that if having a role in the QS signal generation, the putative QS signal synthase is not conserved within the genus *Acinetobacter* and constitutes a potential species genetic marker for QS like sequences in this genus.

MI-P54.
PHENOTYPIC EXPRESSION OF DIFFERENT EFFLUX SYSTEMS TO ANTISEPTICS IN COAGULASE-NEGATIVE STAPHYLOCOCCI

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Health care associated infections constitute one of the greatest challenges of modern medicine. Antiseptics, one of the first barriers, prevent the spread of skin pathogens. A phenotypic and molecular analysis of efflux systems to ethidium bromide, sodium dodecyl sulfate (SDS), sodium deoxycholate, and benzalkonium chloride was performed in 39 coagulase-negative staphylococci isolates. The Minimum Inhibitory Concentration (MIC) in presence/absence of m-chlorophenylhidrazone (CCCP) was performed using *Staphylococcus (S.) aureus* ATCC 29213 and an *Escherichia coli* MG1655 mutant (AcrAB/TolC overexpression). The presence of the genes *smr* and *qacA/B* was determined by PCR and the clonal dispersion was analyzed by PFGE. Of the 39 isolates, 12 *S. epidermidis* (30.8%) and 22 *S. haemolyticus* (56.4%) showed efflux to ethidium bromide, 11 (28.2%) *S. epidermidis* and 21 (53.8%) *S. hemolyticus* to SDS. All the *S. haemolythicus* (56.4%) and 11 *S. epidermidis* (28.2%) extruded sodium deoxycholate. Only 7 *S. epidermidis* and 17 *S. haemolythicus* expulsed benzalkonium chloride. The gene *qacA/B* was found in only 3 *S. epidermidis* and in 5 *S. haemolythicus* whereas *smr* was found in all of them. The results show a wide diversity in the phenotypic expression of resistance to antiseptics, which would indicate various pumps present in a strain. The mechanism of efflux to antiseptics is polyclonal since great variety was found in the two species.

MI-P55.
CONTRIBUTION OF YQIC IN *SALMONELLA TYPHIMURIUM* TO THE VIRULENCE IN MICE AND TEMPERATURE RESISTANCE

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The *yqiC* gene of the pathogenic enterobacterium *Salmonella typhimurium* encodes for a 100-residue protein of unknown function that belongs to a highly conserved protein family in proteobacteria. This protein has homology with the virulence factor IivA of *Brucella abortus*. Similar to IivA, YqiC of *Salmonella typhimurium* has a predicted coiled-coil α-helix domain in the C-terminal half and it displays a trimetric structure. In order to understand the relevance of *yqiC* in *S. typhimurium* virulence, a *yqiC* null mutant, named St-*yqiC*, was constructed and the virulence of this strain was assayed. St-*yqiC* showed a severely attenuated phenotype in BALB/c mice inoculated intraperitoneally as well as orally. This pattern was reverted when St-*yqiC* was complemented in *trans* with the wild-type gene. On the other hand, we studied the behavior of St-*yqiC* in macrophage and epithelial cell lines and in different *in vitro* conditions, such as nutrients deprivation and the presence of H_2O_2, polimixine B or SDS. St-*yqiC* did not display a significantly altered phenotype in any of these conditions. Interestingly, we found that *yqiC* was involved in temperature resistance, due to the fact that St-*yqiC* failed to grow at 42ºC while the parental and the complemented strain grew normally at this temperature. These results contribute to understand the role of *yqiC* in the bacteria-host interaction.

MI-P56.
THE INFECTIVE PHENOTYPE FAVORS *BORDETELLA PERTUSSIS* SURVIVAL IN IMMUNE CELLS

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Bordetella pertussis (Bp), the etiologic agent of Pertussis, expresses adhesins and toxins like Filamentous Hemagglutinin (FHA) or Adenylate cyclase (AC) that play a role in pathogenesis. The expression of these proteins varies in response to environmental signals. Current evidence indicates that the infective phenotype of *Bp* might express FHA but not AC or other toxins are present. The innate interaction of the bacteria with immune cells is not yet clear. We found that CR3 (via FHA) and cholesterol rich domains are critical for *Bp* binding to immune cells and involved in bacterial delivery to non-lysosomal compartments. Several studies indicate that AC has a role in *Bp* survival in immune cells. In this study we investigated the interaction of *Bp* wild type (wt) and AC deficient mutant with human monocytes (U937) by flow cytometry and fluorescence microscopy. We found that the lack of AC expression determined a higher attachment and internalization of *Bp*. Confocal studies indicated that in contrast to IgG-opsonized *Bp*, wt failed to fuse with lysosomas. Interestingly, the lack of AC increased the number of *Bp* colocalizing with lysosmes but survival studies showed an increased number of alive bacteria inside the cells probably due to the higher rate of internalization. These results support the hypothesis that virulence modulation contributes to *Bp* survival in the infected hosts.

MI-P57.
AN *AGROBACTERIUM TUMEFACIENS* TYPE I SECRETION SYSTEM IS INVOLVED IN IRON METABOLISM
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Type I secretion systems (TISSs) deliver proteins to the extracellular media in gram-negative bacteria. The PrsDE TISS of *Rhizobium leguminosarum* secretes NodO, several adhesins and the glycanases PlyA and PlyB. We have previously identified a TISS of *Agrobacterium tumefaciens* (AspDE) which restored protein secretion in the *prsDE* mutant. An extracellular protein encoded by an upstream gene of *aspDE* (*hla*) was overproduced when the *hla aspDE* locus cloned into pIJ7760 was expressed in *R. leguminosarum*. However, Hla was not detected in the extracellular medium of *A. tumefaciens* even in the presence of pIJ7760. Analysis of Hla showed no significant homology with known proteins. To investigate the function of both Hla and AspDE in *Agrobacterium* ecophysiology, we constructed an *aspD* mutant. This mutant showed increased cell clumping when it is grown at low iron concentration. In addition, we detected in the wild type strain a surface associated protein with a molecular weight similar to Hla that was absent in the *aspD* mutant. The mutant was also defective in the secretion of a 40 kDa-surface protein that was present in the wild type when hemoglobin was the iron source. Genomic analysis of the *hla aspDE* context suggests that this locus might be involved in iron acquisition. Taking together, our observations suggest that Hla and AspDE may be involved in the iron uptake.

MI-P58.
DEVELOPMENT OF A FUSION PROTEIN AS A VACCINE ANTIGEN AGAINST LEPTOSPIROSIS
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Leptospirosis, recently categorized as an emerging infectious disease, is a worldwide zoonosis of human and veterinary concern caused by spirochetes of the genus Leptospira. Here we describe the construction of a fusion protein composed by cholera toxin B subunit (CTB) and Lipl 32. Lipl32 is an immunodominant outer membrane lipoprotein that is conserved in most pathogenic leptospires. CTB is currently extensively studied as an immunogen or adjuvant, particularly in mucosa. This fusion protein was cloned in an already described pAE vector and expressed in E. coli BL21 (DE3) and (SI). The protein was purified by Ni2+ affinity chromatography. Recognition by antibodies anti CTB and anti Lipl32 was verified and the ability of pentamer formation was checked by binding assays to GM1 ganglioside and by gel electrophoresis. Its immunogenicity was evaluated by immunoassays using sera from convalescent patients. The ability to elicit antibodies was assessed by intraperitoneal mice immunization. These results allow us to plan the use of this fusion protein as a protective antigen in experimental infections of Syrian hamsters previously immunized with the CTB/LipL32 fusion protein and then challenged with live virulent leptospires to analyze the host immune response and its potential protective properties.

MI-P59.
A SHOTGUN PHAGE DISPLAY LIBRARY OF *BRUCELLA SUIS* TO IDENTIFY NOVEL ADHESINS
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We are interested in discovering the factors that promote adhesion and invasion of the host tissues in the intracellular bacterial pathogen *Brucella*.
It has been shown that *Brucellae* are able to adhere to cultured cells and that sialic acid residues are involved in the interaction of *Brucella* with erythrocytes, macrophages, and epithelial cells. *Brucella* was also able to bind to several extracellular matrix proteins, like fibronectin and vitronectin, as other pathogenic organisms. Shotgun phage display cloning is a useful tool for studying interactions between bacteria and host proteins. Theoretically, these libraries consist of phages that together display all protein domains encoded by the bacterial genome. From such a library, polypeptides with affinity for another molecule can be isolated by affinity selection (panning). This procedure can lead to the selection of clones with a true binding ability. To identify bacterial adhesins, receptins and their minimal binding domains, a *B. suis* phage display library was constructed by cloning shotgun digested genomic DNA into the pG8SAET phagemid vector. The phages were panned several times against immobilized ligands. Fibronectin and fetuin (a sialic-rich protein) were used as ligands in different panning experiments. After enrichment, a number of candidates were selected and the sequences of the inserted foreign DNA were determined.

MI-P60.
BRUCELLA ABORTUS BEARS AN ATYPICAL RIBOFLAVIN PATHWAY THAT IS INVOLVED IN ITS VIRULENCE
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Riboflavin (vitamin B2) is an essential metabolite that is used in the synthesis other vital cofactors. Plants, fungi and prokaryotes can synthesize riboflavin while animals must obtain it from the diet. Lumazine Synthase (LS) catalyzes the penultimate step of the biosynthesis. *Brucella* has two paralog genes coding for LS named ribH1 and ribH2. *B. abortus spp.* mutant strains lacking ribH1 or ribH2 show no auxotrophy for riboflavin. However, only *ribh2*$^-$ shows an important attenuation in its virulence and resistance to oxidative stress. We failed in many attempts to obtain a double mutant, suggesting a lethal phenotype. However, in the presence of a rescue plasmid containing a wild type copy of ribH1, the genomic double mutant could be obtained. Conducting Plasmid-Shuffling experiments with this late strain we clearly showed that both RibH1and RibH2 have LS activity *in vivo*. We also generated an active site mutant W22A of RibH2 and proved that it is unable to complement the LS activity *in vivo*. Auxotrophy for riboflavin is also demonstrated in the *ribh1*$^-$*/ribh2*$^-$ mutant strain. TLC was used to assess flavin cellular content in different strains. Virulence and oxidative stress experiments with different mutant and complemented strains were conducted in order to show the connection between flavins and virulence mechanisms.

MI-P61.
REGULATION OF MYCOLIC ACIDS BIOSYNTHESIS IN *MYCOBACTERIUM*
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The most relevant lipids present in *Mycobacterium* cell envelope are the mycolic acids. These unusual fatty acids are essential for the survival and virulence of *M. tuberculosis*. However, there is no information about the regulation of the synthesis of these lipids, which involves two different fatty acid synthase (FAS) systems. Microarrays and quantitative proteomics studies revealed that inhibition of *M. tuberculosis* mycolate production by isoniazid (INH) induces transcription of the FASII components present in the *kas* operon. We found an ORF (Rv2242) upstream the *kas* operon with homology to transcriptional regulators. This ORF is present in all the actinomycetes genomes analyzed. Therefore, our working hypothesis is that this ORF, proposed to be essential, codes for a transcriptional regulator of mycolic acid synthesis. Rv2242 was successfully expressed and purified by affinity chromatography. Purified protein binds specifically to a 300-base pair fragment containing the *kas* promoter. Moreover, DnasaI footprinting analysis revealed the presence of an inverted repeat in the DNA binding region that is conserved in all the actinomycetes *kas* promoters analyzed. This is the first report of the presence of a protein able to bind the *kas* operon promoter *in vitro*. We are currently studying the physiological role of this transcriptional regulator using *M. smegmatis* as a working model.

MI-P62.
COMPARISON BETWEEN OPTOCHIN AND RIFAMPICIN TESTS TO ASSESS MUTATION FREQUENCY IN *STREPTOCOCCUS PNEUMONIAE*
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Rifampicin test is the most common assay to assess mutation rate in bacteria. Here, we evaluated the subinhibitory concentration (SIC) effects on the mutation rate of *S. pneumoniae* using an additional assay, the optochin test. Three capsulated strains were exposed to SIC of penicillin, chloramphenicol and erythromycin (corresponding to 75% of each minimum inhibitory concentration) for 2 hours, and the mutation rate to optochin and rifampicin was determinated. While optochin test showed that the exposure only to penicillin increased the mutation rate between 2.0-3.2 fold for all three strains, we could not detect this phenomenon using the classical rifampicin test. With the purpose to investigate the cause of this difference, we analysed the *atpAC* and *rpoB* point mutations (that conferred optochin and rifampicin resistance, respectively) in the optochin- and rifampicin-resistant strains generated by penicillin exposure. We found differences in the GC content corresponding to the 20 pair bases surrounding the point mutation that conferred rifampicin or optochin resistance, suggesting that it could influence the repair efficiency, and in consequence, the mutation rate. We proposed that optochin test was more appropriated than the rifampicin test to detect transient hypermutator state in *S. pneumoniae* and it should be considerate for further mutability analysis.

MI-P63.
ADJUVANT CAPACITY OF A MICROBIAL COMPONENT TO ENHANCE IMMUNITY AGAINST *BORDETELLA PERTUSSIS*
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Bordetella pertussis (Bp) is the etiological agent of pertussis or whooping cough, an important respiratory disease, which remains a problem in public health even in countries with high vaccination coverage. Because of that, research on pertussis is mainly focused on development of new strategies towards a better disease control. In this work we analyze the adjuvant capacity of a microbial component flagellin of *Salmonella enterica* serovar typhimurium (Fla)- in a mucosal pertussis vaccination. We analyzed the innate immune response of mice intranasally stimulated with either *Bp* or *Bp* + Fla, by real time RT-PCR. Specific primers for chemokine CCL20 and the cytokines TNF-α and IL-6 were used. We observed that CCL20 and IL-6 expression was enhanced when mice were challenged with *Bp* + Fla (33 ± 2 for CCL20 and 192 ± 29 for IL-6) compared to *Bp* alone (9.7 ± 1.2 for CCL20 and 65 ± 22 for IL-6). We also analyzed the adjuvant capacity of flagellin in lethal-dose pertussis protection assay. In these experiments mice were intranasally immunized with dead whole cell bacteria with or without flagellin as adjuvant and then challenged with live *B. pertussis* (109 CFU per 50 µl). The results obtained showed that flagellin behaves as a good adjuvant since more animals survived in the experiments (44% compared to 33%).

MI-P64.
IMMUNE CHARACTERIZATION OF APA PROTEIN FROM *MYCOBACTERIUM*
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Paratuberculosis (PTB) and bovine tuberculosis (BTB) are significant economic problem in cattle; the causative agents are *Mycobacterium avium subsp paratuberculosis* (MAP) and *Mycobacterium bovis*. Characterization of new potential diagnosis antigens in MAP and *M. bovis* genome can be rationalized by the identification of homologues present in other spp as in *M. tuberculosis* (MT). We chose the Alanine-proline rich protein (Apa) that showed antigenicity in *M. tuberculosis*. Apa was found as a cell surface and secreted glycoprotein. We assayed biochemical purified Apa from *M. tuberculosis* (MT Apa) and the recombinant protein from MAP (MAP Apa) by immunobloting, ELISA (humoral response) and γIFNBovigam^{R} (cellular immune response) in PTB and BTB animals. MAP infected animals showed similar recognition to both proteins by ELISA but OD rates with MT Apa are higher than recombinant MAP Apa protein. In spite of the cross reactivity between the homologues, the *M. bovis* infected animals recognized exclusively the MT Apa. The cellular inmune response of PTB infected animals was significatively higher than the control. The cellular response in BTB animals was high for both MT Apa and MAP Apa recombinant, but MT Apa showed significative high rates than MAP recombinant. These results demonstrated the usefulness of the protein to diagnose PTB y BTB.

MI-P65.
EFFECTS OF PROBIOTIC *BACILLUS SUBTILIS* SPORES ON INNATE IMMUNE RESPONSE, TOLERANCE AND INFLAMMATION
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Generation of a protective barrier against harmful microorganisms by colonizing the intestinal mucosa as well as stimulating the innate immune response are implicated in probiotic effects. A new generation of probiotics are constituted by spores of *Bacillus subtilis*. Complement and Galectins, glycan-binding proteins, represent atractive systems to further explore their response to probiotics and the development of therapies for autoimmune diseases, chronic inflammation and cancer. Galectin-1 (Gal-1) has been localized in skeletal, smooth and cardiac muscle, neurons, thymus, kidney and placenta. Therefore, it was interesting to investigate the effect of probiotic *B. subtilis* spores on tissue-specific galectin expression and complement activation in animal model. Animals were feed daily with supplements of *B. subtilis* spores from 22 to 45, 60 or 90 days. After that, Gal-1 was measured by ELISA and Western Blot in several organs of Wistar rats. *B. subtilis* induced a higher production of Gal-1 in spleen, liver and kidney; organs widely recognize for their key role in health. In addition, probiotic spores induced higher levels of complement components (i.e. C3) and activation of the classic and alternative pathways. Overall these results intoduce new and interesting clues about the probiotic properties of human friendly *B. subtilis* spores.

MI-P66.
***NATRIALBA MAGADII* SERIN PROTEASES AND INHIBITORS: GENE CLONING AND SEQUENCE ANALYSIS**
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Proteolysis in archaeal cells has been poorly investigated. An intracellular serine protease and protease inhibitor (NSP and NSI) have been partially characterized from the haloalkaliphilic archaeon *Natrialba magadii*. To assess the identity of NSP and its cognate inhibitor NSI, in this study a bioinformatic analysis of archaeal genome sequences was performed. A number of serine proteases and protease inhibitors (Serpins and Phosphatidylethanolmine-binding proteins, PEBPs) were found to be encoded in haloarchaeal genomes. Based on similar biochemical features, it was hypothetized that NSP and NSI may correspond to the archaeal Lon protease and PEBP homologs, respectively. The full-length gene encoding *N. magadii* LonB protease, lonBNm, was cloned and sequenced, and the expression of lonBNm mRNA during growth was examined by RT-PCR. The highest expression of lonBNm mRNA was observed during exponential growth and decreased as the cells entered the stationary phase. On the other hand, the complete gene encoding a haloarchaeal PEBP homolog was amplified from *Halobacterium salinarum* NRC-1 genomic DNA and sequenced. The occurrence of PEBP related sequences in *N. magadii* was confirmed by Southern blotting. Our preliminary results suggest that LonBNm may be involved in the cellular protein turnover during active growth in the haloarchaeon *N. Magadii*.
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MI-P67.
FUNCTIONS OF THE OUTER MEMBRANE PROTEIN CARO HOMOLOG IN *ACINETOBACTER BAYLYI*
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Resistance to the last generation of b-lactam carbapenem antibiotics in clinical strains of A. baumannii was associated with the loss of a 29 kDa OMP designated CarO (1). CarO was shown to serve channel functions for L-ornithine and L-arginine in this organism. Since CarO is a member of a family of OMPs restricted to the Moraxellaceae (1), we evaluated the function of an homolog present in the soil bacterium A. baylyi exhibiting 68% primary sequence identity. We thus generated a DcarO deletion mutant by genetic replacement, and found that the absence of this OMP resulted in a selectively impairment of the mutants to grow on L-arginine among all carbon sources tested. Moreover, A. baylyi DcarO mutants not only displayed increased resistance to carbapenems but also exhibited an outstanding ability to grow at high Na+ concentrations that were inhibitory to the wild type strain. Interestingly this effect was not observed in the case of similar K+ concentrations. These findings indicate CarO family members play roles in the specific permeation of basic amino acids. Moreover they also suggest that A. baylyi CarO, in particular, allows the selective permeation of Na+.
(1) Mussi M.A., Limansky A.S., Viale A.M. (2005). Antimicrob. Agents Chemother. 49 (4): 1432-1440.

MI-P68.
TYR9 OF THE MICROCIN J25 MOLECULE IS INVOLVED IN THE OVERPRODUCTION OF SUPEROXIDE
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Microcin J25 (MccJ25) have two intracellular targets: the RNA polymerase and the respiratory chain. The last one is mediated by an increment of superoxide. Previously, we showed that different parts of the molecule are responsible for interaction with each target. Recent studies suggested that tyr, trp, cys and gly residues are involved in the reactive oxygen species production. MccJ25 contain six glycines and two tyrosines (Y9 and Y20). First, we chose Y9 and Y20 for mutation. PA232 harboring a MccJ25-resistant RNAP and transformed with a plasmid carrying the *fhuA* gene (pGC01) was inhibited by MccJ25 and MccJ25(Y20F) under aerobic but not in anaerobic condition. The MccJ25 and MccJ25(Y20F) also inhibited the PA232 (pGC01) when the anaerobic culture was changed to aerobic one. On the contrary, MccJ25(Y9F) did not inhibit this strain either in aerobic culture or when the anaerobic culture was changed to aerobic condition. The MIC of MccJ25 and MccJ25(Y20F) were 2 and 10 µM, respectively, whereas for MccJ25(Y9F) was > 1 mM. In opposite to MccJ25 and MccJ25 (Y20F), MccJ25(Y9F) was unable to inhibit the cell respiration and to increase superoxide generation in vitro. The three peptides inhibited nearly 50% of the in vivo RNA synthesis in AB1133 (pGC01). These results define nature and position of the amino acid in the MccJ25 molecule involved in the superoxide overproduction.

MI-P69.
STRUCTURAL AND FUNCTIONAL ANALYSIS OF THE C-TERMINAL REGION OF *PSEUDOMONAS AERUGINOSA* MUTS

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Escherichia coli MutS, an 853 amino acids (aa) protein, is involved in the postreplicative DNA mismatch repair system (MMRS). This is an oligomeric protein with ATPase activity and capacity to bind to heteroduplex DNA. It is known that the *E. coli* MutS exists as dimer and tetramers and that the 53 C-terminal aa are responsible for oligomerization. We show that *in vitro*, *Pseudomonas aeruginosa* (*PA*) MutS also forms dimers and tetramers. Analysis of different C-terminal deletions and point mutations versions of *PA* MutS, and peptides fused to the monomeric maltose binding protein, indicate that the C-terminal region also drives the oligomerization process. Using *E. coli* and *PA* mutS deletion strains, we tested the capacity of different plasmid encoded C-terminal mutated versions of *E. coli* and *PA* MutS to restore MMRS functionality, by measuring the frequency of cells resistant to antibiotics. We found that *E. coli* and *PA* MutS point mutants with tetramerization defects, were complementation proficient; however the 53 C-terminal aa deleted version was able to complement *E. coli* but not *PA*. Western blot analysis indicates that this difference cannot be attributed to different expression levels of the protein. We conclude that although the tetramer specie of MutS may be dispensable, the C-terminal region is important for the functioning of the MMRS in *PA*.

MI-P70.
BACTERIAL CHEMORECEPTORS: ROLE OF F373 RESIDUE ON THE STABILITY OF TSR TRIMERS OF DIMERS

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Escherichia coli chemoreceptors (MCPs) are organized in a trimer of dimers arrangement that may be important for their signaling activities. The eleven residues involved in contacts between different dimers show a high degree of conservation among MCPs from different organisms, suggesting that the trimer of dimers organization might be also conserved. A comprehensive informatic analysis classified all known MCPs into seven families according to sequence features. That study identified the position number 373 in the serine receptor (Tsr) as a position strongly conserved within families but divergent between families, holding residues predicted to stabilize (F,Y) or destabilize (R,E,D) the trimer of dimers. In line with that interpretation, *E. coli* Tsr crystallizes as a trimer of dimers and has an F at that position while T. maritima MCP1143, holding an E, crystallizes as a hedgerow of dimers. In order to directly assess the influence of different residues at position 373 on the stability of the trimer of dimers, we replaced F373 in Tsr by different aminoacids, including those naturally occurring in other MCP families. We used crosslinking assays to evaluate the stability of trimers made entirely of the mutant Tsr as well as mixed trimers with other wild type receptors. We discuss the obtained results in correlation with the effect that the different replacements have on chemosensing.

MI-P71.
FOLDING OF PERIPLASMIC METALLO-β-LACTAMASE IN SALMONELLA TYPHIMURIUM

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Protein biogenesis in all cells is assisted by different protein systems known as molecular chaperones, which prevent aggregation of non-native proteins favoring their productive folding or degradation. Although cytoplasmic chaperones are well characterized, few data exists on folding assistants present in the periplasm of Gram-negative bacteria. We studied periplasmic protein biogenesis using as a model the metallo-β-lactamase (MβL) GOB-18 from *Elizabethkingia meningoseptica* [Moran-Barrio *et al.* (2007) *J.Biol.Chem.* 282:18286]. We expressed the *gob* gene in *Salmonella typhymurium*, where the enzyme precursor is correctly processed to its periplasmic native form. Folding assistants were searched by MudJ-based mutagenesis and selected for clones with decreased cefotaxime resistance. From more than 10,000 mutants, five showed reduced antibiotic resistance and were further characterized by growth behavior under normal and stress conditions, SDS-PAGE, Southern-blot, and sequencing analyses. In four insertional mutants we found that the disrupted genes were not directly related to antibiotic resistance. On the contrary, the fifth mutant consistently showed much reduced growth rate under stress conditions, no significance differences in heat stress protein patterns, and an insertional disruption which could account for the reduced cefotaxime resistance phenotype and a MβL folding assistant.

MI-P72.
UNRAVELING THE SECRETION MECHANISMS OF METALLO-ENZYMES INVOLVED IN β-LACTAM BACTERIAL RESISTANCE

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Metallo-β-lactamases (MβLs) are Zn(II) enzymes of clinical relevance present in the periplasmic space of Gram-negative pathogenic bacteria, which play a key role in antibiotic resistance. They are synthesized in the cytoplasm as precursors with a cleavable amino-terminal signal sequence, and must be protected from premature folding, aggregation or degradation until secretion to the periplasmic space. Cytoplasmic chaperones of the families Hsp60 (GroEL/S), Hsp70 (DnaK/J) and Trigger Factor (TF), as well as SecB and SecA, are possible candidates for this role. As a model system to analyze the function of these chaperones, we studied the secretion of the recombinant MβL GOB-18 from *Elizabethkingia meningoseptica* (Moran-Barrio *et al.*, JBC, 282(25), 2007) in *E. coli*. Our results show that GOB-18 is secreted to the periplasmic space in a Sec-dependent process, conferring β-lactam resistance to the host bacteria. The secretion in an unfolded conformation suggests that the Zn(II) ion is incorporated in the periplasmic space. Mutant studies indicate that main cytoplasmic chaperones, DnaK and TF, are required for GOB secretion. Conversely, GroEL/S has minor effects on this process. The identification of specific cellular components dedicated to the secretion of MβLs could thus be a possible target for the design of new antibiotics.

MI-P73.
THE EFFECT OF WALL LIPOLISACCHARIDDE DEFECTS UPON THE ANTIBIOTIC ACTIVITY OF MICROCIN J25
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The most significant components of the outer cell envelope in Gram (-) bacteria are the lipopolysaccharide (LPS). LPS are composed by a hydrophobic lipid A, a short non-repeating oligosaccharide core and a distal polysaccharide named O-antigen. *S. typhimurium* mutant strains which produce a LPS which either does not contain heptose or contains heptose residues but no glucose belong to the *rfa* class. These mutants become much more sensitive to a variety of hydrophobic dyes, antibiotics, and detergents. In this communication we have examined the microcin J25 (MccJ25) sensitivity of a series of well-characterized rfa mutants through determination of the minimal inhibitory concentration (MIC) in liquid media. The results indicate that mutants *rfaH, rfaD, rfaI* and *rfaE*, just like the wild type strain, were resistant to MccJ25. On the other hand, *rfaG, rfaL-rfaE*, *rfaF* and *rfaC* were sensitive with a MIC of 62,5; 125; 250 and 500 µg/ml, respectively. Just in opposite to the resistant strains, the sensitive mutants showed a notable uptake of a fluorescent microcine derivative. Some sensible bacteria showed filament formation uncorrelated with their degree of sensitivity. The results provide evidence on the importance of nonspecific interaction of MccJ25 with the cell outer membrane in order to confirm its antibiotic activity.

MI-P74.
OVEREXPRESSION OF *ydiE*, AN ORF OF UNKNOWN FUNCTION, CONFERS HIGH RESISTANCE TO MICROCIN J25
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Previously, we observed an intrinsic, chromosomally-encoded resistance, in several *E. coli* strains to the peptide antibiotic MccJ25. To identify genes responsible for this phenotype, a genomic library in MudII4042 was constructed by thermoinduction of MC4100 Mucts Δ*yojI* (pEG109). Strain AB1133 (sensitive to MccJ25) was infected with the resulting lysate and muductants were selected on medium containing chloramphenicol and MccJ25. Plasmid DNA was extracted from one of several MccJ25-resistant clones, digested with HindIII and cloned into pACYC177. This plasmid conferred resistance to MccJ25 when transformed into a sensitive strain and was named pACAX66. The fragment carried by pACAX66 was sequenced and located on the 38-min region of the *E. coli* chromosome. Additional subclones of this plasmid in the vector pACYC177 were constructed. One of them, containing a 655 bp fragment, was able to confer resistance to MccJ25. The insert should encompass only a single chromosomal ORF, *ydiE*. Interestingly, several experiments showed that YdiE could not export MccJ25 and conferred resistance even in a TolC background. This suggests that *ydiE* neither acts as a MccJ25 pump nor activates one. Whatever the mechanism, this represents a new form of microcin resistance. This is currently under study in our laboratory.

MI-P75.
BACILLUS SUBTILIS COLD ADAPTATION
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Spore development in *Bacillus subtilis* is governed by the master transcription factor Spo0A. Although B. subtilis is a soil bacterium, and hence temperature changes would constitute a common environmental stress, in the past poor attention was devoted to this situation. Our group, nonetheless, recently reported that *spo0A* was dramatically induced after a temperature downshift. This stress correlated with increments in the level and activity of Spo0A and higher cellular survival rates. Here, using deletion mutants, beta-galactosidase reporter-essays and transcriptional fusions to fluorescent proteins, we investigated the circuit that result in the cold-induced upregulation of *spo0A*. We show that cold-induction of *spo0A* depends on its phosphorylation state and the integrity of the phosphorelay signal transduction system. Moreover, similar to what happens at 37ºC, KinA retains an important role during cold adaptation, but interestingly we found that the sporulation efficiency of a *kinB* mutant, even in the presence of KinA, is reduced 200 fold respect to the wild type. Moreover, the expression of key developmental genes (i.e. *spoIIG* and *spoIIA*) was decreased and delayed in the *kinB* mutant during growth at low temperature. Since KinB is an integral membrane histidine kinase that lacks of a "periplasmic" sensor domain, we propose and discuss a novel role of KinB as a "sporulation thermosensor".

MI-P76.
***ESCHERICHIA COLI* MUTATIONS AFFECTING VIABILITY IN STATIONARY PHASE**
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Escherichia coli cultures show a biphasic death curve after entry into stationary phase, with viable counts dropping by one log unit in the first four days of incubation. Afterwards the remaining viable cells die much more slowly. The triggers for the transition from stationary phase to death phase and even the mechanism(s) of cell death are not well understood. We have found that for an *E. coli sbmA* mutant the death phase is dramatically exacerbated. In fact, after 4 days in stationary phase there is a loss of viability of at least three log units. To test whether the reduction in colony forming units (CFU) in the plating assays was due to a loss of viability and not simply to a viable but non culturable state, we employed the Live Dead Bac Light fluorescence-based assay. The results indicated that the reduction in colony counts indeed reflected death of the cels. When the *sbmA* mutation was complemented by a plasmid harboring the *sbmA* gene, the phenotype was not reversed. This suggested that there is probably another mutation in the strain used, MC4100, that, when combined with *sbmA*, led to the increased loss of viability. We are currently testing this hypothesis. This is the first time that a physiological phenotype is described for a *sbmA* mutant. Our results could throw light on the factor(s) that mark the transition between stationary and death phases.

MI-P77.
MICROAEROBIC GROWTH IN A POLYHYDROXY ALKANOATE-PRODUCING *PSEUDOMONAS*
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Pseudomonas sp. 14-3 is an Antarctic strain isolated in our laboratory that accumulates high amounts of polyhydroxyalkanoates (PHA). This reserve polymer is a sink for carbon and reducing equivalents having an important role in stress resistance and redox balance. In *Pseudomonas,* the transition from aerobic to anaerobic growth is regulated by Anr. This regulator controls several genes such as nitrate reductase and the *ArcDABC* operon related with the utilization of arginine. Genes involved in PHA biosynthesis of *Pseudomonas* sp. 14-3 have been characterized, showing a putative Anr-box, site of binding of Anr. It suggests its relationship with PHA biosynthesis. In this work, we identified *anr* and analyzed the growth of *Pseudomonas* sp. 14-3 under microaerobic conditions. This strain was able to growth in nitrate supplemented medium tightly closed bottles, producing until to 1.1 g/l of nitrite after 48 h of culture. Arginine fermentation supported survival and slow growth showing lower arginine deiminase activity compared with *P. aeruginosa*. Amplification by PCR, cloning and sequencing of *anr* showed that it was highly similar (88% of identity) to *P. fluorescens* PfO-1. The amplified fragment complemented an *E.coli* mutant of *fnr*, gene homologous to *anr*. Analysis of the regulation of PHA synthesis under microaerobic conditions will contribute to optimize its production in *Pseudomonas*.

MI-P78.
EFFECT OF OSMOLARITY AND *ppGpp* ON *mdoGH* TRANSCRIPTION AND OPG CONTENT IN *SALMONELLA ENTERICA*
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Osmoregulated periplasmic glucans (OPG) are oligosaccharides composed exclusively of glucose whose level in the periplasm is inversely related to the osmolarity of growth media. Two MudJ insertions that impaired OPG synthesis were isolated by selecting for increased mecillinam resistance: they were identified as an *mdoH* insertion and an *mdoG* transcriptional fusion. To investigate the effect of osmolarity and ppGpp on transcription of the *mdoGH* operon, β-galactosidase (β-gal) activity from *mdoG1*::MudJ cells grown to exponential or stationary phase in LB broth with no salt (NoS) or containing 0.3 M NaCl was assayed. Although OPG content in exponential cultures grown in 0.3 M NaCl was at least 80% lower than in NoS, β-gal activity showed only a 30% decrease. Moreover, transcription of *mdoGH* genes in cells grown in NoS broth was not affected by growth phase; yet, OPG content in stationary cultures grown with no salt suffered a sharp drop to the same low level of 0.3 M NaCl cultures. Absence of ppGpp produced almost no effect on β-gal activity under several growth conditions; however, there was no drop of OPG level in stationary cultures of the ppGpp defective strain grown in NoS broth. It is concluded that osmolarity affects only slightly *mdoGH* transcription but, in contrast, OPG content is strongly modulated not only by osmolarity but also by growth phase and ppGpp.

MI-P79.
OXIDATIVE SHIFT IN LATE STATIONARY PHASE BY PHOSPHATE CONCENTRATIONS IN *ESCHERICHIA COLI*
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Respiratory NADH dehydrogenase-2 (NDH-2) in *Escherichia coli* is a cupric-reductase encoded by *ndh* gene. This gene is expressed in LB medium during exponential phase of growth, decaying in stationary phase. However, we found that *ndh* expression and NDH-2 membrane activity are maintained in late stationary phase by at least 35 mM phosphate salts. In order to study the cellular status in this physiological condition, we determined cell viability, oxygen consumption, NADH and NAD^+ concentration and ROS production. Cells grown for 48 h in high phosphate media were more viable, presented higher oxygen consumption, had a lower NADH/NAD^+ ratio (similar to exponential) and contained lower ROS levels than cells grown in low phosphate media. Moreover, we have observed that the decrease of *ndh* expression in low phosphate media was reversible when phosphate was added at 24h of culture. Our results show the importance of the phosphate concentration in the culture media for the regulation of genes expression, since modifications in the anion concentration can produce a drastic change in the cellular metabolic pathways maybe associated to a protective effect against oxidative stress. Further studies should be done to understand the role of NDH-2 in late stationary phase under this growth condition.

MI-P80.
REGULATION OF SOCIAL BEHAVIOR AND QUORUM SENSING IN *BACILLUS SUBTILIS*
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Bacteria not only behave as self-sufficient individuals but also act as communities capable of cell-cell communication. This social interaction leads to the coordination of communitarian activities that resemble, in their complexity, the behaviors observed in multicellular organisms. This microbial phenomenon is known as quorum sensing (QS), a process by which bacteria monitor their cell population density by measuring the concentration of small secreted signal molecules called autoinducers. AI-2 dependent QS have been reported as a bacterial Esperanto for intra and inter-specific bacterial communication. Recently, we reported that, *B. subtilis* LuxS/AI-2-dependent QS act as an intraspecific bacterial signal required for biofilm formation, sliding motility and structure of sophisticated aerial colonies that behaved as giant fruiting bodies. In this work, we demonstrated the existence of a negative regulator of the luxS gene and its role during the onset of QS, sporulation, biofilm development and fruiting body formation. Additionally, we show evidences of the role of an ABC transporter in the incorporation of AI-2 to the cell and quorum quenching. The AI-2 dependent sliding motility studies bring about an unexpected and interesting role of the master regulator Spo0A in cell-cell recognition on solid surfaces.

MI-P81.

IDENTIFICATION AND CHARACTERIZATION OF A LIPOPROTEIN OF SPORULATION IN BACILLUS SUBTILIS

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In the absence of nutrients *B. subtilis* initiates a differentiation process called sporulation. After an asymmetrical division two compartments are created. The larger one, called mother cell, engulfs and nurtures the smaller one, forespore, and eventually lyses to release a dormant environmentally resistant spore. When exposed to nutrients, the spore resumes vegetative growth in a process called germination. To identify hypothetical lipoproteins involved in sporulation, we labelled *B. subtilis* cultures with tritiated palmitic acid and found a putative lipoprotein expressed under the control of the mother cell specific sigma factor σ^E. We demonstrated that the protein detected is GerM, previously reported to be necessary for both spore formation and germination. A version of GerM mutated in its putative lipidation site (GerM*) was not able to incorporate tritiated palmitic acid. A mutant strain expressing *gerM** showed impaired sporulation efficiency and low dipicolinic acid content in sporulating cells. Addition of germinants to the mutant spores resulted in the triggering of an incomplete germination with a significant deficiency in loss of spore refractility but normal loss of heat resistance. These phenotypes are similar to those of a *gerM* strain, suggesting that the lipobox site of this lipoprotein is critical for its proper function.

MI-P82.

REGULATION OF VIRULENCE FACTORS IN *STAPHYLOCOCCUS AUREUS* IS AFFECTED BY SALICYLIC ACID

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The ability of *S. aureus* to adapt to different environments is due to a global regulatory network comprising the loci *mgrA* and *sae* amongst others. These regulators are involved in the control of the expression of several virulence factors such as *eap* adhesin, which is upregulated by *sae*. Salicylic acid (SAL) negatively affects the expression of bacterial virulence factors. The aim of this work was to study the effect of SAL on *mgrA*, *sae* and *eap* through expression systems including plasmids carrying a reporter gene (green fluorescent protein GFP) driven by target gene promoters as well as by quantitative real time PCR (qRTPCR). We demonstrated a reduced expression of *eap* transcript in *mgrA* mutant through fluorometric assays and RTPCR suggesting that MgrA is a positive regulator of this adhesin. After seven hours of growth, strains treated with SAL showed reduced expression of *mgrA*, none differences in *sae* and increased expression of *eap* as compared with non-treated controls in fluorometric assays. The qRTPCR data shows that SAL down-regulates *mgrA* while up regulating *sae* and *eap* (mean fold change in gene expression by SAL treatment. *mgrA*: 0.75 ± 0.09; *sae*: 1.58 ± 0.19; *eap*: 2.05 ± 0.59). Altogether these results show that SAL does affect *sae* and *mgrA,* which would alter the expression of several virulence factors driven by these regulators and therefore pathogenesis of *S. Aureus*.

MI-P83.

AUXOTROPHIC MUTANT OF *STAPHYLOCOCCUS AUREUS* GENERATED BY ALLELIC REPLACEMENT

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Several strategies have been developed for generating gene replacements in bacterial pathogens. The aim of this study was construct an *aroA* deletion mutant of *Staphylococcus aureus* using a new plasmid (pMAD) for nontransformable bacteria. This termosensitive vector allows a quick colorimetric blue-white discrimination of bacteria that have lost plasmid, greatly facilitating clone identification during mutagenesis. The *aroA* gene of RN6390 strain was amplified with specific primers to generate a fragment with a deletion of 900 bp. The PCR product was cloned into pMAD vector. The pMAD-1 recombinant plasmid obtained was used to transform the RN6390 strain by electroporation. Then, integration of the plasmid into the chromosome by a single crossover event was selected during growth at 44ºC. Subsequent growth of cointegrates at 30ºC led to a second recombination event, resulting in their resolution. White colonies (without pMAD-1) were characterized by PCR and subsequent sequentiation to confirm the deletion of the *aroA* gene. Phenotypic characterization of the mutant SB374 resulted in the loss of growth onto plates lacking aromatic aminoacids suplements. The SB374 mutant had a higher lethal dose 50 than the parental strain demonstrating be attenuated *in vivo*. These results show that the deletion of *aroA* gene obtained by a new tool for Gram-positive, reduced the virulence of *S. Aureus*.

MI-P84.

CHARACTERIZATION OF THE 7,8-DIHYDRO-8-OXO-DEOXYGUANOSINE MUTATION REPAIR SYSTEM OF *PSEUDOMONAS AERUGINOSA*

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The 7,8-dihydro-8-oxo-deoxyguanosine (8-oxodG) Mutation Repair System prevents or repairs the mutations produced by the oxidized base. This system has been mainly studied in *Escherichia coli* where it involves the action of three proteins: the pyrophosphohydrolase MutT, and the DNA glycosylases MutM and MutY. MutT converts 8-oxodGTP to 8-oxodGMP, preventing incorporation of the oxidized base into the DNA. MutM removes 8-oxodG mispaired with A, and MutY removes A mispaired with G or 8-oxodG. We aim to describe this system in *P. aeruginosa*, by studying the influence of these three genes in the mutagenesis rates, and the biochemical characterization of the enzymes. In this work, we used ΔmutT, ΔmutM and ΔmutY *P. aeruginosa* strains, and the three WT genes cloned in expression vectors for complementation assays.

The strains mutation rates was determined by the Rifampicin and Ciprofloxacin resistance tests. Rifampicin resistance mutation frequencies for Δ*mutT,* Δ*mutM* and Δ*mutY* strains showed, respect to the WT strain, an increase of 44-, 3.8- and 26-fold, respectively. Interestingly, the emergence of Ciprofloxacin resistant mutants was, compared with the WT strain, 17- and 136-fold in Δ*mutM* and Δ*mutY* strains, and more than 800-times in Δ*mutT*. This strain was complemented by gene *mutT* confirming that the mutation frequency was dependent of the presence of oxidized bases.

MI-P85.
MCE3R CONTROLS DE EXPRESSION OF A LARGE REGULON INVOLVED IN LIPID METABOLISM IN *MYCOBACTERIUM TUBERCULOSIS*
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*mce*3 is one of the four virulence-related *mce* operons of *M. tuberculosis*. The biological function of Mce proteins is not known, but increasing evidences have demonstrated that they are clearly related to the virulence of *M. tuberculosis* complex species. Gene regulation is considered to play a central role in host-microbe interactions, and many virulence genes are regulated in response to the host. In a previous work we showed that the overexpression of Mce3R, a TetR-type transcriptional repressor, abolishes the expression of LacZ fused to the *mce*3 promoter in *M. smegmatis* and *M. tuberculosis*, indicating that Mce3R represses *mce*3 transcription. Here, combining microarray analyses and real-time quantitative polymerase chain reaction, we found that a knockout of the *mce*3R gene in *M. tuberculosis* results in a remarkable increase in the expression of *mce*3 genes *in vitro*. The expression of the other *mce* operons is not affected by this mutation demonstrating that Mce3R regulates exclusively the transcription of *mce*3 operon among *mce* genes in the conditions tested. We also show that it represses the transcription both of itself and of a large number of genes involved in lipid metabolism and redox reactions. The identification of the mce3R regulon could help to decipher the function of *mce*3 genes and would provide valuable insight into their role on *M. tuberculosis* virulence.

MI-P86.
THE PHOSPHORELAY MECHANISM OF THE RCSC/YOJN/RCSB SYSTEM IN *SALMONELLA ENTERICA*
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The Rcs phosphorelay system involves the sensor protein RcsC, the cognate response regulator RcsB, and the histidin-containing phosphotransfer protein YojN, which serve as an intermediary in the phosphoryl transfer from RcsC to RcsB. Previously, we found that the overproduction of RcsB regulator promotes the Rcs system activation, in the *rcsC* or *yojN* mutants, but not in the double mutant *yojN rcsC*. These results suggested that only RcsB-P, the RcsB active form, is able to induce the RcsB-dependent genes modulation, like *cps* and *flhDC*. We are interested to define the Rcs phosphorelay transduction mechanism. To determinate if RcsC or YojN can independently transfer the phosphate group to RcsB, or if in this process is necessary that both protein act together, is necessary to purify all the Rcs system components, which will be used in an *in vitro* phosphorylation assays. Therefore, we cloned the *rcsB*, *rcsC* and *yojN* genes using the pUHE-21 vector. To facilitate the protein purification procedure, we added in the N-terminal region a sequence that codify for the His-Tag. In these assay we observed that RcsC and YojN proteins appear in the insoluble fraction. In order to overcome this problem we resolved to use the pET41.3 system, which facilitate the generation of a fusion with the *nusA* gene to increment the solubility of our interest proteins.

MI-P87.
THE REGULATION OF FEPE GENE BY THE PMRA/B TWO COMPONENT SYSTEM IN *SALMONELLA TYPHIMURIUM*
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The lipopolysaccharide (LPS) is the outermost component of the cell envelope in Gram (-) bacteria. It consists of the hydrophobic lipid A, a short non-repeating core oligosaccharide and a distal polysaccharide termed O-antigen. We report here the regulation of *fepE gene*, which product determines the O-antigen very long chain (35 to 100 O-antigen subunits) attached to the lipid A-core. We show that the PmrA/PmrB two-component system, which controls the modification of the lipid A, promotes directly the *fepE* transcription, when *Salmonella* experiences PmrA/PmrB-inducing conditions. The PmrA/PmrB system consists of the response regulator PmrA that is activated by Fe^{3+}, the signal sensed by its sensor PmrB; and by low Mg^{2+}, in a PhoP/PhoQ-dependent pathway. The transcriptional induction of *fepE* increased the amount of O-antigen very long chain, but can not modify the resistance to the serum complement-mediated killing. Nevertheless, we show that the deletion of *pbgE2E3* and *wzzB* genes, which are also induced by the PmrA regulator, resulted in an O-antigen without the region containing 1 to 15 or 16 to 35 O-antigen subunits, respectively, and decreased the sensitivity to serum complement. These results indicated that the PmrA/B system is the master regulator of the LPS synthesis, and that both O-antigen formation and the lipid A modification are required at the same time in *Salmonella.*

MI-P88.
THE ACIDIC STRESS-INDUCED LYSIS IS MEDIATED BY STKP AND CIARH IN *STREPTOCOCCUS PNEUMONIAE*
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We have described that acidic stress triggers autolysis in *S. pneumoniae* and that this process is mediated by the response regulator ComE, through a quorum sensing (QS) independent pathway. This constitutes a novel function of ComE, since it has been implicated in the regulation of competence development at alkaline pH, through a QS mechanism. In the present work, we studied the contribution of CiaRH two-component system and the StkP serine/threonine kinase in the acidic stress-induced lysis (ASIL), since they can regulate the comCDE operon and are involved in resistance to several types of stress and lytic conditions. We observed that ciaR and ciaH mutants lysed more rapidly than wild type strain (wt). Double ciaR/comE or ciaH/comE mutants were as susceptible to ASIL as the ciaR or ciaH mutants, indicating that CiaRH protects cells from ASIL by a ComE-independent pathway. Analysis of the stkP mutant showed that ASIL was blocked, and a similar phenotype was observed in stkP/ciaR or stkP/ciaH mutants. To investigate whether StkP was involved in the ComE-mediated ASIL pathway, we constructed a stkP/cup3 mutant, since strain cup3 has high levels of comE transcripts and shows accelerated lysis. Interestingly, ASIL was also abolished in the stkP/cup3 mutant. We conclude that StkP is necessary for the induction of ASIL by participating in both ComE and CiaRH regulatory pathways.

MI-P89.
CITOLOCALIZATION OF THE *SALMONELLA ENTERICA* PhoP RESPONSE REGULATOR
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The *Salmonella enterica* two-component regulatory system controls the expression of several genes necessary for virulence, in response to extracellular (extracel) concentration (cc) of Mg^{2+}. PhoQ interacts with extracel Mg^{2+} and controls the phosphorylation state of PhoP. PhoP overexpression can substitute for PhoQ- and phosphorylation-dependent activation. Many bacterial processes involve asymmetric localization of protein activity (i.e., proteins involved in chemotaxis, development or signal transduction). In order to investigate the localization of PhoP and the effect of the input signal and the phosphorylation state on its spatial distribution, we set up the FlAsH labelling technique, which adds a hexa-aminoacid motif to the target protein. We show in that PhoP was recruited to the cell poles when extracel Mg^{2+} was limiting. This localization disappeared when the cognate component was absent. The non-phosphorylatable PhoP was uniformly localized in the cytoplasm, irrespective of the extracel. Mg^{2+} cc or the presence of PhoQ. In over-expression conditions, both proteins showed a massive polar localization, not altered by the Mg^{2+} cc used or by the absence of endogenous PhoQ. These results indicate that the migration of PhoP to the poles occurs when it is in its activated dimeric state, either due to PhoQ-dependent phosphorylation or to protein-protein interaction forced by over-expression.

MI-P90.
CITRATE METABOLISM IN *ENTEROCOCCUS FAECALIS* IS REPRESSED BY PTS SUGARS
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Enterococcus are involved in the ripening and in aroma development of diverse cheeses. These positive effects have been attributed to specific biochemical traits such as lipolytic activity and citrate utilization. The expression pattern of the *cit* locus showed the presence of two divergent operons, *citHO* and *oadDBcitCDEFXoadAcitMG* encoding the enzymes for citrate metabolism. Northern blot analysis showed activation of both operons by specific addition of citrate to the medium and repression in the presence of glucose. In accordance with that, cell extracts showed repression of citrate lyase activity and the level of OadD protein, determined by western blot, was also repressed when the cells were grown in the presence of citrate and glucose. Carbohydrate repression was only observed with PTS sugars suggesting a Carbon Catabolite Repression (CCR). Moreover, the repression was partially relieved when transcriptional fusions of the promoters were analyzed in an *Enterococcus ccpA* mutant strain. Sequence analysis of the promoter regions revealed the presence of catabolite responsive elements (*cre*). Band shift experiments demonstrated that *Bs*CcpA could bind to these *cre* sites downregulating the expression of the operons. In order to establish which one is involved in the CCR observed, the *cre* sites were systematically mutated and the promoter activities were determined.

MI-P91.
INFLUENCE OF GLYOXYLATE AND ALLANTOIN PATHWAYS ON ANTIBIOTIC PRODUCTION IN *STREPTOMYCES COELICOLOR*
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In *Streptomyces* sp. acetyl-CoA is a key metabolite in the interaction between primary and secondary metabolism since it can be incorporated into celular structures and also it is a precursor of polyketide antibiotics. In this work we have identified and characterized the components of glyoxylate and allantoin pathways from *S. coelicolor*. Two clusters of putative genes involved in these pathways have been identified in the genome: *sco0982* and *sco0983* genes encoding isocitrate lyase and malate synthase are next to *sco0981* gene which encodes a putative protein homologue to RamB, a negative regulator of acetate metabolism in *C. glutamicum*; *sco6243*, *sco6247* y *sco6248* genes encoding proteins involved in allantoin pathway are next to *sco6246* gene which encodes a protein homologue to AllR, the allantoin regulator of *E. coli*. Genetic studies with mutant strains in each of these genes demonstrated that deregulation of these pathways strongly affect antibiotic production. These results could suggest that availability of precursors is a checkpoint for the biosynthesis of secondary metabolites. Enzyme activity profiles have been characterized to confirm the role of putative regulator proteins in each metabolic pathway. These studies will allow us to understand better how enzymes involved in the primary metabolism affect the production of secondary metabolites in *S. Coelicolor*.

MI-P92.
TRANSCRIPTIONAL REGULATION OF THE FATTY ACID BIOSYNTHETIC GENES IN *STREPTOMYCES COELICOLOR*
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Fatty acid biosynthesis is a vital facet of bacterial physiology and is carried out by a series of enzymatic steps, each encoded by a different gene known as the type II fatty acid synthase (FAS). In *Streptomyces coelicolor* the essential FAS genes are clustered at a single location within its genome (*fab* operon). Bioinformatics analysis of the *fab* genes locus revealed the presence of an open reading frame located upstream of *fabD*, named *sco2386*, which has homology with transcriptional regulators of polyketides biosynthesis in *Streptomyces ssp*. Sco2386 is highly conserved among several actinomycetes like *Mycobacterium*, *Nocardia*, *Frankia*, etc. In this work we investigated the functional role of Sco2386 in the transcriptional regulation of the *fab* operon and the physiological and morphological consequences of its mutation in *S. coelicolor*. For this, we constructed a *sco2386* disruption mutant strain; evaluated its ability to growth under different carbon source and analyzed its fatty acid and TAG composition in different growth conditions. We also examined by reporter fusions the *fab* transcriptional profile in the *sco2386* mutant as well as in the wild type strain. Finally we studied Sco2386-*fabD* promoter interactions by electrophoresis shift mobility assays. All the results obtained strongly suggest that Sco2386 mediates the transcriptional regulation of *fab* operon.

MI-P93.
REGULATION OF EXPRESSION OF SUC METABOLISM GENES IN *ANABAENA* BY ALTERNATIVE RNA POLYMERASE SUBUNITS
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One strategy employed by eubacteria to regulate gene expression is the use of alternative sigma subunits of RNA polymerase. The expression of one group 2 sigma factor, in *Anabaena* sp. strain PCC 7120, was induced under N-limitation conditions; although it was not required for heterocyst differentiation. It was demonstrated that this factor is homolog to stationary phase sigma subunits of other cyanobacteria. At present, the function of most principal sigma-like genes is not known. In the *Anabaena* sucrose (Suc) is synthesized by Suc-phosphate synthase and Suc-phosphate phosphatase, and cleaved by Suc synthase. These enzymes are encoded by *spsA* and *spsB*, *sppA*, and *susA*, respectively. Since Suc metabolism genes were upregulated in stationary phase, the aim of this work was to examine the involvement of the group 2 sigma factor in this regulation. By RTPCR and primer extension experiments we demonstrate that the expression of this factor was increased in the stationary phase. In addition, band shift assays were performed to analyse its binding to Suc metabolism gene promoter regions. We concluded that this sigma subunit might regulate gene expression in the stationary phase since its expression was increased under this condition and that Suc metabolism genes may be included into the repertoire of its regulated genes given that this factor bound to Suc gene promoter regions.

MI-P94.
TRANSCRIPTIONAL AND POSTTRANSCRIPTIONAL CONTROL OF SUCROSE GENES IN *SYNECHOCOCCUS* SALT-TREATED
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Compatible solutes accumulate during cellular acclimation to environments with low water potentials. *Synechococcus* sp. PCC 7002 synthesizes glucosylglycerol (primary osmolite) and sucrose (Suc) in response to salt stress. Recently, it was elucidated that Suc synthesis in this strain occurs through the sequential action of sucrose-phosphate synthase (SPS) and sucrose-phosphate phosphatase (SPP). This strain presents a single gene that encodes SPS, in agreement with the proposed pattern of Suc metabolism evolution. SPS gene (*spsA*) is superimposed 8 nucleotides with the SPP gene (*sppA*), suggesting a transcripcional unit with two overlapped cistrons. We investigated the effect of NaCl and stationary phase on Suc gene expression in *Synechococcus*. Northern blots revealed independent transcripts for both genes. We found that rifampicin abolished *spsA* and *sppA* transcript synthesis after 3 h of its addition. RT-PCR assays using intergenic primers were carried out to determine the possible presence of a bicistronic transcript. Only in the presence of chloramphenicol was detected the *spsA/sppA* transcriptional unit, suggesting that protein synthesis is involved in the primary transcript degradation, and that both genes are cotranscribed. Putative promoters were identified by primer extension analysis. This is the first report of a operon coordination of Suc synthesis gene expression.

MI-P95.
PROTEIN-DNA INTERACTIONS IN THE REGULATION OF LIPID BIOSYNTHESIS IN *BACILLUS SUBTILIS*
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FapR is a global transcriptional repressor that controls the expression of many genes involved in the biosynthesis of lipids (the fap regulon) in *Bacillus subtilis*. FapR has highly conserved homologues in many Gram-positive bacteria, including several human pathogens. As well, in all these organisms the consensus binding sequence of FapR is largely invariant in the putative promoter regions of the fapR gene, indicating that the regulation mechanism is conserved. FapR belongs to a new class of bacterial repressors in which malonyl-CoA operates as the direct and specific inducer of FapR-regulated promoters. Despite genetic and biochemical studies, specific details of the interaction between FapR and its operator sequences remain unknown. To obtain a high resolution profile of the contacts between FapR and its target DNA we resorted to hydroxyl radical footprinting analysis and gel shift assays. For each promoter analyzed, four protected regions spaced from 6 to 9 nucleotides were detected. Also, in each case, two different complexes were observed in gel shifts assays. To confirm the recognition motifs, these assays were repeated with promoter variants carrying specific mutations. Our results strongly suggest that FapR binds to its operators as a tetramer or two dimers in contact with the DNA backbone at the same face of the DNA helix.

MI-P96.
THE METAL-BINDING LOOP DETERMINES METAL SELECTIVITY IN CUER-LIKE TRANSCRIPTIONAL REGULATORS
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Metal-dependent transcriptional regulatory systems are the major cellular mechanism that bacteria employ to control metal homeostasis or resistance to harmful non-essential transition elements. *Salmonella* has two transcriptional regulators of the MerR family that respond to monovalent metal ions: CueR, which is involved in copper homeostasis, and GolS, which is responsible for gold detoxification. Both proteins share 42% amino acid sequence identity but, unlike CueR, GolS can discriminate between Au and Cu ions. To understand how GolS discriminates between these two related metal ions we first performed a comparison of the protein regions that can influence metal binding: the metal-binding loop (between C_{112} and C_{120}), the C-terminal region after the loop, and the amino acid residues surrounding S_{77} in the dimerization domain. These regions were modified in GolS by loop- or site-directed mutagenesis to resemble these of CueR, and introduced into the chromosome, replacing the wild-type copy of *golS*. The response of each mutant regulator to either Au or Cu ions was analyzed by monitoring expression of GolS-controlled genes. Our results indicate that the amino acid residues surronding the two conserved Cys at the metal binding loop are essential for the discrimination between the two metal ions. These findings can be used for the construction of Group IB metal-specific biosensors.

MI-P97.
EXPRESSION OF *SINORHIZOBIUM MELILOTI* NFRA GENE CODING A PUTATIVE CHAPERONE OF RNA TRANSACTIONS
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Riboregulation refers to post-transcriptional regulatory processes that operate on mRNAs with the participation of other RNA molecules, notably small non-coding RNA molecules (sRNAs), and in some cases of collaborator RNA binding proteins, which usually act together to modify the stability or translation rate of target mRNAs in response to regulatory cues. In the genome of the plant root symbiont and nitrogen-fixing bacteria *Sinorhizobium meliloti* 1021, there is an 80 aa-ORF annotated as NrfA having 47% identities to Hfq, the master riboregulator chaperone of *Escherichia coli*. As we are interested to study riboregulatory mechanism in this bacterium, we asked: ¿Does nrfA express in *S. meliloti*? And if so, ¿Is its expression regulated? We first located the putative *nrfA* promoter region in silico and used this information to generate a chromosomal transcriptional fusion to the promoterless *lacZ-accC1* cassette in the closely related strain *S. meliloti* 2011. We found that the identified promoter region is expressed *in vitro* and that it is induced ca. 3-fold at late-exponential phase. We next studied the *in vitro* response of the fusion to different stress conditions and to conditioned growth medium. *nrfA-lacZ* expression was also monitored along different stages of the interaction between bacteria and alfalfa roots.

MI-P98.
STUDY OF THE *sbmA* GEN REGULATION IN *ESCHERICHIA COLI*
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E. coli SbmA transports MccB17, MccJ25, and bleomycin into the cell cytoplasm. Homologs of the *sbmA* gene are found in a variety of bacteria suggesting an important physiological role might exist for these genes however it remains unknown. In this work we described the study of the *sbmA* regulation and the *sbmA* mutant sensitivity to different agents. Using the GPS bioinformatical program, we demonstrated that the SlyA regulator has a putative binding site on the *sbmA* promotor region. To confirm these results we performed β-galactosidases assays, and found that *sbmA* transcription is negatively regulated by SlyA. We also probed the expression in conditions of MccJ25 accumulation studying *sbmA*::*lacZ* expression in a *tolC* mutant. Interesting, in the absent of the antibiotic we still observed high level of β-galactosidase activity, indicating a positive regulation by *tolC* mutation. We also observed that *sbmA* is expressed mainly during the stationary phase and this expression levels was increased when the mutant growth in minimal medium containing low Mg^{2+} concentration. We only found a partial resistance to polymyxin B in *sbmA* mutant when the sensitivity of this strain was probed to several agents. Ours preliminary results led us to propose a hypotetical regulation of *sbmA* by SlyA and PhoP/PhoQ in *E. Coli*.

MI-P99.
AUTOINDUCER-2 REGULATES CELL-CELL COMMUNICATION AND DEVELOPMENT IN *BACILLUS SUBTILIS*
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Cell to cell communication in bacteria is regulated by quorum sensing (QS). In particular, LuxS / AI-2 dependent QS has been proposed to act as a universal lexicon that mediates intra- and inter-specific bacterial behavior. Here we report that the model organism *B. subtilis* operates a luxS-dependent QS that regulates its morphogenesis and social behavior. We show that luxS is a growth-phase regulated gene that produces active AI-2 to mediate the inter-specific activation of light production in *Vibrio harveyi*. LuxS expression is under the control of a novel AI-2-dependent negative regulatory feedback loop pointing out AI-2 as a key signaling molecule. AI-2 production was negatively regulated by the master regulatory proteins of pluricellular behavior, SinR and Spo0A. Interestingly, wild and undomesticated *B. subtilis* cells require the LuxS-dependent QS to form robust and differentiated biofilms and also to swarm on solid surfaces. Furthermore, LuxS activity was required for the formation of sophisticated aerial colonies that behaved as giant fruiting bodies where AI-2 production and spore morphogenesis were spatially regulated at different sites of the developing colony. We proposed that LuxS constitutes a novel form of intraspecific quorum sensing where AI-2 behaves as a morphogen-like molecule that coordinates the social and pluricellular behavior of *B. Subtilis*.

MI-P100.
INDUCTION OF RpoS DEGRADATION BY THE TWO-COMPONENT SYSTEM REGULATOR RSTA IN SALMONELLA
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Bacteria sense environmental conditions using a variety of strategies. Among them, two-component systems are the predominant form of signal transduction used by prokaryotes to respond to different kind of stresses.
We found that the expression of RstA, the regulator of the two component system RstA/RstB, is able to down-regulate σ^S (RpoS) levels in *Salmonella*. We determined that this regulation does not occur neither at the transcriptional nor at the translational level, but it alters RpoS protein turn-over. We showed that RstA expression increases three times RpoS degradation rate, using a mechanism that remains unknown. However, we found that it is independent of the ClpP/MviA pathway, the unique RpoS proteolytic route described up to now. We also established that none of the other three mayor proteases of *Salmonella* (Lon, HslV and HlfB) are involved in the RstA-dependent degradation of RpoS.
Besides, we found that a strain overproducing RstA decreases biofilm formation, while an *rstA* mutant strain shows a moderate increase in this process. As biofilm biogenesis is RpoS-dependent, we attribute this effect to the lower content of RpoS displayed by the RstA-producing strains.
In sum, we unraveled the existence of a new mechanism for RpoS degradation mediated by the expression of RstA in *Salmonella*.

MI-P101.
WHAT IS THE ORIGIN OF LARGE GENOMIC SIGNATURES? THE MUTATIONAL BIAS HYPOTHESIS
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Although it is generally known that cellular genomes differ in specific features like G+C content and codon usage, the specific causes of such differences are not always clear. Using a measurement of nucleotide aggregation .DNA Homogeneity Index. Miramotnes *et al.* (1995) showed that genomes differ significantly in the their tendency of aggregation of different kinds of nucleotides along the DNA sequence. Here we propose the hypothesis that such differences are due to a mutational bias rather than natural selection or genetic drift. If this is the case, there are some predictions relating evolutionary rates and structural parameters in DNA (aggregation parameters) that should be measurable. We also discuss the possibility that our method could detect xenologs genes.

CB-P01.
MORPHOLOGICAL CHARACTERISTIC OF PERITUBULAR MYOID CELLS FROM SHR RATS
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Spermatozoa in the tubular lumen are transported to the rete testis by the contractile activity of peritubular myoid cells (PMC). The PMC form a layer that surrounds the seminiferous tubules (ST) in the testis. These cells have a hexagonal shape that can be visualized by silver stain. Our interest was to know whether PMC are affected by hypertension. PMC from testis of SHR rat (hypertense) and WKY rat (normotense) were analysed in semiferous tubules. The area and the axes (x ± SE) of PMC of ST before and after contraction with 50 nM endothelin-1 were assayed. The surface of PMC from WKY was of 1,973 ± 110 μm2 and 1,556 ± 95 μm2 before and after contraction respectively. While the surface of PMC from SHR was of 1,571 ± 123 μm2 and 1,264 ± 144 μm2 before and after contraction respectively. The x - y axes of WKY PMC were of 47 ± 8 - 44 ± 9μm and 46 ± 9 - 38 ± 9 μm before and after contraction respectively. While the x-y axes of SHR PMC were 42 ± 3 - 43 ± 2 μm and 39 ± 1 - 34 ± 3 μm before and after contraction respectively. From these results we infer that in the rat, hypertension affects the morphology of MPC, a kind of not vascular smooth muscle cells.

CB-P02.
EFFECT OF ANTI-ZPA ANTIBODIES ON SPERM BINDING AND INDUCTION OF THE ACROSOME REACTION BY BaPZA
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Bufo arenarum oocyte vitelline envelope is an extracellular matriz composed by glicoproteins. One of them, named baZPA, has been cloned. It presents homology with mouse and human ZP2. It has been demonstrated that this protein is synthesized in the ovary by both the oocytes and the follicular cells surrounding them, by in situ hybridization and immunocytochemistry. During the process of fertilization it takes place the proteolytic cleavage of the baZPA by the action of the cortical granule contents released by the oocyte. A 28 aminoacid N-terminal peptide remains bound to the rest of the protein by disulfide bridges. Specific antibodies directed against the peptide were used in an inhibitory assay testing the binding capacity of sperm to the protein extracted from the vitelline envelope, showing a great decrease in the binding with the increasing concentration of antibody. It was also studied the capacity of the protein extracted from vitelline envelope or from the fertilization envelope to induce acrosome reaction in homologue sperm, showing that none of them is able to trigger this reaction by itself, and that is necessary the complete vitelline envelope in order to induce it.

CB-P03.
A PIG OVIDUCTAL GLYCOPROTEIN BINDS TO SPERM PROTEINS HOMOLOGOUS TO HUMAN PSORIASIN AND DERMCIDIN
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The oviduct is a dynamic organ which modulates gamete physiology. Sperm-oviduct interaction provides the formation of a sperm storage and allows the selection of sperm with certain qualities. Pig (*Sus scrofa*) oviductal SBG (Sperm Binding Glycoprotein) is present at the apical surface of the epithelial cells of the oviduct and binds to sperm producing in vitro acrosome alteration in capacitating conditions. Its presence induces also an increase on the tyrosine-phosphorylation of a polypeptide of apparent molecular mass 97 kDa. Altogether, these results suggest that SBG might be involved in sperm selection by alteration of the acrosome of sperm that have already begun the capacitation process when they arrive to the oviduct. In this study, we evaluate the interaction of SBG with boar sperm proteins. We show that SBG interacts with at least nine peptides in a direct or indirect manner. Three of them were sequenced by LC-MS/MS. One of the peptides presents homology to dermcidin and the other two represent isoforms of psoriasin. The presence of these proteins on sperm or in pig has not been reported previously. The interaction of these proteins with SBG may be partially responsible of triggering the intracellular signal required for the increase on tyrosine-phosphorylation of the 97 kDa polypeptide or another signal involved in acrosome alteration.

CB-P04.
PUTATIVE STAPS CD4-LIKE RECEPTOR BINDING DOMAIN IS INVOLVED IN StAPs/SPERMATOZOA SURFACE INTERACTION

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Potato aspartic proteases (StAPs) are plant proteases with antimicrobial activity. Previous results have shown that StAPs are spermicidal compounds. These proteins are able to interact with capacitated spermatozoa surface in the post-acrosomal region. Likewise, another aspartic protease from human seminal plasma, named gp17, interacts with spermatozoa in the same region that StAPs. This binding could be associated with gp17 capacity to interact with CD4-like receptor. Additionally, gp17 interferes with gp120 (HIV-type I envelope glycoprotein)/CD4 binding, although it binds to a different site but close to the gp120-binding site. The aim of this work was to analyse if the capacity of binding of StAPs to spermatozoa surface is associated to StAPs/CD4-like receptor binding. Besides, the presence of a putative StAPs-binding domain to CD4-like receptor was estimated by bioinformatic analysis. Results obtained from immunological analyses using monoclonal CD4 IgG antibodies show that StAPs are able to bind to CD4-like receptor of capacitated spermatozoa surface. Bioinformatic analysis shows a putative CD4 binding domain in StAPs structure. According with these results, StAPs interact with CD4-like receptor in a different domain that those described for gp17 or for gp120. The results obtained here suggest that StAPs could be analysed as a possible new gp120/CD4 blocker.

CB-P05.
HSP70 EXPRESSION AND PROTEIN TYROSINE PHOSPHORYLATION IN *BUFO ARENARUM* OOCYTES DURING FERTILIZATION

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We recently provided evidence for the involvement of HSP70 in sperm-oocyte plasma membrane interaction. On the other hand, although protein tyrosine phosphorylation plays a crucial role in many biological processes, little is known about its participation in fertilization. Our aims were to analyze HSP70 expression in the oocyte and if protein tyrosine phosphorylation is a feature of *B. arenarum* fertilization. We performed *in vitro* fertilization assays at the end points 1, 5, and 10 minutes and fractions enriched in oocyte plasma membranes (PM), microsomes, and cytosol were prepared by differential centrifugation. Before and after fertilization, HSP70 expression was assessed by Western blot using α-DnaK antibodies and protein tyrosine phosphorylation by using the PY350 antibody (Santa Cruz Biotech., against phosphotyrosine residues). We found that HSP70 is exclusively detected in PM and that is rapidly down-regulated after fertilization. Protein phosphorylation was found to be increased in cytosol, microsomes, and PM after fertilization. In particular, we detected four bands with increased tyr-phosphorylation in cytosol (approx. 42, 33, 30, and 27 kDa) and only one in PM and microsomes (approx. 42 kDa). Our results suggest that in *B. arenarum*, fertilization involves changes in protein tyr-phosphorylation and removal of HSP70 from oocyte plasma membranes.

CB-P06.
ACROSOMAL SWELLING IS REQUIRED FOR THE ACROSOME REACTION IN HUMAN SPERMATOZOA

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The acrosome is a large secretory granule underlying the sperm nucleus, whose exocytosis (acrosome reaction, AR) is required for fertilization. During the AR fusion between the outer acrosomal membrane (OAM) and plasma membrane (PM) occurs releasing the acrosomal contents. Our main objective is to characterize the molecular mechanisms underlying the morphologicals changes that take place during the AR. In resting sperm the acrosome is a flat structure with a dense content and a smooth OAM. Upon stimulation the acrosome swells so that the OAM contacts the PM at multiple points. We propose that fusion will occur in these contact sites leading to the formation of hybrid vesicles consisting of OAM and PM. We used transmission electron microscopy (TEM) to quantify changes in morphology elicited by acrosomal Ca^{2+} efflux blockers and/or AR inducers in human sperm. We found that AR inducers, such us progesterone, Ca^{2+}, ionophore A23187 and the small GTPase Rab3A cause acrosomal swelling (AS), whereas AR blockers such us PTK inhibitors inhibits it. These data support the notion that AS is required for the AR.

CB-P07.
RAB3A EFFECTORS IN ACROSOMAL EXOCYTOSIS

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Rab3A is a monomeric GTPase required for regulated exocytosis. However, the detailed mechanism of Rab3A action is not completely understood. Previous results from our laboratory indicate that Rim is a Rab3A effector in acrosomal exocytosis. Recent experiments have shown that Munc13, a Rim interacting protein participates in this process. The ZF domain of Rim2 (residues 82-142, which binds Rab3A and Munc13) and MuncC2A (Rim-binding domain of Munc13) strongly affected exocytosis in SLO permeabilized sperm. Interestingly, the inhibitory effect of MuncC2A was competed by the Rim ZF wild type domain but not by a Munc13 binding-defective mutant (K97E / K99E). The antiMuncC2A antibody recognized the endogenous protein in the sperm acrosomal region, and inhibited acrosomal exocytosis. By using a non prenylated RabGTPγS and a light-sensitive calcium chelator we demonstrated that Munc13 and Rim affect the Rab3A dependent stage of the acrosomal exocytosis. Taken together our data support a model where Munc13 directly interacts with N-terminal domain of Rim, and this interaction is required for the role of Rim as Rab3A effector in acrosomal exocytosis.

CB-P08.
ACROSOMAL EXOCYTOSIS IS REGULATED AT DIFFERENT STAGES BY COMPLEXIN AND SYNAPTOTAGMIN

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Regulated secretion is a fundamental process for many cell types. In particular, acrosomal exocytosis in mammalian sperm is essential for egg fertilization. Regulated secretion requires SNARE proteins and, in neurons, also synaptotagmin I and complexin. Complexin imposes a fusion block that is released by Ca^{2+} and synaptotagmin I. However, no direct evidence for this model in secreting cells has been provided and whether this complexin/synaptotagmin interplays functions in other cells is unknown. We show that the C2B domain of synaptotagmin VI and anti-complexin antibody blocked the formation of trans SNARE complexes in permeabilized human sperm, and this effect was reversed by complexin. In contrast, an excess of complexin stopped exocytosis at a later step, when SNAREs were in loose trans complexes. This blockage was released by addition of the synaptotagmin VI C2B and Ca^{2+}. Previously we demonstrated that this domain is regulated by PKC-mediated phosphorylation. Here, we show that a phosphomimetic mutation in the polybasic region of the C2B strongly affects its Ca^{2+} and phospholipids binding properties. This mutation abrogates its ability to rescue the complexin block. Our results show that the functional interplay between complexin and synaptotagmin has a central role in a physiological secretion event, and this interplay can be modulated by phosphorylation of the C2B domain.

CB-P09.
DAG-INDUCED ACROSOME REACTION REQUIRES PHOSPHATIDIC ACID AND PHOSPHATIDYLINOSITOL 4,5-BIPHOSPHATE

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The acrosome reaction (AR) is a particular Ca^{2+}-regulated exocytosis. We have developed a SLO-permeabilization model where Ca^{2+} activates spermatozoa AR resembling the opening of SOC channels. By using this approach, we determined that PKC activators like diacylglicerol (DAG) and phorbol esters (PMA), triggered the AR in permeabilized spermatozoa. This effect was not inhibited by chelating extracellular Ca^{2+} suggesting that DAG plays a role after the opening of SOC channels. DAG-induced AR was abrogated by PKC inhibitors (cheleritrine, Ro317549) and PLD inhibitors (1-butanol, anti-PLD). These inhibitions were reversed by phosphatidic acid (PA) suggesting that PLD activity is required during the AR downstream PKC activation. In other cell types, PA activates a specific kinase leading to phosphatidylinositol 4,5-biphosphate (PIP2) production. We demonstrated by western blot that PMA stimulation leads to PLD membrane recruitment confirming the previous result. We determined that PIP2 depletion leads to the inhibition of PMA- and Ca^{2+}-induced AR by using PLCδ4-PH domains and anti-PIP2 antibodies. To confirm this hypothesis, we performed indirect immunofluorescence by using anti-PIP2 antibodies. We observed that PIP2 increases upon PMA stimulation. Altogether these results indicate that DAG, PA and PIP2 are part of the signaling cascade activated downstream the opening of SOC channels.

CB-P10.
ARF6: A KEY MOLECULE REGULATING ACROSOMAL EXOCYTOSIS

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Sperm acrosome reaction (AR) is a special type of Ca^{++}-regulated exocytosis. Our previous results suggested that AR needs phosphatidylinositol 4,5-bisphosphate (PIP2) and phosphatidic acid(PA) after the opening of SOC channels. ADP-ribosylation factors (Arfs) are a family of monomeric GTP-binding proteins. In chromaffin cells, Arf6 activates both PI4P5Kinase and PLD1; both enzymes are thought to be coupled during signaling to support simultaneous increases in PIP2 and PA. We demonstrated that anti-Arf6 antibodies abrogated Ca^{++}-triggered AR. This inhibition is reversed by PIP2 remarking that Arf6 and PIP2 are essential molecules in AR. To study the role of Arf6 in AR, we co-transformed *E. coli* with plasmids encoding 6His-Arf6 and N-myristoyltransferase, to obtain myristoylated Arf6. Recombinant Arf6 loaded with GDPγS inhibits calcium-triggered AR confirming its involvement in the calcium pathway. Surprisingly, Arf6 activated with GTPγS induced AR per se in permeabilized cells. No effect was observed when cells were challenged with Arf6GDPβS or non-myristolated Arf6. Arf6GTPγS-induced AR was abrogated by anti-PLD antibodies or the PLC-PH domain (specifically binds PIP2) suggesting that Arf6 is upstream PLD1 and PIP2 production probably regulating their function and production, respectively. Our results constitute the first evidence suggesting a key role of Arf6 in regulating AR.

CB-P11.
INTERNALIZATION AND INTRACELLULAR FATE OF ANTIBODIES TO GM1 GANGLIOSIDE IN EPITHELIAL CELLS

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Gangliosides are glycolipids mainly located in the plasma membrane. Antibodies to ganglioside have been associated with a wide range of clinically identifiable acute and chronic neuropathy syndromes. Particularly, antibodies to GM1 ganglioside are present in patients with Guillain-Barré syndrome (GBS) and in a rabbit model of this disease. In this work, we investigated the binding and intracellular fate of internalized IgG antibodies to GM1 (from rabbit model of GBS) in CHO-K1 cells expressing GM1. We demonstrate that antibodies to GM1 are rapidly and specifically endocytosed by CHO-K1 cells. After internalization, the antibodies transit sorting endosomes to accumulate at the endocytic recycling compartment, almost no colocalizing with lysosomes marker. About 50% of the endocytosed anti-GM1 was recovered from the culture medium. Interestingly, this endocytic recycling route was only partially followed by cholera toxin (CTx), a toxin which tightly binds GM1. Briefly, endocytosed anti-GM1 colocalized with co-endocytosed CTx in recycling endosomes but not in Golgi and ER, where CTx was also found to reside. Moreover, CTx did not recycle to the culture media. Thus, the results indicate that anti-GM1 is endocytosed, accumulated in recycling endosome and transported back to the plasma membrane in a fate different from the intracellular transport of another GM1 binding molecule, as Ctx.

CB-P12.
SUBCELLULAR DISTRIBUTION AND INTRACELLULAR ROUTES FOR TRAFFICKING OF DIACYLATED GAP-43
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GAP-43 is a palmitoylated protein in Cys residues at positions 3 and 4 that mostly localizes in plasma membrane (PM). We have examined membrane association, subcellular distribution and intracellular trafficking of GAP-43 in CHO-K1 cells. Using biochemical assays and confocal and video microscopy in living cells we demonstrate that GAP-43, at steady state, localizes at the recycling endosome in addition to the cytoplasmic leaflet of the PM and Golgi complex. Single or double mutation of Cys-3 or 4 of GAP-43 completely disrupts PM and recycling endosome association. A combination of selective photobleaching techniques and time-lapse fluorescence microscopy reveals a dynamic association of GAP-43 with recycling endosomes in equilibrium with the PM pool. Newly synthesized GAP-43 is found mainly associated with the Golgi complex, but not with recycling endosomes, and traffics to PM by a BFA-insensitive pathway. Impairment of PM fusion and internalization by treatment with tannic acid does affect the trafficking of GAP-43 from PM to recycling endosomes which reveals a vesicle-mediated retrograde trafficking of GAP-43. Taken together, these results suggest that the recycling endosome-associated pool of GAP-43 represents a post biosynthetic fraction in equilibrium with the PM-associated GAP-43 and support new roles for GAP-43 in membranes from the pericentriolar endosomal compartment.

CB-P13.
***IN VIVO* STABILITY OF GOLGI RESIDENT GLYCOLIPID GLYCOSYLTRANSFERASES**
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The glycolipid glycosyltransferases (GGTs) are N-glycosylated integral membrane proteins of the Golgi complex. Information about the degradation pathways that operate on proteins resident of Golgi complex membranes, in particular the GGTs is lacking. Here we show by pulse chase and Western blot analyses, that the half-life of full length SialT2, GalT2 and GalNAcT stably transfected in CHO-K1 cells, was about 7h. Inhibition of protein synthesis by cycloheximide shortened the half-life to 1,5 h and this decrease was not reverted by inhibitors of lysosomal and proteasomal degradation. Redistribution of these enzymes to the ER by either treating cells with brefeldin A or by impairing the N-glycosylation, lead to their stabilization even in the presence of cycloheximide. Similar results were obtained with constructs in which the lumenal domain was replaced by GFP, suggesting that degradation of these enzymes is dictated by the N-terminal domain. These results suggest that GGT are degraded by non-classical pathway(s) and that their stability depends on ongoing protein synthesis while in the Golgi but not when re-localised to the ER.

CB-P14.
EXPRESSION OF SIALYLTRANSFERASE2 INCREASES THE ACTIVITY OF OTHER GOLGI RESIDENT GLYCOSYLTRANSFERASES
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The activity of the endogenous galactosyltransferase 1 (GalT1) and sialyltransferase 1 (SialT1) of CHO-K1 cells were increased 1.4 and 2.3 fold, respectively, in a cell clone (ST18) stably expressing sialytransferase 2 (SialT2). Here we show, by biochemical assays, that this activation was neither due to the appearance of activators (i.e. glycolipid products) nor to evident stabilization of GalT1 and SialT1. Real-time PCR experiments failed to demonstrate transcriptional activation of GalT1 and SialT1 genes. Since SialT2 forms a complex with GalT1 and SialT1 with participation of their N-terminal domains (Ntd), we looked for the activation of GalT1 and SialT1 in a cell clone that stably express the Ntd of SialT2 fused to the GFP. Results showed a poor activity of the Ntd of SialT2 to activate GalT1 and SialT1. Taken together, these results indicate that activation of GalT1 and SialT1 by SialT2 is associated to its lumenal domain, and reinforce the possibility previously advanced of an effect on the topological organization of these enzymes along the Golgi complex (Uliana *et al*., 2006).

CB-P15.
NONE OF BOTH ER GTS IS ESSENTIAL FOR VIABILITY IN *C. ELEGANS*
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Quality control mechanisms are in place to ensure that newly synthesized proteins reach their properly folded conformation. N-glycans contribute to folding efficiency in the ER by a series of oligosaccharide processing and lectin binding reactions. The UDP-glycoprotein glucosyltransferse (GT) functioning as a conformational sensor is the key element in this mechanism. The two human GT homologues HUGT1 y HUGT2 share 55% identity, but only HUGT1 is active. We undertook a functional characterization of the two putative GTs in the *C. Elegans* encoded by the F48E3.3 y F26H9.8 genes. F48E3.3 shows a higher degree of identity (40%) with both human GTs than F26H9.8 (35%). We examined the consequences of depleting F26H9.8 and F48E3.3 proteins by RNA interference in wild type animals (WT), in calnexin and calreticulin mutants (cnx-1 y crt-1), in KP948 (RNAi supersensitive strain) and in SJ4400 containing an ER stress reporter. We found that RNAi against F26H9.8 and F48E3.3 does not cause morphological phenotypes or affect brood size, viability in WT. However, F48E3.3 RNAi elicits expression of the ER stress marker in SJ4400, suggesting the involvement of F483.3 in ER quality control. RNAi against both GTs in crt-1 and cnx-1 mutants cause some minor pleitropic effects in the larvae and adults, and RNAi against both protein produce an increase in the lethality of KP948.

CB-P16.
PALMITOYLATION OF TYPE-TWO MEMBRANE PROTEINS IN YEAST AND MAMMALIAN CELLS
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Many proteins are modified by the addition of a palmitate to a cysteine residue. Hydrophilic proteins such as G proteins or Ras, use this modification to attach to membranes. However, many transmembrane proteins are also palmitoylated and the function of this modification is less clear. A family of transmembrane proteins sharing a motif called DHHC-CRD (Asp-His-His-Cys Cysteine Rich Domain) is involved in most protein S-acylation events described so far. Swf1, a yeast member of the DHHC family has been implicated in membrane-associated palmitoylation of SNARE proteins and Golgi Glycosyltranferases (GTs), both type-two membrane proteins. We have focused our study into the C-terminal domain of Swf1 predicted to be cytosolic. Within this region, we have identified a novel motif, conserved in some DHHC proteins, that is essential for Swf1 function. Site-directed mutagenesis allowed us to identify a tyrosine residue within this motif as critical for Swf1 function. We have also shown that this C terminal domain is able to bind palmitoyl-CoA. Palmitoylation of SNARE proteins or GTs has not been described in mammalian cells nor has the Swf1 orthologue. We have evidence that at least some SNARES are similarly palmitolylated. Experiments are underway to extend this observation to GTs, to identify the Swf1 orthologue and to study the functional consequences of this modification.

CB-P17.
GANGLIOSIDES AND GDNF PATHWAY IN DIABETIC SMALL INTESTINE
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Chronic diabetes gives rise to multiple complication including those of gastrointestinal tract. Diabetic intestinal motility dysfunction is associated with pathological lesions that occur in the enteric neurons. However the mechanisms underlying these changes in the enteric nervous system are not well defined. Gangliosides (Gls), sialic acid-containing glicoesphingolipids are present at cell surface. Recent studies indicate that they are implicated in many biological functions including cell-cell interaction, cell activation, and signal transduction through modulation of growth factors. In addition Gls play role in neuron survival and death. In this study we investigated the pattern of Gls and GDNF/GFRα1-Ret signal pathway in the small intestine of diabetic mice. Samples were analyzed by TUNEL, immunohistochemistry, thin layer chromatography and RT-PCR. A significantly increase of apoptotic cells was observed at the myenteric plexus level of diabetic animals. Also we observed quantitative and qualitative changes in the pattern of intestinal Gls. Diabetes produces a significant decrease in GD1a, GD3, GM1 and an increase expression of GM3. Semi-quantitative variations on GDNF/GFRα1-Ret mRNA levels were observed under diabetic state. In conclusion we suggest that the impairment of enteric neurons could be mediated by alterations in both GLs pattern and in consonance with GDNF pathway.

CB-P18.
EXPERIMENTAL DIABETES ALTERS CELL-CELL CONTACTS IN INTESTINAL SMOOTH MUSCLE LAYER
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Intestinal dysfunction is a clinical complication of diabetes; however the molecular mechanisms of the intestinal motility disorders remain unclear. The smooth muscle layer of the intestine has a critical role in the motility. In diabetes altered intercellular communications may account for some aspects of the enhanced contraction and/or impaired relaxation of intestinal muscular layer resulting in a disturbed motility. To understand the impact of diabetes on muscle layer dysfunction, we analyzed gap junction and N-cadherin/β-catenin complex in an experimental model of diabetes in rodents. Jejunum samples from normal and diabetic animals were analyzed by RT-PCR, immunohistochemistry, western-immunoblotting and confocal microscopy in order to determine these junctions. Our results show a decrease in Cx43 expression between smooth muscle cells during diabetes. Studies of colocalization of Cxs and c-kit suggested that Cx43 localization in the intersticial cell of Cajal network remain unchanged. In contrast, Cx45 shows a cytoplasmic strong reactivity in the diabetic myenteric plexus cells. Diabetes produces a significantly reduction of N-cadherin/β-catenin complex. The alterations in gap junctions and N-cadherin/β-catenin complex suggest an impaired intercellular communication between cells of the muscle layer which could be responsible for the diabetic motility disfunction.

CB-P19.
BRADYKININ INDUCES VINCULIN-STAINED FOCAL ADHESIONS (FA) DISSIPATION BY VESICLES INTERNALIZATION
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FA are structures of cell attachment to the extracellular matrix. Previously, we showed that bradykinin (BK) induces dissipation of vinculin-, but not talin-, staining FA by a mechanism that involves activation of PLC, suggesting that BK could be inducing a restructuring of FA. Now, we investigated the mechanism of vinculin - containing FA dissipation. We treated cultured rat renal papillary collecting duct cells with BK (1, 5 and 10 minutes) and analyzed the cells by confocal microscopy to study the route of vinculin internalization. After BK stimulation, the number of FA per cell decrease concomitant with the appearance of vesicles containing vinculin and PIP_2, in the citosol. To ascertain whether these vesicles are formed via caveolin - coated intermediates, we analyzed the distribution of caveolin and vinculin by double immunofluorescence. Caveolin is detected in punctuate arrays but is not present in vinculin-containing vesicles in BK treated cells. To test another endocytic route, we pre-incubated cultured cells with the fluid-phase endocytosis marker fluorescent dextran (F-Dex) (5 min) before BK stimulation. Vinculin-containing vesicules do not internalized F-Dex after BK treatment. Taken together, BK induces vinculin - stained FA dissipation by the internalization of vesicules containing vinculin and PIP_2, by a caveolae -, and fluid phase-independent endocytic pathway.

CB-P20.
ANCHORAGE OF MICROTUBULES TO PLASMA MEMBRANE THROUGH INTERACTION WITH NA,K-ATPASE

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Na,K-ATPase is an integral membrane protein that transports 3 Na+ out of the cell in exchange for 2 K+ by using the energy of ATP hydrolysis. We had previously found that this enzyme interacts with acetylated tubulin resulting in inhibition of its catalytic activity. In the present work we found that, during molecular filtration chromatography, the complex eluted at volume corresponding to higher MW than those of each protein component alone. We determined that tubulin forming part of microtubules can interact with Na,K-ATPase because: 1) native microtubules depleted of membranes have associated Na,K-ATPase and 2) microtubules assembled from purified tubulin were able to associate Na,K-ATPase when incubated with a detergent-solubilized membrane preparation. Furthermore, we tentatively identified CD5 (cytoplasmic domain 5) as the cytoplasmic fragment of Na,K-ATPase capable of interacting with acetylated tubulin. We tested the six cytoplasmic fragments and only CD5 bound to acetylated tubulin. Two of the peptides were obtained commercially and four as recombinant proteins. Taken together, our results are consistent with the idea that Na,K-ATPase may act as an anchorage point of microtubules on plasma membrane.

CB-P21.
LOCALIZATION OF RAB24 IN K562 HUMAN LEUKEMIC CELLS IN BOTH CONTROL AND STARVATION CONDITIONS

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The Rab family of small GTPases plays an important role in the regulation of membrane trafficking along the endo/lysosomal and secretory pathways. The exact role of Rab24 is currently unknown. We have previously reported a partial colocalization of GFP-Rab24 with autophagic vacuoles in nutrient-starved CHO cells, suggesting that Rab24 may participate in macroautophagy. During the maturation of erythroid cells, autophagy of organelles such as mitochondria is necessary for erythrocyte generation. K562 human leukemic cells have a default high level expression of LC3 (autophagy marker) and Rab 24. In order to investigate the possible role of Rab24 in K562, cells cotransfected with different GFP-chimeric proteins and labeled with fluorescent compounds were analyzed by confocal microscopy. Initially, we confirmed that the Rab24 and LC3 colocalization increases under starvation conditions. We have also observed a partial colocalization of Rab24 with Lysotracker-Red and DQ-BSA, markers of acidic and lysosomal/degradative compartments, respectively. In addition, there was a considerable colocalization with Rab7, a protein that labels late endosome/lysosomes, which is also recruited to autophagic vacuoles. In contrast, no colocalization with markers of multivesicular bodies was observed. These data suggest that in K562 cells Rab24 localizes in a compartment with autolysosomal features.

CB-P22.
EXOSOME-DEPENDENT TRAFFICKING OF INSULIN DEGRADING ENZYME (IDE) MODULATES AMYLOID β (Aβ) CLEARANCE

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IDE degrades Aβ in the brain and its expression/activity is impaired in Alzheimer's disease. IDE lacks typical signal peptide needed for secretory pathway but was detected extracellularly. IDE is located in lipid rafts and its secretion is stimulated by calcium without the involvement of ER-Golgi pathway, mechanism compatible with exosomes (EX)-mediated release. Here we detected in living N2a cells by laser confocal microscopy IDE associated to multivesicular bodies (MVB) labeled with a fluorescent lipid (NR-h-PE). MVB are intracellular structures containing EX. EX isolated from N2a conditioned supernatants by sequential ultra-centrifugation contained IDE (detected by immunoelectron microscopy and western-blotting). EX-associated IDE degraded ^{125}I-Aβ. Stable transfection with Rab11wt or Rab11S35N (which unable the fusion of MVB to the plasma membrane) showed that EX are mainly involved in IDE secretion and that impairment of EX release significantly reduces Aβ clearance. The amount of EX was quantified by acetylcholynesterase activity assay. N2a cell were exposed to acute hypoxia (0.1%O_2) and IDE was quantified intra- and extra-cellularly. Our results show under hypoxia the size of MVBs and the release of EX increase but the secretion of IDE is impaired suggesting that stress factors may directly impact on the translocation of IDE to EX and its further proteolytic activity.

CB-P23.
DIFFERENT PKC ISOFORMS PHOSPHORYLATE THE SMALL GTPASE RAB11

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The Rab family of monomeric GTPases regulates intracellular traffic. Some Rabs can be phosphorylated by specific kinases. This post-transductional modification changes Rab`s affinity for effectors as well as its function. Rab11 is localized to the endosomal recycling compartment and we have previously demonstrated that Rab11 regulates phagocytosis and recycling from phagosomes. It has been shown that upon sustained stimulation with phorbol esters, PKC α and βII translocate towards Rab11-enriched vesicles. Taken together, these observations prompted us to investigate the possibility that Rab11 might serve as a substrate for PKC. We used on line predictive software to analyze Rab11`s putative phosphorylation sites and we found different consensus sites for basophilic serine/threonine kinases, like PKC. Then, we performed *in vitro* phosphorylation assays by use of [γ^{32}P]ATP, GST-Rab11 and cytosol. The samples were resolved by SDS-PAGE and protein phosphorylation detected by autoradiography. Our results show that Rab11 is phosphorylated by kinases present in cytosol. To further analyze the role of PKC on Rab11 phosphorylation, we used different pure PKC isoforms. These experiments demonstrated that Rab11 is phosphorylated by the classic PKCα, PKCβII and the novel PKCδ. The phosphorylation of Rab11 is likely to be a regulatory event that affects the interaction with its effectors.

CB-P24.
RAB11, RAB14 AND RAB22: THREE SMALL GTPASES JOINED AT THE ENDOCYTIC/RECYCLING PATHWAY
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Rabs GTPases regulate intracellular trafficking and maintain structural identity by overseeing the vectorial transport of proteins and membranes between organelles. Rab proteins function as molecular switches by cycling between inactive GDP and active GTP bound forms. Each Rab protein controls a specific membrane transport step. Rab11 is present at the endocytic recycling compartment and TGN derived vesicles. Rab14 is localized at the Golgi/TGN and early endosomes. Rab22 is associated to early/sorting endosomes. As these Rabs share similar intracellular distribution, our goal was to analyze the degree of overlapping vesicular localization and function. These Rab proteins and some mutant forms were over-expressed fused to different fluorescent tags to assess their intracellular localization by confocal microscopy. Rab11wt and Rab14 wt almost completely co-localized at the endocytic recycling compartment and TGN vesicles. Rab14 wt and Rab22 wt were present in the same vesicles at the perinuclear region and also co-localized in some peripheral endosomes. The Rab14 GDP-bound form (Rab14S25N) and a truncated that does not associate to membranes (Rab14 ΔGCGC) did not co-localize with Rab22wt.The results using mutants and different expression levels indicate that these proteins influence the localization of the each other and that they are present at the same domains on the membrane vesicle. These Rabs acting coordinately would control molecular trafficking between endocytic/biosynthetic pathways.

CB-P25.
RAB11-FIP2 AND Rme-1 ARE INVOLVED IN PHAGOCYTOSIS
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Macrophages internalize microorganisms by a process called phagocytosis. We have demonstrated that Rab11, a small GTPase, regulates phagocytosis and recycling from the phagosomal compartment. A Rab11- Family of Interacting Proteins known as FIPs have recently been cloned. All these proteins possess a conserved motif of 20 amino acids at its C-terminus, the Rab Binding Domain. Among the members of Rab11-FIPs, it has been described FIP2. FIP2 has three motifs NPF that interact with EH proteins (Eps15-Homology domains) like Rme-1 (Receptor Mediated Endocytosis). Our objective is to study the involvement of FIP2 and Rme-1 along the phagocytic pathway. We over-express these proteins and several mutant forms fused to fluorescent proteins to analyze *in vivo* their intracellular distribution by confocal microscopy as well as their participation along the phagocytic pathway. In macrophages, FIP2 presents a punctuate pattern throughout the cytosol resembling little endosomes and Rme-1 displays a tubule-vesicular network. Our results demonstrate that both proteins are recruited to early phagosomal membranes in patches like microdomains. The C-terminal domain of FIP2 is necessary for its binding to phagosomes. Both proteins, FIP-2 and Rme-1, might have a role in the internalization step in macrophages.

CB-P26.
THE ACTIN INTERACTING PROTEIN, CORTACTIN, IS INVOLVED IN THE *COXIELLA BURNETII* PHAGOCYTOSIS
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Coxiella burnetii (CB) is an obligate intracellular pathogen. After has been phagocytosed, the bacteria mature forming large vacuoles where the bacteria multiply. It is known that cortactin plays a role in several phagocytic proccesses and its function is regulated by phosphorylation. To test if cortactin is involved in CB phagocytosis, HeLa cells were transfected with plasmids encoding EGFP-cortactin wild type (WT) and -cortactin phosphorylation defective mutant, and subsecuently infected with CB. In some experiments prior infection the cells were treated with vanadate, a general phosphatase inhibitor. Also, the vanadate effect on internalization was tested in tranfected cells. CB was detected by indirect immunofluorescence and the cells were analyzed by confocal microscopy. We have observed that the level of infection in cell overexpressing EGFP alone or EGFP-cortactin mutant was similar, however in cells overexpressing EGFP-cortactin WT the infection level was significantly reduced. This result suggests that cortactin phosphorylation is important for CB uptake. We observed that vanadate inhibited infection what suggets that protein dephosphorylation is involved in that proccess. Interestingly, the vanadate effect was reverted by overexpression of the phosphorylation defective cortactin. We conclude that cortactin and its phosphorilation state regulate *C. burnetii* phagocytosis.

CB-P27.
***MYCOBACTERIUM MARINUM* CONTROLS ITS INTERACTION WITH THE AUTOPHAGIC PATHWAY**
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Mycobacterium marinum is a natural pathogen of fish that occasionally affects humans causing the “fish tank granuloma”. These granulomas are very similar to the ones caused by *M. tuberculosis* in the lungs of humans. Since *M. marinum* is a useful model to study pathogenic mycobacteria we have analyzed the interaction of this bacterium with the autophagic pathway. Raw macrophages overexpressing the autophagy protein GFP-LC3 were infected with RFP-*M.marinum* and analyzed by confocal microscopy. At 2h post infection (p.i) a clear association of *M. marinum* with GFP-LC3 was observed. Infected cells were subsequently incubated for two hours in full medium with or without rapamycin (a classical enhancer of autophagy) or in starvation media (plus or minus the autophagy inhibitor wortmannin). The percentage of bacteria associated to GFP-LC3 varied depending on the conditions used, and reached the highest association when rapamycin was used. To determine at which time the autophagic response was more significant we analyzed the recruitment of LC3 at different p.i. times, Interestingly the highest level of association was found between 15 and 30 minutes p.i. Unexpectedly, heat killed bacteria, was unable to recruit GFP-LC3. Our results indicate that at early p.i. time *M. marinum* interacts with the autophagic pathway and importantly, that this interaction is regulated by the bacterium itself.

CB-P28.
TCVPS34, A PI3-KINASE INVOLVED IN OSMOREGULATION AND MEMBRANE TRAFFICKING IN *TRYPANOSOMA CRUZI*
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During its developmental stages in different hosts, *T. cruzi* faces extreme fluctuations in osmolarity and in the uptake of nutrients that must incorporate through its endocytic system. In this work, we report the identification of TcVps34, a class III phosphatidylinositol 3-kinase of *T. cruzi*. Recombinant TcVps34 was able to phosphorylate only phosphatidylinositol (PI) to produce phosphatidylinositol 3-phosphate (PI 3-P) and complemented yeast Vps34 knockout strain, indicating that TcVps34 is a functional PI 3-kinase. Microscopy analysis of TcVps34 over-expressing epimastigotes cells revealed functional and large contractile vacuoles as well as some defects in the region near of the citostome and the flagellar pocket. Pre-incubation of wild-type epimastigotes with PI 3-kinase inhibitors reduced the recovery of the parasites to hypo-osmotic stress. Kinetics of proton uptake in TcVps34 over-expressing cells showed higher H+-ATPase and lower H+-PPase activities than in wild-type cells. In addition, endocytosis of FITC-transferrin was reduced in TcVps34 over-expressing cells. Finally, a yeast two-hybrid assay showed interaction of TcVps34 with TcVps15, a ser/thr kinase that interact as a component of a protein complex with Vps34 in yeast. Taken together, these results suggest a potential role for TcVps34 in osmoregulation, vacuolar acidification, and membrane trafficking in *T cruzi*.

CB-P29.
HISTONE DEACETYLASE INHIBITOR TRICHOSTATIN A AFFECTS *T. CRUZI* EPIMASTIGOTE STAGE GROWTH RATE
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Regulation of gene expression in higher eukaryotes is closely related to post translational modification of histones bound to DNA. Lysine residues of the N-terminal tails of histones are acetylated by histone acetyltransferases (HATs) and deacetylated by histone deacetylases (HDACs). We have previously reported the cloning and expression in *E. coli* of two *Trypanosoma cruzi* sequences with HAT identity named TcHAT1 and TcElp3. Expression in epimastigotes was confirmed by Northern blot but no HAT activity could be measured to date. In this work we used different approaches to further characterize these enzymes. TcElp3 was cloned into pYES2 and used to transform an Elp3 deficient yeast strain. The transformed cells were not able to rescue the thermosensitive phenotype. We also analyzed expression and localization of TcHAT1 and TcElp3 using specific antibodies obtained after immunization of mice with the recombinant proteins. Subsequently we analyzed the effect of HDACs inhibitors Trichostatin A (TSA) and sodium butyrate in epimastigotes grown in vitro. When parasites were treated with 50 nM TSA strong proliferation inhibition was observed but also a sub G1 peak appeared on DNA histogram when flow cytometry was performed. This evidence not only supports a functional mechanism of histone acetylation in *Trypanosoma cruzi* but also opens new insights in drug therapy against Chagas' disease.

CB-P30.
POLY(ADP-RIBOSE)POLYMERASE IS INVOLVED IN DNA BREAK SIGNAL MECHANISMS IN *TRYPANOSOMA CRUZI*
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DNA damage signaling is crucial for the maintenance of genome integrity. In higher eukaryotes a NAD dependent signal transduction mechanism protects cells against the genome destabilizing effects of DNA strand breaks. The nuclear enzyme poly(ADP-ribose)polymerase has been implicated in this pathway. We demonstrated that the recombinant enzyme from *Trypanosoma cruzi* (TcPARP) is strongly activated in presence of nicked DNA and, subsequently, polymers of ADP-ribose (PAR) are attached to PARP itself and to *T. cruzi* histones. According to this, PAR synthesized could dissociate histones from DNA granting the DNA repairing machinery access to damaged DNA. Nucleic acid damaging agents, such as H_2O_2, beta-lapachone, methyl methane sulfonate and UV radiations, which trigger different repair mechanisms, were used to study PARP response. The results by using Western blot analysis with anti PAR antibodies showed that PAR synthesis was increased in the presence of these agents. However, when the damage induced led to cell death, the amount of PAR detected decreased. *In vivo* assays by indirect immunofluorescence confirmed the results obtained by Western blot. We also tested the response in trypanosomes over-expressing PARP. These results are in agreement with the previous proposed mechanisms for PARP in DNA strand break signaling.

CB-P31.
THE SUMOylation SYSTEM IN *TRYPANOSOMA CRUZI*
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SUMOylation is a post-translational modification present in eukaryotic organisms that involves the covalent attachment of the ubiquitin-like protein SUMO to other cellular proteins. Unlike ubiquitination, which targets proteins for degradation, SUMOlylation participates in a number of cellular processes such as nuclear transport and transcriptional regulation. The SUMO conjugation pathway is biochemically similar to the ubiquitin conjugation pathway. A bionformatic analysis of the *T. cruzi* genome led to the identification of all components of the SUMOylation system. SUMO seems to be presents as a single copy gene encoding a polypeptide consisting of 107 aminoacid residues that conserved Gly-Gly motif. Anti-SUMO polyclonal antibodies were generated using the purified recombinant protein expressed in a bacterial system. Western blot analysis carried out with these polyclonal antibodies allowed us to detect free-SUMO protein as well as SUMO-conjugated proteins in cell extracts of all main cellular stages. In vivo experiments using trasnfected stable cell of epimastigote form showed that SUMO is attached to the targets through its GG C-terminal motif. In addition, SUMO's targets might be predominantly nuclear proteins. Then, we confirmed that SUMOylation system is funtional in *T cruzi*.

CB-P32.
PARTICIPATION OF Agp1 PERMEASE IN THREONINE UPTAKE IN *SACCHAROMYCES CEREVISIAE*
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The old concept that yeast transports amino acids by multiple specific permeases and a general amino acid permease had to be modified with the demonstration that many individual amino acid permeases are capable of transporting several different amino acid Most notable is the permease Agp1p that under appropriate conditions can transport virtually all the nonpolar amino acids. Physiological expression of "secondary" specificities can be manipulated by changes in growth medium and by mutation in other permeases. The relative contribution to the transport of a single amino acid by more than one permease is an area that has not been fully explored. In this paper we report experiments in which gap1 mutants defective individually in Agp1p. Agp2p, Agp3p, and Bap3p were used in investigating low-affinity leucine transport and transport of threonine. The cells grew in synthetic media with leucine or theonine 0.5 mM as the only nitrogen source. Our results show: 1) The LET2 gene appears to encode the most significant threonine transporter after Gap1p, 2) The following candidate genes are not allelic to LET2: GNP1, BAP3, AGP2 and AGP3 3) BAP3, and possibly AGP2, contribute to threonine transport in gap1 strains. Current phenotypic evidence favors the hypothesis that let2 is an allele of agp1. If this hypothesis is correct, low-affinity leucine transport is primarily due to Agp1p activity.

CB-P33.
PROTEIN C4 IS A PUTATIVE SMALL HEAT SHOCK PROTEIN IN THE INTESTINAL PARASITE *GIARDIA LAMBLIA*
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Giardia lamblia is a medical important protozoan with a low position in the eukaryotic lineage. *G. lamblia* trophozoites are transmited through infected hosts by differentiation into cyst forms. By screening a *G. lamblia* WB 1267 cDNA expression library, we cloned the previously isolated gene Glorf-C4, considered specific of this organism. It encodes a 22 kDa protein that assembles in 60-66 kDa and high molecular mass complexes during the whole life cycle. 66 kDa complex formation is favored under some stress conditions. C4 localizes in cytosol of trophozoites and cysts and forms large spherical aggregates when it is overexpressed, without affecting viability and encystation. No homologous proteins nor conserved domains or features arise from sequence analysis of the predicted protein. Nevertheless, C4 presents local tertiary structure similarity with the α-crystallin domain of small heat shock proteins Hsp16.9B from *Triticum aestivum* and Hsp16.5 from the hyperthermophilic archaea *Methanococcus jannaschii*. Here, we discuss C4 putative role as a small heat shock protein in *G. lamblia* biology.

CB-P34.
IgA PRODUCTION AFTER ADMINISTRATION OF A PROBIOTIC
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Probiotics are live microorganisms that exert health beneficial actions to the host after their oral administration if they are given in suitable amounts. They have a stimulatory effect on humoral immunity by increasing the amount of available local and circulating IgA. However, it is possible that continuos administration might not be the optimal way to administer them. Our objective was to compare two doses of probiotics and study their effect on local immunity in rat intestine. For this purpose we used 27 rats that were divided in three groups: Control group (3 animals), Group A (15 animals) and group B (9 animals). Group A was given 1 ml of BIOFLORA (SIDUS Lab) and Group B 0,2 ml. Animals were sacrificed at 3,5,8,10 and 15 weeks of treatment and intestine was resected and processed by freezing technique. Immunofluorescence was performed in order to study the amount of IgA+ cells. Results: BIOFLORA stimulated the production of IgA secretory cells in group A with a peak at 3 days whereas in group B it did not exert significant effects with respect to control animals. The amount of IgA+ cells returned to basal values after 8 days of treatment. The results suggest that the increase in IgA secretory cells is dependent on the dose of probiotic and the period of treatment and that it is possible that a cyclic treatment would be a better way of administering the probiotic.

CB-P35.
ISOLATION AND MOLECULAR CHARACTERIZATIONS OF MARINE BACTERIA ISOLATED FROM BEAGLE CHANNEL
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Marine microorganisms play an important ecological role in the biological process of transformation of marine environment. Marine bacteria exhibit different hydrolytic enzymes that degrade fractions of organic matter in the sea. Marine microorganisms are the extremophiles carry out main a role in the biodegradation of the organic matter in diverse Antarctic ecosystems. Those culturing efforts have yielded new species within the divisions of γ- and α-Proteobacteria. The aims of this work were the isolation and taxonomy characterization of marine microorganisms from Antarctic region. Samples of benthonic organisms and seawater were taken from different coastal areas of the Beagle Channel (55ºS; 67ºW), Tierra del Fuego, Argentina. Samples were spread in LB medium and growth at 4 and 15ºC. The 95 isolated colonies were characterized by 16S rDNA and gyrB PCR amplifications. Isolates were separated into groups according to their ARDRA profiles and RFLP analysis. 16S rDNA and gyrB gene were sequenced from one isolate from each group, and the phylogenetics trees were constructed. The gene sequences allowed us to determine their association with the class *Proteobacteria*; members of genera *Pseudomonas*, *Pseudoalteromonas*, *Serratia*, *Halomonas*, *Alteromonas*, *Psychrobacter*, *Shewanella*. These studies provide a new source of strains, genes, enzymes and metabolites for biotechnological applications.

CB-P36.

REGULATION OF APOPTOSIS BY ΔNP63 DURING *XENOPUS LAEVIS* DEVELOPMENT

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The p63 gene is a transcription factor and in mammals has two promoters that generate two types of protein isoforms, TAp63 and ΔNp63. The TA isoforms contain a transcription activation domain and are able to induce apoptosis. The ΔN isoforms lack the transactivation domain and can function as "dominant negative" proteins having an anti-apoptotic role. Although the p63 sequences are conserved among organisms, in *Xenopus*, only the orthologue of mammalian Δnp63γ has been cloned and its role in development and apoptosis remains unknown. We analyzed the participation of ΔNp63 regulating apoptosis in *Xenopus embryos*. First, we analyzed the expression pattern of ΔNp63 using whole mount *in situ* hybridization. The main expression of ΔNp63 is located in the epidermis and also is detected as a defined line in the limit between neural folds and epidermis. Then, we proceeded to overexpress ΔNp63 in whole embryos and in animal caps to determine whether ΔNp63 was able to modify apoptosis. In both experiments, the TUNEL analysis showed that ΔNp63 produced a decrease of apoptosis. Finally, we analyzed whether ΔNp63 was able to regulate the transcription of caspasa 2, 3 and 9, and Bcl2. Our results suggest that ΔNp63 is acting as an antiapoptotic factor during the development of *Xenopus laevis* regulating the transcriptions of some apoptotic and anti-apoptotic factors.

CB-P37.

THALLIUM INDUCES PC12 CELLS APOPTOSIS THROUGH THE ACTIVATION OF MULTIPLE MOLECULAR PATHWAYS

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Thallium (Tl) is a non-essential heavy metal, with two oxidation states (Tl^+ and Tl^{3+}). Tl is highly neurotoxic through still poorly understood mechanisms. We previously demonstrated in PC12 cells that Tl^+ and Tl^{3+} altered cells redox state and increased the number of apoptotic nuclei (~26% at 100 μM Tl^+ or Tl^{3+}). To elucidate the molecular pathways of cells death, PC12 cells were incubated with Tl^+ or Tl^{3+} (10-100 μM) for 24h. We found higher PARP cleavage (~70% at 100 μM Tl^+ or Tl^{3+}), cytochrome c release (~60% at 100 μM Tl^+), and AIF translocation (~70% at 100 μM Tl^+). Total Bcl-2 content remained unaffected. Accordingly to the higher PARP cleavage, a significant increase in caspase 3 activity was found (100 μM Tl^+ or Tl^{3+}). Phosphatidylserine exposure was only found when PC12 cells were treated with 100 μM Tl^+. Besides, an increase in caspase 8 activity was observed when the cells were incubated in the presence of 50 μM Tl^{3+}. Significant lysosomal labilization as determined by catepsin D activity in cytosol was observed in cells treated with Tl^+ or Tl^{3+} (50-100 μM). Together, present results indicate that Tl^+ or Tl^{3+} induce apoptosis in PC12 cells through the activation of multiple cellular pathways, effect that depends on metal speciation and concentration.

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CB-P38.

VENOM OF *BOTHROPS* SPECIES FROM NORTH EAST OF ARGENTINA INDUCES APOPTOSIS IN C2C12 MYOBLAST CELLS

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In Latin America, the majority of snakebites are caused by species of *Bothrops* genus. *B. alternatus* (yarará grande) and *B. neuwiedii* (yarará chica) are responsible for most snake poisonings in North East of Argentina. Acute skeletal muscle damage is a frequent manifestation in envenomations induced by these snakes. Therefore, C2C12 Myoblast Cell culture is an appropriate in vitro model system to evaluate the events involved in the cell death. In this study we report a first-hand investigation about morphological features induced by these venoms. Previously the cytotoxicity and the corresponding DC50 were determined (*B alternatus*: DC50: 5.8 μg/ml; *B neuwiedii*: DC50: 2 μg/ml, 3h incubation). The assays were performed by phase-contrast (PCM) and fluorescence (FM) Microscopy. Briefly, C2C12 myoblast cells were split into six-well plates containing cover slides and growth for 24 h. The cells were incubated with 10 μg/ml of *Bothrops* venoms for 30 min. The cover slides were observed by PCM and then stained with Acridine Orange and Ethidium Bromide and examined by FM. Our results demonstrate that *B. alternatus* and *B neuwiedii* venoms are potent promoters of apoptosis in C2C12 cells. Typical features of apoptosis, including membrane blebbing and nuclear condensation were observed. Further studies are needed for identification of the molecular mechanism by which these venoms induces apoptosis.

CB-P39.

PRO-APOPTOTIC EFFECTS OF PTH IN INTESTINAL CELLS

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Depending on the cell type involved, parathyroid hormone (PTH) has been shown to inhibit or promote the apoptosis. The objective of this study was to further delineate the role of PTH in the intestinal apoptosis using the human colonic Caco-2 cells. To that end, Caco-2 cells were exposed during 3 to 5 days to PTH (10^{-8} M). The hormone increases spontaneous Caco-2 cell apoptosis according as DNA laddering formation by agarose gel electrophoresis. Analysis of the reduction in the nuclear size by the nuclear-specific colorant DAPI reveals that PTH treatment increases the number of apoptotic nuclei in a time-dependent fashion, with the maximum effects achieved at 5 days (+600%). In addition, assessment of dead cells after PTH treatment by propidium iodide shows that the hormone increases the number of red-stained cells (+ 500%, 5 days). Evaluation of the cell survival by quantification of cell number using crystal violet staining reveals that PTH treatment diminishes the number of cells (- 58%, 5 days). Cell viability assays using resazurin, a dye that measure the metabolic capacity of cells, shows that PTH diminishes the number of viable cells (- 52%, 5 days). Taken together, our results confirm that PTH promotes the apoptosis of Caco-2 intestinal cells.

CB-P40.

ANTIAPOPTOTIC EFFECTS OF 17β-ESTRADIOL THROUGH ESTROGEN RECEPTORS IN SKELETAL MUSCLE CELLS

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In this study we report that 17β-estradiol (E2), through estrogen receptors with non-nuclear localization, e.g. mitochondria, endoplasmic reticulum and Golgi, can regulate apoptosis in mouse skeletal muscle C2C12 cells. E2, at physiological concentrations, abrogates DNA damage, PARP cleavage and cytochrome c release induced by H_2O_2 or etoposide. This protective action of the steroid involves fast activation of the PI3K/Akt/Bad pathway. Blocking experiments with specific antibodies and siRNAs against the estrogen receptor α (ER α) and β (ER β) isoforms, revealed that ER β mediates mitochondrial protection by the hormone to a greater extent than ERα. On the other hand, both ER isoforms mediate to the same degree PI3K/Akt activation, suggesting different levels of participation of each isoform depending on the step of the apoptotic/survival pathway evaluated. Furthermore, it was shown that the protective role of the hormone also involves heat shock protein 27 (HSP27). E2 at longer exposure times increased the expression of HSP27. Immunocytochemistry and co-immunoprecipitation assays demonstrated co-localization and interaction of HSP27 with ERs in mitochondria. Altogether, these results suggest that the antiapoptotic signal triggered by 17β-estradiol in muscle cells is mediated by ER β and α and involves rapid activation of PI3K/Akt and the participation of HSP27.

CB-P41.

EFFECTS OF PLANTS POLYPHENOLS ON MAMMALIAN CELL VIABILITY, PROLIFERATION AND DIFFERENTIATION

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Experimental evidence suggests that most herbs and spices possess a wide range of biological and pharmacological activities. The objectives of our study were: first, to determine the effects of plant extracts on viability of normal cell and second to investigate their possible effects on cell proliferation and differentiation. The plant extracts examined were rosemary (*Rosmarinus officinalis L.*) and sage (*Salvia officinalis L.*). Isolated compounds such as rosmarinic acid, carnosol and carnosic acid, the main polyphenolics compounds that are strong dietary antioxidants, were also evaluated. The MTS test was used to determine cell viability. Our results indicated that no significantly changes in the viability of quiescent 3T3-L1 cells were observed. However, in proliferating cells there is a significantly reduction in cell viability that depends on the dose. The effect of plant compounds on cell differentiation was evaluated using the 3T3-L1 cell line as a model, since inducers and various transcription factors that trigger the differentiation programme are known like C/EBPs and PPAR gamma. We observed that rosemary compounds regulate the expression of C/EBPbeta, a key player for adipocyte differentiation. This approach will be valuable to bring inside into the understanding of the mammalian cell targets of phytopolyphenols.

CB-P42.

GALECTIN-8 INDUCES T CELL PROLIFERATION VIA CD45 SIGNALLING PATHWAY

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Galectin-8 (Gal-8) was recently shown to be present in mouse thymus and to induce apoptosis of the CD4high CD8high subpopulation. In the present work, we wanted to explore its role on mature lymphocytes. First, the two splice-variants (Gal-8S and Gal-8L) were cloned from mouse splenocytes and protein expression was confirmed by western-blot. When recombinant Gal-8 was added to splenocyte cultures, a strong proliferation was observed. The highest proliferation rate was achieved with T cell enriched cultures, thus identifying them as the principal target. Furthermore, a Th1/Th2 profile was obtained with Gal-8-treated splenocytes by RT-PCR. In order to find potential binders, mouse splenocyte extracts were subjected to Gal-8-affinity cromatography followed by MALDI-Tof assay. Interestingly, CD45 a known coestimulator in the TCR signalized activation was one of the identified binders. Therefore, its possible involvement in Gal-8 proliferation induction was tested. An inhibitor of the protein tyrosine phosphatase activity of the CD45 included in splenocyte cultures together with Gal-8, substantially reduced the proliferation rate. Moreover, Gal-8 was able to induce p44 and p42 MAPK phosphorylation in Jurkat T cells, demonstrating the activation of the CD45 intracellular cascade. Clearly, these results make evident the involvement of CD45 in Gal-8-induced T cell proliferation.

CB-P43.

TNF-α AND NO PRODUCTION IN LPS-ACTIVATED HUMAN MONONUCLEAR CELLS WITH DISRUPTED-LIPID RAFTS

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Lipopolysaccharide (LPS) stimulates TNF-α production in immune cells through receptors localized in lipid rafts. Previously we found that *L. reuteri* CRL 1098 inhibited TNF-α production in control (27%) and disrupted-lipid rafts cells (51%). Here we studied the effects of *L. reuteri* CRL 1098 on TNF-α and nitric oxide (NO) production of normal and disrupted-rafts cells activated with LPS. Rafts of peripheral blood mononuclear cells were modified by cholesterol depletion with 10 mM methyl-β-clyclodextrin treatment. Control (no treated) and disrupted-lipid rafts cells were incubated with 100 ng/ml LPS at different times. TNF-α and NO production were measured by chemiluminiscence and Griess assays respectively. TNF-α value in the supernatant of $1x10^6$ cells exposed to LPS was 514 pg/ml after 4 h incubation at 37ºC; 17% of inhibition in TNF-α level was observed in disrupted-lipid cells in the same conditions. When *L. reuteri* or the supernatant of its culture was added to disrupted-rafts cells, 30% and 11% inhibition on TNF-α production was observed respectively. This effect was increased at 24 h of incubation. In presence of *L. reuteri* NO synthesis is time dependent and was correlated with TNF-α produced by both LPS-activated control and disrupted-lipid rafts cells. Studies are currently in progress to further define the role of rafts in the mechanism involved in this response.

CB-P44.
PARAXIS EXPRESSION IN NEURAL CREST CELLS MIGRATION DURING POST-SOMITIC *XENOPUS LAEVIS* EMBRYOS

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Morphogenesis somite and neural crest (NC) ontogeny are two important processes of vertebrates. In embryogenesis the paraxial mesoderm (PM) becomes segmented and gives rise to the somites. Paraxis is a bHLH-transcription factor which is expressed in PM and then in the somites of mouse, chicken and amphibian embryos. Later its expression declines after sclerotome formation. In *Xenopus laevis*, paraxis is also expressed in the neural tube and the head mesoderm. We analyzed by RT-PCR, whole mount *in situ* hybridization and histology the paraxis expression in post-somitic stages of *Xenopus laevis* embryos. Several markers were used in order to determine the different embryo regions. In the present study for a first time we observed the expression of paraxis transcription factor associated with migrating NC cells, under cranial, cardiac and trunk pathways. In relation to cranial migration stream paraxis is expressed in the craniofacial mesenchyme, otic vesicle and pharyngeal arches. Paraxis is also expressed in aortic arch arteries and the septum between the aorta and pulmonary artery corresponding to the cardiac NC. In trunk NC cells which migrate to the dorsal fin along the dorsal lateral pathway to form melanocytes, paraxis is expressed. Our results suggest that paraxis transcription factor may comprise part of regulatory cascade involved in the migration of NC cells.

CB-P45.
ANTI-PROLIFERATIVE EFFECTS OF 1α,25-DIHYDROXY-VITAMIN D3 ON KAPOSI SARCOMA CELL-LIKE MODEL

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The aim of this work was to study the effects of the steroid hormone 1α,25-dihydroxy-vitamin D3 (1,25D) on the proliferation and apoptosis of endothelial cells transformed by the viral G protein-coupled receptor (vGPCR), the constitutively active chemokine receptor encoded by human herpesvirus 8 (HHV8), also known as Kaposi sarcoma herpesvirus. For this purpose, SKSV-40 immortalized murine endothelial cells (SVECs; kindly provided by Dr. Silvio Gutkind, NIH- USA) stably expressing vGPCR full-length were used. MTS proliferation assays and FACS cell cycle analysis showed that 1,25D decreased SVEC-vGPCR proliferation, in a time and dose-dependent manner, induced G1 phase arrest and increased subG0/G1 population. VDR protein levels were induced upon treatment with 10 nM 1,25D, whereas cyclin D1 protein levels decreased and p53 levels remain unchanged. Induced VDR protein levels were blocked by cycloheximide. Confocal microscopy studies indicated that under basal conditions native VDR has cytoplasmic and nuclear localization. VDR translocation to the nucleus was observed within 15 min and also at 48 h of 1,25D exposure. Taken together, these results indicate that 1,25D exerts antiproliferative effects in the Kaposi sarcoma cell-like model regulating cyclin D1 and VDR protein expression.

CB-P46.
LIPIDS MODULATE CELL EICOSANOIDS RELEASE, APOPTOSIS AND PROLIFERATION IN A MURINE BREAST CARCINOMA

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Dietary polyunsaturated fatty acids (PUFAs) and eicosanoids influence neoplastic cell (NC) proliferation, differentation and apoptosis although their mechanisms remain unclear. We investigated PUFAs effects and some cycloxygenase (COX) and lipoxygenase (LOX) products on breast carcinoma in mice fed with different lipid diets. Two groups were fed on a basic diet plus 6% of oil: walnuts oil (W), rich in 18:2,n-6 and 18:3,n-3 or peanut oil (P), rich in 18:1,n-9 and 18:2, n-6. Control group (C) received comercial diet. We analysed membrane fatty acids of NC by GLC; eicosanoids via LOX:12-HETE, 15-HETE, 13-HODE and via COX:12-HHT, PGE2 by HPLC. Apoptosis by flow citometry and counted in a light microscopy as the mitosis. Membrane arachidonic acid (AA) was higher on P (14.96%) than W (3.15%) and C (1.92%)($p<0.05$). The 12-HHT was higher on P (50.3ng) than W (21.3ng) and C (23.1ng)($p<0.05$). 12-HETE and 15-HETE were lower on P(9.3; 8.9ng) than W (9.8; 9.1ng) and C (10.3; 9.2ng), respectively. 13-HODE/12-HETE ratio was higher on P (1.13) than W (1.11) and C (1.04). Apoptosis was higher on P compared to W and C (33 vs 25 and 21%) ($p<0.05$). Mitosis was lower on P than W and C(1,6 vs 2.6 and 3.3)($p<0.05$). High levels of AA, 12-HHT, 13-HODE/12-HETE and low levels of 12-HETE and 15-HETE could be involved in anti-tumoral mechanisms by increasing NC apoptosis and decreasing cell proliferation.

CB-P47.
EFFECT OF THE RETINOIDS (IE:ATRA) ON DIFFERENTIATION AND GROWTH IN THE MURINE MAMMARY CELL LINE LM38

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Retinoids (Rtds) are responsible for cell growth and differentiation through its binding to RAR/RXR nuclear receptor. LM38 composed by luminal (LEP) and myoepithelial (MEP) cells, express all RAR/RXR and ATRA inhibited their metastatic dissemination. We studied the effect of Rtds in growth and differentiation of LM38 cells and the mechanisms involved in the interaction LEP/MEP. ATRA increased RARb2 level (PCR) confirming system functionality. Rtds treatment inhibited LM38 cell growth, effect reversed by a RARa antagonist: Ro415253. 3D culture developed polarized colonies with acinar structure. ATRA maintained the polarity and induced E-cadherin reorganization as well as a decrease of MEP (CK14-) in colonies periphery. ATRA increased activated caspase-3 level and promoted p27 nuclear translocation in the colony centre. In 2D, MEP was p27+ and LEP PpRB+. Rtds increased the number of arrested MEP and growth inhibition in LEP islets. A cell layer around the islets kept cycling (confocal microscopy/ WB). Besides Rtds did not affect LM38 clonogenic capacity. Cell cycle analysis (ATRA/ Ro415253) by flow cytometry showed G1 arrest and RARa implicance. Conclusion: Rtds, mainly through RARa, are able to inhibit growth of a mammary cancer cell line LM38 through apoptotic events together with cellular arrest, also suggesting the existence of a clonogenicy population resistant to retinoids.

CB-P48.
DIFFERENT SUBCELLULAR LOCALIZATION OF FOS PROTEIN DURING MAMMARY CARCINOGENESIS

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We have demostrated that three ip injections of N-Nitroso N-Methylurea (NMU) at 50, 80 and 110 days of animals life, induce malignant mammary tumors on normal rats and benign lesions on diabetic ones. The aim of this study was to investigate the cytoplasmic and nuclear expression of Fos protein in the mammary gland during the carcinogenic period and both malignant and benign tumors in these animals. Four groups of rats were used: control, injected with NMU, diabetic rats (STZ), diabetic rats with NMU. Gland mammary specimens were processed at 60, 90 and 120 days. In all samples Fos and PCNA was determined by immunohistochemical and western blot analyses in citosolic and nuclear fraction. We observed a differential expression of Fos protein in cytoplasm and nucleus during carcinogenic process. Cytoplasmic expression of Fos diminishes as the tissue progresses to malignancy, and its expression is elevated in normal tissue and scarce in agressive carcinomas. Conversely, the expression of this protein increases as the tissue becomes more malignant, being low in normal tissue and elevated in agressive carcinomas. PCNA follows the same pattern expression as Fos. In conclusion, this results suggest that there is a possible relationship between the different subcellular localization of Fos protein and the degree of differentiation of mammary gland and mammary tumors.

CB-P49.
INSULIN-LIKE GROWTH FACTOR- I RECEPTOR EXPRESSION AND LOCALIZATION IN DIABETIC RAT MAMMARY TUMORS

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The aim of this study was to investigate the expression and localization of the Insulin Growth Factor-I Receptor (IGF-IR) in mammary glands and tumors of normal and diabetic rats. For tumor induction, three doses of N-Nitroso-N-methylurea (NMU) were injected into rats at 50, 80 and 110 days of age. The diabetic disease of rats were obtained by streptozotocin (STZ) injection at 36 hours of life. Four group of rats were employed: Control (C), STZ, NMU and NMU+STZ. Mammary glands and tumor samples were collected at 140 and 180 days of age for immunohistochemistry and western blot analysis of IGF-IR and RE alfa using specific antibodies. In parallel, specimens were processed for histopathological studies. The results shown: a) at 140 days, IGFI-R levels remained unchanged in STZ vs. C and no differences were found in NMU vs NMU+STZ; however all the tumors had IGF-IR levels higher than C ($p< 0.001$); b) at 180 days, values changed: IGF-IR levels increased in STZ vs C ($p< 0.05$) and in NMU+STZ vs. NMU ($p< 0.001$). The RE alfa content determined at 140 and 180 days was decreased in NMU+STZ vs. NMU ($p<0.01$). Immunohistochemical results are in concordance with these observations. In conclusion, our observations suggest that IGF-IR level may be involved in the promotion/progression phases of the mammary carcinogenic process.

CB-P50.
ROSIGLITAZONE INHIBITS PROLIFERATION OF MDA-MB-231 BREAST CANCER CELLS

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Rosiglitazone (Rosi) is an antidiabetic drug extensively used for insulin-resistant type 2 diabetes mellitus treatment, and also ehxibits antitumoral action in several malignant cell lines. We have previously demonstrated the antitumoral effect of Rosi on mammary tumors induced in rats. The aim of this work was to study the in vitro effect of Rosi alone or combined with tamoxifen (Tam) on MDA-MB-231 breast cancer cells. Cell growth was determined using the clonogenic assay and results indicated that Rosi inhibited proliferation in a dose dependent manner (IC50=30 μM). Rosi also produced a significant cell-cycle arrest in G0/G1 phase (69% vs. 50% in controls) analyzed by flow cytometry. In accordance, the doubling time increased after Rosi treatment (29.4 h vs. 24.9 h in controls), while the expression of the well known proliferation marker, PCNA, decreased significantly (63%). Rosi-induced inhibition of proliferation was associated with an increase in the number of senescent cells (3.7% vs 1.2% in controls) as assessed by β-Galactosidase staining. Although we observed an imbalance of the Bax/Bcl-2 ratio, no significative effect on induction of apoptosis was found. Similar effects were observed when Rosi was combined with 1 μM Tam. Further studies are needed to elucidate the potential therapeutic use of Rosi in hormone independent breast cancer.

CB-P51.
SPHINGOSINE KINASE-1 EXPRESSION IN HUMAN TUMORS

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Sphingosine kinase-1 (SK1) is a key enzyme that regulates the balance between cellular levels of the pro-apoptotic molecules sphingosine (SPH) and ceramide (CER) and the growth-promoting lipid sphingosine-1-phosphate (S1P). SK1 has been shown to be up regulated in lung cancer and in glioblastoma and to confer resistance to chemotherapy in some cell types. However, few studies have been performed showing expression in human tumors. Therefore, we focused on studying the role that this protein plays in human tumor progression by analyzing its expression in breast and colon adenocarcinomas, gliomas, head and neck squamous cell carcinomas (HNSCC) and clear cell renal carcinomas (CCRC). We first performed a screening of SK1 expression in HNSCC samples by using the recently developed tissue array: we observed that the enzyme was expressed in 85.7% of tumors (162 out of 189). To obtain more qualitative information we studied different tumor tissues by regular immunohistochemistry and observed that the staining was stronger in tumor cells when compared with adjacent tissue and that it had a cytoplasmic localization. We also studied protein expression on a mouse model of breast cancer (MMTV-PyMT). The results shown confirmed that SK1 may play a role in human tumor progression.

CB-P52.
HEME OXYGENASE-1 EXPRESSION IN HUMAN TUMORS

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Heme oxygenase-1 (HO1) enzyme is responsible of catalyzing the rate-limiting step in heme catabolism. However, it has been very recently suggested to be involved in oncogenesis both as a promoter and as an inhibitor of tumor progression. Thus, it has been shown to transform cells in culture and to induce tumor formation in a mouse model of Kaposi Sarcoma. It has also been shown that its expression levels are associated with a better survival in colorectal cancer. Most of the data has been obtained mainly by using mouse models of cancer and cell lines. Little work analyzes protein expression in human tumors. Therefore, we focused on studying protein expression in various human tumors. We first performed a screening of HO1 expression in HNSCC samples by using the recently developed tissue array: we observed that the enzyme was expressed in 79% of tumors. To obtain more qualitative information we studied angiosarcomas, head and neck squamous cell carcinomas (HNSCC), gliomas, breast and colorectal carcinomas by immunohistochemistry and observed that the staining was stronger in tumor cells when compared with adjacent tissue. Staining was cytoplasmic for all the specimens assayed except for gliomas where nuclear staining was also observed. In a glioma-derived cell we observed nuclear staining as well. The results shown confirmed that HO1 may play a role in human tumor progression.

CB-P53.
HISTAMINE (HA) ACTION IN RAT MAMMARY GLAND MALIGNANT TRANSFORMATION

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We have previously shown that ranitidine (Ran, H2 antagonist), decreased while Loratadine (Lor, H1 antagonist) increased growth of NMU-induced mammary carcinomas in rats. The aim of this work was to evaluate the effect of HA, Ran and Lor in mammary gland of NMU injected rats during malignant progression. Four groups of rats were used: NMU (control); NMU/HA; NMU/Lor; NMU/Ran. NMU was injected at 55, 80 and 110 days of age and treatments began 7 days before. Histological differences were observed starting at 55 days: Control, HA and Lor groups showed important ductal proliferation with ramifications, frequent terminal end buds, anisocariosis and multi-stratified layers. Instead, Ran group showed milder ductal proliferation and anisocariosis as well as mono-layered ducts. At 110 and 160 days the difference between Ran and control was even more pronounced. Meanwhile, HA and Lor treated tissues exhibit signs of transformation: ductal dysplasia, hyperplasia and papilomatosis as well as foci of carcinoma; changes that were observed in Controls to a lesser extent. In addition, HA and Lor treatment produced a significant increase in plasma insulin levels. Ran decreased IGF-1 and insulin receptor expression in ductal cells. Results suggest that HA may play an important role in mammary gland carcinogenesis not only by a direct action on cell growth but also modulating the IGFs system.

CB-P54.
INVOLVEMENT OF H_2O_2 IN WM35 HUMAN MELANOMA CELLS PROLIFERATION

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We have reported that histamine (HA) regulates signaling processes in melanoma cell lines. Accumulating evidence suggests that H_2O_2 plays an essential role in cancer development and metabolism. In addition H_2O_2 is related to cellular damage produced by ionizing radiation (IR). The aim of this work was to investigate the involvement of H_2O_2 in the modulation of WM35 cell proliferation. The expression of Catalase (CAT) and Bax were determined by western blot; intracellular H_2O_2 by flow cytometry employing specific fluorescent staining and cell proliferation by clonogenic assay. Cells were irradiated with doses ranging from 0 to 10 Gy (137Cs source of 189 TBq). Results indicate that 10 μM HA significantly reduced WM35 cell proliferation (50%) while increased intracellular H_2O_2 levels; this effect was completely reverted by the addition of CAT (125 UI). CAT treatment enhanced proliferation ($p<0.001$), decreasing Annexin V positive cells and the expression of Bax. In accordance, H_2O_2 decreased proliferation in a dose dependent manner (IC50=2.5 μM). The surviving fraction after a dose of 2Gy was SF2Gy=0.35 and it was decreased after treatment with 1 μM H_2O_2 (SF2Gy=0.20). IR induced apoptosis and senescence. We conclude that H_2O_2 levels are critical for WM35 melanoma cell proliferation and may be involved in the inhibitory action of HA as well as in the cellular response against IR.

CB-P55.
RAB1 GTPASE REGULATES GENE EXPRESSION VIA P38 MAP KINASE PATHWAY

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The GTPase Rab1 is essential for protein transport between the Endoplasmic Reticulum and the Golgi complex. In previous studies we have demonstrated that changes in Rab1 protein levels regulate gene expression of specific genes (among them KDELR3, GM130 and c-JUN) by modulating their gene promoter activity. In this work we aim to understand the molecular mechanism that can explain this Rab1-mediated function. Using a luciferase reporter assay, we show that changes in the previously mentioned gene promoter activity levels occurred at very short times after Rab1 induction. This data suggest that phosporylation events participate in these genes activation. To test if MAP kinase pathways participate in Rab1 induced response, specific pharmacological inhibitors of the p38, JNK and ERK MAP kinase pathways were used. Rab1-dependent activation was inhibited only by the p38 MAPK inhibitor. Furthermore, deletions of the 5'-flanking regulatory region of KDELR3, GM130 shows that the consensus binding site for the transcription factor CREB (cAMP responsive element-binding protein) is required to response to Rab1changes. These results suggest that Rab1 modulates gene expression by activating the p38 MAPK signaling pathway and that CREB could be the transcription factor that response to this activation.

CB-P56.
INTERLEUKIN 10 ENHANCE THE EXPRESSION OF FORMYLPEPTIDE RECEPTOR 2 IN ACTIVATED MICROGLIAL CELLS

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Microglial cells are important participants in inflammatory responses in the central nervous system. We previously observed that tumor necrosis factor alpha (TNFalpha) induces the expression of the formylpeptide receptor mFPR2 on microglial cells. This chemoattractant receptor mediates microglial cell chemotaxis in response to a variety of peptides, including amyloid beta peptide (Abeta42), a major pathogenic factor in Alzheimer's disease (AD). In search for agents that regulate microglial cell activation, we unexpectedly found that IL-10 enhanced the expression of mFPR2 on TNFalpha-activated microglial cells. This was associated with a markedly increased microglial chemotaxis to Abeta42 and its endocytosis via mFPR2. Mechanistic studies revealed that the synergistic effect of IL-10 on TNFalpha-induction of mFPR2 in microglial cells was dependent on activation of p38 MAPK. Our results suggest that IL-10 may affect the pathogenic process of AD by up-regulating mFPR2 and thus favoring the recognition and internalization of Abeta42 by activated microglial cells.

CB-P57.
PARTICIPATION OF C-FOS IN ACTIVATING PROTEIN SYNTHESIS IN DIFFERENTIATING PC12 CELLS

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We have found that c-Fos, in addition to being a member of the AP-1 family of transcription factors, associates to the endoplasmic reticulum (ER) and activates the synthesis of phospholipids in events that require membrane biogenesis. The complex process of membrane biogenesis requires not only lipids but also other components such as proteins. It was examined if c-Fos also activates protein synthesis by an AP-1 independent mechanism. PC12 cells in culture induced to differentiate by feeding with nerve growth factor (NGF) and labeled with ^{35}S-methionine showed that blocking c-Fos expression inhibits NGF-activated protein synthesis; culturing cells with a peptide that specifically blocks the nuclear import of AP-1-c-Fos shows that AP-1 is required to trigger protein synthesis whereas cytoplasmic c-Fos is necessary for this process to continue. To establish if the activation of protein synthesis by c-Fos is a direct effect on the ER we performed in vitro translation. Increasing concentrations of recombinant c-Fos were incubated with the rabbit reticulocyte system in the presence of canine pancreatic microsomal membranes and a signal sequence mRNA. Preliminary results show activated protein biosynthesis that was dependent on the concentration of recombinant c-Fos. It is hypothesized that c-Fos, in addition to activating lipid synthesis in the ER, activates that of proteins.

CB-P58.
TREATMENT WITH ACE INHIBITORS MODULATES AT1 RECEPTOR AND VEGF EXPRESION IN RAT LUNG DEVELOPMENT

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Vascular endothelial growth factor (VEGF) is related to vasculogenesis and angiogenesis. The VEGF gene is alternatively transcribed into four isoforms of VEGF (120, 144, 164, 188). The lung is one of the organs with high expression of VEGF. Previous studies suggest that AT1 receptors are involved in Ang II-mediated VEGF synthesis. The aim of this study was to investigate the effect of prenatal ACE inhibition on the expression of VEGF isoforms, in correlation to AT1 receptor expression in postnatal lung tissue. Mini-osmotic pumps with enalapril, captopril or saline solution, were implanted in pregnant Wistar rats during the last week of pregnancy. The expression of VEGF isoforms and AT1 receptors was analysed by RT-PCR. Lungs of animals born from treated mothers, at different ages, PND0, PND8, PND15 and PND30, were evaluated. We found expression of all the VEGF isoforms at the different stages. Expression of VEGF188 increased during lung development ($p<0.001$) for the different treatments. VEGF164 and VEGF120 expression increased at PND8 and PND15, encompassing AT1 receptor expression. Treatment with ACE inhibitors induced an increase of AT1 expression early on development (PND0, PND8), while enalapril treatment led to a lower expression of VEGF at PND0. These results suggest a correlation between AT1 receptor and expression of VEGF isoforms that might affect lung development.

CB-P59.
THE NUCLEIC ACID CHAPERONE PROTEIN CNBP BINDS G-RICH NUCLEIC ACIDS THAT MAY FORM G-QUARTETS

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Cellular nucleic acid binding protein (CNBP) is a single-stranded nucleic acid binding protein made of seven CCHC Zn knuckles and a RGG box. CNBP is conserved among vertebrates and plays broad-spectrum functions. It is required for rostral head development during vertebrate embryogenesis and acts as a nucleic acid chaperone, remodeling and stabilizing the secondary structure of its targets. Neither the consensus sequence of these targets nor their structural features were still elucidated. Our main goal was to gain insights into the identification of common features among the preferred CNBP molecular targets. We studied CNBP binding to single-stranded nucleic acid probes representing its main reported putative targets. Analysis of mutant and artificial probes shows that the preferred CNBP targets contain unpaired guanosine-rich stretches. Some of these probes contain the consensus sequence for the formation of stable G-quartet structures. Besides, experimental data from a “Systematic Evolution of Ligands by Exponential Enrichment” (SELEX) suggest that CNBP preferred nucleic acid targets may contain G-quartets consensus. Therefore, these results begin to dissect how CNBP performs its essential biological function: recognizing and probably acting as a nucleic acid chaperone on G-quartets structures, which have been described as pivotal elements for gene expression regulation.

CB-P60.
REGULATION OF ZEB1 PROMOTER
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ZEB1 is a transcription factor involved in lymphopoiesis T, neurogenesis and myogenesis. Deletion analysis of the ZEB1 promoter localized a proximal G-string critically required for transactivation. Analysis of the G-rich element with TRANSFAC identified consensus binding sites for Runx1, Sp1 and MZF1 and two ZEB1 binding sites. We demonstrated that ZEB1 is able to repress expression of its own gene by binding to one of these sites. In addition, we observed that the activity of ZEB1 protein is regulated by changes of phosphorylation. The aim of this work was to test the transcriptional effect of the putative ZEB1 regulators Runx1, Sp1 and MZF1 on ZEB1 promoter activity. Cell lines expressing ZEB1 (CHO-K1, COS-7) were transfected with Runx1, MZF-1 or Sp1 expression vectors and ZEB1 luciferase promoter (Z1p.212Luc) by Ca-P precipitation. Data are expressed as activation relative to Z1p.12Luc (control). Z1p.212 was activated 3-fold above Z1p.12Luc by Runx1 and 5-fold by Sp1. MZF1 repressed ZEB1 promoter to 50%. No effect of the factors was observed in absence of the G-string in ZEB1 promoter. Runx1 (absolutely required for the T and B cell lineages) and Sp1 (ubiquitous factor implicated in lymphopoiesis) may act in part by activating the ZEB1 gene through the G-string. MZF1 is a repressor involved in granulopoiesis that may prevent expression of ZEB1 in the myeloid lineage.

CB-P61.
SOLUBLE ppGAlNAc-T2 INTERACTS WITH GLYCOSYLATED RNA POLYMERASE II
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O-linked N-acetylglucosamine (O-GlcNAc) glycosylation is an abundant post-translational modification of cytosolic and nuclear proteins on Ser and Thr residues. This modification often acts in a reciprocal manner to O-phosphate modification of proteins and together they can synergistically control the activity of many cellular processes. RNA polymerase II is one of nuclear proteins that change between glycosylated to phosphorylated state. C-terminal of polypeptidil GalNAc transferase 2 (ppGalNAc-T2) is a glycan-binding domain. In the present work we initiate the study of binding specificity of ppGalNAc-T2 to different glycoconjugates and cellular extract. By western blot, we saw that soluble ppGalNAc-T2 interacts with RNA pol II among others nuclear matrix components. Immunoprecipitation using anti-ppGalNAc-T2 and anti-RNA pol II antibodies also shows the interaction. ppGalNAc-T2 recognizes YSP(GlcNAc)TSPS glycopeptide that is commonly present in tandem repeats of C-terminal of RNA pol II. The ppGalNAc-T2 interaction with RNA pol II and YSP(GlcNAc)TSPS is lost when ppGalNAc-T2 is chemically acetylated; suggesting that post-translation modification can regulates ppGalNAc-T2 functions. The glycan-binding ability of ppGalNAc-T2 could be involved in gene transcription and cell proliferation through glycosylated RNA pol II interaction.

CB-P62.
REGULATION OF THE DROSOPHILA PROTEIN HIF-BETA/TANGO DURING THE CELLULAR RESPONSE TO HYPOXIA
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HIF is a mammalian transcription factor involved in the adaptation of cells to hypoxia. It is an α/β heterodimer of two bHLH-PAS proteins, where the alpha-subunit is oxygen-regulated and the beta-subunit is constitutive. Previous work from our laboratory has led to the identification of a HIF system in Drosophila, being Sima and Tango the homologues of the alpha and the beta subunit respectively. We show here that, in contradiction with accepted models, levels of the beta-subunit Tango are modulated by the presence of the alpha subunit Sima. We over-expressed Sima in transgenic embryos and analyzed tango protein levels by either immunofluorecence or western blot. By both techniques, we observed a clear increase of both endogenous and over-expressed Tango protein, revealing that the presence of Sima regulates Tango. Tango mRNA levels are unaffected by the presence of Sima, revealing that the regulation is exerted at a post-transcriptional level. We found a correlation between Sima subcellular localization and Tango protein levels, as a nuclear but not a cytoplasmic variant of Sima provoked the upregulation of Tango. Finally, Tango protein levels are upregulated by heat-shock but not by hypoxia. A model for Tango regulation is discussed.

CB-P63.
NCAM MINIGENE AS A TOOL TO STUDY TRANSCRIPTION INFLUENCE ON SPLICING REGULATION
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The nervous system shows many examples of alternative spliced-mRNAs relevant to neuron function. The NCAM gene is a suitable model to investigate transcriptional control of alternative splicing (AS). Exon 18 (E18) AS leads to two of the main isoforms of this protein. Their proportions change in models of synaptic plasticity, suggesting a role in learning and memory. We previously determined that endogenous E18 splicing changes upon membrane depolarization in both primary cultured neurons and N2a cells. We have developed a minigene reporter, consisting of NCAM exons 17, 18 and 19 under the control of NCAM promoter. This construct mimics endogenous splicing response to depolarization in N2a cells. It also duplicates the effects on NCAM AS of drugs that modulate transcription such as HDAC and PolII elongation inhibitors. These responses are abolished when cells are depolarized, suggesting a link between transcriptional control and depolarization. In addition, we found that transcription by a mutant RNA polymerase II with low elongation rate causes higher E18 inclusion, further supporting a kinetic control of E18 splicing. Looking for trans-acting factors that might play a role in E18 regulation, we found that the SR protein SRp20, drastically increases E18 inclusion. This tool will help to elucidate the transcriptional components of a physiologically relevant splicing regulation.

CB-P64.
UV IRRADIATION AFFECTS ALTERNATIVE SPLICING THROUGH A CO-TRANSCRIPTIONAL MECHANISM
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DNA damage caused by UV irradiation is known to trigger a panoply of cell responses, including apoptosis and cell division arrest, that involve signal cascades that control gene transcription, DNA repair and pre-mRNA cleavage and polyadenylation. We found that DNA damage that follows UV-irradiation of cells deeply affects alternative splicing of the fibronectin and Bcl-x pre-mRNAs. Upon irradiation with doses that range from 10 to 40 Joules/m^2, we observed higher inclusion levels of the fibronectin EDI cassette exon and an increase in the proportions of the shorter mRNA isoform of Bcl-x, known to be pro apoptotic. Transfections of reporter minigenes, either prior or after UV irradiation, clearly indicate that the UV effect on alternative splicing is independent from the damage in cis of the actual reporter DNA template. Furthermore, when cells are transfected directly with *in vitro* synthesized pre-mRNA, capped and polyadenylated, alternative splicing takes place but the UV effect is not observed. This indicates that the UV effect is mostly co-transcriptional. Results with different promoters also support the idea that the UV effect is linked to global changes in transcription. FRAP experiments to measure transcription in vivo showed that UV irradiation causes an extended residency time for nascent RNA, which is consistent with a reduced elongation rate.

CB-P65.
EXTENSIVE ROLE OF UNPHOSPHORYLATED CREB ON GENE EXPRESSION IN ABSENCE OF EXTERNAL STIMULI
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Transcription factor CREB induces the activity of CRE-containing gene promoters through PKA-mediated S133 phosphorylation. However, it has been reported that its binding to DNA is independent of such modification. This fact opens a question about the role, if any, of unphosphorylated CREB on gene regulation. By northern blot analysis and transfection experiments in HepG2 cells, we have demonstrated that ALAS basal transcription is diminished by CREB overexpression. This inhibition is stronger after overexpressing a CREB mutant that can not be phosphorylated or by incubating cells with the PKA inhibitor, H89. These results suggest that basal phosphorylation significantly increases the steady state level of ALAS expression. Overexpressing CREB with a mutation in its DNA-binding domain or a CRE-mutated ALAS promoter, we demonstrated that this inhibitory effect is dependent on the CREB-binding and the presence of CRE sites on the promoter. The H-89 inhibition is released by oligodeoxynucleotide CREB decoy only when wild type CRE-ALAS promoter was used. We selected 30 hepatic genes harboring CRE regulatory regions and reported to be cAMP responsive. Interestingly, several of such genes are repressed by unphosphorylated CREB in a similar way that ALAS did. These results support a widespread role of transcription factor CREB controlling gene expression in the absence of external stimuli.

CB-P66.
CELL CYCLE INHIBITOR, p19INK4d, IS UP-REGULATED IN CELLULAR SENESCENCE TRIGGERED BY CAMPTOTHECIN
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Cellular senescence is a state of stable cell cycle arrest triggered by stress strimuli. Disruption of DNA damage response prevents senescence and promotes transformation. Given the function of cyclin dependent kinase inhibitor, p19INK4d, in cell cycle arrest and DNA repair, the aim of this work is to elucidate if this protein is involved in this process. Senescence was induced with camptothecin. Treated cells exhibit characteristic of senescence such as flat and enlarged morphology, increase β-galactosidase activity, cell cycle arrest and upregulation of p16INK4a and p21Cip1. Northern blot analyses show that p19 is upregulated after camptothecin treatment in BHK cells. Levels of p19 peak at 12 h after camptothecin addition. This induction is dose dependent and starts at 20 nM. Reporter gene experiments with a construct harboring 2250 bp of p19 promoter indicate that camptothecin is acting at the transcriptional level. Abrogation of ATM kinase activity severely impairs genotoxic action. Transient expression assays with a mutagenized promoter construct reveal that up-regulation of transcription requires E2F sites. A significant increase in the percentage of cells undergoing senescence is observed in BHK stably overexpressing p19 when compared to wild type or p19 deficient counterparts. Our results suggest that p19 may influence the induction of senescence triggered by camptothecin.

CB-P67.
CDK INHIBITORS p19INK4d AND p21Cip1 PROTECT FIBROBLASTS FROM APOPTOSIS INDUCED BY STAUROSPORINE
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Apoptotic cell death and withdrawals from cell cycle are tightly coordinated. Essential mechanisms for regulation of the cell cycle also influence processes of cell death. An important mechanism for cell cycle control involves inhibition of cyclin dependent kinases (CDKs) by CDKs inhibitors that in mammalian cells fall into two families: Cip/Kip and INK4. The aim of this work is to elucidate the role of the structurally unrelated CKIs, p21Cip1 and p19INK4d, in apoptosis triggered by staurosporine. Northern blot analyses show that p19 and p21 are upregulated after staurosporine treatment in normal BHK fibroblasts. These inductions resulted dose dependent and started at 100 nM. Reporter gene experiments with a construct harboring 2250 bp of p19 promoter show that staurosporine was acting at the transcriptional level. Importantly the presence of caffeine, an ATM/ATR inhibitor abrogated staurosporine action upon p19 regulatory region. Clonogenic assays developed after 7 days post-treatment reveal that BHK cells stably overexpressing either p19 or p21 are more resistant to this proapoptotic inductor. Accordingly, these clonal cell lines exhibited reduced caspase-3 activity when compared to wild type or p19 deficient counterparts. Taken together these results suggest that the overexpression of a CKI can influence the sensitivity of cells to staurosporine mediated programmed cell death.

CB-P68.
ANALYSIS OF THE MECHANISMS CONTROLLING hDlg GENE EXPRESSION

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There is a great deal of evidence suggesting that hDlg expression is lost during tumour development in different tissues, associated with lack of cell polarity and tissue architecture. However, the mechanisms controlling hDlg expression are not fully understood. We performed the cloning and analysis of the genomic 5´flanking region of hDlg ORF with promoter activity, and determined minimal and cis elements required for efficient transcription. Using RACE techniques, we identified an alternative splicing event in the 5' UTR of hDlg mRNA. We showed by reporter assays that the Dlg 5'-UTR containing the alternatively-spliced exon interferes with the translation of a downstream ORF, suggesting that this splicing event can contribute to regulate hDlg levels. Moreover, we found within hDlg promoter consensus binding sites for the Snail family of transcription factors that repress the expression of epithelial markers and are up-regulated in tumours. By co-transfection experiments we demonstrated that Snail proteins repressed transcriptional activity of hDlg promoter constructs and, in addition, stable expression of Snail in Caco2 cells leads to a consistently reduction of hDlg protein levels. To assess the direct interaction of Snail proteins with hDlg promoter sequences at the chromatin level, Chromatin immunoprecipitation (CHIP) assays are currently being performed.

CB-P69.
Wnt-β-CATENIN SIGNALING AND HIGH GLUCOSE LEVELS REGULATE StarD7 EXPRESSION

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StarD7 belongs to the START domain proteins involved in intracellular transport and lipid metabolism. To understand the molecular mechanisms that regulate the StarD7 expression, we characterized the 5´ region of the gene. We reported that StarD7 promoter is activated by SF-1 and this effect is increased by cAMP. Here we demonstrate that an additional regulatory element corresponding to TCF-4 binding site appears important for StarD7 expression, since a reporter gene expression is reduced when this site is deleted, implicating Wnt/β-catenin signaling. Co-transfection assays in JEG-3 and COS-7 cells demonstrated that StarD7 promoter is activated by β-catenin S33Y. Since this activation implies the inactivation of GSK-3β and a glucose storage diminution, we study whether glucose level modify StarD7 expression. A significantly increased StarD7 mRNA, determined by quantitative real time PCR, was found in JEG-3 and HepG2 cells cultured in high glucose compared to the cells cultured in low glucose medium. Also, both StarD7 mRNA and a reporter gene driven by StarD7 promoter were up-regulated by inhibition of GSK-3β. Furthermore, the transcript levels of a list of factors involved in transcriptional control of glucose and lipid metabolism were evaluated. These studies provide novel insights into the regulation of StarD7 gene by Wnt-β-signaling associated to glucose and lipid metabolism.

CB-P70.
CHARACTERISTICS OF THE rDNA CLUSTER FROM *ANASTREPHA FRATERCULUS* AND *ANASTREPHA LUDENS*

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Fruit flies of the *Tephritidae* group are important world orchard pests. Due to the observed phenotypic variablility (morphology, karyotype, habits) *Anastrepha fraterculus*, the South American fruit fly has been postulated as a complex of cryptic species ("fraterculus complex") and their immature stages are difficult to discriminate from those of other *Anastrepha spp*. Nuclear rDNA genes (18S, 5,8S, 2S y 28S) are highly conserved among related species, whereas the internal spacers (ITS) are polymorphic. These non-coding regions show a rapid evolution, allowing discrimination of close species. We previously showed that ITS1 from *Tephritidae* show a highly conserved size and sequence within a species but is quite different among closely related species. We have sequenced and compared, for the first time, the rDNA clusters of *A. fraterculus* (*A.f.*) and *A. ludens* (*A.l.*). The size of the 5.8S subunit is identical in both species whereas the 18S subunit is 1980 pb long in *A.f.* and 2003 pb long in *A.l.*, with a 99% homology. The respective ITS1s show similar length but their sequences diverged 16% whereas the length of ITS2 from A.f. is 374 pb and of A. l. is 435 pb, with a divergence in sequence of 73%. Therefore the data from both ITS provide a useful tool to discriminate quarantine-related immature stages (or damaged adult specimens) otherwise difficult to identify by non-specialists.

CB-P71.
RELATION BETWEEN THE POLYMORPHISM ALA12 OF PPAR GAMMA 2 AND METABOLIC PROFILE IN OBESE WOMAN

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The Pro12Ala polymorphism of peroxisome proliferator-activated receptor-gamma (PPAR-γ) has been associated with decreased risk of diabetes and obesity. Our objective was study the putative relationship between metabolic parameters and PPARγ2 polymorphism in lean and obese woman concurrent to Endocrinology Service of Hospital San Luis. Twenty five obese woman in fertile age were studied. Insulin, Cortisol, TSH, T3, T4, T4L, Prolactine, FSH, LH, DHEAS, 17OH progesterone and testosterone (T) , were determine in serum by RIA. The levels of glucose was determine by available commercial kits. The Insulin Resistance was estimated according to HOMA-IR following the formula (Fasting insulin x fasting glucose)/22.5. Analysis of PPAR-γ2 was performed by Taqman Allelic Discrimination using a STANDARD 7500 termocycler (Applied Biosystems).The frequency of allele Ala12 was 20%, significantly higher than informed in other populations. The levels of glucose, insulin and HOMA with allele Ala 2 were lesser than those woman that show allele Pro12Pro. The 75% of obese woman with Pro12 Pro shown HOMA higher than normal value. In the total of obese woman study, 24% have Cortisol value lesser than the normal, the 20% TSH and 36% T higher than the normal value. There was not correlation between the polymorphism and the levels of determined hormone in the population studied.

CB-P72.
CIRCADIAN VARIATION OF GLUTATHIONE METABOLISM IS MODIFIED IN THE VITAMIN A DEFICIENCY

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Glutathione (GSH) is essential for the maintenance of an optimal cellular redox state and the production of NADPH,H^+, which are critical for the transcriptional activity of the biological clock. Retinoid receptor binding sites and E-box sites have been found in the regulatory regions of Glutathione peroxidase (GPx) and Glutathione reductase (GR) genes. Our objective was to investigate the daily variation of RARs, RXRs, GPx and GR mRNA levels as well as GR activity and GSH levels in the liver of control, vitamin A-deficient and vitamin A-recovered rats. Liver total RNA was extracted using the Trizol reagent (Invitrogen). Transcript levels of RARalpha, RXRalpha, RXRbeta, GPx and GR were determined by RT-PCR and normalized to β-actin as endogenous control. GR activity and GSH levels were determined following Schaedle & Bassham (1977) and Akerboon & Sies (1981), respectively. Interestingly, we observed a daily rhythmicity in the expression of RXRalpha and beta, GPx and GR activity as well as in the GSH levels which were differentially modified by the vitamin A deficiency. Circadian regulation of glutathione metabolism and cellular redox state could be mediated, directly or indirectly, by retinoids receptors.

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CB-P73.
METABOLISM OF β-ALANYL DERIVATIVES OF CATECHOLAMINES IN INSECTS

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We study the metabolism of β-alanyl derivatives of catecholamines in insects. The best known of these is N-β-alanyldopamine (NBAD) the main precursor of sclerotization of insect brown cuticles. The enzyme responsible for its synthesis is NBAD-synthase. We demonstrated that this enzyme is capable of conjugate beta-alanine with other catecholamines such as norepinephrine. Furthermore we showed that, NBAD-synthase can also accept as substrate other biogenic amines like octopamine, tyramine, serotonin and histamine, or aminoacids such as tyrosine to synthesize β-alanyl derivatives. The epidermal enzyme is induced by 20-0H-ecdysone only at the time of pupariation and of adult ecdysis, when the insect needs to sclerotizing its cuticle. On the other hand it appears to be present in nervous tissue in a constitutive manner. Finally we ascertained that NBAD-synthase is activated in epidermis in response to microbial infection, showing NBAD antimicrobial properties in vitro. This result indicates that the synthesis of NBAD is a novel aspect of the overall innate immune response in insects. We study the activity of this enzyme in three different metabolic pathways: cuticle sclerotization, innate immune response and neurotransmition/neuromodulation. We characterized the melanic mutant niger of medfly *Ceratitis capitata* and ebony of *D. melanogaster*, lacking this enzymatic activity.

CB-P74.
RAT PROSTATE CYTOSOLIC XANTHINE OXIDASE MEDIATED METABOLISM OF ACETALDEHYDE TO ACETYL RADICALS

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Repetitive alcohol drinking is known to lead to deleterious effects on prostate epithelial cells from humans and experimental animals. In previous studies from our laboratory we provided evidence about the presence in the rat ventral prostate of cytosolic and microsomal pathways of metabolism of ethanol to acetaldehyde and 1-hydroxyethyl radical and about the poor to null presence of alcohol dehydrogenase and aldehyde dehydrogenase. Acetaldehyde accumulation in prostate tissue and oxidative stress promotion were also observed. In the present study we report that in the ventral prostate cytosolic fraction, xanthine oxidoreductase is able to metabolize acetaldehyde to acetyl radical. The identification of the acetyl was performed by GC-MS of the silylated acetyl-PBN adduct. Comparison was made against reference adduct from acetyl generated in two chemical model systems. Acetyl was also detected using pure xanthine oxidase from butter milk. The generation of acetyl by the prostate cytosol was inhibited by allopurinol, oxypurinol, diphenyleneiodonium chloride, folic acid and ellagic acid at low concentrations. Previous and present results suggest that metabolism of ethanol to acetaldehyde and to 1-hydroxyethyl and acetyl radicals could be involved in the deleterious effects of alcohol drinking on prostate epithelial cells.

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CB-P75.
METABOLISM OF ALCOHOL TO ACETALDEHYDE IN THE RAT UTERUS AND ITS REPRODUCTIVE TOXICITY

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It is known that alcohol drinking can lead to impairment in reproductive function in women. In this study we analyze the possibility that part of these effects were mediated through alterations of uterus function related to ethanol oxidation to acetaldehyde occurring in that tissue. We found that biotransformation in the cytosolic fraction is mediated by xanthine oxidoreductase (XOR), required a purine cosubstrate and was inhibited by allopurinol and pyrazol. By histochemistry XOR activity was detected in the epithelium and aldehyde dehydrogenase activity was detected in the muscular layer and serosa. The microsomal process did not require NADPH but was of enzymatic nature, sensitive to oxygen and was inhibited by diethyldithiocarbamate, diphenyleneiodonium and partially by esculetin and nordihydroguaiaretic acid. Ultrastructure of uterus from rats treated with standard Lieber & De Carli liquid diet for 28 days revealed extensive vacuolization in cytoplasm and loss of cell content. In addition we observed the promotion of oxidative stress as evidenced by increased response in the t-butylhydroperoxide induced chemiluminiscence and the depletion of the protein sulfhydryl content. Results suggest that in the rat uterus, metabolism of ethanol to acetaldehyde may play a role in alcohol effects on female reproductive function.

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CB-P76.
METABOLISM OF ETHANOL TO ACETALDEHYDE IN THE RAT OVARY AND ITS INDUCED REPRODUCTIVE TOXICITY

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It is known that alcohol drinking can lead to reproductive problems in women. In this study we analyze the possibility that part of these effects were mediated through alterations of ovarian function related to ethanol oxidation to acetaldehyde occurring in that tissue. We studied the bioactivation to acetaldehyde in rat ovary and found that biotransformation in the cytosolic fraction is partially inhibited by allopurinol, suggesting the participation of xanthine oxidoreductase (XOR) in the process. The microsomal pathway was also of enzymatic nature, requiring NADPH and sensitive to oxygen. In both cases, metabolic ability to oxidize ethanol was compared with those of post lactation young mother (two weeks after weaning) rats, showing to be more intense in the case of virgin rats. By histochemistry XOR and aldehyde dehydrogenase activities were detected in both ovary and oviduct. No alcohol dehydrogenase was detected. Ultrastructure of ovary from rats treated with standard Lieber & De Carli liquid diet for 28 days revealed alterations at the level of the granulosa, theca interna and pellucida zones. In the oviduct, intense vacuolization and partial loss of cell content were observed. Results suggest that in the rat ovary, metabolism of ethanol to acetaldehyde might play a role in alcohol effects on reproductive function.
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CB-P77.
5ALPHA-REDUCTASE AND GLUCOCORTICOID ACTION IN TOAD TESTES

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In the toad, breeding levels of corticosterone (CORT) decrease testosterone (T) synthesis by inhibiting the enzyme Cyp450c17. The effect of non-breeding levels of CORT is blocked by the activity of 11β-hydroxysteroid dehydrogenase (11βHSD), enzyme located in Leydig but not in Sertoli cells. Thus, that enzyme cannot counteract the effect of CORT on Sertoli cells, which express glucocorticoid receptors (GR) and 5α-reductase (5αRed). As in Sertoli cells 5αRed could be involved in the regulation of CORT access to GR, its rol in *Bufo arenarum* testes was studied by analyzing spermiation and T production after 24-hr incubation under different conditions. T synthesis was measured by RIA and hCG-induced spermiation by counting spermatozoa in incubation media. CORT decreases T secretion, the effect being abolished by RU486 but enhanced by glycyrrhetinic acid (GA), signifying that 11βHSD modulates CORT effect in Leydig cells. After 24-hr incubation, hCG-induced spermiation decreases but in the presence of physiological concentrations of CORT (15 or 150 nM), it was similar to time 0 values. Finasteride decreases CORT action, suggesting that CORT effect in spermiation is mediated by its conversion in 5α-dihydrocorticosterone (5αDHB). As with T synthesis, RU486 completely abolished the effect of 5αDHB suggesting that its action on spermiation is due to the interaction with GR in Sertoli cells.

CB-P78.
FERRITIN EFFECT ON THE LABILE FE POOL IN RAT LIVER

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Ferritin (Ft) is understood as an antioxidant protein by its ability to store Fe but other functions should be analyzed. Commercially available horse spleen Ft was passed through a Sephadex G25 column to avoid adventitious Fe. Fe released from Ft was assessed spectrophotometrically (λ = 562 nm) in the presence of ferrozine. Fe was not actively released spontaneously from Ft ($0.6\ 10^{-4}$ OD/min), however the supplementation with 5 mM ascorbate significantly increased the Fe release rate ($8.11\ 10^{-4}$ OD/min). The labile-Fe pool (LIP) is defined as a low-molecular-weight pool of weakly chelated Fe and it likely consists of Fe^{2+} and Fe^{3+}. Male Wistar rat liver samples were homogenized in 10 mM Tris-HCl, 120 mM KCl, pH 7,4 containing 1 mM desferal. Liver homogenates were incubated for 10 min at 25ºC to assay for LIP content by electron paramagnetic resonance. LIP content was 2.1 ± 0.3 μM and was not affected by the addition to the medium of either 0.1 mM NADPH, or 0.1 mM EDTA. The supplementation during incubation with 50 μM Fe-EDTA increased the LIP to 83 ± 6 μM. Liver samples added with 50 μg/ml Ft increased the basal LIP to 8 ± 1 μM. The simultaneous addition of Ft, EDTA and NADPH raised the LIP to 15 ± 2 μM. These results suggest that Fe released from Ft can be incorporated to the cellular LIP, increasing the risk of free radical-dependent alterations to lipids, proteins and DNA.

CB-P79.
EFFECT OF CADMIUN IN THE DRINKING WATER ON THE EXPRESSION OF PROLACTIN GENE AND NAPH OXIDASE IN PITUITARY GLAND

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Cadmium (Cd(2+)) is an ubiquitous toxic metal that is involved in a variety of pathological conditions. Our previous result have shown that Cadmium (Cd) expression modify the lipid metabolism of pituitary gland. On the other hand it is known that Cd(2+)-mediates cytotoxicity and the inhibition of prolactin release, supporting the involvement of oxidative stress in the mechanism of Cd(2+) action. NADP oxidase is a generator of species oxygen reactive. Our objective was determine if Cd modify the expression of NADP oxidase, as generator of oxidative stress. Also, the relation of NADP oxidase and prolactin gene expression, was studied. Wistar adult male rats were exposed to 15 ppm of Cd as Cl2Cd in the drinking water during 8 weeks. Then, rats were sacrified and pituitary gland was extracted. The mRNA was isolated by Trizol reagent and the expression of NADP oxidase and prolactin genes were determine by RT-PCR. We observed that Cd did not modify the expression of mRNA NAPH oxidase and decreased the expression of mRNA Prolactin. We have previously shown a decreased secretion of prolactin, in this experimental condition, which could be attributed to a decreased expression of mRNA prolactin independent of oxidative stress.

CB-P80.
DO YOU WANT A DRINK? CARDIOVASCULAR OXIDATIVE STRESS PRODUCED BY DRINKING Cd CONTAMINATED WATER

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Cadmium intoxication increases oxidative stress parameters in several tissues. Objectives: The aim of this study was to assess the cardiovascular effect of drinking cadmium contaminated water. Experimental model: Male Wistar rats were separated into four groups: 1 (Controls, tap water), 2, 3 and 4 (tap water with 15 ppm Cd ion, during 15, 30 and 60 days respectively). Food and water were administrated ad libitum. Afterwards, animals were decapitated, under light ether anesthesia. Samples were kept at -20ºC, until processed. Methods: Determinations in serum: TBARS (Jentzsch *et al*., 1969), Proteins (Layne *et al*., 1957), NO (Schulkz *et al*., 1999), and Paraoxonase-1 (PON-1) (Beltowsky *et al*., 2002). TBARS and iNOS expression (Western Blot) was assessed in heart. Results: Serum: TBARS were elevated in groups 3 and 4, ($p<0.05$). PON-1 activity in group 3 was the highest ($p<0.001$). PON-1 activity augmented in group 4 compared to controls ($p<0.001$). NO determination showed no differences among the groups. Heart: TBARS increased in group 4, ($p<0.05$). It was seen a decrease in iNOS production in groups 2 and 3. Conclusions: Rats exposure to oral cadmium intoxication increases oxidative stress parameters in serum and heart. It also seems to be an induction of antioxidant enzymes (PON-1) in serum at the first stages of intoxication, with a decreased iNOS concentration in heart.

CB-P81.
ANTIOXIDANT METABOLISM AND DEVELOPMENT IN *BUFO ARENARUM* EMBRYOS EXPOSED TO AZYNPHOS METHYL

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Amphibians are very susceptible to environmental contaminants, being the toad *B. arenarum* particularly threatened during its embryonic development by the massive application of the organophosphate azynphos methyl (AM) used for pest control in Norpatagonia valleys. Our objective is to determine if reactive oxygen species (ROS) in connection with altered polyamine (PA) metabolism contribute to altered embryonic development. Embryos were continuously exposed until complete development to 0.5, 2 and 9mg/l AM. Antioxidant enzyme activities were measured. Catalase was early increased by 0.5mg/l AM returning to control levels thereafter. GSH reductase was also early increased by all doses of AM, while GSH peroxidase tended to decrease at late stages. GST was not affected by AM. Putrescine levels increased throughout development, rising significantly in exposed embryos, while spermidine and spermine were decreased in late embryos exposed to 9mg/l AM. These effects are accompanied with a dose dependent increase in the number of embryonic malformations. The results suggest that catalase and GSH reductase are early induced in response to mild exposure to AM, while in later embryonic stages the antioxidant enzymes are altered by the increase of oxidative stress. PA levels suggest an oxidative metabolization to putrescine, contributing to oxidative stress and altered development.

CB-P82.
EFFECT OF Cd, Pb AND As ON *BIOMPHALARIA GLABRATA* OVIPOSITION, EMBRYONIC SURVIVAL AND HATCHING

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Biomphalaria glabrata is a widespread freshwater gastropod mollusc. *B. glabrata* was exposed to cadmium, lead and arsenic using acute laboratory bioassays. Snails were exposed 96 h to different concentrations of the following contaminants: 0.1 and 0.05 mg Cd/L; 0.5, 0.1 and 0.05 mg Pb/L; 0.5, 0.1 and 0.05 mg As/L. Snails were removed from glass aquarium and, egg-masses were left in the same contaminant concentrations. The impact of these contaminants was tested on the number of laid eggs, survival of the embryos and hatching time.
At 0.10 mg/L cadmium significantly decreased the total number of laid eggs (TNLE) ($p<0.05$) and there was no embryo survival and no hatching. The lowest assayed level (0.05 mg/L) delayed the hatching time twice the control time ($p<0.01$). Lead decreased the TNLE at 0.5 mg/L level ($p<0.01$). The other assayed doses (0.05 and 0.10 mg/L) also decreased significantly embryo survival ($p<0.05$ and $p<0.01$ respectively) while extended twice the hatching ($p<0.01$). The 0.50 mg/L level killed all the embryos. Arsenic at all studied levels decreased the TNLE ($p<0.05$) while the hatching time increased 50%. Embryo survival only decreased at the highest level (0.5 mg/L).

CB-P83.
PORPHYRIC PHOTOINSECTICIDES AGAINST PEST FLIES

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In recent years, attention has been focused on the photosensitizing properties of porphyrins since they produce reactive oxygen species (ROS) when illuminated. These have the ability to absorb in most of the wavelengths from UV to visible and to generate long live triplet states. To establish a proof of principle, we tested the larvicidal properties of Haematoporphyrin (HP) in immature stages of the Medfly, *Ceratitis capitata* and the Hornfly *Haematobia irritans*. A LD50 value of 0.14 mM was obtained for *C.c*, lower than for *H.i.* and lower than those obtained with the xanthene dye Phloxine B (PhB) too (LD50 of 0.5 mM). HP light-induced mortality was higher during the dispersal stage of the larvae (whereas larvae reared with PhB died during de pupal stage). We examined the presence of ROS in different tissues of larvae exposed to light, using a ROS detection test based on a fluorogenic marker (carboxy-DCFH). Only the midgut and the brain showed increased intensity of fluorescence, due to the oxidated state of the reagent. Total activity of catalase increased in HP treated larvae, whereas superoxide dismutase activity remained unchanged. We also found that larvae treated with HP presented increased levels of lipid peroxidation. Our results demonstrate that HP is a more effective larvicide than PhB and generates a lower lethal time.

CB-P84.
ANALYSIS OF *Ebaf* AND CFC IN THE OVIDUCT DURING THE EARLY PREGNANCY
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Growth factors and their receptors are expressed by the preimplantation embryo and the reproductive tract. Moreover, they affect the rate of embryo development and they are involved in the maternal-embryo cross-talk. In previous work we described the isolation and cloning of Ebaf, a member of the TGF-B family, in the rat oviduct. In the present work we study the expression of *Ebaf* in the oviduct during the early pregnancy (EP) and pseudopregnant (SP) stage and compare it with the expression of CFC (Cripto-FRL1-Cryptic), a molecule that acts like a receptor. Western blots (WB) reveal that the mature form of Ebaf (26 kDa) was present in the oviduct during the EP showing a significant increase in the day 4º that was maintained until the day 6º, also showed that Ebaf is present in the oviductal fluid at day 4º, when the embryo is still there. RT-PCR demonstrated that *Ebaf* and *CFC* expression levels were higher during the 4th day of EP, in contrast with SP where the transcript levels present minimal variations. These results suggested that Ebaf up-regulation is independent of sexual hormones, indicating that the embryo or/and other factors present in the EP are responsible of its regulation. The presence of CFC and the mature form of Ebaf in the organ and the fluid would indicate that Ebaf is active and that could act in an autocrine and/paracrine way on the oviduct and/or on the embryo.

ST-P01.
STUDY OF PHOSPHORYLATED RESIDUES OF LEPRK2 IN TOMATO AND THEIR FUNCTION IN POLLEN-PISTIL INTERACTION
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LePRK1 and LePRK2 are two pollen-specific receptor kinases from Solanum lycopersicum, of pollen tubes and potentially involved in pollen-pistil interactions. Transduction, by their kinase activity, of a pistil signal might initiate a signalling cascade in the pollen tube that regulates its growth. LePRK2 is phosphorylated in vivo in pollen membranes.
To determine which LePRK2 residues are phosphorylated in vivo, we separated proteins from germinated pollen, by 2D gels. To visualize the phosphorylated isoforms, we performed western blots using an antibody against LePRK2. Spots corresponding to those isoforms were isolated from a Coomassie blue stained 2D gel.
Another strategy involves computational approaches to predict phosphorylation sites in LePRK2. We selected 8 serines or treonines of LePRK2: S277, S279, S282, S304, S307, T308, T358 and S396. To study their functional relevance during pollen tube growth, we substituted them by Alanines using wild-type pLAT52-LePRK2-eGFP vector in mutagenesis, followed by transient expression in tobacco pollen. We previously showed pollen tubes that received wild type LePRK2-eGFP, displayed aberrant ballon tip morphology at germination. On the contrary, pollen tubes transfected with the construct that had A277, 279, 282, recovered the wild type phenotype. These results will be further studied and complemented by the proteomic approach.

ST-P02.
ISOLATION AND CHARACTERIZATION OF GROWTH HORMONE RECEPTORS SUBTYPES IN ZEBRAFISH (DANIO RERIO)
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Growth hormone (GH) and somatolactin (SL) are evolutionary related hormones coming from the same ancient gene, being SL an exclusive fish hormone. Though both hormones have been related to numerous physiological processes, little is known about their mechanism of action. In various fish species two types of receptors for GH (GHRI and GHRII) and one type for SL (SLR) have been characterized, being the last one, homologue to GHR type I. By in silico analysis of the zebrafish genome, we identify two configs containing putative GHRs sequences; one of them situated in the chromosome 8 and the other in the chromosome 21. We cloned the full-length cDNA sequence of GHR I and a partial sequence of GHR II as deduced from a phylogenetic study using known fish GHR sequences. The GHR I transcript have 2339 pb, and the genomic structure comprise 9 exons and 8 introns. Although a gene prediction is available in the Ensemble web site we characterized two new unpredicted exons. The protein sequence of zGHR I have 41% of identity with predicted zGHR II and 80% with other cypriniformes GHRI. We studied the tissue specific expression patterns of both receptors in adult zebrafish and during zebrafish development using RT-PCR. The data obtained showed that both genes are expressed during early development and adult tissues, and that there exist differences in the expression pattern between them.

ST-P03.
ISOLATION AND CHARACTERIZATION OF THE GROWTH HORMONE RECEPTOR IN PEJERREY (ODONTESTHES BONARIENSIS)
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Growth hormone (GH) is a pluripotent regulator that is involved in metabolism and linear growth in fish. It exerts its functions interacting with a GH receptor (GHR) on the cell membrane of the target tissues. GHR belongs to the hematopoietic cytokine receptor superfamily, it has a single extracellular domain with conserved cysteine residues (Cys) that are involved in the GH binding, one transmembrane domain, and one intracellular domain with conserved regions and conserved tyrosine residues (Tyr). There are two types of GHRs in fish (GHRI and GHRII) that differ in the number of Cys and Tyr between each other. It has been proved that the effect of GH in growth is closely related with the GHR transcription level in liver. Our group is interested in the endocrinology of growth in Pejerrey (Atheriniformes). We are characterizing the GH receptor in this species. We isolated a 1500 bp cDNA sequence that covers almost the entirely open reading frame of the receptor. It presents a 70% homology with perciformes GHRs, and it has all the features of GHRs type II. We detected transcripts of the receptor in 14 organs. ghrII expression was measured in fish injected with recombinant pjGH using a RT-PCR semiquantitative assay. Although the level of IGF-I mRNA increased significantly 9 h post injection, no variation in the transcription level of GHR was found up to 24 h post injection.

ST-P04.
COMPARATIVE EXPRESSION PROFILING OF MYCOBACTERIUM BOVIS STRAINS WITH VARIABLE VIRULENCE

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Bovine tuberculosis (TBB) remains an important animal and zoonotic disease in many countries of the world causing significant economical losses and a public health hazard. *Mycobacterium bovis*, the causative agent of TBB, is closely related to *M. tuberculosis*, the agent of humanTB.
A range of different global techniques for identifying large numbers of genes that are related with virulence has been applied since last years. Among these techniques, DNA microarray analysis has been applied to defining the repertoire of genes expressed in macrophages infected with *Mycobacterium tuberculosis*.
To extend the repertoire of these virulence related genes we therefore undertook a whole transcriptomic microarray analysis of *M. bovis*. We compared the expression profile of a *M. bovis* strain that have showed a hipervirulent phenotype in mice with that of an attenuated strain. We found that during in vitro culture condition, the expression of Rv3496 and Rv3681 was upregulated in the hipervirulent strain while the expression of Rv3249, Rv3251 and Rv1048 was upregulated in the attenuated strain. These genes encoded probable transcriptional regulatory proteins, virulence-related gene and genes involved in fatty acid metabolic process.

ST-P05.
EFFECTS OF HEME-OXYGENASE/CARBON MONOXIDE SYSTEM (HO/CO) ON TESTICULAR STEROIDOGENESIS

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Previous results from our lab, showed that nitric oxide (NO) inhibits steroidogenesis in MA-10 Leydig cells, and it is believed that CO, generated from heme in the reaction catalyzed by heme oxygenase, might have similar regulatory effects as NO.
The aim of this study was to investigate the effects of HO/CO on steroidogenesis (P4 synthesis) and StAR protein expression in MA-10 Leydig cells. Hemin, an HO inducer, lowered basal and dibutiryl-cAMP-stimulated (db-cAMP: 1 mM) P4 levels. SnPPIX, an HO inhibitor, increased basal and hCG-stimulated P4 synthesis (hCG: 1 ng/ml, submaximal concentration). Moreover, SnPPIX reverted hemin effects. The incubation of the cells with the permeable analog of cholesterol, 22R-OH-cholesterol or pregnenolone in the presence of SnPPIX suggested that HO/CO inhibits the P450-scc activity. The steroidogenic acute regulatory protein (StAR) regulates the limiting rate step in steroidogenesis: cholesterol transport to the inner mitochondrial membrane. In agreement with P4 results, hemin inhibited the db-cAMP-stimulated StAR protein expression. In summary, CO, generated by HO, inhibited P4 synthesis and diminished StAR protein expression in MA-10 Leydig cells, in a similar way to that observed in the presence of NO. In conclusion, CO and NO might have similar effects on steroidogenesis, involving a regulation of StAR protein expression and P450-scc enzyme activity.

ST-P06.
SPHINGOSINE KINASE 1 IS INVOLVED IN THE ACROSOMAL EXOCYTOSIS OF HUMAN SPERMATOZOA

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The acrosome is an exocytic granule overlying the sperm nucleus. In response to progesterone or zona pellucida, it undergoes calcium-regulated exocytosis. We demonstrated that sphingosine 1-phosphate (S1P), produced by sphingosine phosphorylation catalyzed by sphingosine kinase (SK), triggers acrosome reaction (AR) in human sperm. The fusion process triggered by S1P was inhibited by BAPTA suggesting a dependency of extracellular calcium. We hypothesized that S1P activates exocytosis via a receptor-mediated mechanism that opens SOC channels. Ni^{2+}, an inhibitor of SOCCs, blocked S1P triggered exocytosis. Furthermore, voltage-dependent calcium channel inhibitors, such as verapamil and nifedipine, blocked S1P-induced AR. No effect of S1P was observed in permeabilized sperm suggesting that S1P exerts its effect through S1P receptors (S1PR). We blocked S1P-induced AR by using Pertussis Toxin, confirming the presence of a Gi-coupled S1PR. S1P stimulus could proceed from the oocyte or from sperm cells during fertilization. To unveil this riddle, we used DMS, a SK1 inhibitor, in functional assays. DMS blocked S1P induced-AR, and this effect was overcome by exogenously added S1P. We detected SK1 by western blot and indirect immunofluorescence assays. We conclude that SK1 is present in human sperm, its distribution pattern changes after a stimulus and actively participates in sperm AR.

ST-P07.
ROLE OF CALCINEURIN DURING ACROSOMAL EXOCYTOSIS OF HUMAN SPERM

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Synaptotagmins (syt) are transmembrane proteins with two cytoplasmatic calcium- and phospholipids-binding domains (C2A and C2B). Synaptotagmins have been involved in exocytosis as calcium sensors. The acrosome exocytosis (AE) is a calcium regulated exocytosis essential for fertilization. We have demonstrated that syt VI is required for the AE and that Syt VI is phosphorylated in resting sperm, and its dephosphorylation after stimulation requires calcium. Calcineurin is a calcium/calmodulin-activated serine/threonine protein phosphatase whose role in exocytosis is still unclear. Our aim is to unveil whether calcineurin is involved in dephosphorylation of syt VI in human sperm. To this end, we have used a streptolysin O permeabilized sperm model. Cyclosporin A and FK 506, two calcineurin inhibitors, blocked the RA at the same early step where syt is dephosphorylated. In an *in vitro* phosphorylation/dephosphorylation assay calcineurin dephosphorylated syt VI C2B domain when it was activated with calcium and calmodulin. Our results indicate that calcineurin dephosphorylate syt VI during acrosomal exocytosis.

ST-P08.
MARCKS PHOSPHORYLATION: A NEW COMPONENT OF THE SIGNAL TRANSDUCTION PATHWAY IN ACROSOMAL EXOCYTOSIS

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MARCKS is a prominent substrate of PKC in many cell types; nevertheless the presence of MARCKS in sperm, as a possible PKC substrate has not been investigated. Using a specific antibody against MARCKS, Western blot analysis revealed the presence of MARCKS in human sperm. Furthermore, immunocytochemistry assays showed that MARCKS localized at the acrosomal region. This localization prompted us to investigate if MARCKS participate in acrosomal exocitosis (AE). To test this hypothesis, we expressed MARCKS effector domain (ED). Using the Streptolysin O-permeabilized sperm model, we investigated the effect of MARCKS ED on the AE stimulated by a PKC activator, phorbol 12-myristate 13-acetate (PMA). MARCKS ED inhibited specifically AE stimulated by PMA. To investigate if MARCKS phosphorylation might affect the AE stimulated by PMA, we generated two different mutants of MARCKS ED: the constitutively phosphorylated and the constitutively unphosphorylated MARCKS by replacement serines of the ED with aspartic acid and alanine, respectively. When these mutants were assayed in the AE, only the constitutively nonphosphorylated MARCKS inhibited the exocytosis stimulated by PMA suggesting that MARCKS phosphorylation is required during the progression of AE. These results show that MARCKS is expressed in human sperm and its phosphorylation is a new component of the signal transduction pathways in AE.

ST-P09.
THE TRANSCRIPTION FACTOR KLF6 IS REGULATED BY JNK AND P38 PATHWAYS

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KLF6 is a potential tumor suppressor whose expression is responsive to growth factors, DNA-damage and infections. This factor interacts with the c-Jun oncoprotein and induces its degradation leading to inhibition of cell proliferation. MAP kinases are main regulators of the c-Jun activity and stability. However, the biochemical response to extracellular stimuli and its effects in c-Jun-sustained proliferation are still unknown.
We observed that KLF6 protein levels were markedly reduced in *jnk-/-*cells upon expression of JNKs. Moreover, JNK2 activity was associated to KLF6 translocation from the cytoplasm to the nucleus. Additionally, activated p38 isoforms also decreased KLF6 protein levels. Interestingly, KLF6 was phosphorylated in vivo in *jnk-/-*cells under minimal ERK and p38 activities, indicating a constitutive KLF6 phosphorylation independently of MAPKs.
These results show that JNK and p38 pathways are mainly involved in the regulation of protein stability and cell localization of KLF6. Thus, increased activity of JNK1 and p38 diminished KLF6 protein levels whereas allowed high stability and activation of c-Jun. Conversely, JNK2 substantially increased KLF6 targeting to the nucleus whereas c-Jun activity was low, suggesting that activated c-Jun and KLF6 are mutually exclusive, correlating with enhanced or reduced cell proliferation rate, respectively.

ST-P10.
INVOLVEMENT OF C-SRC (SFK) IN AT2 SIGNALING PATHWAY IN RAT DEVELOPMENT

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Besides the classical effects of Ang II, this peptide is involved in growth control. The aim of the present study was to evaluate the involvement of c-Src in AT2 receptor signaling pathway during rat development in hindbrain (PDN15) and fetal (E20) membrane preparations. Aliquots (500 μg) of membranes were stimulated with Ang II in presence or not of competitors and the c-Src inhibitor PP2. Immunocomplexes obtained with anti-AT2 or anti-SHP-1 antibodies were used to measure SHP-1 activity (colorimetric assay) and interaction with c-Src by western blot. Following Ang II (10^{-7}M) stimulation, SHP-1 associated to AT2 receptors but not to AT1 subtype. Immunocomplexes obtained with anti-AT2 or anti-SHP-1 exhibited PTPase activity, which was blocked by PD123319 (AT2 antagonist) or PP2. We correlated this activity with tyr-phosphorylation level of SHP-1 in immunocomplexes by western blot. Both immunocomplexes contained c-Src. Thus, Ang II stimulation via AT2 subtype, induced tyr-phosphorylation of SHP-1 and the formation of immunocomplexes AT2-SHP-1-Src. These results suggest an activation of SHP-1 by tyr-phosphorylation mediated by Ang II through AT2 subtype. PP2 blocked tyr-phosphorylation as well as SHP-1 activation. Altogether, these data suggest the involvement of c-Src in the activation of SHP-1 via AT2 receptors in the models studied.

ST-P11.
PTP ACTION AND ARACHIDONIC ACID RELEASE AS COMMON STEPS IN DIFFERENT SIGNAL PATHWAYS

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We have previously demonstrated the existence of a tyrosine phosphorylated protein necessary for the expression of an essential gene that promotes intramitochondrial cholesterol transport. This protein must be dephosphorylated by a protein tyrosine phosphatase (PTP) regulated through the cAMP/PKA pathway. Once dephosphorylated, the protein induces the expression of ACS4, an arachidonic acid (AA) preferring acyl-CoA synthetase, which acts in concert with Acot2, a long chain fatty acyl-CoA thioesterase, to promote AA release from mitochondria. Since compartmentalized AA release is activated by different pathways, we aimed to study if the PTP is a common step activated by these different pathways. We used H295R cells, a human adrenal cell line that responds to three different signal transduction pathways to release aldosterone. Using two different PTP inhibitors we have shown that PTP action was necessary for the stimulation of aldosterone synthesis by ACTH as well as by angiotensin II and K^+.
Inhibition of PTP activity was overcome by the addition of exogenous AA, supporting the notion that this activity is involved in AA release and aldosterone synthesis.
These results suggest, by first time, that the PTP action and compartmentalized AA release are common steps in the mechanism of activation of steroid synthesis by different signal transduction pathways.

ST-P12.
FUNCTIONAL CHARACTERIZATION OF THE PROMOTER OF THE ACYL-COA SYNTHETASE 4
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We have described that, in steroidogenic tissues, hormonal action prompts the synthesis of a labile protein, an acyl-CoA synthetase (ACS4), which is involved in the regulation of arachidonic acid (AA) release and is essential for steroidogenesis. We demonstrated that ACS4 protein levels are rapidly induced by steroidogenic hormones and cAMP in Y1 adrenocortical and MA10 Leydig cells. The aim of the present work is to characterize the promoter sequence of ACS4 and to study its transcriptional regulation. In order to determine the minimum sequence required for maximal promoter activity, unidirectional progressive 5´ deletions of the promoter sequence were performed. Obtained constructions were used to measure promoter activity by dual-luciferase assay in transfected MA-10 cells stimulated for 3 h with 8Br-AMPc. The minimum sequence that maintains basal and cAMP-induced promoter activity is contained from base -150. This sequence includes consensus binding sites for CREB, and Sp1 transcriptions factors. Specific binding to CREB and SP1 sites within the promoter sequences was detected by EMSA and binding was augmented when the cells were stimulated by cAMP. We also detected an increase in ACS4 protein and mRNA levels in MA10 cells over-expressing CREB. Our results demonstrate the transcriptional regulation of ACS4 by cAMP.

ST-P13.
MAP KINASES IN PROLIFERATING HUMAN COLON CANCER CACO-2 CELLS
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Cells adapt to environmental changes by monitoring and reacting quickly to extracellular stimuli. The mitogen-activated protein kinase (MAPK) cascade is one of the most ubiquitous signal transduction systems and is rapidly activated by various stimuli, such as cellular stress and death. The Caco-2 cell line is an in vitro model for colon cancer studies. We investigated the activation status of the ERK1/2, p38, JNK1/2 and ERK5 kinases and their respective upstream intracellular activators in Caco-2 cells induced to proliferate by 10% fetal bovine serum (FBS). The states of phosphorylation of the above MAPKs and their upstream kinases, MEK 1/2, MKK3/6, MKK4 and MKK7, respectively, were studied by Western blot analysis. Phosphorylation was barely detectable before serum stimulation, and the stimulation of cell proliferation by the addition of FBS increased MEK1/2 and ERK1/2 phosphorylation 2- to 3-fold after 5 min. FBS stimulated p38 and MKK3/6 to the same extent within 2 min of treatment and JNK1/2 and its upstream kinases MKK4 and MKK7 5-fold (3 min). Addition of FBS also rapidly phosphorylated ERK5 (2 - 3.5-fold between 2-5 min). Studies with pharmacological inhibitors are under progress to establish interactions and the relative role of individual MAP kinases in the regulation of Caco-2 cell proliferation.

ST-P14.
INSULIN-STIMULATED PHOSPHATIDIC ACID (PA) FORMATION IN RAT CC SYNAPTOSOMES. IS DAGKε ACTIVATED?
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Insulin (I) plus vanadate (V) increased DAGK activity in rat CC synaptosomes. A phospholipase-dependent activation and a phospholipase-independent effect (PIE) have been reported. PIE was studied with ATPγ^{32}P in a micellar assay using octylglucoside (OG) and di 16:0 or 18:0-20:4 DAG (250 μM). It was suggested that DAGKε participates in IR signaling (SAIB 2006). DAGKε was reported to be negatively modulated by PIP_2. Potential regulators of PIP_2 levels through G protein activation (GTPγS and NaF) were used. R59022 and R59949, type I DAGK inhibitors, were also used to evaluate other DAGK activities involved. In assays performed with DMSO, PA synthesized with 18:0-20:4 DAG (PA-u) or di-16:0 DAG (PA-s) represented about 700 and 200% respectively with respect to endogenous DAG. I+V similarly stimulated PA-u or PA-s synthesis. In the presence of DAGK inhibitors, PA synthesis was unchanged. GTPγS and NaF are potent stimulators of PA-u or PA-s synthesis. However, they failed to stimulate the increased PA-u or PA-s synthesis when they were added after a 5-min exposure to I+V. PIP_2 labeling was increased by GTPγS and NaF when PA-u was increased. However, and although PA-s was increased, PIP_2 labeling was strongly decreased. I+V increased PA formation through predominant DAGKε activity that could be modulated by PIP_2 depletion. A potential role in PIP_2 resynthesis is therefore suggested.

ST-P15.
INTERACTION BETWEEN THE PHEROMONE AND THE OSMOLARITY MAPK PATHWAYS IN SACCHAROMYCES CEREVISIAE
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Yeast has systems to sense and respond to environmental signals, including five phylogenetically conserved mitogen-activated protein kinase (MAPK) pathways. Two of these, the mating pheromone pathway and the HOG pathway (which mediates the response to high osmotic stress), share essential components (e.g., the MAPKKK Ste20 and the MAPKKK Ste11). To study in single living cells how these pathways maintain signal specificity, we made strains that express mCherry and YFP controlled by a pheromone-inducible or a HOG-inducible promoter. Stimulation with pheromone causes a continuous increase in mCherry, and stimulation with NaCl causes a transient 30 min increase in YFP, after which cells adapted. We found that co-stimulation with pheromone and salt causes a 30-minute delay in pheromone response, without altering salt response. However, pretreatment for 90 min. with pheromone, followed by addition of salt results in a sustained salt response for at least 3 hours. This effect is not observed with shorter pretreatments, indicating that long-term changes must occur. Long term exposure to pheromone is known to activate the cell wall integrity MAPK pathway. We hypothesize that this pathway partly counteracts osmolarity response, thereby forcing the continued activity of the Hog pathway. These results highlight the complex interaction of signaling pathways in this "simple" model organism.

ST-P16.
LEU3 IS A KEY FACTOR IN UGA4 REGULATION BY CARBON SOURCE IN SACCHAROMYCES CEREVISIAE
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The expression of *UGA4* gene, that encodes the GABA and ALA permease in yeast, is highly regulated and its promoter contains two activating sequences, UAS_{GATA} and UAS_{GABA}. We have already shown that the quality of the carbon source regulates *UGA4* expression. Different elements present in the regulatory region of *UGA4* are involved in this regulation: the transcription factor Gln3, acting on the UAS_{GATA}, and another factor, yet unknown, acting on the UAS_{GABA}. Sequence analysis revealed a consensus site for the transcription factor Leu3 in the UAS_{GABA}. The aim of this work was to establish the role of Leu3 on *UGA4* regulation by the carbon source. For this purpose, beta-galactosidase activity was measured in wild type and *leu3* mutant strains carrying *UGA4* regulatory region or mutated versions of this sequence fused to *lacZ* gene grown in the presence of glucose or acetate. Both wild type cells transformed with a UAS_{GABA} mutated promoter and cells lacking Leu3 transformed with the whole promoter showed high *UGA4* expression levels in both carbon sources being higher in acetate than in glucose. These results indicate that Leu3 is the unknown factor acting on the UAS_{GABA} element and that it has a negative effect on *UGA4* transcription. We conclude that the main mechanism involved in the regulation of *UGA4* by carbon source is mediated by Leu3 through the UAS_{GABA}.

ST-P17.
MOLECULAR MECHANISMS OF UGA4 REGULATION BY EXTERNAL AMINO ACIDS IN SACCHAROMYCES CEREVISIAE
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The yeast *UGA4* gene encodes the ALA and GABA permease. The expression of this gene is highly regulated and its promoter contains two activating sequences, UAS_{GATA} and UAS_{GABA}. Recently we described that *UGA4* expression is regulated by the availability of extracellular amino acids through the amino acid sensor complex SPS. Stp1 and Stp2 transcription factors are required for amino acid-induced expression of other SPS regulated genes. The aim of this work was to study the molecular mechanisms of *UGA4* regulation in response to leucine, an SPS activator. For this, beta-galactosidase activity was measured in strains carrying *UGA4* regulatory region or mutated versions of this sequence fused to *lacZ* gene in the presence or absence of leucine. Results showed that Stp1 and the UAS_{GABA} element are involved in *UGA4* response to amino acids and that this regulation is mediated by Leu3. Here we report for the first time that *UGA4* transcription is regulated by Leu3. This transcription factor, firstly described as a key regulator in branched chain amino acid biosynthesis, is acting as a repressor of *UGA4* expression. Altogether our results show that the signal triggered by external amino acids, such as leucine, depends on SPS components at the plasma membrane, Stp1 and Leu3 as the transcription factors acting downstream SPS and UAS_{GABA} as the DNA target sequence.

ST-P18.
ACTIVATION OF PKA BY DIFFERENT SUBSTRATES FROM SACCHAROMYCES CEREVISIAE
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The effectiveness of protein phosphorylation by kinases is believed to depend on the primary structure of the protein around the phosphorylation site. Protein kinase A is the kinase cAMP dependent and it is widely accepted that the cyclic nucleotide activates the kinase. However recent experiments suggest that the substrate plays an important role in the activation of the holenzyme. Among all the yeast ORF which have a consensus RRXS sequence for PKA phosphorylation, we chose 10 and probed their phosphorylation *in vitro* by yeast PKA. Only three of them were effectively phosphorylated: Pyk1, Pyk2 and Nth1. Synthetic peptides including the consensus phosphorylation sequences from these proteins were phosphorylated *in vitro*. Although all of them present the canonical RRXS, only three of them were substrates. Small differences in peptide sequences resulted in important Km and Vmax differences. The substrate role in the activation of the holoenzyme was investigated. Holoenzyme PKA was purified and its activation in presence of different cAMP amounts and different peptides was assayed. The cAMP A0.5 were different for each substrate, and different with the substrate concentration indicating that the substrate primary sequence plays an important role in the activation mechanism. The activation of PKA was also different when the activation assays were made with Pyk1 whole protein.

ST-P19.
PDK1 HOMOLOGUE DELETION IMPAIRS THE REGULATION OF PKA IN SACCHAROMYCES CEREVISIAE
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In mammalian cells, the PDK1 protein kinase controls numerous processes through substrate phosphorylation on a Thr in a conserved motif. The yeast S.cerevisiae contains three PDK1 homologues: Pkh1, Pkh2 and Pkh3. Yeast cells lacking both Pkh1 and Pkh2 are nonviable and a temperature-sensitive mutant, pkh1D398Gpkh2 delta showed defects in actin organization, endocytosis and trehalose levels. Whereas in mammalian and yeast cells Pdk1/Pkh substrates have been identified, the phosphorylation of the conserved Thr in the catalytic loop of PKA still remains enigmatic, particularly the role of this phosphorylation on in vivo regulation of PKA activity. S.cerevisiae PKA encodes three catalytic subunits: Tpk1, Tpk2 and Tpk3. Here we show that Pkh1 is capable of phosphorylating Tpk1 *in vivo* increasing the interaction with the regulatory subunit Bcy1. Tpk1 and an inactive version tpk1dead were phosphorylated by Pkh1 and Pkh2. Using pkhD398Gpkh2 delta mutant we could study the effect of inactivation of Pkh activity over several phenotypes controlled by Tpk1, namely, a mislocalization of Tpk1-GFP under fermentative growth, a reduction in glucose induced decrease of trehalose and glycogen levels, and an increase on the PKA dependent phosphorylation state of Msn2 transcription factor. These results point to the Pkh kinase as upstream regulator of the cAMP dependent response of PKA in S. Cerevisiae.

ST-P20.
REGULATORY SUBUNIT ANCHORING PROTEINS OF PKA FROM SACCHAROMYCES CEREVISIAE
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The cAMP-dependent protein kinase (PKA) is activated by cellular events that induce the cAMP synthesis and is involved in many specific signalling transduction pathways. The specificity of PKA is determined not only by the molecular recognition of a specific peptide sequence surrounding the phosphorylated residue of a substrate; the substrate recruitment also plays a critical role in determining substrate preference. PKA localization in subcellular compartments by anchoring proteins (AKAPs) contributes to this specificity. The amino terminus of regulatory subunits (BCY1) contains a dimerization/docking (D/D) domain that mediates the dimerization between two R subunits and the binding to AKAPs. Our aim is to identify and characterize yeast AKAPs to study PKA localization and to evaluate their participation in PKA activation. In silico analysis of BCY1 amino terminus predicts a classical D/D domain for this protein. We have isolated and identified putative anchoring proteins through MALDI-TOF-TOF using three strategies: Purification of BCY-TAP and analysis of associated proteins; purification of TPK1-TAP and dissociation of the bound complex containing BCY1 and associated proteins with cAMP and NaCl and further identification of proteins in the flow-through; purification of overexpressed BCY through cAMP agarose and analysis of bound proteins.

ST-P21.
COMPARISON OF EXPRESSION, LOCALIZATION AND KINETICS OF PKA CATALYTIC SUBUNITS IN S.CEREVISIAE
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Our aim is to study the complexity of the activation mechanism of PKA in S.cerevisiae focusing on the contribution of specificity of the three different isoforms (Tpk1, Tpk2 and Tpk3) of catalytic subunit in this process. Our data suggests a specificity of signaling through the three PKA catalytic subunits in yeast. We found differences in the subcellular localization of each Tpk isoform in response to carbon sources and phase of growth. In exponential phase on glucose both Bcy1 (regulatory subunit of PKA) and Tpk2 displayed a nuclear-cytoplasmic localization whereas Tpk1 and Tpk3 showed a pattern with a higher proportion of cells with exclusive cytoplasmic localization. In exponential phase on glycerol and stationary phase of growth, the PKA subunits showed a cytoplasmatic localization, except Tpk3 that with a cytoplasmic punctuate pattern on stationary phase. Analysis of expression levels followed the order: Bcy1>Tpk1>Tpk2>Tpk3 during the exponential phase on glucose, whereas Tpk3=Tpk2 on glycerol and Tpk1> Bcy1 on stationary phase. The kinetic properties of purified Tpk1 and Tpk2 showed differences in the specificity constant toward synthetic peptide substrates, suggesting that Tpk2 has a higher catalytic efficiency.
We propose that the combination of different properties of each Tpk isoform could operate as a control mechanism for the diversification of the PKA response.

ST-P22.
DELETION OF REGULATORY SUBUNIT OF PROTEIN KINASE A IN MUCOR CIRCINELLOIDES ALTERS MORPHOLOGY
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In M. circinelloides pkaR and pkaC genes, encode the regulatory and catalytic subunits of the PKA. Expression analysis during the dimorphic shift suggested a role for pkaR in the control of morphology and branching. To determine the pkaR gene functions we have constructed a mutant of M. circinelloides that lacks the pkaR, and analyzed its growth and development. Null mutant was generated by gene replacement. The knock-out vector contained the pyrG gene selective marker, flanked by sequences of the corresponding pkaR gene. Restriction fragments from the plasmid containing the pyrG gene and sequences of the corresponding pkaR gene to allow homologous recombination were used to transform protoplasts from the MU402 strain, which is wild-type for PKA but auxotrophic for uracil and leucine. The disruption of pkaR gene was confirmed by PCR, Southern, Northern and Western blot analysis. PKA and cAMP binding activities were measured in wt and mutant strains. Binding activity is reduced but not abolished and PKA activity is lower and cAMP independent in null mutant. The growth and germination rates of the mutant are reduced. The switch from isodiametric growth to tip growth in null mutant is delayed. The germ tubes are wider than those of the control. The results indicate that more than one gene codes for cAMP binding activity and that PKA is involved in Mucor morphology.

ST-P23.
***YARROWIA LIPOLYTICA* PKA ACTIVITY VARIES DURING GROWTH AND WITH DIFFERENT CARBON SOURCES**
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It is known that cAMP/PKA signal transduction pathway regulates physiological processes in fungi including growth, metabolism, stress resistance and cell differentiation. In this study, we analyzed the role of this pathway on growth of *Yarrowia lipolytica*, a dimorphic yeast of biotechnological and basic interest. We measured PKA specific activity (PKAsa) during growth in different carbon sources. It was found that in dextrose, the activity was low at the exponential phase reaching maximal expression when cells entered the stationary state. At this stage the specific activity was around 2.13 pmol μg-1 min-1 and was strongly dependent on cAMP (ratio with/without cAMP=15). We also assessed the relationship between PKAsa and growth in different carbon sources. We found that with glycerol PKAsa and the activity profile were similar to the values in dextrose medium, while with hexadecane PKAsa was maximal at the exponential stage, strongly decreasing when cells entered the stationary phase. Finally, we compared the PKAsa of cells growing under normal conditions with that of cells deprived of nitrogen (citric acid production medium). We found that under this stressing condition the PKAsa was two-fold higher compared with that of rich medium. Our results suggest that the cAMP/PKA signaling pathway is involved in the metabolism and stress response in this non conventional yeast.

ST-P24.

PKA CATALYTIC SUBUNITS ARE OF GREAT IMPORTANCE FOR *CANDIDA ALBICANS* GLYCOGEN STORAGE

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In *C. albicans*, regulation of MAPK and the cAMP/PKA transduction pathways is essential for growth, mycelial development, and glycogen storage. It is well known that *S. cerevisiae* cells with low PKA levels accumulate carbohydrates and become more resistant to heat and oxidative stress; on the opposite, cells with elevated kinase activity are unable to stock up reserves. We have addressed the participation of PKA subunits in glycogen metabolism in a set of *C. albicans* mutant strains deleted in PKA subunits genes. Previous results showed that strains devoid of one or both *TPK1* alleles were defective in glycogen accumulation and mutants that lack the Tpk2p isoform, which represents 90% of total phosphotrasnferase activity, accumulated at least twice the amount than mutants harboring this isoform. We here report that triple mutant *tpk2 TPK1/tpk1 BCY1/bcy1*, which is unable to store glycogen, displayed the highest kinase activity at logarithmic phase, being the activity dramatically diminished at stationary phase. Our experiments suggest that glycogen absence in this strain is due in part to the unusual higher catalytic activity due to over-expression of the unique *TPK1* isoform. We also studied the expression of different putative ORFs involved in glycogen metabolism. In a wild-type strain we found that these genes are differentially expressed during vegetative growth.

ST-P25.

IDENTIFICATION OF PROTEIN COMPLEXES ASSOCIATED TO p8, A PROTEIN RELATED TO TUMOR PROGRESSION

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p8 is an 8 kDa protein involved in tumor progression. In different mammalian cell lines p8 migrates between nucleus and cytoplasm, via a functional NLS, depending on cell culture density, ATP availability, cell cycle arrest and deacetylase inhibition. It is remarkable that being small enough to diffuse between nucleus and cytoplasm passively, p8 should possess an NLS and a controlled localization. These results suggest that p8 is forming part of multiprotein complexes and that it could even be the mediator of the translocation of these complexes. The aim of this study is to identify the members of these complexes. A HEK 293 cell line, that stably expresses p8 fused to N-terminal histidine-FLAG tags, was generated. Tandem affinity purification, using anti-flag antibodies and metal affinity chromatography (Nickel and Cobalt) was performed. The purified complexes were proteolitically digested and analyzed by tandem mass spectrometry to identify p8 associated proteins and to detect any possible post translational modifications on p8. This approach has allowed us to identify a small group of putative partners of p8, the most interesting one being Ying Yang 1, a ubiquitous transcription factor which has no NLS but presents the same sub-cellular localization pattern as p8. Further studies are in progress in order to find p8's PTMs and to verify the specificity of the identified partners.

ST-P26.

RSUME, A NEW RWD DOMAIN-CONTAINING PROTEIN, ENHANCES PROTEIN SUMOYLATION

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Sumoylation, a post-translational modification similar to ubiquitination, regulates cellular processes including regulation of transcription, protein trafficking and degradation, and chromatin structure. In this work, we studied the function of a new RWD domain-containing protein, RSUME (for RWD-containing Sumoylation Enhancer), a gene cloned in our laboratory. We demostrate that RSUME enhances overall and substrate specific sumoylation by enhancing Ubc9 - SUMO E2 conjugase- function, as observed in cultured cell lines by Western blot analysis. RSUME co-localizes with Ubc9 in the nucleus when analyzed by fluorescent confocal microscopy and interacts with a recombinant GST-Ubc9 fusion protein in pull-down experiments. RSUME enhances the Ubc9-SUMO non covalent interaction and enhances the interaction between Ubc9 and the E1 activating enzyme, as observed by pull-down experiments with GST-Ubc9 and GST-E1 proteins. RSUME enhances Ubc9-SUMO thioester formation and in a two step experimental setting with recombinant IκBα protein as reporter substrate, we observed that in addition to the main action on the thioester-Ubc9 bond formation, RSUME increases the transference of SUMO to a specific substrate. Together, these results indicate an instrumental role of RSUME regulating the SUMO machinery.

PL-P01.

SECONDARY STRUCTURE CHANGES OF 2-Cys PEROXIREDOXIN ARE ASSOCIATED TO THE FORMATION OF AGGREGATES

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2-Cys peroxiredoxins (2-Cys Prx) constitute a subfamily of ubiquitous peroxidases. The composition of the milieu influences the assembly of 2-Cys Prx dimers into decamers, and the latter into high molecular mass oligomers that form large filamentous oligomers in mammalian cells. Although a key to understanding the biological significance of aggregation is the characterization of the underlying mechanism, factors that drive 2-Cys Prx out of the solution are barely known. We found that the rapeseed counterpart aggregates at pH 6.0 but returns to solution either by the reversal of pH to 8 or at pHs beyond 5.5. Circular dichroism spectroscopy reveals that the aggregation was accompanied by changes in the proportion of secondary structures; β-enriched structures form at pH 6.0 while the contribution of α-helical structures increases when the pH is above 6.5 or falls below 5.5. In line with these transitions, the dynamic light scattering analysis of 2-Cys Prx uncovers that small sized oligomers build up high molecular mass species at pH 6. On the other hand, transmission electron microscopy reveals that the whole architecture is represented by globular structures of variable diameter extending for several nanometers in length. Taking together these data indicate that the formation of β-sheet structures may be essential for the reversible formation of aggregates.

PL-P02.
PURIFICATION AND PHYSICOCHEMICAL CHARACTERIZATION OF A NOVEL TRYPSIN INHIBITOR FROM *Pinus ponderosa*

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Plants synthesize a variety of molecules including proteinase inhibitors, which play an important role as defense proteins against plant predators. To isolate a trypsin inhibitor from *Pinus ponderosa* (PPTI), seeds were ground and extracted with 150 mM NaCl, 5 mM $CaCl_2$. The extract was submitted to affinity chromatography on a trypsin-agarose column and a purified fraction of PPTI was obtained. SDS-PAGE under non reducing conditions showed two bands of 12,000 and 14,000 Da while in reducing conditions only one band of around 6,000 Da was observed. Only one peak was obtained by HPLC on a C_{18} column and on a Superdex S75 column, confirming the purity of the protein so that the two bands are considered as isoforms. Further separation of the reduced and modified chains by HPLC revealed two fractions with a mass of 6,800 and 4,600 Da by mass spectrometry analysis, indicating that PPTI is an heterodimer. Two amino-terminal sequences were determined by Edman degradation: SCDPQRLSACRDYLQR and GREEEE showing high homology with a 2 S albumin-like protein from *Picea glauca* and *Pinus strobus*. PPTI was able to inhibit trypsin and quimotrypsin. Furthermore, viability of human leukemia Jurkat cells was evaluated in the presence of increasing concentrations of PPTI. Results demonstrated that this protein (640 μg/ml) has a cytotoxic effect on Jurkat cells.

PL-P03.
FUNCTIONAL ANALYSIS OF THE TOMATO GDH PROMOTER

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In ripening tomato fruits a strong induction of NAD(H)-dependent glutamate dehydrogenase (GDH) paralleled the increase of glutamate concentration. The role of GDH during this process is still a matter of discussion. GDH catalyses a reversible reaction for the reductive amination of α-ketoglutarate to glutamate. Previously in our lab a full-length gene encoding the β-subunit of GDH and its corresponding promoter were isolated. In *silico* analysis of 3.7-kb *gdh* putative promoter revealed two classes of motif, one associated with physiological processes like ripening, nitrogen and carbon metabolisms, and another class associated with stress processes like drought and pathogen infection. We cloned several promoter length fragments fusion to the β-*glucuronidase* (GUS) reporter gene in order to test the *in vivo* response of this promoter. Fragments of 200, 500 y 900 bp of *gdh* promoter were introduced in the pBI101 plasmid. Arabidopsis plants were transformed by floral dip with these clones and the transgenic lines were subjected to several treatments including ABA, SA, ethephon, carbon and nitrogen sources. We found that the *gdh* promoter clones reacted differently to the assayed treatments, corroborating some of the predictive cis-acting elements. This analysis revealed that physiological conditions and stress factors are involved in the regulation of gdh expression.

PL-P04.
ON THE SHELF TOMATO RIPENING DIMINISHED GLUTAMATE CONTENT OF RED FRUIT

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Free L-Glu is known as an enhancer of the natural flavour of the foods. It is also important in human diet as short chain of folate polyglutamate. Glu levels increased markedly in the pericarp of tomato fruits when the ripening process is carried out *in-planta*, while no changes were observed *ex-planta*. GABA (a non protein amino acid) levels decreased in the ripening transition of fruits kept both *in-* and *ex-planta*. We investigated the activity of GABA metabolism enzymes of tomato fruits under both ripening conditions. The activity of GABA transaminase (GABA-T), which catalyses the interconversion of GABA into Glu, increased only in *in-planta* ripening fruits. Succinic semialdehyde dehydrogenase (SSAdH) which catalyzes the oxidation of SSA (a GABA-T product) showed a decrease during ripening transition both, *in-* and *ex-planta*. Glutamate decarboxylase (GAD), which catalyzes the irreversible decarboxylation of Glu to GABA, also manifested a drop in its activity during *in-planta* ripening transition. The transcript levels of these enzymes were followed by Real Time-PCR showing different changes in mature green to red fruit transition. These results suggest a direct relationship between Glu and GABA levels through GABA-T activity and the importance of *in-planta* ripening in order to have high Glu content in ripe tomato fruits.

PL-P05.
ECTOPIC EXPRESSION OF MITOCHONDRIAL gamma CA2 CAUSES MALE STERILITY BY INDEHISCENT ANTHERS

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AtCA2 is a member of a small gene family of putative Carbonic Anhydrases in Arabidopsis. The proteins are located into mitochondria and bonded to mitochondrial Complex I, forming an extra domain exclusive of green organisms. Ectopic expression of AtCA2 causes male sterility by non anther dehiscence. In these plants respiration levels are comparable to those of rotenone-treated Col-0 plants. Furthermore, Reactive Oxygen Species (ROS) levels are reduced in overexpressing anthers. In normal anther development, secondary thickenings of endothecial cell wall allow anthers to open during the final dehydratation process. Histological analyses revealed that, in 35SCaMV::AtCA2 anthers, an abnormal thickening occurs, consequently, anthers are unable to open and plants are male sterile. A very similar phenotype is found in MYB26 knockout plants suggesting a relation between CAs and MYB factors. Subcellular localization analyses with AtCA2-GFP protein revealed that, besides of mitochondria, AtCA2 is located into nucleus, according to its extended C-terminus with transcription factor features. Moreover, *ca2* null mutant plants show 80% Complex I reduction. Taken together, these results suggest a double function for CA2 and strengthen the idea that this mitochondrial protein is involved in retrograde regulation (mitochondria-nucleus) affecting nuclear-encoded Complex I gene expression.

PL-P06.

EXPRESSION, PURIFICATION AND PRELIMINARY STRUCTURAL STUDIES OF gamma CARBONIC ANHYDRASES FROM PLANTS

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Mitochondrial electron chain Complex I of Arabidopsis includes five structurally related plant-specific subunits representing γ-type carbonic anhydrases termed AtγCA1, AtγCA2, AtγCA3, AtγCAL1 and AtγCAL2. These proteins share relatively high similarity to CAM, the only fully characterized and crystallized γCA from *Methanosarcina thermophila*. Crystallographic studies of CAM have shown a quaternary homotrimeric structure coordinating a Zinc ion in the catalytic site.

In silico modelling analysis shown that AtγCAs possess all important aminoacid needed for catalysis, and the possibility to form homotrimeric and heterotrimeric functional structures. To make an attempt to test the results obtained by informatics analyses, the gene encoding carbonic anhydrase 3 from *Arabidopsis thaliana* was over-expressed in *Escherichia coli*, and the heterologously produced enzyme was purified from the inclusion bodies to apparent homogeneity, refolded, and subjected to cryoelectromicroscopy. Furthermore, a robot screening of 590 distinct conditions of crystallization was performed. This screening allowed us to obtain eight conditions, most of them containing divalent cations, useful for crystallization of AtγCA3.

PL-P07.

CHARACTERIZATION OF ASCLEPAIN cI AND ASCLEPAIN cII BY PMF MALDI - TOF ANALYSIS

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The aim of this work was to obtain the peptide mass fingerprints (PMF) of asclepain cI and asclepain cII, two plant proteases isolated from latex of *Asclepias curassavica*, by MALDI-TOF MS in order to compare them. The enzymes have similar physical and biochemical features. Purified proteases were separated by SDS PAGE. Selected bands were cut, washed and dried under vacuum. The gel fragments were treated with 0.1M NH_4HCO_3 and 10 mM DTT, centrifuged, washed with acetonitrile, and then incubated in darkness in 0.1M NH_4HCO_3 with 50 mM iodoacetamide for 20 min at 25ºC. Digestions were carried out with Trypsin and Lys-C during 12 h at 37ºC. The peptides obtained were recovered by centrifugation, washed with milli Q water and acetonitrile, dried in a SpeedVac, redissolved in 0.1% (v/v) TFA and analyzed by MALDI-TOF MS. PMF analysis showed the enzymes have some equivalent peaks, revealing a high degree of homology among them, which would reflect the presence of conserved domains in these plant peptidases belonging to *Asclepiadaceae* family. By means of MASCOT tool searches were made in order to identify the studied proteins with these tryptic maps. No identification was possible due to the slight information of plant cysteine proteinases consigned in the available databases. Results obtained represent an interesting contribution for PMF databases containing information on plant endopeptidases.

CYTED, ANPCyT and AECI.

PL-P08.

CHARACTERIZATION OF ISOENZYMES BY PMF MALDI - TOF ANALYSIS

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The objective of the present work was to characterize by peptide mass fingerprints (PMF) three cysteine peptidases from latex of Araujia hortorum, araujian hI, hII and hIII, the three enzymes share physical and biochemical characteristics.

Crude extract as well as purified proteases from latex were run by SDS PAGE. Bands corresponding to proteases were cut, washed and dried under vacuum. Gel fragments were treated with 0.1M NH_4HCO_3 and 10 mM DTT, centrifuged, washed with acetonitrile, and left in darkness in 0.1M NH_4HCO_3 with 50 mM iodoacetamide for 20 min at 25ºC. Digestions were carried out with Trypsin and Lys-C during 12 h at 37ºC. The peptides obtained were recovered by centrifugation, washed with milli Q water and acetonitrile, dried in a SpeedVac, redissolved in 0.1% (v/v) TFA and analyzed by MALDI-TOF MS.

Enzymes analyzed by PMF have some equivalent peaks, revealing the presence of conserved domains and a high degree of homology among them. The obtained results suggest that the proteases are isoenzymes. PMF obtained with lys-C does not contribute to differentiate the isoenzymes. PMF obtained with trypsin can be adopted as an excellent tool to differentiate in fast and unequivocal way proteases with very similar physicochemical and functional properties. It is also useful for the detection of cysteine proteases in vegetal extracts.

CYTED, ANPCyT & AECI.

PL-P09.

MOLECULAR CLONING AND EXPRESSION OF AN *Arabisopsis thaliana* PHOSPHOENOLPYRUVATE CARBOXYKINASE

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Phosphoenolpyruvate (PEP) carboxykinase (PEPCK) is an ubiquitous enzyme that catalises the ATP-dependent decarboxylation and phosphorylation of oxaloacetate to yield PEP, ADP and CO_2. Plant PEPCK has been assigned roles in gluconeogenesis and N assimilation. The study of the regulation of this key enzyme has been hampered by the extreme lability of its N-terminus (containing two phosphorylation sites of uncertain function in enzyme regulation) to proteolysis, that renders an active, yet truncated enzyme. With the goal of obtaining an active, intact form of PEPCK, we attempted the cloning and heterologous expression of a cDNA coding for a PEPCK from *A. thaliana.* The source of cDNA was a fragment cloned in the PCMV-Sport6-PEPCK vector (INRA, CNRGV, Toulouse, Francia). This fragment was subcloned in pET-32a(+) with which *E. coli* strains BL21(DE3) and K12 JM109(DE3)pRIL were transformed, with poor expression or expression directed to the insoluble fraction being accomplished, respectively. Finally, expression in *E. coli* K12 JM109(DE3)pRILpKJE8 (using 30 µM IPTG) yielded a soluble form of the enzyme. Purification of the recombinant enzyme was accomplished with low yields, although activity could be measured. Further work aiming to enhance expression yield is on the way, paving the way for further studies on the impact of phosphorylation on enzyme activity and regulation.

PL-P10.
MOLECULAR CLONING, EXPRESSION AND PURIFICATION OF ORANGE FRUIT CYTOSOLIC PYRUVATE KINASE
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Pyruvate kinase (PK) catalyzes a reaction situated at a metabolic branchpoint in plant cytosolic carbon metabolism. In this energy conserving reaction ATP and pyruvate are generated from phosphoenolpyruvate and ADP, thus playing a pivotal role in the energy generation of plant cells. In addition, plant cytosolic PK (PK_c) has been implicated in providing carbon skeletons for N assimilation and secondary metabolism. Pyruvate kinase cDNA was obtained by reverse transcription of mRNA isolated from orange fruit endocarp (*C. sinensis* var. Valencia late) followed by PCR using specific primers. The cDNA fragment was cloned in the expression vector pET-32a(+) as a 6xHis fusion protein. PKc was expressed in *E. coli* JM109(DE3)pRILpKJE8 cells and purified by one step Ni-chelating chromatography. The expressed protein was recognized by anti-His tail and *B. napus* anti-PKc antibodies. The heterologously expressed PK_c, after treatment with enterokinase, migrates as a single band of about 56 kDa as assessed by SDS-PAGE. This work will allow the study at the molecular level of plant PK_c during development and in the mature fruit and perform kinetic and structural studies on the heterologously expressed enzyme.

PL-P11.
PURIFICATION OF A SECRETORY PHOSPHOLIPASE A_2 FROM SOYBEAN *(Glycine max)*
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We previously described the catalytic properties of a PLA_2 from soybean *(Glycine max)* obtained by dye-ligand affinity chromatography, which were similar to those of the secretories PLA_2. The purpose of this study was to optimize the purification of this enzyme. Active fractions from affinity chromatography were concentrated by lyophilization and then loaded onto a DEAE-Cellulose column. The elution was carried out applying a stepwise NaCl gradient from 0.1 to 0.4 M. The enzyme activity eluted with 0.2 M in coincidence with its pI of 5.4 determined by IEF. Two principal bands of 22.4 and 15.1 kDa were detected by SDS-PAGE. The enzyme activity was spectrophotometrically (Δ A340) determined using lecithin liposomes as substrate. A purification factor of 8.9 was calculated in comparison with the enzyme obtained by affinity chromatography. It yielded a Vmax = 5.9 x 10^3U/mg, representing six orders of magnitude of PLA_2 from affinity chromatography, whereas the KM = 1.1 mM was similar to that previously reported. The high catalytic efficiency reached by this purification procedure could be associated with the separation of substances that partially interfere with the interfacial activity of PLA_2. On the other hand, high ionic strength could induce to the pure enzyme to act in a strictly *hopping* mode.

PL-P12.
REVERSIBLE GLYCOSYLATED POLYPEPTIDE IS DIFFERENTIALLY EXPRESSED IN PLANTS
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Reversible glycosylated polypeptides (RGPs) are highly conserved plant specific proteins, essential in plants and they perform self-glycosylation. Its precise function remains unknown. Here, we report features of RGP expression and activity regulation. Using specific probes for two RGP proteins, RGP1 and RGP2 thought to be involved in different metabolic pathways, we demonstrated a differential expression pattern. RGP1 is expressed earlier than RGP2 during plant development. Also, hormonal induction of RGP protein in a sugar mobilization context such as stress and elongation may happen. To establish what factors are involved in the regulation of the RGP activity, complex formation was reported earlier. Now, we show that these complexes are active since are capable of perform self glycosylation. Interestingly, their presence depends on the plant developmental stage, almost in rice. Complex formation modulates the RGP self glycosylation activity. To summarize, all data suggest that RGPs have a critical function in plants and show that are more than reversible glycosylated polypeptides.

PL-P13.
MOLECULAR CHARACTERIZATION OF CHLOROPLASTIC HSP100 CHAPERONES FROM *Arabidopsis thaliana*
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Molecular chaperones of the Hsp100 family have been identified in chloroplasts of *Arabidopsis thaliana*, namely ClpC1, ClpC2 and ClpD. By the use of knockout plants, it has been proposed that these proteins may have multiple roles such as protein folding assistance, protein disaggregation, proteolysis and precursor import into chloroplasts. The study of these activities is still in its infancy due to the fact that, to date, none of the chaperones have been purified. The main goals of this work were to purify them using a bacterial expression system and study their function *in vitro*. The cDNAs were obtained from the RIKEN *A. thaliana* cDNA bank, amplified by PCR and cloned into the vector pET28a. ClpC2 and ClpD were expressed and purified by affinity chromatography. They were recovered as properly folded dimers with expected molecular weight. The proteins have basal Mg^{2+}-dependent ATPase activity. While both proteins have similar Km values, the Vmax value of ClpD was almost two-fold higher than that of ClpC2. The influence of temperature, pH, ionic strength and divalent cations was also assessed. Interestingly, ClpC2 was found to be the most heat stable member of the Clp family with a temperature optimum of 55ºC. By contrast, ClpC1 could not be purified since its expression causes growth retardation and death to the host as judged by growth curves and viable cell counts.

PL-P14.
INTERACTION OF THE TRANSIT PEPTIDE OF A CHLOROPLAST PRECURSOR PROTEIN WITH HSP100 CHAPERONES

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Transit peptides (TP) are N-terminal extensions that route nuclear encoded proteins into plastids. Although Hsp100 chaperones have been implicated in precursor import into plastids, evidences of Hsp100-TP interaction are lacking. We have analyzed the interaction between the TP of pea ferredoxin NADP+ reductase (FNR) and several Hsp100 chaperones from *A thaliana* and *E. coli*. Four plasmids were constructed for the expression of FNR, FNR precursor (preFNR), GFP and an amino terminal TP fusion to GFP (TP-GFP). We also constructed two plasmid for the expression of two Hsp100 chaperones ClpC1 and ClpC2 from *A. thaliana*. In vivo *E. coli* fluorescence plates, protein accumulation in soluble fractions and protein aggregation into inclusion bodies were analyzed using several comparative assays. Ours results show that *E. coli* mutants defective in Hsp100 contained more aggregated TP-GFP and preFNR than the wild type strains. Co-expression of TP-GFP or preFNR and *A. thaliana* chaperones resulted in a reduction of aggregates. Controls expressing GFP and FNR showed no significant differences between wild type and Hsp100-defective *E. coli*. These results indicate that the TP directs the fusion proteins to degradation, in which Hsp100s are involved. We suggest that this effect is produced by an interaction between the FNR TP and Hsp100s from *E. coli* and *A. Thaliana*.

PL-P15.
PHOSPHORYLATION OF NON-PHOSPHORYLATING GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE FROM WHEAT

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Non-phosphorylating $NADP^+$-dependent glyceraldehyde-3-phosphate dehydrogenase (EC: 1.2.1.9; NP-GAPDHase) is modified by phosphorylation in heterotrophic plant cells. Phosphorylation is a prerequisite for interaction with regulatory 14-3-3 proteins, followed by a decrease in activity. We heterologously expressed the wheat NP-GAPDHase in *Escherichia coli*. The recombinant enzyme was purified and utilized to study its phosphorylation by wheat endosperm extracts, under reaction conditions specific for SnRK, GSK-3, and MAPK kinases. NP-GAPDHase was better phosphorylated by a SnRK kinase. The latter was partially purified and characterized. The kinase required Mn^{2+} or Mg^{2+} for activity, with optimal conditions reached at 2.5 mM or 5 mM of the respective essential divalent cation. Ca^{2+} was ineffective. Interestingly, as previously reported for plant SnRK kinase, it was inhibited by glucose-6-P. NP-GAPDHase has two putative phosphorylation sites, Ser404 and Ser447. Studies with single mutants S404A and S447A, showed that Ser404 is critical for phosphorylation of the enzyme. These results are consistent with the Ser404 being located in a protein domain characteristic of a site for phosphorylation by SnRK kinase. Also, it is suggested that NP-GAPDHase could be regulated *in vivo*, together with other enzymes, in agreement with a system for the control of carbon and energy metabolism.

PL-P16.
CHARACTERIZATION OF SORBITOL-6-PHOSPHATE DEHYDROGENASE FROM LEAVES

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Sorbitol is the major photosynthetic end-product in many crop trees, including apple and peach. Despite this relevance, relatively few studies have been performed on the kinetic, regulatory and structural characterization of the enzymes involved in sorbitol synthesis in plants. Recently, we developed the recombinant expression of sorbitol-6P dehydrogenase (S6PDHase, EC 1.1.1.200) from apple and peach leaves. In order to improve the level of expression and purification, we placed a His-tag in the protein N-term instead of the C-term previously utilized. The specific activity of the N-term tagged enzyme is near 50-fold higher compared with the C-term tagged one. This result is in good agreement with a homology model based in the crystal structure of xylose reductase from *Candida tenuis*, a member of the group 2 of the aldo-keto reductase superfamily. According to the model, the C-term appears to be involved in the interaction between both subunits in the dimer. Oxidation assays showed complete inactivation of S6PDHase after incubation with 2 mM diamide. The oxidation is prevented by NADPH, but glucose-6P showed no effect. Results suggest the relevance of cysteine residues in catalysis and the interaction of the enzyme with substrates. Also, they establish models to understand structure-function relationships in a key enzyme for carbon partitioning in plants accumulating sugar-alcohols.

PL-P17.
ANALYSIS OF THE BINDING PROPERTIES OF BLH3-STM HETERODIMERS AND THE INDIVIDUAL PROTEINS WITH DNA

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Plant TALE homeodomain (HD) proteins from the KNOX and BEL families interact through conserved domains present in the N-terminal portion of the respective proteins. In the present study, we have analyzed the interaction of the complex formed by the Arabidopsis proteins STM (KNOX) and BLH3 (BEL) with DNA sequences containing one or two binding sites. EMSA assays indicate that complex formation produces an increase in binding affinity for both types of sequences. However, missing nucleoside experiments suggest that this is not related with the establishment of new detectable contacts. In addition, site-directed mutants in the HD indicated that only one functional homeodomain is required for high affinity DNA binding. In vivo DNA binding assays using the yeast one-hybrid system showed that the complex is able to interact with DNA, but that sequence-specific differences exist in the interaction of the complex and individual proteins with DNA that depend on the protein expression level. Moreover, the BEL proteins ATH1 and BEL1 are able to produce complexes with STM that have similar properties as those observed with BLH3. Taken together, our results reflect the existence of different regulatory modes by KNOX and BEL proteins, either alone or in combination, depending on specific sequences present in their target genes and on the relative concentrations of the two proteins within the cell.

PL-P18.
SEQUENCES INVOLVED IN THE RESPONSE OF THE ARABIDOPSIS COX5B-1 GENE TO METABOLIC AND HORMONAL SIGNALS
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The promoter of the Arabidopsis thaliana nuclear gene COX5b-1 (At3g15640), encoding subunit 5b of mitochondrial cytochrome c oxidase, was analyzed using plants transformed with different promoter fragments fused to a reporter gene. Quantitative measurements in extracts prepared from 21-day-old plants carrying the entire promoter region (2.0 kpb) showed that the inclusion of sucrose, 6-BAP (cytokinin), abscisic acid (ABA), gibberellic acid (GA), the ethylene precursor ACC, phosphate and H_2O_2 in the culture medium induced COX5b-1 promoter-dependent β-glucuronidase expression. The analysis of progressive upstream deletions of β the promoter led us to identify fragments required for these responses: nucleotides -2000 to -600 from the translation start site for GA and phosphate responses, -600/-400 for ethylene response, -400/-350 for cytokinin response and -350/-260 for sucrose and ABA responses. Site-directed mutagenesis showed that over-represented TCAT elements between nucleotides -330 and -290 are required for sucrose response and two elements that resemble the distal portion of the B-box (CCACTTG), present at -281 and -263, are required for ABA-enhanced expression, maybe in combination with a G-box/ABRE (GACACGTG) present at -228. Finally, an ARE-like/CAAT-box (GTGACCAATT) element at -169 is required for H2O2 response.

PL-P19.
SITE II MOTIFS IN ARABIDOPSIS GENES ENCODING COX6 SUBUNITS INFLUENCE RESPONSES TO INDUCING FACTORS
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Cytochrome c oxidase (COX) is composed of 3 subunits encoded in the mitochondrial genome and several subunits encoded in the nuclear genome. In plants, 4 polypeptides encoded in the nuclear genome are associated with the enzymatic core: COX5b, COX5c, COX6a and COX6b. In this work we focus in the analysis of the response of the single COX6a and the three COX6b gene promoters from *Arabidopsis thaliana* towards different factors. We incubated in different conditions transgenic plants that express the gus (β-glucuronidase) gene under the control of COX6a and COX6b promoter sequences and analysed GUS enzymatic activity by fluorometric assays. In this way, we found that the four genes were induced when plants were grown in darkness and in the presence of sucrose. However, each gene showed a different response pattern: COX6a was induced with H_2O_2, salicylic acid, jasmonic acid, ethylene, abscisic acid, phosphate and auxin; COX6b-1 increased gus expression in the presence of gibberellins, auxins and cytokinins while COX6b-2 showed induction with jasmonic acid and COX6b-3 with ultraviolet light and cytokinins. In transgenic plants that contained COX6a and COX6b promoters with mutations in the site II motifs the induction by most factors disappeared, suggesting that these motifs convey the responses of COX6 genes towards several inducing factors.

PL-P20.
DIMERS OR TETRAMERS: OLIGOMERIC STATES OF MAIZE NADP MALIC ENZYME
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NADP dependent Malic Enzyme (NADP-ME) is widely distributed in nature. It has been reported that in the native state the most active forms are usually tetramers. Dimers and monomers can also be found but they are present in lower amounts. Maize NADP-ME protein family has long being studied due to the essential role of one of the isoforms in carbon fixation. Even though the sequences in maize are well conserved, the photosynthetic isoform (ZmME1) is more closely related to a plastidic enzyme associated with housekeeping functions (ZmME2). Both isoforms have been compared with regard to their kinetics and structural features. Interestingly, the most abundant form of ZmME2 is dimeric, as has been shown by studies in recombinant systems. The aim of this work was to study the structural bases related to the oligomeric state of these isoforms. First, the native conformation of ZmME1 and ZmME2 were assayed in plastids and cytosol of transgenic *Arabidopsis thaliana*. Both proteins conserved the same oligomeric states as previously shown for the recombinant proteins, but ZmME2 displayed multiple conformations in the cytosol, most probably due to the association with another protein. Additionally, based on previous reports, we identified a small group of residues that are most probably responsible for the differences in the subunits interactions in ZmME1 and ZmME2.

PL-P21.
EXPRESSION OF miRs AND GENE TARGETS IN CEREAL GRAINS
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Our previous work has shown the expression of several conserved miRs in maize and wheat immature grains among other tissues. The level of some of these miRs changes abruptly during grain development. Thus miR167 expression increased more than 10 times in maize going from 7 to 14 DAP while miR159 and miR396 behaved otherwise dropping 10 and 3 times, respectively. Otherwise, in wheat, we observed a slight increase in miR156 and miR167 both in embryo and endosperm going from 7 to 14 DAP, in contrast to miR159, which showed an increase in embryo but maintained its expression levels in endosperm going from 7 to 14 DAP. We determined by 5´ RACE that the targets of miR167 were, in maize, ARF6 and ARF8 while processing of ARF8 was also observed in wheat, although ARF6 was not assayed. Measuring the level of these targets by Real-Time PCR we found that ARF6 and ARF8 expression decreased in immature maize grains from 7 to 14 DAP while the expression of miR167 increased during the same period of time. This inverse correlation was also observed in wheat embryos and endosperms from 7 to 14 DAP. Recently Yang et al. (NAR (2006) 34:1892-1899) showed that, in rice, miR167 levels respond to auxin concentration, down regulating ARF8. Thus in developing grains of maize and wheat the auxin signal may involve modification of miR167, ARF6 and ARF8 levels.
**They equally contributed to this work.*

PL-P22.
CLONING, EXPRESSION AND PURIFICATION OF A KEY *A. thaliana* DNA MISMATCH REPAIR PROTEIN
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The mismatch repair (MMR) system has been highly conserved during evolution and is critical for maintaining the overall integrity of the genetic material, essentially through its post-replicative correction activity. In eukaryotes mismatch recognition is achieved by two different heterodimers which share one subunit, called MSH2. These complexes, MutSα (MSH2-MSH6) and MutSβ (MSH2-MSH3), are implicated in the first stage of the MMR system and have a special importance in the process. Arabidopsis and other plants present a third heterodimer complex, MutSγ, which is formed by an MSH2 subunit and a unique ortholog called MSH7. In this study, we describe the cloning, expression and purification of MSH2 from *A. thaliana* using *E. coli* as an expression host. The cDNA fragment of MSH2 was cloned into pET32b, an IPTG-based expression system vector. The protein was expressed as a fusion protein of 120 kDa in *E. coli* JM109(DE3)RIL strain. Subsequent purification methods had been applied obtaining pure recombinant MSH2. Afterwards, enterokinase treatment generated an intact protein with only one extra amino acid residue at the N-terminal end of the sequence which was used for the induction and subsequent affinity purification of rabbit antibodies against MSH2. The recombinant protein obtained and the antibodies generated will allow further functional and structural studies on plant MMR.

PL-P23.
ALTERNATIVE SPLICING AND ITS REGULATION IN PLANTS
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Alternative splicing (AS) is a mechanism that leads to more than one mRNA from a single gene, generating protein diversity. Although the fraction of genes with AS is nearly 70% in humans, less than 22% of Arabidopsis genes display AS and little is known about AS regulation in plants. With the aim of understanding the mechanisms that regulate AS in plants, we studied the RUBISCO Activase and the RSp31 SR protein genes, which AS pattern varies dependent on light conditions. Light intensity, but not its quality, seems to be the factor that elicits changes in splicing patterns. This is evidenced by realizing that blue or red light produce similar effects as white light and that different photoreceptor mutant plants behave as the WT in the AS response to white light. Consistently, drugs that inhibit the electronic photosynthetic transport abolish the effects of light on AS. The involvement of a chloroplast signal was explored by using chloroplast signaling mutants. Furthermore, H_2O_2 treatment of seedlings in the dark duplicates the effects of light on AS, which suggests that peroxides (or ROS) might act as signal intermediates. A correlation between the proportions of splicing isoforms and RUBISCO Activase´s gene expression suggests a transcriptional regulation of AS. By using splicing reporter minigenes in BY-2 cells, we are investigating a possible coupling to transcription in plants.

PL-P24.
HETEROLOGOUS EXPRESSION OF *A. thaliana* MutL-alpha USING A DUAL-VECTOR STRATEGY
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The highly conserved post-replicative mismatch repair (MMR) system is crucial for maintaining genomic stability in all organisms. The main proteins involved in this repair pathway have already been identified, among which are the MutL-type proteins, whose role as molecular switches is critical for the repair reaction to take place. For all plants in general, including *Arabidopsis thaliana*, little is known about this process. Our aim is to study the main MutL activity of this organism, MutLα, which consists in a heterodimer of two proteins, PMS1 and MLH1. To the moment, MLH1 and PMS1 DNA sequences have been obtained in our laboratory by reverse transcription followed by PCR via specific primers, using total plant RNA as a template. The cDNA fragments were then cloned into compatible expression vectors and used to transform *Escherichia coli* JM109(DE3)pRIL strains in order to achieve the recombinant expression of the plant protein. Once the desired expression was observed, we proceeded to purify the recombinant heterodimer and asses its functionality. As an initial step in this direction, purified intact PMS1 protein was used to raise polyclonal rabbit antibodies. The tools generated so far should allow the development of a variety of further studies in *Arabidopsis*, consequently shedding some light on how the phenomenon of DNA MMR repair takes place in plants.

PL-P25.
ANALYSIS OF SPROUTING IN POTATO PLANTS
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Growing and freshly harvested potato tubers do not sprout and are dormant, even when environmental conditions are favourable. Sprouting is initiated after a period of dormancy and hormones play an important role in this process: ABA and ethylene are required for the initiation and maintenance of dormancy, cytokinins (CK) are involved in its termination and gibberellins (GAs) are thought to regulate sprout growth after termination of dormancy. Tubers were grown in the dark at 4ºC in MS-agar with: hormones (GAs, ABA, CK), sucrose or a combination of hormones and sucrose. In addition, control and GA-treated tubers were exposed to continuous light at 18ºC. Early sprouting (10 days) was observed in light conditions but it was evident 1 month later in dark-grown tubers: GA-treated ones had numerous sprouts buts CCC, ABA or CK-treated tuber had only incipient sprouts confirming GA as a sprouting-promoting signal. Ca^{2+}-dependent protein kinases (CDPKs) and lipid kinases could be involved in GA signalling. Up-regulation of StCDPK2 expression was observed in GA-treated tubers (light conditions) and in dark-incubated tubers treated with GAs and sucrose. In addition, lipid kinase activity and StCDPK2 expression increased in GA-treated (2-6h) potato plants. To understand how GAs affect sprouting we are making microarrays (TIGR, Potato Functional Genomics Expression profiling Project).

PL-P26.
PHYSIOLOGICAL AND BIOCHEMICAL ANALYSIS OF PEACHES MAINTAINED UNDER CONTROLLED ATMOSPHERE

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The storage of peaches at 0ºC increases the time between the harvest and consumption but can cause the disorder called chilling injury. Different strategies have been applied to prevent it, including genotype selection, harvest time and postharvest handling. In this work, we evaluate the effect on the organoleptic and biochemical properties of peaches "Dixiland" kept in controlled atmosphere (N_2) during 3 days at 20ºC (TN) and stored for another 3 days in regular atmosphere (TN+3). These samples were compared with fruits of the same maturity stage: harvest (C), or kept during 3 or 5 days under normal air (C+3, C+5, respectively). After the treatment, TN samples presented much lower ethylene content than samples (C+3). This was correlated with a maintenance of the firmness and in the malic acid content with respect to the fruits recently harvest (C). It was also found in TN an increase in fermentation as accounted by and increase in the activity of pyruvate decarboxylase and alcohol dehydrogenase, and an increase in the ethanol and acetaldehyde content. A decrease in NAD-malic enzyme, UDP-Glc-pyrophosphorylase and aldolase activities was also measured. In summary, this treatment is beneficial in terms of quality for consumption, keeping almost the same parameters than the fruits recently harvest.

PL-P27.
CLONING AND FUNCTIONAL ANALYSIS OF THE PROXIMAL PROMOTER REGION OF FaEXP2 GENE IN STRAWBERRY FRUIT

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Studies of promoter regions of ripening specific genes are highly relevant due to their biotechnological implications in fruit quality improvement. Knowledge of either promoter strength or the length of its best regulatory regions is therefore essential. Transient expression systems allow a fast analysis especially valuable in fruits like strawberry. Expansins are cell wall proteins that have been related to fruit softening. FaEXP2 is a strawberry expansin gene whose expression is fruit specific and its hormonal regulation has not been determined yet. No promoter region of a strawberry expansin gene had been described until this report. In order to find the possible hormones in control of the expression of this gene and to characterize an expansin promoter, we isolated a 650 bp fragment of the promoter region of FaEXP2 gene. The prediction of cis-acting regulatory element was performed using PLACE and PlantCARE databases. Deletions of the promoter fragment were constructed in pGL3 Basic vector. Fruit discs were transiently transformed by the biolistic method and luciferase activity was detected in protein extracts using a luminometer. Regulatory elements in response to light (G-box; I-box) and hormones (GARE y ABRE) were predicted. By transient transformation we determined that two fragments of 650 pb and 500 pb have the elements required to conduct satisfactory gene expression.

PL-P28.
ENZYMATIC SOURCES REQUIRED FOR NITRIC OXIDE PRODUCTION DURING LATERAL ROOT FORMATION IN ARABIDOPSIS

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Lateral root (LR) formation is a postembryonic developmental process induced by auxin. We have shown that nitric oxide (NO) is required for the auxin-induced LR formation. NO is a signal molecule produced by several enzymatic and non-enzymatic pathways in plants. Among enzymatic pathways, nitrate reductase (NR) and nitric oxide synthase (NOS) activities are the most studied sources of NO production. In this work we evaluate the contribution of NR and NOS during LR development induced by auxin. Lateral root development induced by the application of the auxin NAA could be abolished by the NOS inhibitors L-NAME, L-NNA and L-NMMA. These inhibitors drastically reduced NO production (assayed with the specific NO fluorescent probe DAF-2 DA) induced by auxin in roots. Accordingly, Arabidopsis *noa1* mutant (knock out of a protein associated to a NOS-like activity) failed to respond to NAA treatment. On the other hand, the NR inhibitor sodium tungstate was not able to prevent the NAA-induced LR formation. Moreover *G-4'3* mutant (NR deficient) has a normal response to auxin. Altogether, these results indicate that a NOS-like activity is the major source of NO production during LR development induced by auxin. Studies at the molecular level by analyzing the expression of two transcription factors (NAC1 and NAC2) involved in LR formation are in progress.
Supported by ANPTyC, CONICET and UNMdP.

PL-P29.
***HAHB6*, A SUNFLOWER HD-ZIP ENCODING GENE INVOLVED IN LIGHT SIGNALLING**

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HAHB6 is a member of the subfamily II of HD-Zip transcription factors previously isolated in our laboratory. In addition to the HD and LZ domains, combination unique to plants, it presents the CPSCE motif, involved in redox regulation. Members of this subfamily already characterized are involved in specific developmental processes related to light signalling. An EST containing the complete coding sequence was obtained from the Arizona Genomics Institute and used as probe to isolate the entire gene from a genomic library constructed in BACs. Hahb6 exhibits high homology with other members of sunflower and *Arabidopsis thaliana* HD-Zip II subfamily. A recombinant protein was expressed in bacteria and purified. This protein binds in vitro with high affinity the sequence CAAT(G/C)ATTG probably present in the promoter sequence of its target genes as other HD-Zip II members. Transgenic Arabidopsis plants over-expressing this TF under the control of the 35S CaMV promoter were obtained and their phenotype analyzed in normal growth conditions as well as under different illumination conditions. A differential phenotype was observed under FR enriched light indicating the participation of this TF in the shade avoidance response. Expression studies in sunflower and with transgenic plants bearing *HAHB6* promoter fused to GUS will aid to elucidate the role played by this TF in its genomic environment.

PL-P30.

AtSCO1: A COPPER CHAPERONE ESSENTIAL DURING EMBRYOGENESIS IN *Arabidopsis thaliana*

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The assembly of cytochrome c oxidase (COX) involves a number of auxiliary proteins. Some of these proteins are implicated in metallation of the two copper centers present in COX. Yeast Sco1p is a constituent of the mitochondrial inner membrane involved in the formation of the CuA center and mutant sco1 cells are respiratory-deficient. We have identified Arabidopsis plants with T-DNA insertions in two genes that encode proteins with homology to yeast Sco1p. Homozygote mutant plants with insertions in one of these genes, located in chromosome 4, have no obvious phenotype. In turn, segregation analysis of heterozygote mutant plants in the other gene (named AtSCO1, located in chromosome 3) suggests that its function is required at early stages of plant development. Siliques of these plants contain 25% abnormal seeds with embryos arrested at the torpedo stage. To establish a causal link between genotype and phenotype, we used complementation with the AtSCO1 cDNA. This approach allowed us to obtain plants homozygous for the T-DNA insertion in AtSCO1. Sequences upstream from the translation start site of AtSCO1 were introduced into plants in front of the gus gene. Expression of the reporter gene was localized in meristematic tissues, anthers and embryos at different developmental stages. Our results suggest that AtSCO1 function is essential during plant embryogenesis.

PL-P31.

FUNCTIONAL CHARACTERIZATION OF THE *HAHB10* GENE

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Hahb-10 sunflower gene encodes a transcription factor from the HD-Zip family. Its expression is transcriptionally regulated by etiolation and GA, and its overexpression in *Arabidopsis thaliana* produces a shortening of the life cycle. Here we report the isolation from a sunflower genomic library of a 1270 bp Hahb-10 promoter region. *In silico* analysis of its sequence revealed the presence of cis-elements putatively involved in etiolation and gibberelin signalling among others. Aiming to investigate the functionality of such cis-elements we prepared constructs bearing fractions of this promoter fused to GUS and transformed Arabidopsis plants with them. The expression patterns obtained by histochemistry showed high expression in the reproductive state, essentially in stem and anthers. Moreover, the activity of the promoter was strong in etiolated seedling in accordance with the expression studies done in sunflower. Additionally, plants transformed with the whole gene show a phenotype similar to the one shown by those transformed with the construct 35S:*HAHB10* indicating that this promoter is well recognized in Arabidopsis. We can conclude that HAHB10 participates in light signaling pathways specially in process like etiolation and flowering.

PL-P32.

IN VITRO* MANIFESTATION OF SELF-INCOMPATIBILITY IN *NICOTIANA ALATA

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Self-incompatibility is studied in a natural population of *Nicotiana alata (Solanaceae)*. Pollination assays were used to establish the phenotype of compatibility in 8 individuals, by pollen tube migration through the style and fruit production criteria. All plants were self-incompatible and compatible when they were crossed each other. The compatibility genotype is being analyzed by PCR-generated fragments of *S-RNase* gene, responsible of pollen recognition and rejection. Self-incompatibility was also studied in an *in vitro* system by pollen cultivation with pistil extracts. Pollen growth in compatible extract doubled the one reached in incompatible extract. 61% of pollen cells cultivated in compatible extract showed normal morphology and vesicular movement. In contrast, pollen challenged with incompatible extract produced 70% of dead cells and 23% of pollen tubes with morphological alterations. Endomembrane traffic in cultivated pollen tubes was investigated using a fluorescent marker of endocytic route. Pollen tubes challenged with incompatible extracts showed remarkable alterations in the labeling pattern of the apical zone. The *in vitro* manifestation of self-incompatibility offers a useful system to study the pathway followed by S-RNase when pollen rejection occurs.

PL-P33.

JASMONIC ACID INDUCES DEVELOPMENT AND DEFENSE RESPONSES IN SUNFLOWER SEEDLINGS

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Stress induced morphogenic responses have been recently identified after applying different abiotic stimulus to the roots and specific phytohormones seem to be signaling each particular process. Interestingly, jasmonic acid (JA) is known as a growth inhibitor as well as a defense signal molecule. Evidences presented in model species indicate that JA is involved in both local and systemic defense responses. However, its participation in development-related responses is poorly understood. We have previously described that 80 μM JA induces root growth inhibition of sunflower seedlings. Here we report that JA also inhibits the growth of sunflower leaves and cotyledons after local (cotyledons) or systemic (roots) application. Moreover, in local treatments growth inhibition was dose-dependent. Defense responses triggered by JA were assessed by studying the expression of marker genes selected on the basis of bibliographic references and sunflower EST databases information. RT-PCR experiments indicate that PR5-1, NPR1 and SAD expression in aerial organs was reduced upon local or distal application of JA. On the other hand, LOX and PDF show different responses for local and distal JA treatment.

Together, these results indicate that JA modulates sunflower morphogenic and defense responses both locally and systemically.

PL-P34.
PLD alpha1 AND PLD delta ARE INVOLVED IN NO INDUCED STOMATAL CLOSURE
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Previously we showed, in *Vicia faba* guard cells, that nitric oxide (NO) induces stomatal closure via activation of phospholipase D (PLD) with the consequent production of phosphatidic acid (PA). Since these evidences are biochemical and pharmacological, our current goal is to unravel the signaling players by means of genetics. Multiple PLD isozymes exist, Arabidopsis has 12 with distinguishable biochemical and regulatory properties, and also with little functional redundancy. Two isoforms (PLDα1 and δ) had been related to stomatal closure and drought response upon ABA treatments. Thus, our aim is to determine whether PLDα1 and PLDδ are required for NO induced stomatal closure. As a measure of stomatal closure the relative water content (RWC) and the loss of fresh weight (LFW) were determinate. NO treated wild type plants show an increase in RWC and less LFW than non-treated plants, reflecting the fact that NO induces stomatal closure. The same NO treatment has no effects on RWC and LFW in *pldα1* and δ mutants, which indicates that NO fail to induce stomatal closure in these plants. These results suggest that during stomatal closure NO could be activating PA accumulation via PLDα1 and PLDδ. Futures studies will be done to directly measure stomatal closure and *in vivo* PLD activity upon NO treatments in Arabidopsis.
Supported by ANPCyT, CONICET, Fundación Antorchas TWAS, UNMdP.

PL-P35.
EXTRACELLULAR ATP ACTIVATES PHOSPHOLIPID SIGNALLING IN TOMATO CELLS SUSPENSIONS
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ATP is a vital molecule used by living organisms as a universal source of energy. In animals, extracellular ATP (eATP) is a well-documented regulatory signal involved in numerous physiological processes. eATP release Ca^{2+} from internal stores via the phospholipase C (PLC) derived inositol-3-phosphate (IP_3). In plants, eATP induces increases in citoplasmic Ca^{2+}concentration, however it is not known whether PLC-derived IP_3 is regulating this process. PLC has a role in signalling generating two-second messengers IP_3 and diacylglycerol (DAG). In plants, DAG is converted to phosphatidic acid (PA) via DAG kinase (DGK). Still another source of PA is phospholipase D (PLD). The aim of the present work is study whether eATP induce phospholipid signalling in tomato cell suspensions. Our results show that eATP induces a biphasic PA response at different eATP doses. Time course experiments showed that PA is generated within minutes and the contribution of PLD and/or PLC pathway is being studied. Another second messenger that is induced by eATP in tomato cells is Nitric Oxide (NO). We studied the cross talk between NO and PLC signaling during eATP treatment. Our results resembled the ones already described in animals where eATP induces PLC and then NO productions takes place.Supported by CONICET, ANPCyT and UNMdP.

PL-P36.
REGULATION OF THE AUXIN RECEPTOR, TIR1, BY NITRIC OXIDE
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Many environmental and hormonal stimuli are transmitted by nitric oxide (NO)-regulated signaling cascades. Accumulating evidence indicate that NO interacts with auxin to regulate plant growth and development, particularly in the root system. Despite the exciting recent advances in the understanding of auxin signal transduction, many aspects of auxin signaling remain poorly understood. Previously, we reported that NO regulates the expression of auxin-response reporter genes in Arabidopsis transgenic lines *BA3::GUS* and *DR5::GUS* by modulating the TIR1-Aux/IAA protein interaction. In this work, we showed that NO was also necessary for the auxin-induced degradation of AXR3/IAA17 protein in *HS::AXR3NT-GUS* transgenic line. *In silico* analysis of TIR1 sequence revealed that Cys140 and Cys480 residues contains putative S-nitrosylation motifs. We have generated TIR1 mutant versions in which one of the Cys residues was mutated and its interaction with GST-IAA3 protein was tested. Both mutant proteins were deficient in GST-IAA3 binding. In order to evaluate the functional significance of these results, we have generated *tir1* transgenic plants carrying mutated versions of TIR1. Further investigations are in progress to elucidate the role of S-nitrosylation in TIR1 function.
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PL-P37.
ASR1 PROTEIN LEVELS ASSOCIATED WITH CHANGES IN GROWTH AND DEVELOPMENT OF TOMATO PLANTS
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ASR transcription factor family (abscicic acid -ABA-, stress and ripening) is widely distributed in higher plants such as tomato, potato, grape and others. Isolated evidences suggest these proteins to be involved in a common transduction signaling pathway of carbohydrates and ABA. Understanding the functionality of these proteins will render in their use for metabolic engineer approaches. Transgenic tomato plants silenced and overexpressing *Asr1* were obtained. An exhaustive phenotypic analysis of these plants showed dramatic alterations in their growth and development patterns both during vegetative and reproductive stages. Plant high and dry matter accumulation were significantly reduced in silenced lines. Moreover, flowering and fruit set patterns resulted significantly reduced in these transgenic in comparison with the overexpressing lines and their controls. These changes correlated with reductions in transpiration rate and stomata conductance measurements recorded during early vegetative stages. In addition, silenced lines showed a decrease of total yield and an increase in soluble solid contents of the fruits (SSC). Preliminary analysis of total phenolic contents of these fruits showed no differences between the lines and their corresponding controls. A detailed biochemical analysis of sink and source organs should allow to understand the proposed cross-talk mechanisms.

PL-P38.
EXTRACELLULAR PHOSPHOLIPIDS OF SUNFLOWER SEEDS ARE MODULATED BY ABA AND JASMONIC ACID
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Phospholipids (PLs) are well known intracellular messengers involved in plant development and stress responses. We have recently reported the presence of PLs in extracellular washing fluids (EWF) of sunflowers seeds, demonstrating that phosphatidic acid (PA) and phosphatidylinositol (PI) are the major components of this fraction. This finding has suggested their putative involvement in intercellular signaling events. The aim of this work was to determine if plant hormones involved in seed germination and seedling growth were able to induce changes in the composition of extracellular phospholipids. PLs levels in EWF were determined by electrospray ionization tandem mass spectrometry and lipid overlay assays. Imbibition of sunflower seeds with 100 μM jasmonic acid (JA) induces a decrease in PI content and accumulation of phosphatidylinositol 4-phosphate (PI4P) and specific PA species in EWF. On the other hand, the EWF from seeds treated with to 50 μM abscisic acid (ABA) exhibit an increase in PA and phosphatidylglycerol levels. The existence of signaling PLs such as PA, PI and PI4P as extracellular components of seeds and the modulation of their levels by hormonal treatments suggest their contribution to intercellular communication in planta.

PL-P39.
EFFECT OF CYTOKININS ON THE ULTRASTRUCTURE OF CHLOROPLASTS AND RUBISCO CONTENT IN WHEAT PLANTS
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To advance in the knowledge of the role of cytokinins in N remobilization from wheat leaves we investigated the effects of the supply of the synthetic cytokinin bencylaminopurine (BAP) on the RuBISCO content and ultrastructure of chloroplasts. Young wheat plants were supplied with 20μM BAP and after 48 h of treatment the last fully expanded leaves were sampled. RuBISCO were analyzed by SDS-PAGE and its expression by semi quantitative RT-PCR. Amino acids and sugars were measured in phloem exudates. Leaf material was fixed and ultrathin sections were evaluated using a transmission electron microscope. After 48 h of BAP treatment, an increase of proteins and RuBISCO contents were observed, but not differences in its expression was detected. Amino acids and sugars export were inhibited in BAP supplied plants. The chloroplasts were significantly altered by the addition of BAP. A swollen chloroplast was noticed in the BAP plants with more plastoglobuli and starch content and less granal system than the control ones. These results suggest that cytokinin-mediated signaling is preventing amino acids N and sugars export to the phloem, stimulating N accumulation in the chloroplasts.

PL-P40.
REGULATION OF GLUTAMINE SYNTHETASE ISOFORMS BY NITROGEN AND CYTOKININS
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Glutamine synthetase plays a central role in the assimilation and re-assimilation of ammonia. We studied the regulation of platidic (GS2) and cytosolic (GS1) isoforms in leaves of wheat plants supplies with the synthetic cytokinin 6- benzylaminopurine (BAP). Plants were cultivated for 15 d in controlled conditions with 10 mM N in the nutrient solution before the beginning of 48 h of N deficiency and/or BAP. The expression analysis was carried out by semi-quantitative RT-PCR, proteins were visualised by western blot using a pine antibody anti-recombinant GS1 and the activity was determined by the transferase method after the separation of the isoforms in an anion exchange chromatography (Q-sepharose). When N deficiency was imposed to plants (N-), GS2 mRNA, polipeptide and activity decreased. These effects could be reverted with the addition of 20 μM BAP. However BAP supply to N+ plants did not produce any significant change. However, N starvation and BAP addition both induced an increment in GS1 mRNA, polipeptide and activity, which was higher in BAP plants, but no additive effect between treatments was observed. The data support the concept that GS1 and GS2 play a central but different role in N allocation and highlight the need for further studies of its physiological role in wheat.
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PL-P41.
MOLECULAR CLONING AND CHARACTERIZATION OF StCDPK3
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Ca^{2+} is a crucial signalling molecule in plants involved in the transduction of environmental, hormonal and developmental stimuli. Ca^{2+}-dependent protein kinases (CDPKs) play distinct roles in mediating Ca^{2+} signalling. CDPKs are monomeric proteins, with an N-terminal variable domain, a kinase domain, an autoinhibitory domain and a calmodulin-like domain (CaM-LD). Different CDPK isoforms are expressed during stolon to tuber transition in potato plants: StCDPK3 in early stolons and StCDPK1 in induced stolons. *StCDPK3* was isolated as a partial clone containing the autoinhibitory domain, the CaM-LD and part of the 3´UTR region; it is 98% homologous to *LeCPK1* and *NtCDPK1*, two CDPK from tomato and tobacco. *StCDPK3* was used to probe a genomic potato library prepared in Dr Glenn Bryan´s lab at SCRI. A BAC clone, B15, containing the *StCDPK3* gene, was isolated. The 5´end of the gene was amplified using primers directed against the 5´UTR region of *LeCPK1* and *NtCDPK1*, subcloned into pGEM-T and sequenced. StCDPK3 presents an N-terminal myristoylation and palmitoylation consensus. Expression of StCDPK3 was analysed in different tissues, the complete coding sequence was cloned and the recombinant StCDPK3-6xHis protein will be expressed in bacteria. The structure of the gene is being analysed. We expect to obtain promoter sequences to understand the transcriptional regulation of *StCDPK3*.

PL-P42.
BIOCHEMICAL DEFENSE RESPONSES OF POTATO CULTIVARS TO PHOSPHITE COMPOUNDS
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It has been reported that Phosphite compounds (Phi) can stimulate plant defense responses and that they are active against oomycetes. In the present work different biochemical responses in potato after Phi treatment were analyzed. Calcium and Potassium phosphites (CaPhi and KPhi) were applied to foliage under field conditions in cvs Spunta (SP), Kennebec (KN) and Shepody (SH). Phytopathological responses and activities of pathogenesis related proteins were measured in harvested tubers coming from Phi treated and non treated plants. Disease symptoms against *Phytophthora infestans* were reduced in KN and SP by KPhi and CaPhi. *Fusarium solani* colonization was reduced in SP and KN by CaPhi and KPhi, respectively. Peroxidase activity increased in SH by KPhi treatment, in SP after CaPhi treatment and a slight increase was observed in KN by both Phi. Serin protease inhibitor activity increased mainly in SP by CaPhi and KPhi and a minor response was observed in KN. Activity of glucanases also changed by Phi treatments showing a major increase by KPhi in all cultivars. The behaviour of these activities in infected tubers treated with different Phi is being evaluated. Our results show that the response to Phi treatment depends on both the type of Phi and the cultivar analyzed. This is of interest to further utilizations of Phi in integrated crop management strategies.

PL-P43.
BIOCHEMICAL STUDY OF DISEASE RESISTANCE MOLECULES IN *Solanum tarijense*
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Horizontal resistance is a valuable tool for potato Late Blight control, caused by the oomycete *Phytophthora infestans*. In Argentina several sources of horizontal resistance were identified and the wild species *Solanum tarijense* have shown high levels of it. Many studies have demonstrated that the expression of pathogenesis related proteins (PR) differ with the level of disease resistance in potato cultivars. These results suggest that the constitutive or inducible expression of PR proteins may contribute to the resistance against *P.infestans*. In this study, we measured the enzymatic activity of different PR proteins in the specie *S. tarijense*. Two potato clones with different levels of resistance were used. Both clones were more resistant to Late Blight than Bintje and Pampeana cultivars, used as control. The activity of glucanases, chitinases, peroxidases and poliphenoloxidases were 8, 1.5, 2.5, and 3 times higher respectively, in *S. tarijense* than in Bintje and Pampeana. The results described and others needed in the future related to horizontal resistance to *P.infestans* in *S. tarijense* could lead this species to be introduced in potato breeding programs.
Supported by RIO-SANTANDER and ANPCyT.

PL-P44.
INTERACTION BETWEEN SERIN AND ASPARTIC PROTEASES INVOLVED IN PLANT DEFENSE
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In previous reports it has been showed that FESP, a *Fusarium eumartii* extracellular serin protease, is able to degrade, *in vitro*, a potato aspartic protease, *St*AP1, detected in intercellular washing fluids (IWF) after 1 day of wounding (1dW). Such interaction was analyzed in Pampeana, a cultivar susceptible to *F. eumartii* infection. In order to know the role of this interaction in the potato-*F. eumartii* pathosystem, the accumulation of both proteases was analyzed in Spunta, a moderately resistant cultivar. Results indicated that the pattern of accumulation of FESP and *St*AP1 in Spunta was similar to the one previously obtained in Pampeana, though the timing of expression differed, being *St*AP1 more persistent in Spunta than in Pampeana. On the other hand, in an attempt to determine if this proteolytic interaction affects *St*AP1 antimicrobial activity, *F. eumartii* conidia were incubated in the presence of *St*AP1 or *St*AP1 plus FESP and germination was evaluated. As expected, conidia failed to germinate when incubated with 3.75 μM *St*AP1, although they did in the presence of each single protease. This result show that the proteolytic interaction between *St*AP1 and FESP affects *St*AP1 inhibitory effect on *F. eumartii* conidia, suggesting a possible *in vivo* role, in which the *St*AP1 antimicrobial activity could be regulated by the accumulation of FESP in infected tissue.

PL-P45.
ANTIOXIDATIVE RESPONSE TO SALINITY IN SOYBEAN LEAVES: THE ROLE OF HEME OXYGENASE
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In plant cells, salinity causes the induction of the endogeneous synthesis of reactive oxygen species. Oxidative stress parameters were analysed in soybean leaves subjected to different NaCl concentrations (0, 50 and 200 mM).
Loss of chlorophyll content as well as the enhancement of ion leakage and TBARS formation were only evidenced under 200 mM NaCl treatment. Accumulation of hydrogen peroxide and superoxide anion were tested *in situ* by histochemical methods. While treatment with 100mM NaCl caused an enhancement of catalase activity respect to controls, no difference was observed under 50 mM NaCl, and an inhibition (33%) was detected in 200 mM NaCl treated plants. Superoxide dismutase activity was augmented under 50 mM NaCl treatment, no differences were observed under 100 mM NaCl and 200 mM NaCl caused a diminution in both parameters. RT-PCR analysis revealed that all these activities were positive correlated with gene expressions. Heme oxygenase (HO) is involved in heme degradation and participates in the antioxidant machinery of the cells. Our results indicate that HO activity, protein synthesis and gene expression were significantly increased under 50 and 100 mM NaCl, whereas no differences were observed under 200 mM NaCl. These findings indicate that, under salinity, heme catabolism is up-regulated as a means of defence against oxidative injury.

PL-P46.
ANALYSIS OF A 2-Cys PEROXIREDOXIN B OVEREXPRESSING *Arabidopsis thaliana* LINE
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The 2-Cys peroxiredoxin B (2-Cys Prx B) is a chloroplastic protein that reduces a broad spectrum of peroxides like H_2O_2, lipid hydroperoxide and peroxinitrite. Its peroxidase activity was extensively analyzed *in vitro* in the literature, but studies are lacking about its *in vivo* function. To understand the role of this protein in the antioxidant defence of plants we overexpressed the *2-Cys Prx B* gene (At5g06290) under the control of the cauliflower mosaic virus 35S promoter (CaMV 35S promoter) in *Arabidopsis thaliana* (Ecotype Col-7). We analyzed the constitutive transcript levels of several defence related genes in an overexpressing line (OE-line). Apparently, overexpression of 2-Cys Prx B in *Arabidopsis thaliana* did not significantly affect the mRNA levels of genes of the antioxidant system.
We also studied the participation of 2-Cys Prx B in the tolerance to oxidative stress in *Arabidopsis thaliana* by treatment with methyl viologen (MV). We determined electrolyte leakage, chlorophyll content and levels of antioxidant enzymes. Results indicated higher tolerance of the OE-line to this oxidative challenge. These results suggest that 2-Cys Prx B could be involved not only in keeping redox homeostasis of chloroplasts but also in the antioxidant response of *Arabidopsis thaliana*.

PL-P47.
MODULATION OF HEME OXYGENASE RESPONSE BY NITRIC OXIDE IN ULTRAVIOLET-B IRRADIATED SOYBEAN PLANTS
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We have previously shown that heme oxygenase (HO) is up-regulated by ROS and protects soybean leaves against UV-B radiation. In the present study, we evaluated whether nitric oxide (NO) could enhance this protection mechanism by modulating HO response to UV-B-induced oxidative stress. Soybean (*Glycine max* L.) plants were treated with different sodium nitroprussiate (SNP) concentrations before UV-B irradiation (30 kJ m^{-2}). Pretreatments with 0.8 or 1.2 mM SNP prevented chlorophyll loss and ion leakage in UV-B treated plants. These SNP doses reduced by 80%, both the accumulation of H_2O_2 and the production of $O_2^{\cdot-}$. *HO-1* transcript levels were diminished by 63% after irradiation, whereas SNP pretreatment enhanced *HO-1* gene expression up to 100%, respect to controls. HO activity showed a positive correlation with *HO-1* transcript levels. The presence of cPTIO, a specific NO scavenger, avoided *HO-1* up-regulation. On the other hand, 0.4 or 1.6 mM SNP doses did not fully prevent the effects elicited by UV-B. Treatments with SNP in the absence of UV-B demonstrated that NO itself enhances *HO-1* gene expression. Our data indicate that NO acts as an antioxidant and modulates HO activity. Moreover, *HO-1* overexpression involves not only ROS, but also NO. This mechanism could explain, at least in part, the protective role of NO against UV-B radiation.

PL-P48.
PROTEIN PHOSPHATASES TYPE 2A PARTICIPATE IN STRESS RESPONSES IN *SOLANACEAE*
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Protein phosphorylation/dephosphorylation plays critical roles in stress responses and development in plants, being serine/threonine phosphorylation the predominant postranslational modification. Ser/Thr phosphatases in plants include members of the type 1, 2A and 2C subfamilies. PP2As contain a highly conserved catalytic subunit (c), a structural subunit (a) and a regulatory subunit (b). The aim of this study was to characterize the catalytic subunit of PP2As in potato (*Solanum tuberosum*) and tomato (*Solanum lycopersicum*) plants and its participation in the responses to diverse stress conditions and in tuber development. Sequence homology analysis revealed six isoforms in potato and five in tomato. Protein and RNA expression profiles of PP2Ac isoforms in potato and tomato under different environmental conditions were determined. The stimuli studied were related to tuber development, abiotic stress (cold, salinity and wounding) and biotic stress (fungal elicitors). These analysis together with experiments using the PP1/PP2A inhibitor okadaic acid yielded evidence on the physiological roles of different isofoms of PP2Ac in potato and tomato. The results obtained suggest that certain isoforms of PP2Ac play important roles in the response to cold, salinity, fungal elicitors, and tuber development in *Solaneceae*.

PL-P49.
EXPRESSION OF PEA CYTOSOLIC THIOREDOXINS UNDER OXIDATIVE STRESS INDUCED BY COPPER
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Thioredoxins (Trx) are ubiquitous proteins that participate actively in tolerance to oxidative stress in procaryotes and cellular compartments of eucaryotes. Among the large number of isoforms found in subfamilies of plant tissues, the functions of cytosolic Trx-h isoforms are barely known. Therefore, we deemed it desirable to analyze the contribution of four pea Trx-h isoforms to the tolerance of the oxidative stress. To this end, we selected copper, a micronutrient essential for growth, as the environmental perturbant because high concentrations are extremely toxic due to the capacity to generate reactive oxygen species (ROS). Leaves from plants exposed to an excess of copper were analyzed for (a) the uptake of the cation, (b) the enzyme activities related to the detoxification of ROS and (c) the expression (RT-PCR) of the four pea isoforms of Trx-h. In these studies, we found higher levels of cooper intracellular concentrations, greater activities of antioxidant enzymes and differential expression of the Trx-h isoforms. But more importantly, the treatment with the cation increased transcript levels of Trx-h1 and Trx-h2 while those of Trx-h3 and Trx-h4 did not show significant changes. These results are in line with the view that isoforms of cytosolic Trx-h in pea leaves play specific roles during oxidative stress.

PL-P50.
ENDOGENOUS NO PROTECTS FRATAXIN DEFICIENT ARABIDOPSIS PLANTS FROM OXIDATIVE STRESS

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Frataxin (FH) is a mitochondrial protein highly conserved throughout evolution. The function of FH remains essentially unknown. We studied the *A. thaliana* mutant line *atfh-1*, which is deficient in FH. Previously results indicated that FH is a regulatory component on iron homeostasis in Arabidopsis. atfht-1 plants present a constitutively high iron content likely accumulated in mitochondria, suggesting this mutant has a defect in iron distribution. Probably, as a consequence of iron accumulation, *atfh-1* plants present a higher sensitivity to hydrogen peroxide compared to wt. In addition, frataxin deficiency produces constitutively high NO levels. The aim of this work is to investigate the role (protective/toxic) of NO in atfh-1 plants. We present evidences that the high levels of NO produced in *atfh-1* roots could be abnormally accumulated in mitochondria. Furthermore, the NO acts in a citoprotective way against oxidative stress by induction of mitochondrial-predicted ferritin (FER4) which could contribute to diminish free-Fe levels within the organelle. Preliminary results suggest that endogenous levels of NO in wt roots are necessary for the basal expression levels of FH. We conclude that in *atfh-1* plants the higher levels of NO could be a compensatory response that may attenuate the oxidative damage generated by FH deficiency.

PL-P51.
ANALYSIS OF DEFENSIVE RESPONSES IN POTATO AND TOBACCO PLANTS

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Plants evolved several mechanisms to avoid or resist invasion by a pathogen or insect foraging. This capacity depends on early warning followed by activation of wound/defense-response genes. An increase in cytosolic calcium is a key regulator of many defense pathways triggered and Ca^{2+}-dependent protein kinases (CDPKs) are active players in these processes. In addition, nucleotide diphosphate kinases (NDPKs) expression and tyrosine phosphorylation are enhanced in response to wounding and pathogens. Tobacco and potato plants were exposed to wounding and viral infection with PVX. Viral infections were confirmed with antibodies against viral coat protein. Controls, wounded or infected leaves (local and systemic) were harvested and RNA and proteins were extracted. Semiquantitative RT-PCR assays show that the expression of two potato CDPK isoforms, StCDPK1 and StCDPK2, is upregulated in response to wounding while a similar response was observed with NtCDPK2 in tobacco. Anti-NDPK antibodies against human A and B subunits detected NDPK subunits in control potato soluble extracts; while an anti-phosphotyrosine antibody detected tyrosine phosphorylation in tobacco and potato extracts. The immunologic assays have been optimised and will be used with protein extracts from treated leaves. Cloning of NDPK is currently being performed by RT-PCR using mRNA from potato leaves.

PL-P52.
TRANSCRIPTOME MODULATION OF *Nicotiana benthamiana* BY *Xanthomonas campestris* pv. *campestris* DSF-REGULATION

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Xanthomonas campestris pv. *campestris* (Xcc) is a virulent pathogen that causes disease on cruciferous crops worldwide. The quorum sensing system in *Xcc* is controlled by *rpf* genes, and mediated by DSF (Difussible Signal Molecule) that is synthesized by the RpfF and RpfB proteins. The DSF signal plays a key role in pathogenesis by modulating of wide range of virulence factors (VF) that contributes to virulence and disease symptoms development. Recent studies suggest that glucan and xanthan gum are involved in the suppression of host basal defense. However, little is known about the interplay between the host gene expression changes associated with basal defenses and the virulence activities of the VF during infection. In this study, we used, in collaborations with The Institute for Genomic Research (TIGR), a cDNA array representing 15000 genes of *Solanum tuberosum* to determine the transcriptome associated with basal defense of *Nicothiana benthamiana* to *Xcc rpfF* mutant compared with *Xcc* 8004 (wild type). Our global gene expression analysis identified 200 reproducibly genes regulated in response to DSF-regulated VF. Our results demonstrate that VF module systemic immunity at the transcriptional level, and they provide insight into the coordinated transcriptional regulation of several plant defense genes.

PL-P53.
GENOME WIDE ANALYSIS OF GENE EXPRESSION IN SOLANACEAE BY CHALLENGE WITH A NON VIRULENT MUTANT

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The genus xanthomonas mainly consists of phytopathogenic bacteria, which infect a wide variety of economically important plants. *Xanthomonas campestris* pv *campestris* (Xcc) is the causal agent of black rot of crucifers. We previously described a non virulent mutant of Xcc defective in xanthan synthesis, the major EPS in this bacterium (Yun et al, Plant Phys). The mutant showed an important penality for in planta growh, and elicits a strong defense response in *Nicotiana benthamiana*, compared to the wild type strain. To dilucidate the genetic basis of this difference, we used the microarray approach, comparing the transcriptome of mock inoculated plants against plants inoculated with the wild type strain and the EPS defective mutant.
The analisis of the microarray data revealed an important number of differentially expressed genes between the wild type and the mutant strain, some of wich are transcription factors, PR proteins, cellulose sinthetases, ionic channels and cold induced proteins. This result allows us to speculate about the molecular basis of the xanthan function in the plant-pathogen interaction, and provide more data useful for dissect the complex apparatus of plant defense pathways.

PL-P54.
OVEREXPRESSION OF *WRKY30* TRANSCRIPTION FACTOR CONFERS TOLERANCE TO OXIDATIVE STRESS IN PLANTS
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In plants, oxidative stress is one of the major causes of damage as a result of various environmental stresses. Oxidative stress arises from an imbalance in the generation and removal of reactive oxygen species (ROS). Despite the deleterious effects of ROS, recent studies indicate that ROS act as signalling molecules. *WRKY30* transcription factor, which was early induced under oxidative conditions, was overexpressed in Arabidopsis plants (35S::WRKY30). Seeds from these lines were germinated in MS/2 agar plates containing MV (0.25 and 0.50 μM), NaCl (150 mM) and ABA (0.50 μM) and per cent of seedlings at growth stage 0.7 was scored. Under these conditions, 35S::WRKY30 plants showed to be more tolerant than wild type plants, being able to germinate and develop cotyledons. The antioxidant response of Arabidopsis plants under MV treatment was followed by determining ascorbate peroxidase (APX), glutathione reductase (GR) and catalase (CAT) activities in solution. These activities were markedly increased in 35S::WRKY30 after 6 h MV treatment, while wild type plants were unaffected. Tolerance of transgenic plants could be correlated with an increment in antioxidant enzymes activity. We suggest that WRKY30 could regulate downstream genes involved in the defense against ROS damage during Arabidopsis seed germination.

PL-P55.
OXIDATIVE STRESS AND MORPHOLOGICAL DAMAGE IN *Chlorella kessleri* EXPOSED TO GLYPHOSATE
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The herbicide glyphosate toxicity is relatively low but coadyuvants present in commercial formulations could increase it. Effects of one formulation were studied in the green microalga *Chlorella kessleri* (Chlorophyta), exposed to increasing concentrations of glyphosate (0-70mg/l) and coadyuvant (50%) added to the medium, at 24º ± 1ºC and under continuous light and agitation. After 96 hr, there were evaluated cellular growth by cell counting in Neubauer chamber; CI50 using the software Probit; the malondialdehyde content (MDA, lipid peroxidation parameter) using the technique of TBARS; antioxidant defences, measuring catalase and superoxide dismutase (SOD) activities and reduced glutation (GSH) levels. The cellular morphology was analized by fluorescence microscopy. Glyphosate caused inhibition of cellular growth and the IC50 was 54.37 mg/l. The activity of antioxidant enzymes and the GSH/10^6 cells increase in a doses dependent way. The MDA/10^6 cells content was significant higher from 50 mg/l ($p<0.05$). Morphological studies showed an increase in vacuolization and disorganization of the chloroplast with the increase toxic concentration. The used formulation of glyphosate has nocive effects on this cosmopolitan genus of microalgae, commonly present in aquatic communities and soils, triggering oxidative stress and thus a significant decrease of the growth and morphological damage.

PL-P56.
THE ROLE OF POLYAMINES IN MAIZE LEAF GROWTH UNDER SALINITY
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The reduction of leaf expansion is one of the primary effects of salt stress. In previous works, we showed that apoplastic reactive oxygen species (ROS) are necessary for leaf extension in maize. Decreased ROS concentration in the elongation zone (EZ) contributes to the reduction in maize leaf growth under salinity. Also, the polyamines (PAs) are low Mr organic polycations, involved in plant growth and development. Apoplastic PAs oxidation produce H_2O_2 (ROS). The PAs levels increase in plants under salinity. The purpose of this work was to assess the role of PAs in elongation growth in the expanding zone (EZ) of maize leaf blades, subjected to saline stress. Analysis of PAs in EZ segments (EZS) with highest elongation in control and significant difference with salinized plants did not show changes in total PAs. However, the levels of individual PAs changed, being observed lowest and highest level of putrescine and spermine (spm) respectively in salinized EZS, indicating a displacement of status to superior PAs, like spm. Parallelly, in elongation assays, salinized SEZ tried with BE-4-4-4-4, an inhibitor of polyamine oxidase (PAO), catalytic enzyme of spm, reduced elongation in 25%. These preliminary results could indicate that the H_2O_2 from the spm oxidation in apoplast, contributes to the maize leaf growth under salinity.

PL-P57.
ANTHRAQUINONES ELICITATION AND OXIDATIVE BURST IN *Rubia tinctorum* CELL SUSPENSIONS
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In *Rubia tinctorum* cell suspensions, anthraquinones (AQs) production is induced by elicitors such as chitosan. This defense response also includes generation of reactive oxygen species (ROS) and cell death. It has been recently showed that chitosan induction of anthraquinone synthesis in *R. tinctorum* involves the stimulation of PLC, intracellular Ca^{2+} mobilization and PI3K, which mediates MAPK activation. In the present work we study the relationship between elicitation, oxidative burst and cell death. Elicitation with chitosan (200 mg/l) stimulates AQs synthesis in *R. tinctorum* L. cultures at 24 and 48 hr. An increase in H_2O_2 production at 6 and 24 hr was observed and correlated with higher levels of cell death. Addition of chitosan and diphenylene iodonium (DPI), an inhibitor of H_2O_2 generation, resulted in a decrease in cell death and H_2O_2 production. In both treatments similar levels of AQs accumulation were observed. In cultures treated with chitosan and lanthanum chloride, an intracellular calcium channel blocker, cell death and AQs production decreased showing that both processes require an increase in cytosolic calcium levels. The relationship between elicitation and ROS generation is under study.

PL-P58.
EFFECT OF PHOSPHOROUS DEFICIENCY ON CHLOROPHYLL FLUORESCENCE OF COTYLEDON AND ROOT EXUDATES OF RAPE

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The study of the spectroscopic properties is a non destructive procedure monitoring energy transfer and, consequently, plant health. In particular, Chlorophyll-a fluorescence (Chl-F) emitted by leaves brings information concerning the plants response to external factors. Cotyledons of rape (*Brassica rapa* L.) young plants under phosphorous (P) deficiency showed a Chl-F ratio in the red/far red region (F685/F737) lower than the corresponding value for non-stressed plants. Moreover, minimal differences in F685/F740 were detected in leaves. These results showed that P deficiencies may better be detected by measuring changes in Chl-F emission in cotyledons than in leaves. Stressed cotyledons showed different emission spectra in the blue-green (maxima at 469 and 555 nm) compared to non-stressed cotyledons. The use of the spectroscopic correction, as described by Cordon et al. (Photochem. Photobiol. Sci., 2006, 5, 735-740), allowed us to present here F spectra that are not distorted by differential energy absorption by pigments. On the other hand, we also studied the F in root exudates emitting approximately at 410 nm in both the stressed and non-stressed plants. The intensity of those emission signals are identical in stressed and non-stressed plants.

PL-P59.
A PLANT NATRIURETIC PEPTIDE-LIKE PROTEIN FROM A BACTERIAL PHYTOPATHOGEN INVOLVED IN HOST RESPONSE

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Natriuretic peptides are peptide hormones present in vertebrates involved in the regulation of salt and water homeostasis. In plants, natriuretic peptides (PNP) have been discovered with similar biological activities modulating plant water and solute homeostasis. *Xanthomonas axonopodis* pv. *citri*, the bacterial phytopathogen responsible for citrus canker, has a PNP-like protein (XacPNP) that shares significant sequence similarity and identical domain organization with PNPs. In this study we expressed and purified XacPNP in order to investigate whether it is biologically active and able to modify plant cell homeostasis during the pathogenic process. XacPNP showed a role in citrus leaves stomatal aperture and photosynthetic efficiency. XacPNP expression in citrus leaves during the infection process was observed by RT-PCR. Our results show that XacPNP acts as an active peptide, that it is expressed during the interaction with the host plant and thus has a role in the plant-pathogen interaction. Together, these findings provide strong evidence that XacPNP has a role in plant cell homeostasis suggesting that this probably laterally acquired protein assists the pathogen in the manipulation of plant responses in order to create favourable conditions for its survival on the host.

PL-P60.
SUBCELLULAR LOCALIZATION OF ASR1, A PROTEIN RESPONSIVE TO WATER DEFICIT STRESS IN PLANTS

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Asr1, a gene induced by stress in plants, belongs to a family composed by at least four members. The biochemical function of the encoded protein remains unknown although two alternative and not mutually exclusive possible roles have been postulated: 1) protective against water loss, based on its high degree of hydrophylicity. 2) transcription factor, from evidence on nuclear localization and DNA-binding activity. To test the possible mechanism of ASR1 in protecting cells from water loss, the protein from tomato was expressed in the heterologous expression system *S. cerevisiae* under control of a galactose-inducible promoter. We have earlier demonstrated that ASR1 complements the hyperosmotic sensitivity of mutants deficient in the HOG (High Osmolarity Glycerol) pathway. To validate our experimental design, we analysed by flux cytometry (FACS) the expression of the GFP reporter gene driven by the osmotic stress-responsive Hsp12 promoter when Asr1 was overexpressed. We made sure that the accumulation of Asr1 within the cells did not trigger any stress response.
We showed that Asr1 led to the accumulation of glycerol in HOG-deficient strains. These results suggest that Asr1 acts as a positive transcription factor specific for genes involved in glycerol accumulation. However, our preliminary results using confocal microscopy indicated that Asr1 is mainly cytoplasmatic.

PL-P61.
PI4P ACCUMULATES EXTRACELLULARLY AND TRIGGERS DEFENCE RESPONSES IN TOMATO CELL SUSPENSIONS

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Various phospholipid molecules have recently been implicated in plant defence signalling. Until now, such molecules have been exclusively related to intracellular signalling. Here evidence is provided for a role of phosphatidylinositol 4-phosphate (PI4P) in intercellular signalling. We have analyzed and compared the intracellular and extracellular phospholipid profiles of [^{32}Pi]-prelabeled tomato cell suspensions challenged with the fungal elicitor xylanase. These phospholipid patterns were found to be different from each other, being phosphatidylinositol phosphate (PIP) the most abundant phospholipid in the extracellular medium. There are three natural PIP isoforms occurring: PI3P, PI4P and PI5P. Two experimental approaches (PIP kinase assays and lipid overlay assays) have allowed the identification of extracellular PIP as PI4P. Exogenous application of PI4P to tomato cell suspensions triggered typical defence responses such as the production reactive oxygen species. However other negative phospholipids such as PA, phosphatidylinositol (PI) and phosphatidylinositol biphosphate (PIP2) also induced defence responses in tomato cell suspensions. The biological relevance of these data is discussed.

PL-P62.
HEAT-SHOCK INCREASES THE ACTIVITY OF THE GERMIN-LIKE PROTEASE INHIBITOR OF WHEAT LEAF APOPLAST

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Previously, we found that leaf apoplast serine proteases take part in the wheat defence against the pathogenic fungus *Septoria tritici.* Their action either increases or decreases after inoculation with the fungus to plants of cultivars either resistant or susceptible to septoriosis, respectively. Also, we proved that these behaviours depend on the presence of an inhibitor, which was purified and partially sequenced. Its N-term sequence is homologous with germin-like proteins, being the first Germin Serine Protease Inhibitor (GPI) found. Its C-term was also determined by HPLC-MS/MS. To know more about the control of its expression, the effect of high temperatures was explored. For it, 12d seedlings of the susceptible cv. ProINTA Molinero were put under 40ºC for 1, 2, 3 and 4 hours. After 3 hours, GPI activity increased ~30 %, while proteolytic activity decreased ~40%. These results suggest a link between GPI activation and heat-shock proteins induction (McElwain & Spiker. Plant Physiol 99: 1455, 1992). Also, whether GPI gene transcription changes under heat-shock or other stresses exist is explored. Thus, to earn GPI cDNA, primers based on its N-term and C- term sequences were prepared. The cDNA that encodes for GPI was isolated by RT-PCR and is used in Northern blot essays.
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PL-P63.
INDUCTION OF PHENYLALANINE AMMONIA LYASE IN STRAWBERRY FRUIT BY UV-C TREATMENT

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Phenylalanine ammonia lyase (PAL) is a key enzyme in the shikimic acid pathway, which derives partially in the accumulation of anthocyanin pigments, determining strawberry fruit quality. On the other hand, the mentioned pathway is linked to the accumulation of phytoalexins and the formation of lignin-like polymers, both associated with fruit resistance to pathogenic fungi. It has been demonstrated in several fruits that the treatment with low doses of UV-C light (hormesis), induces the synthesis of a number of anti-fungal compounds in the fruit, like phytoalexins, peroxidase enzymes, PR-proteins (chitinases and β-1,3-glucanases) and PAL. In this work, we have designed specific primers to generate 249 bp strawberry PAL gene fragment by PCR amplification. This gene fragment was used as a probe to analyze PAL expression in control and UV-C irradiated fruit at several times after the treatment. We found an increased PAL expression at 4, 18 and 24 h after the irradiation. We also studied PAL enzyme activity and observed the same pattern than in the gene expression, with an increase in the irradiated fruit even 48 h after the treatment.

PL-P64.
IDENTIFICATION OF DIFFERENTIALLY EXPRESSED TRANSCRIPTS IN PEACHES AFTER HARVEST AND HEAT TREATMENT

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One goal in postharvest fruit technology is to increase the time between harvest and consumption. Fresh peaches are stored at 0ºC to preserve the quality and nutritional properties. Low temperatures reduce enzymatic and microbial activity; diminish the respiratory rate and the loss of water pressure. Nevertheless, under these conditions, some varieties are affected by physiological disorders called chilling injury. The strategies for diminishing this injury include heat treatment, among others, to maintain fruit quality and safety. However, no conclusive research about the transcriptome expressed after this treatment have been performed. In the present work, the "differential display" technique has been applied to identify differentially expressed transcripts in Dixiland peaches during 3 or 7 days after harvest; as well as after 3 days at 39ºC and ulterior ripening for 3 days at room temperature. After the heat treatment, the induction of transcripts related to the synthesis and response to auxin, as well as in the metabolism of reactive oxygen species, mRNA and phytochrome, were identified. On the other hand, after 7 days of harvest a lipocalin transcript is induced, which is repressed after heat treatment. The results obtained in the present work will help in the identification of the molecular factors involved in the protection against chilling injury after heat treatment.

PL-P65.
EFFECTS OF SUGAR PRECURSORS AND PLANT DEFENSE HORMONES ON VITAMIN C SYNTHESIS IN TOMATO HAIRY ROOTS

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Previously, we reported that *in situ* and *de novo* ascorbic acid (AsA) biosynthesis in tomato hairy roots (HR) could occur via uronic acid pathways and that AsA production can be enhanced about 3-fold by overexpression of *GalUR* combined with precursor feeding with D-galacturonic acid. In the present work, we used the same experimental system, consisting of a transgenic UR4 HR clone overexpressing *GalUR* gene which encodes a D-galacturonic acid reductase involved in an alternative pathway of AsA biosynthesis described in fruits, but we have tested other sugar precursors and also plant defense hormones. Precursor treatments consisted in addition of D-glucuronic acid, methyl D-galacturonic acid and myo-inositol at final concentration of 30 mM. Plant hormones assayed were salicylic acid (SA) and jasmonic acid (JA) at final concentration of 1 mM. All treatments were applied on exponential growth phase of HR clones. D-glucuronic and methyl D-galacturonic acid were more effective in increasing AsA content, while myoinositol has a negative effect. Regarding plant hormones treatments, SA caused a decrease in biomass and AsA production, and a change of colour in the medium; while JA induced an increment of 50% in AsA content after 48 h. Taken together, these and previous results indicate that at least two of the AsA biosynthetic pathways described so far in plants occur in the system studied.

PL-P66.
IS ABA PLAYING A ROLE IN ARABIDOPSIS RESPONSES TO IRON DEFICIENCY?
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Iron is an essential mineral nutrient for plant growth and development. When faced with iron deficiency, all plants except the grasses induce a set of responses: (i) ATPase-mediated acidification of the rhizosphere, (ii) enhanced activity and expression of a plasma membrane-bound reductase (FRO2), (iii) increased expression of a Fe^{+2} transporter (IRT1) and (iv) root hair proliferation. Control of genes expression in response to different stresses is often mediated by transduction pathways which involve the plant hormone abscisic acid (ABA). In maize, it has been demonstrated that ABA promotes the induction of ferritin synthesis. Ferritins play a key role because of their ability to sequester several thousand iron atoms in their central cavity. In this work, we treated Arabidopsis plants with ABA to induce the ferritin expression. We hypothesized that this could generate a reduction of available iron and then induce the characteristic responses associated to iron deficiency. Accordingly, we found that *FRO2* and *IRT1* expression, and root hair proliferation were induced by ABA. Previously, we have demonstrated that Nitric Oxide (NO) participates in iron homeostasis in plants. Experiments are in progress to show whether a link between ABA and NO takes place during the induction of plant responses to iron deficiency.
Supported by ANPCyT, CONICET and UNMdP.

PL-P67.
DIFFERENTIAL EXPRESSION OF PI-PLC IN TOMATO CELLS EXPOSED TO SALT STRESS
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Salt stress is known to generate phosphatidic acid via the concerted action of phosphoinositide-specific phospholipase C (PI-PLC) and 1,2-diacylglycerol kinase. Both enzymes are encoded by large gene families, e.g. six PI-PLCs genes have been identified in tomato so far. We are interested in elucidating the gene-specific expression profiles of individual *PI-PLC* during salt stress in tomato (*Solanum lycopersicum*). We studied the expression patterns of *PI-PLC*s by RT-PCR in tomato suspension cells exposed to different doses of NaCl and KCl. The results show a differential expression depending on the dose and the duration of the treatment. Activation of signaling enzymes is often followed by a quick, transient increase in corresponding transcript levels, supposedly in order to replenish enzymes lost by turnover. Thus, the *PLC* genes that are up-regulated might encode for the enzymes that are involved in salt stress signaling. Nitric Oxide (NO) is a second messenger related to abiotic stress responses in plants. Our laboratory showed that treatments with salt both induced NO production and activated PI-PLC signaling in tomato cells. Future analysis will be performed to unravel the role of NO in the activation of PI-PLCs during salt stress and the unequivocal identification of the responsible genes.
Supported by ANPCyT, CONICET and UNMdP.

PL-P68.
MECHANISMS OF NITRIC OXIDE PROTECTION AGAINST THE UV-B INDUCED OXIDATIVE STRESS IN MAIZE SEEDLINGS
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The objective of this work was to characterize the effect of Nitric Oxide (NO) against the oxidative damage produced by UV-B in plants. Maize seedlings were maintained 10 days in normal conditions (H), or were pre-treated with the NO donors, Sodium Nitroprusside (N) or ABA (A), which apparently induce antioxidant enzymes and NO production. Seedlings mantained in normal conditions or pre-treated were irradiated 8 h with 3 W/m^2 of UV-B (named Hi, Ni and Ai, respectively). Twenty four hours after the irradiation, NO concentration was higher in Ni and Ai than in Hi. DAB staining showed a high concentration of ROS in Hi seedlings. Catalase and ascorbate peroxidase activities were slightly increased during the first 4 h of UV-B irradiation, but then decreased to the standard level observed under non-irradiated conditions, suggesting that the antioxidant enzymatic activity is not responsible for the ROS drop in Ni seedlings. Ni seedlings presented an increased concentration of flavonoids (other ROS scavengers). Interestingly, the flavonoids localization is coincident with the NO presence, in the irradiated surface of the Ni leaves. Our results indicate that NO increase does not influence the antioxidant activity, but reduces the oxidative stress, probably raising the concentration of others ROS scavengers like flavonoids.
Supported by CONICET and ANPCyT.

PL-P69.
***HAHB-11*, A SUNFLOWER HD-ZIP TRANSCRIPTION FACTOR INVOLVED IN DROUGHT RESPONSE**
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Homeodomain-leucine zipper proteins constitute a family of transcription factors found only in plants involved in abiotic stress adaptive response. HAHB-11 is a member of the Helianthus annuus subfamily I. An EST containing the complete coding sequence was obtained from the Arizona Genomics Institute. It encodes a 175 amino acids open reading frame. Bioinformatic analysis indicates that it exhibits 51% homology in the HD-Zip domain with HAHB-4, another HD-Zip member previously characterized in our lab. Taking the whole protein this homology descends to 31%, still being the more related known genes in sunflower while higher homology was detected in Arabidopsis members of this family. Transgenic Arabidopsis plants overexpressing HAHB-11 were obtained and their phenotype analyzed, both in control growth conditions or under severe water stress. Transformed plants present shorter stems and compact inflorescences as well a strong tolerance to drought. Although the resemblance between HAHB-4 an HAHB-11 and their corresponding transgenic plants, they were not identical. HAHB11 plants show an improved development and a healthier appearance compared with HAHB4 ones while the tolerance to water deficit was more pronounced. We propose that HAHB-11 participates in the drought adaptive response via a still unknown mechanism. Further studies will carried out in order to elucidate this mechanism.

PL-P70.
ROLE OF CHROMATIN REMODELING PROTEINS IN THE UV-B RESPONSE OF PLANTS
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UV-B radiation affects plant development and physiology. UV-B causes the formation of photoproducts in DNA, which affects the structural and dynamic properties of chromatin. In maize, genes encoding chromatin remodeling proteins are regulated by UV-B, including *chc101*, *nfc102* and *sdg102*. *A. thaliana chc1*, *nfc4* and *sdg26* genes are the homologues to those from maize; we measured mRNA levels after 4 h-UV-B exposure: transcripts are induced for all genes. To investigate the role of each protein in UV-B responses, T-DNA mutants and RNAi plants were analyzed; they showed null or decreased transcript levels compared to WT plants. Flavonoid levels increased in WT plants by UV-B (54%). However, *chc101* deficient plants showed a lower increase (24%); while in *nfc 102* and *sdg10*, flavonoids were unchanged. Thus, these plants have altered regulation of UV sunscreen accumulation. Chlorophyll levels also varied among lines: *chc1* mutants showed a decrease of 55% by UV-B, while WT, *sdg26* and *nfc4* deficient plants show unchanged chl levels by UV-B. However, although preliminary, CO_2 fixation in *sdg26* mutants seems to be more affected by UV-B than in WT plants. Together, Arabidopsis plants with null or decreased levels of transcripts encoding chromatin remodeling factors exhibit increased UV-B sensitivity. DNA damage and UV-B regulation of marker genes in these plants are under study.

PL-P71.
ANALYZING RIBOSOMAL PROTEINS AS PART OF THE POTATO DEFENSOME
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In plants, an essential step in the study of the induced defense response against environmental stresses is to describe the global changes that occur at the transcriptional level. In our laboratory, approximately 460 cDNA clones were obtained from a differential screening of a cDNA library from *F. eumartii*-infected potato tubers. These cDNA potato clones were classified in different functional categories. In order to extend the study of these cDNAs we undertook an *in silico* expression analysis by using microarray data available at TIGR. In particular, 44 cDNAs belonging to different functional categories were analyzed under different experimental conditions, including biotic and abiotic stresses. A total of 28 cDNAs were specifically up-regulated under biotic stress conditions. Among them, 7 potato cDNAs encode for ribosomal proteins. Extraribosomal functions of the ribosomal proteins have been postulated. However, their roles in the plant defense response are still discussed. In conclusion, the *in silico* and experimental expression analysis allowed us to select them as a set of genes with potential interest to further studies on the regulation of plant defense response.
Supported by UNMdP, ANPCyT and CONICET.

PL-P72.
DEVELOPMENT OF STRESS TOLERANCE IN CROP PLANTS BY INTRODUCTION OF A CYANOBACTERIAL FLAVODOXIN
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Plants growing under natural conditions unavoidably face episodic situations of environmental stress in the course of their life times. They have developed numerous strategies to survive in such adverse conditions. Crops, instead, are selected by humans for their high productivity in agriculture, but this is usually not accompanied by increased resistance to hostile environments (diseases, unfavorable climates or inappropriate soils) that are responsible for most agricultural losses. Oxidative stress leads to decreased levels of the electron transport protein ferredoxin (Fd). Under these conditions, cyanobacteria and some algae induce the synthesis of the flavoprotein flavodoxin (Fld), which can provide a functional substitution of Fd in many of its crucial functions in the chloroplasts. Transgenic tobacco plants expressing *Anabaena* Fld in chloroplasts displayed enhanced tolerance to oxidative stress conditions and iron deficiency. Transformation of related *Solanaceae* (tomato, potato) yielded essentially the same tolerant phenotypes when challenged with the redox cycling herbicide paraquat and exposed to drought conditions. Protocols to incorporate Fld into several other crops, including cereals and *Brassicaceae* are currently under way. The Fld gene-based strategy could be a useful biotechnological tool to improve crop yields and to gain at least some wastelands for agriculture.

PL-P73.
NITRIC OXIDE IS NOT RELATED TO CADMIUM-INDUCED OXIDATIVE DAMAGE IN WHEAT LEAVES
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The mechanisms involved in cadmium toxicity have not been completely elucidated. In wheat roots, cadmium induced nitric oxide (NO) formation and this molecule seemed to be related to the oxidative damage caused by the metal. The aim of this work was to evaluate if the same process that occur in roots is operating in leaves. Wheat leaf segments were incubated for 3 or 21 h in a rotatory shaker with either 500 μM Cd_2Cl or 10, 100 or 500 μM SNP, used as NO donor. Electrolyte leakage was increased 105% by Cd^{2+} after 3h or 21h, but NO increased cell membrane damage only when used at 500 μM. However, whereas at 21h Cd^{2+} increased TBARS by 25%, NO 100 or 500 μM reduced damage to lipids by at least 50%. Chlorophyll content was slightly diminished by all treatments at 21h, except by 10 μM NO. Detoxification of H_2O_2 was evaluated by estimating different H_2O_2 decomposing enzymes. Nitric oxide significantly increased ascorbate peroxidase at higher concentrations, while Cd^{2+} reduced its activity by around 20% at 3h and 21h. Catalase activity was also increased by 100 and 500 μM NO after 21h of treatment. These preliminary results suggest that NO is performing a protective role by increasing the activity of H_2O_2 detoxifying enzymes and decreasing TBARS formation compared to non- treated leaf discs, and seemed not to be involved in the oxidative damage produced by cadmium

PL-P74.
NITRIC OXIDE AND SALT STRESS TOLERANCE IN WHEAT-AZOSPIRILLUM ASSOCIATION
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Salinity is one of the main constraints for expansion of cultivated areas and yield increases. There are reports showing higher performance under salt stress for wheat plants inoculated with the plant growth promoting rhizobacterium Azospirillum. Nitric oxide (NO) can be synthetized by Azospirillum and was involved as signaling molecule in plant salt stress adaptation. To detect the biochemical basis underlying this differential response, uninoculated and Azospirillum-inoculated wheat seeds were grown for 8 days in Hoagland nutrient solution alone (control) or containing 200 mM NaCl or 200 mM NaCl+100 μM methylene blue (MB), a NO trapping agent. Plant height, chlorophyll content, fresh and dry weight of shoots and roots, and root morphology were assessed. Whereas chlorophyll contents did no vary, plant height and total plant biomass were significantly reduced upon salt exposure in both uninoculated and inoculated plants. This decrease, however, was less pronounced for inoculated plants. MB addition reverted this protective effect in inoculated plants. After salt treatment, roots were shorter (mainly in control plants), brownish and intensively branched. MB and salt co-treatment resulted in a significantly less brownish. We conclude that NO is probably involved in Azospirillum-mediated alleviation of salt stress. Changes in phenolics/oxidized products should also be considered.

PL-P75.
EFFECT OF POLYAMINES PRE-TREATMENT ON Cd- AND Cu-CHANGES IN SUNFLOWER AND WHEAT MEMBRANE FLUIDITY
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Polyamines (PAs) are organic cations that regulate many physiological events. They bind to membrane phospholipids, nucleid acids and proteins. The objectives of this work were (a) to investigate whether Cd and/or Cu could alter plants membrane fluidity, and (b) whether these alterations could be prevented by PAs. Membrane fluidity was evaluated in leaves and roots of sunflower and wheat, watered for 15 days with 1 mM Cd2Cl or Cu2Cl. While Cd2+ decreased leaves and roots fluidity (evaluated with the fluorescent probe DPH), no significant effects were observed for Cu2+, although a small but significant fluidification was observed in wheat leaves. Next, plants were pre-treated with putrescine (Put), spermidine (Spd) or spermine (Spm) (0.1 mM) for either 5 or 10 days, and then watered with the metals until day 15. A significant decrease in membrane fluidity was observed at days 5 and 10 of PAs pre-treatment, followed by a significant increase in the fluidity at day 15. Put did not prevent Cd-mediated decrease in membrane fluidity, while Spd and Spm partially or totally prevented the effects of this metal. Together, experimental results demonstrate that during plant development (a) Cd have noxious effects on plants membrane rheology that could be partially responsible of its toxicity, and (b) this deleterious effect could be partially or totally prevented by PAs pretreatment.

PL-P76.
CADMIUM AND PARAQUAT TOXICITY IN TRANSGENIC TOBACCO CATALASE DEFICIENT PLANTS
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Cadmium produces oxidative stress in several plant species. The aim of this work was to evaluate Cd^{2+} toxicity in transgenic tobacco catalase-deficient plants, giving a deeper insight in Cd^{2+} toxicity mechanisms by comparing its effect with that of paraquat, a well known oxidative stress inductor. Leaf discs from 40-day old transgenic CAT AS1 and wild type SR1 plants were incubated in a rotatory shaker for 3, 14 or 21 h with 500 μM Cd_2Cl or 100 μM paraquat. Paraquat greatly increased electrolyte leakage from 14 h on, but TBARS only increased at 21h, both in CAT AS1 and SR1 plants. Cadmium slightly increased electrolyte leakage and did not evidence lipid peroxidation neither in CAT AS1 nor in SR1 plants. CAT expression decreased in CAT AS1 plants as expected, but Cd2+ and paraquat increased its expression by 50% at 3 h. Surprisingly, O^{2-} and H_2O_2 were significantly reduced by paraquat in both type of plants whereas Cd^{2+} slightly increased O^{2-} but reduced H_2O_2. However, other peroxidases, like ascorbate and guayacol peroxidases, are also decreased by both stressors and did not seem to be involved in H_2O_2 detoxification to compensate CAT deficiency. The results suggest that CAT deficient plants are damaged by paraquat, but not by Cd^{2+} used in concentrations that are toxic to other species, revealing that different mechanisms are involved in Cd^{2+} and paraquat toxicity in tobacco.

PL-P77.
SEQUENCING OF THE *PSB*, A GENE IN *Lolium multiflorum* AND ATRAZINE TOLERANCE ASSESSMENT
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Some atrazine resistant plant species have agricultural application, which turns them into candidates for different phytoremediation strategies of this herbicide. A sequence change of D1 protein in the Photosystem II Reaction Center is one of the reported mechanisms of resistance to triazines. The substitution of the base A by G in the position 790 of psb A gene, causes the amino acid serine to be replaced by glycine in D1 protein, which is associated with resistance to triazine herbicides. The aim of this work is the selection of atrazine resistant plants with agronomical application and the characterization of the resistance mechanisms for their future use in phytoremediation. Tolerance assays were carried out and, among several candidates, *Lolium multiflorum* exhibited tolerance to atrazine at concentration above of the agronomical application rate. Total DNA was extracted in CTAB medium and purified. The fragment between position 612 and 835 was amplified by PCR, purified and sequenced in an automatic platform. Further, the whole gene was amplified, purified and sequenced. *Lolium m.* showed no mutation at the 790 position, hence, resistance is based on another mechanism, which is been investigated. Primers designed for the fragment can be used for assessment of triazine resistance in multiples vegetal species. Sequence of psb A gene will be registered into international databases.

PL-P78.
DNA DEMETHYLATION IN ARABIDOPSIS PLANTS INFECTED WITH PSEUDOMONAS
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DNA methylation affects the structure and function of genomes generating stable epigenetic marks. Nevertheless, genomes can become demethylated under particular replicative or nonreplicative conditions. We have recently demonstrated that the genome of *Arabidopsis thaliana* plants displays chromatin decondensation and DNA hypomethylation upon the attack of *Pseudomonas syringae* pv. *tomato* (*Pst*). This demethylation response affects repetitive sequences and single copy genes and it occurs in the absence of DNA replication suggesting it is mediated by an active process involving enzymes. We here analyze the effect of *Pst*-induced DNA demethylation on basal transcriptional activities of repetitive sequences and single copy genes, identifying genomic regions increasing transcription under infection. We also evaluate if MBD4, a potential DNA glycosidase, affects the host epigenetic changes described above. We found that chromatin condensation is normal in naïve *mbd4/mbd4* mutant plants and that these plants displayed abnormal chromatin decondensation in response to *Pst*. In addition, we observed that *mbd4/mbd4* plants have reduced vegetative growth and enhanced resistance against *Pst*. These results indicate that *Pst*-induced DNA demethylation affects the transcripcional activity of the host genome and suggest that MBD4 may participate in the phenomena of *Pst*-induced chromatin decondensation.

PL-P79.
UBIQUITIN-PROTEASOME SYSTEM AND CELLULAR CYCLE PROTEINS IN WHEAT UNDER COPPER STRESS
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Ub-proteasome system is responsible for the degradation of proteins in cytoplasm and nucleus, controlling the regulatory short-life proteins that affect processes such as cellular proliferation. The aim of the present work was to evaluate the effect of copper on Ub-proteasome system and on proteins related to cellular cycle. Seeds of *Triticum aestivum* L. were floated for 48 h on distillated water (control) or adding with 1, 10 y 100 µM $CuCl_2$. The highest Cu concentration used inhibited the germination. Growth was evaluated measuring the length of the primary root. While 1 µM Cu induced the growth (15%), 10 µM Cu inhibited it (54%), effect knew as hormesis. Membrane permeability was unchanged with 1 and 10 µM Cu treatments. Total oxidized proteins were increased with treatments, but total soluble and Ub-proteins contents were unaltered respect to control. In Cu treatments, 20S proteasome protein abundance was similar to control. Althought cyclin D protein abundance in treated seedlings was similar to control, with 10 µM Cu decreased Ub-cyclin (30%) and increased oxidized-cyclin level (117%) with respect to control values. CDKD2 protein was unaffected by Cu. In conclusion, Cu did not show effect on the proteasome system but produced oxidation of specific proteins involved in the cellular cycle that could be responsible of growth inhibition observed in the treated seedlings.

PL-P80.
ROLE OF THE L10 RIBOSOMAL PROTEIN IN THE UV-B RESPONSE IN PLANTS
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Plant development and physiology are affected by UV-B radiation. In maize, a number of genes encoding ribosomal proteins are regulated by UV-B, one example is L10. In *A. thaliana*, three genes encode putative L10s (*rpl10 A-C*). We measured mRNA levels by real time RT-PCR: *C* mRNAs are increased, *B* is decreased and *A* is not changed by UV-B. In order to investigate the role of each RPL10 protein in UV-B responses, *A. thaliana* T-DNA mutant plants in the *rpl10* genes were analyzed. In *rpl10C* mutants, *RPL10A* mRNAs are strongly increased in UV-B treated plants. Heterozygote *rpl10A* mutant plants expressing 5-fold lower transcript levels than WT showed a similar pattern of *rpl10B* and *C* expression compared to WT under UV-B, but surprisingly, *rpl10A* expression is induced. We further assayed the expression of marker genes regulated by UV-B (*CHS*, *HY5*, *UGT*): there are no significant differences between *rpl10C* and WT plants, but in *rpl10A* deficient plants, the induction of these genes by UV-B is reduced. These data suggest a possible involvement of RPL10A in regulation of gene expression as it has been described in other organisms, this role is under investigation.

PL-P81.
CADMIUM AFFECTS WHEAT PLANTS GROWTH THROUGH OXIDATIVE DAMAGE OF CELULAR CYCLE PROTEINS
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In this work we analyze if oxidative stress produced by Cd alters the Ub-proteasome system, causing modifications in the cellular cycle, and thus influencing plant growth. *Triticum aestivum* L. seeds were floated for 48 h on distillated water or exposed to 1, 10 and 100 µM Cd or 0.5 and 1 µM paraquat or 100 and 1000 µM H_2O_2, under dark conditions. The highest Cd concentration inhibited seed germination. All treatments caused inhibition of primary root length. Root membrane permeability increased 41, 99 and 66% only with 1, 10 µM Cd and 1000 µM H_2O_2, respectively. Total soluble protein level was unaffected by Cd, but it diminished the Ub-conjugated protein level and increased oxidized proteins. Proteasoma activities varied with the metal addition. Under these treatments, abundance of 20S proteasoma protein remained constant and no protein fragmentation was observed. The abundance of cyclin D protein was unaffected by Cd, but the level of Ub-cyclin D decreased and increased oxidized cyclin level. Analysis CDKD2 showed that this protein abundance, degree of Ub-conjugation and oxidation were not affected significantly with 1 µM Cd, whereas 10 µM Cd increased only protein oxidation. These results suggest that Cd and other prooxidants affects plant growth by oxidation of cell cycle proteins that regulate proliferation and protein recycling.

PL-P82.
PROTEINS SECRETED BY AN AVIRULENT STRAIN OF *Colletotrichum spp.* INDUCE RESISTANCE IN STRAWBERRY

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We have previously reported that the avirulent isolate M23 of *C. fragariae* protects the cv Pájaro of strawberry against anthracnose and that the conidial extracts derived from that strain induced HR, autofluorescence and oxidative burst. The aim of this work is to investigate the proteins secreted by the isolate M23. Proteins were obtained from free-cell liquid cultures that were concentrated by ultrafiltration and then fractioned by acetone precipitation and anionic-exchange (monoQ-FPLC) and hydrophobic-interaction chromatographies (Phenyl Superose-FPLC). The elicitor activity of the fractions was monitored by phytopathological test with the virulent pathogen M11 and ROS production. Proteins were visualized by 2D-PAGE and subjected to MALDI-TOF/TOF MS/MS. Results showed that elicitor activity was found in the flow-trough of the mono Q (pH 7.5), indicating the presence of basic active proteins. When this unbound protein fraction was further applied to the PS matrix, the ROS-inducing activity was distributed in 4 peaks that bound weakly to it. None of the active proteins presented high score in the searches with PMF in databases, only enzymes scavengers of ROS were identified in fractions that lacked elicitor activity. These results indicate that the strain M23 secrets proteins with different functions, some of which are involved in the activation of plant defense mechanisms.

PL-P83.
IDENTIFICATION OF MRCV PATHOGENICITY DETERMINANTS USING A PVX VECTOR SYSTEM

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Mal de Río Cuarto virus (MRCV, Fijivirus, Reoviridae) causes the most important maize disease in Argentina. MRCV genome consists in ten linear segments (S1-S10) of double-stranded (ds)RNA that theoretically code for a total of 13 proteins. We analyzed the influence of MRCV pS3, pS4, pS5.1, pS5.2, pS6, pS7.1, pS7.2, pS8, pS9.1, pS9.2 and pS10 on PVX accumulation and disease symptoms in systemically infected Nicotiana benthamiana plants. The MRCV open reading frames were amplified by RT-PCR from maize infected plants and cloned into a recombinant PVX binary vector. As controls we used a PVX vector carrying the YFP reporter or two well known viral suppressors of gene silencing such as AC2 protein of African cassava mosaic virus and Rice yellow mottle virus P1 protein. Virus accumulation was assessed by indirect DAS-ELISA. Our results showed that recombinant PVX carrying MRCV pS7.1, pS7.2 and pS8 accumulate significantly more virus than PVX carrying YFP or AC2 and P1 proteins. Accordingly, these infected plants showed more severe symptoms. These results suggest that MRCV pS7.1, pS7.2 and pS8 may act as pathogenicity determinants during disease development. We are now testing their activity as suppressors of post-transcriptional gene silencing and obtaining transgenic Arabidopis plants expressing these candidate MRCV proteins to assess their effect on phenotype.

PL-P84.
CARBON METABOLISM AND DIGE PROTEOMIC STUDIES OF "DIXILAND" PEACHES AFTER HEAT TREATMENT

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Fresh peaches can develop chilling injury during storage at low temperatures. In order to prevent this disorder, heat treatments have been applied after harvest of several fruits and vegetables. In the present work, the level of activity and expression of key enzymes involved in carbon metabolism, as well as 2-D Difference Gel Electrophoresis (DIGE) Proteomics analysis, have been performed after heat treatment of "Dixiland" peaches and during ripening at room temperature. Peaches with the same maturity grade were harvest and stored for ripening during 3 and 7 days or treated with 39ºC for 3 days. The analysis of enzyme activities and Western blot of several enzymes involved in carbon metabolism in the different peach samples, indicates that during ripening, enzymes involved in sucrose metabolism and sugar utilization are induced. On the other hand, after heat treatment, up- and down- regulation of enzymes involved in organic acid synthesis and consumption, respectively, has been observed. Fifty-four polypeptide dots that are differentially expressed in the different samples have been identified among the peach samples analyzed. Identification of some of these dots have been performed by MS/MS. The understanding of proteins and metabolic routes involved in protective processes against chilling injury will have a great impact in a way to improve the treatments to avoid it.

PL-P85.
TWO NEW ISOFORMS OF SOYBEAN THIOREDOXIN-H AND THE RESPONSE TO OXIDATIVE STRESS

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Thioredoxins (Trx) are ubiquitous small (ca. 12 KDa) proteins characterized by the conserved motif -WCGPC- that participates actively in thiol-disulfide exchange with other proteins. Among the plethora of plant Trx (20 isoforms in Arabidopsis), cytosol Trx-h (9 isoforms) play an important role in the tolerance to oxidative stress even though the contribution of individual isoforms is barely known. On the basis of known nucleotide sequences of plant orthologues, we designed oligonucleotides that were employed in the amplification by RT-PCR of mRNA prepared from mature soybean leaves. We isolated two open reading frames that were 87% and 98% identical to pea Trx-h1 and Trx-h4, respectively, but only 75% and 71% identical to Arabidopsis Trx-h1 and Trx-h2, respectively. Subsequently, we constructed specific oligonucleotides that matched the internal region of mature Trx-h for using in the RT-PCR analysis of gene differential expression. When different tissues were subjected to oxidants (e.g. H_2O_2, methyl viologen), the expression of Trx-h4 relative to soybean Trx-h1 not only was higher in non-photosynthetic tissues (seeds, roots) but also exhibited a different response. These results are in line with the view that soybean has developed mechanisms to tolerate the oxidative stress wherein isoforms of cytosol Trx-h play specific roles.

PL-P86.
COMMON BEAN WRKY AND BHLH TFS DIFFERENTIALLY REGULATED IN STRAIN-SPECIFIC SYMBIOSIS

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Common beans (*Phaseolus vulgaris*) establish symbiosis with the soil rhizobia resulting in nitrogen fixation within a newly formed organ, the nodule. We have previously shown coevolution of the symbiotic partners resulting in a preference in nodulation by strains of rhizobia from the same geographical region. In order to identify genes of the plant involved in this strain selection, 11502 ESTs were generated from root hairs of Mesoamerican common beans inoculated with *Rhizobium etli*. One hundred and ninety genes for signal perception and transduction, transcriptional activation and hormone responses, out of 2108 independent sequences we examined, were selected and spotted in a macroarray. Membranes were hybridized with cDNA of roots inoculated with different *R. etli* strains to identify genes differentially induced in the more efficient interaction. Two of the differentially identified genes that were confirmed by qRT-PCR are predicted to encode transcription factors belonging to the WRKY and helix-loop-helix family, involved in pathogen perception and cell differentiation respectively. Transcript levels of these genes in response to different strains of rhizobia and regulation by plant hormones were analyzed by qRT-PCR. Understanding competitiveness and strain specificity is of vital importance to develop inoculants that can out-compite the indigenous strains present in the soil.

LI-P01.
SREBP EXPRESSION ON FATTY ACID METABOLISM IN VITAMIN A DEFICIENT LIVER

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We know that vit A deficiency decreases liver Acetyl CoA Carboxylase (ACC-L) activity, which is involved in malonyl-CoA formation. Here we determine the mRNA levels of ACC-L, Fatty Acid Synthetase (FAS), Carnitine Palmitoyltransferase I (CPT-I), a key enzyme in fatty acid beta-oxidación, and SREBP-1c, a key transcription factor that regulates lipid enzyme genes. Also, mitochondrial CPT-I activity was measured. Wistar female 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. Also, a -A group was refed for 15 days with control diet (-A refed). Serum and liver Vit A was measured by HPLC. Liver mRNA levels were determined by RT-PCR, and CPT-I activity, by using [^{14}C]carnitine as substrate. Statistical analysis was performed by ANOVA. In -A rats the mRNA expression of SREBP-1c decreased, compared to control. This was associated to a decrease in ACC and FAS mRNA levels ($p< 0.01$) due to SREBP-1c regulation of gene expressions. Vit A deficiency increased the mRNA levels and activity of CPT-I ($p< 0.001$). Vit A restitution to the diet reversed mRNA levels and enzyme activity. The changes in ACC suggest a low availability of malonyl-CoA, endogenous inhibitor of CPT I, that could produce a stimulation of the fatty acid β-oxidation process. Vit A regulates hepatic fatty acids synthesis and degradation.

LI-P02.
EXPRESSION OF RETINOIC ACID RECEPTORS (RAR AND RXR) AND PPARS IN HEART: EFFECT OF VITAMIN A DEFICIENCY

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We have showed that vit A deficiency increases the activity of Carnitine Palmitoyltransferase I (CPT I), key enzyme in heart fatty acid β-oxidación. Now, the effects of vit A on expressions of genes that are involved in the regulation of β-oxidación 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. Also, a -A group was refed for 15 days on control diet. Serum, liver and heart Vit A levels were measured by HPLC. The mRNA expression of 9-cis-retinoic acid receptors (RXR α and β, and RARα), peroxisome proliferator activated receptor α and β (PPARα and PPARβ), CPT I and Acetyl CoA carboxylase β (ACCβ), and also the ACC activity, were determined. Statistics was analyzed by ANOVA. In -A rats the mRNA expression of RARα, RXRα, and RXRβ decreased ($p< 0.01$), while that of PPARα, PPARβ and CPT I increased ($p<0.05$), compared to control. Vit A restitution to the diet reversed all those mRNA levels. High expression of PPARα and β genes were associated to the increase of CPT I mRNA. The low RXRα mRNA levels were related to the increased ACC mRNA levels. Vit A deficiency induces fatty acid β-oxidation at gene levels. In addition, PPARβ can activate AMPK which inhibits ACC. The low ACC activity observed in -A rats could reduce malonyl-CoA levels, an endogenous inhibitor of CPT I.

LI-P03.
n-3 FATTY ACID SUPPLEMENTATION IN BURN PATIENTS: BIOCHEMICAL AND CLINICAL EFFECTS

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Burn injury induces a systemic response that leads to the development of alterations, including lipid metabolism. Diet is part of therapy and lipids must be included to provide energy and to prevent essential fatty acid (EFA) deficiency. EFA are involved in wound healing and immune function. In the present study we studied the effect of supplementation of the diet of burn patients with n-3 fatty acids on the fatty acid composition of plasma and erythrocyte phospholipids and its correlation with several clinical parameters. Pediatric patients admitted at the Children Hospital of La Plata with burn injury were selected and assigned into two groups. One of them received a dietary supplement of n-3 fatty acids for four weeks. No burn patients were selected as control. Blood samples were collected immediately after burn injury and during recovery. Neutral and polar lipids were separated from erythrocytes and plasma, and phospholipid fatty acid composition was determined. We also analyzed biochemical parameters related to the clinical evolution. Results showed an increase of saturated and monounsaturated fatty acids in plasma phospholipids, and a decrease in polyunsaturated fatty acids in the early postburn profile when compared with control. In n-3 fatty acid supplemented group these changes were further reverted. A favorable response in some biochemical parameters was also shown.

LI-P04.
METABOLIC ALTERATIONS IN CONGENITALLY ATHYMIC MALE MICE: EFFECTS OF NEONATAL THYMULIN GENE THERAPY

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Adult congenitally athymic (nude) mice display endocrine imbalances and a moderate hyperglycemia. Here we determined the impact of congenital athymia on lipid and glucose plasma levels as well as on hepatic lipid composition in male nude mice. We also assessed the ability of neonatal thymulin gene therapy (NTGT) to prevent the metabolic effects of athymia. An adenoviral vector, RAd-metFTS, expressing a synthetic DNA sequence encoding the thymic peptide thymulin was used. On postnatal day 1-2 nu/nu and nu/+ mice were i.m. injected with 108 pfu RAdmetFTS or RAd-βgal (control vector). The animals were processed at 71 days of age. Circulating thymulin, glucose, cholesterol (CHOL) and triacylglycerides (TAG) as well as hepatic TAG, phospholipid (PL) fatty acid composition and free, esterified and microsomal CHOL was determined. The nu/nu controls were moderately hyperglycemic when compared with their nu/+ counterparts (109.4±11.8 vs. 87±5.0; $P<0.05$). NTGT fully prevented hyperglycemia in nu/nu mice. Athymic males showed significantly reduced levels of plasma CHOL and TAG, alterations that were not prevented by NTGT. Hepatic levels of TAG were reduced in control nu/nu but this alteration was prevented by NTGT. We conclude that adult nu/nu mice develop multiple alterations in lipid and glucose metabolism. Thymulin may modulate some of these metabolic variables, notably glucose homeostasis.

LI-P05.
L-FABP STIMULATES ENDONUCLEAR ARACHIDONIC ACID MOBILIZATION

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We have already shown that endonuclear lipid pools have a more active metabolism than that in whole nuclei. Thus endonuclear arachidonic acid (20:4n-6) pools could provide an endogenous source of PPARá ligands and/or be metabolized to prostaglandins or leukotrienes.
Taking into account that the nucleus is considered a control site for 20:4n-6 incorporation and subcellular redistribution; the aim of the present work was to determine if L-FABP is involved in endonuclear 20:4n-6 mobilization and release.
With this purpose, endonuclear 20:4n-6 pools were labeled by in vitro incubation of isolated nuclear matrix (Mx: membrane depleted nuclei) with [1-^{14}C]20:4n-6, ATP and CoA. [^{14}C]Mx was reincubated without 20:4n-6, plus ATP, CoA and delipidized L-FABP.
We observed that increasing concentrations of L-FABP determined a dose-dependent release of 20:4n-6 to the incubation mixture, and an increase of 20:4n-6 in PL and a decrease in FFA from endonuclear lipids.
In conclusion, we showed that L-FABP stimulates endonuclear 20:4n-6 mobilization. L-FABP delivers 20:4n-6 to an acyl-CoA synthetase located in the nuclear matrix, and then to those enzymes (acyl-tranferases) responsible for its esterification in PL. In the same time frame, L-FABP would deliver 20:4n-6 to another fate, by promoting fatty acid release from endonuclear pools to different cellular compartments.

LI-P06.
PHOSPHATIDIC ACID METABOLIZATION IN LIVER NUCLEI

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Evidences not only confirm the presence of lipids in the nucleus as a part of the chromatin structure but also demonstrate the existence of numerous enzymes which modulate changes in their composition. Phosphatidic acid (PA) is metabolized to diacylglycerol (DAG) by PAP1 and LPPs. DAGs are partially metabolized to monoacylglycerols (MAG) by diacylglyceride lipase (DGLs) activities, and MAG are metabolized to water soluble products (WSP) by MAGL activities. LPPs dephosphorylate PA, lysophosphatidic acid (LPA), sphingosine 1-phosphate (S1P) and ceramide 1-phosphate (C1P). The aim of the present work was to evaluate which PAP isoforms are associated to liver nuclei and how PA is metabolized by LPPs in the presence of LPA, S1P and C1P. To this purpose, adult (4 mo) rat liver was homogenized and highly purified nuclei were isolated by sucrose-density ultracentrifugation. Nuclear preparations were checked for purity by electron microscopy. Our results indicate that LPPs are present in nuclei from liver. In addition, it was observed that nuclear DGL and MAGL activities represent 70% and 28% of LPPs activities, respectively. At equimolecular concentrations (100μM) of PA/LPA or PA/S1P, LPPs diminished PA metabolization by 25% and 21%, respectively. PA metabolization in nuclei by LPPs seems to be an important signalling pathway in this fraction.

LI-P07.
SIMVASTATIN AND GERANIOL SHOW A SYNERGISTIC EFFECT ON A549 CELLS

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Simvastatin is a competitive inhibitor of HMG-CoA reductase activity whereas geraniol is a natural monoterpene with multiple effects on mevalonate metabolism. Both of them inhibit growth and proliferation of many cell lines. We have previously reported that a combination of simvastatin and geraniol inhibited the growth rate of an hepatoblastoma cell line. Here we investigated the alterations produced by simvastatin (S) and geraniol (G) on lipid metabolism in a human lung adenocarcinoma cell line (A549). Cells were treated with 20mM S, 100 mM G or 20mM S + 100 mM G (SG) for 24 h. Three hours prior to starvation, ^{14}C-acetate was added. Total lipids were extracted and ^{14}C incorporation in non saponifiable lipids, fatty acids, neutral lipids and phospholipids were determined. The addition of either 20 mM S or 100mM G did not significantly inhibit A549 cell proliferation, while their combination inhibited by 20% the growth rate of this cell line. Simvastatin incremented ^{14}C incorporation in phospholipids specially phosphatidylcholine whereas G and SG groups showed a diminished ^{14}C incorporation in non saponifiable and neutral lipids as well as in phospholipids. These results demostrated that also a non hepatic cell line model shows a synergistic effect of the combination of a statin and a nonnutritive dietary component which would provide a new appoach to chemotheraphy treatment.

LI-P08.
EFFECT OF PESTICIDES ON THE ANTIOXIDANT DEFENSE SYSTEM IN VARIOUS RAT TISSUES
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The effect of intoxication with dimethoate (D), glyphosate (G) or zineb (Z) injected alone or in combination (i.p 1/50 to 1/250 LD50, three times a week for 5 weeks) was studied on the antioxidant defense system of plasma, liver, kidney and brain rat homogenates. In all preparations TBARS and [NOx] were augmented by D, Z o G treatment. The association of pesticides increased these effects. Concomitantly, glutathione content was incremented in all tissues except brain in which it was decreased. Tocopherol also decreased in a direct proportion to the degree of intoxication. As a result, FRAP in plasma was diminished up to 5 times in the group in which the three drugs were combined. Catalase activity was incremented by administration of D, G o Z alone; however, association of drugs inhibited the enzyme probably due to overproduction of ROS. GSH-Tr and -Rd activities were both inhibited while SOD was decrease in the group treated with Z+G+D. In brain, only GSH-Px was inhibited. Protein was also affected since carbonyls were incremented. In conclusion, we demonstrated that low doses of pesticides were able to produce an oxidative stress that affects main organs such as SNC, liver and kidney. This scenario was aggravated by simultaneous administration of more than one toxic. This biomarkers may be useful to prevent human illnesses, especially neurodegenerative disorders such as Parkinson disease.

LI-P09.
TESTICULAR STEROIDOGENESIS IN RATS INTOXICATED WITH DIMETHOATE, ZINEB OR GLYPHOSATE
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We studied the biosynthesis of testosterone (T) in testis from Wistar rats chronically intoxicated by i.p. injection of dimetoathe (D), zineb (Z) or glyphosate (G) (1/50 to 1/250 DL50 three times a week for 5 weeks). In all groups that received D the level of T (free or bound) was decreased compared to control values. This effect was augmented by association with G o Z. Concomitantly, plasma concentrations of LH and FSH were increased and estradiol level simultaneously decreased. Concentration of T was also decreased in testicular homogenates. These hormonal changes were directly related with increments in the production of [NOx], ROOHs, total glutathione, protein carbonyls, and protein thiols. Total antioxidant capacity (FRAP) and tocopherol content were diminished in intoxicated rats. The activities of the androgenic enzymes were decreased slightly in rats treated with G, but they were strongly inhibited in those groups receiving D (alone or in combination with G or Z). In a separate study we demonstrated that hydroxysteroid deshydrogenases were inhibited by overproduction of ROS. Thus, we conclude that organophosphorated pesticides (especially D) decreased the biosynthesis of T through a mechanism that involved ROS and subsequent damage of the androgenic enzymes. This conclusion may be relevant in monitoring steroidogenic function in man professionally exposed to this kind of pesticides.

LI-P10.
DIMETHOATE INHIBITS TESTOSTERONE BIOSYNTHESIS TROUGH A MECHANISM THAT INVOLVES COX-2 AND STAR
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We studied the mechanism through which intoxication of Wistar rats with dimethoate (D) (1/50 DL50, i.p., three times a week for 5 weeks) inhibits testosterone (T) biosynthesis. Expression of COX-2 was incremented by 44% over control data in Leydig cell suspension from intoxicated rats, while transcription of StAR protein was decreased by 45 to 52% and the expression of the protein was diminished by 36 to 41% compared to control values. F2α and E2 prostaglandins were incremented by 61 and 78% respectively after D intoxication. T level decreased 49% in cellular homogenates. Concomitantly, plasma concentrations of LH and FSH were increased. Arachidonate content in Leydig phospholipids decreased by 28% and the docosapolyenoic acids (n-3 and n-6 series) were diminished by 19 to 30%. Protein carbonyls and ROOHs were increased whereas total antioxidant capacity (FRAP) of sonicated Leydig cells decreased under D treatment. Stimulation of Leydig cells with h-CG (10 nM, overnight) failed to overcome the inhibition caused by D on T production. In conclusion, we demonstrated that decreased T biosynthesis caused by D may be the consequence of: (i) inhibition of StAR biological activity due to stimulation of COX-2 and overproduction of PGF2α, (ii) decreased stimulatory effect of arachidonate on StAR with subsequent alteration in the availability of cholesterol for androgenic pathway, (iii) inhibition of steroidogenic enzymes by direct oxidative injury or indirectly by damage due to a slower transcriptional rate caused by elevated PGF2α levels. However, other factors such as alterations in phospholipase A2, ACS4 or Acot2 activity should not be ruled out.

LI-P11.
ANDROGENIC FUNCTION IN TESTIS FROM RATS FED DIFERENT COMMERCIAL OILS: CORRELATION WITH FREE RADICALS
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We studied the fatty acyl composition, testosterone (T) biosynthesis, and 3-HSD/17-HSD enzyme activities in Wistar rats fed on diets supplemented with commercial oils (C: coconut, S: soybean, O: olive, and G, grape-seed) for 60 days. Various oxidative stress biomarkers were also determined. S- or G-supplemented diets incremented protein carbonyls, TBARS, [NOx], conjugated dienes and ROOHs. Protein thiols, α-tocopherol and total antioxidant capacity (FRAP) were significantly decreased in testicular homogenates. C or O diets produced the opposite effects, being O the less pro-oxidant supplement. Unsaturation indexes in phospholipids were decreased in the order G>S>O>C; however, testicular PUFA levels were maintained in all diets except C in which a slight deficiency was observed. Changes were correlated with T levels (plasma and testicular homogenates) showing C or O groups the highest values and S or U the lowest ones. An opposite effect was observed for LH and FSH plasma levels. Activities of androgenic enzymes were inversely proportional to the oxidative stress condition generated by each diet. In vitro generation of free radicals (ACCN or Fe/ascorbate) demonstrated that both enzymes were inhibited by overproduction of ROS. In conclusion, we demonstrated that dietary lipids modify both, T biosynthesis and the balance between androgens and gonadotrophines. These effects are likely to occur due to overproduction of ROS derived from the lipid metabolism and subsequent inactivation of the key androgenic enzymes.

LI-P12.
PHOSPHATIDYLCHOLINE SPECIFIC-PHOSPHOLIPASE C AND PHOSPHOLIPASE D ARE ACTIVATED BY OXIDATIVE INJURY

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Our purpose was to characterize the state of phospholipase D (PLD) and phosphatidylcholine-specific phospholipase C (PC-PLC) pathways in cerebral cortex synaptosomes (Syn) from adult and aged rats exposed to oxidative stress. Oxidative injury induced by FeSO4 increased diacylglycerol (DAG) generation by 77% with respect to control conditions both in adult and aged animals. Experiments carried out in the presence of ethanol demonstrated that both PLD and PC-PLC pathways contributed to DAG generation in the presence of iron.
For determining the cellular fate of DAG produced from PC, assays were performed in the presence of either RHC80267 (DAG lipase inhibitor) or R59022 (DAGK inhibitor) or U73122 (PIP2-PLC inhibitor). Under control conditions, these inhibitors did not modify DAG levels generated from PC. Similar results were observed in the presence of free iron. U73122 decreased DAG levels by 23% after 60 min of iron exposure in adult animals. This effect was not observed in senile animals. Preincubation of Syn with Genistein or Herbimycin A, two tyrosine kinase inhibitors, did not modify the DAG increase induced by Fe^{2+}. The presence of sodium vanadate (tyrosine phosphatase inhibitor) strongly inhibited DAG generation under control and stimulation conditions.
Our results show that oxidative stress activates DAG generation pathways in synaptic endings from adult and aged rats.

LI-P13.
TYROSINE PHOSPHORYLATION REGULATES PI3K/AKT/ERK ACTIVATION DURING SYNAPTOSOMAL OXIDATIVE INJURY

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The aim of this work was to study the mechanisms involved in phosphatidylinositol 3-kinase (PI3K)/Akt activation during oxidative injury in rat cerebral cortex synaptosomes. We have previously demonstrated the activation of PI3K in synaptosomes exposed to $FeSO_4$ (50 µM) (Uranga et al., 2007). For evaluating downstream pathways activated by PI3K, Akt and Erk phosphorylation levels were determined by Western Blot. Akt phosphorylation in Ser473 and in Thr308 was increased upon a 5 min exposure to Fe^{2+}. However, pErk levels were increased after 30 min of oxidative insult exposure. Experiments with vanadate and herbimycin A let us determine that tyrosine phosphorylation is involved in the mechanism of PI3K activation by free iron. PI3K activation was evident when we measured phosphatidylinositol bis-phosphate (PIP2) as product in whole synaptosomes. Additionally, immunoprecipitates (IPs) with anti-phosphotyrosine antibody showed an increase in PI kinase (namely PI-4 kinase) activity induced by iron. Viability assays demonstrated that LY294002 (a specific PI3K inhibitor) was not able to prevent LDH leakage and diminution of MTT reduction induced by Fe^{2+}. Our results demonstrate that free iron provokes the activation of PI3K/Akt/Erk pathways in cerebral cortex synaptosomes. However, PI3K activation is not sufficient for protecting synaptic endings from oxidative damage.

LI-P14.
EGF REGULATES THE GEN EXPRESSION OF ENZYMES EVOLVED IN AA RELEASE

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The epidermal growth factor (EGF) promotes cell growth by AA release. In MA10 mouse Leydig cells EGF promotes AA release and its metabolization to lipoxygenated products. However the mechanism by which EGF releases AA in these cells was unknown. Steroidogenic hormones release AA for steroids synthesis by a concerted action of an acyl-CoA synthetase (ACS4) and an Acyl-CoA thioesterase (Acot2). These hormones increase ACS4 protein levels and regulate Acot2 by phosphorylation and substrate availability.
Here we show that EGF produces a mitochondrial AA increment which is released by Acot2 action, as was demonstrated in Acot2 down or overexpression experiments. This AA, in turn, regulates StAR protein expression, an indirect evidence of its metabolization to lipoxygenated products.
EGF increased ACS4 and Acot2 mRNA in MA10 and Y1 adrenal cells. The Acot2 induction was described before in liver by peroxisome proliferator action and fasting, and in diabetic rat heart. ACS4 is overexpressed in colon and hepatocellular carcinoma. This is the first time that a growth factor regulation of these enzymes was described.
Since EGF is involved in cell transformation to a tumoral state through AA release and metabolization by lipoxygenases, these results suggest that EGF-stimulated AA tumorigenic effects may be induced by the AA release in a specific compartment of the cell, i.e. the mitochondria.

LI-P15.
C-FOS ACTIVATES GLYCOLIPID SYNTHESIS BY ACTIVATING THE FIRST GLYCOSYLTRANSFERASE OF THEIR SYNTHESIS

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It has been demonstrated that c-Fos has, in addition to its well recognized AP-1 transcription factor activity, the capacity to associate to the ER and activate key enzymes involved in the synthesis of phospholipids required for membrane biogenesis during cell growth and neurite formation (Gil et al, Mol Biol Cell, 2004). As membrane genesis requires the coordinated supply of all its integral membrane components, the question emerges of whether c-Fos also activates the synthesis of glycolipids, another ubiquitous membrane component. We show that c-Fos activates the metabolic labeling of gangliosides in differentiating PC12 cells. Specifically, c-Fos activates the enzyme Glucosylceramide synthase (GlcCer S), the first glycosylated intermediate in the pathway of glycolipid synthesis. By contrast, the enzymes Galactosyl transferase 1 (GalT1) and Sialyl transferase 1 (SialT1) are essentially unaffected by c-Fos. The stimulatory effect of c-Fos on GlcCer S is most noticeable in subcellular fractions containing ER membrane markers.
As c-Fos is not a constitutive component of cells, its expression is tightly regulated by specific environmental cues. This strict regulation assures that lipid metabolisms activation will occur only when required thus pointing to c-Fos as an important regulator of key membrane metabolisms during neuronal differentiation.

LI-P16.
LIPID SYNTHESIS IN TESTIS: GPAT2 ACTIVITY AND EXPRESSION

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Four different isoforms of glycerol-3-phosphate acyltransferase have been described in mammalian cells. GPATs 1 and 2 are located in the mitochondria. GPAT2 is sensitive to sulfhydryl reagents (NEM), has no acyl-CoA substrate preference and was first recognized in liver from GPAT1 null mice. GPAT2 cDNA cloned from mouse testis encodes a protein of 797 aa (89 kDa) and 27% aa identity to GPAT1. Because GPAT2 mRNA expression was very high in testis, the aim of this study was to characterize the GPAT2 activity in highly purified mitochondria from wild type mouse and rat testis. The purity of the subcellular fractions was confirmed by marker enzymes and proteins. For the first time, NEM-sensitive GPAT activity was detected in purified mitochondria from wild type mouse and rat testes. We assume that this activity corresponds to the GPAT2 isoform because GPAT2 mRNA expression was more than 10 times higher in testis than in any other tissue. Although GPAT1 mRNA was 5-fold higher in liver than in testis, NEM-resistant GPAT activity in testis was 3-fold higher than in purified mitochondria from liver. In contrast, GPAT2 mRNA was 50-fold higher in testis than in liver, and, consistently, the NEM-sensitive activity in purified mitochondria was high in testis and was not detectable in liver.

LI-P17.
PHOSPHATIDYLCHOLINE BIOSYNTHESIS AND CELL GROWTH

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Phosphatidylcholine (PC) is the major phospholipid in mammalian cells. The CTP:phosphocholine cytidyltransferase (CTα) catalyze the limiting step that governs the Kennedy pathway. The understanding of the mechanisms that regulate its expression during the cell cycle will contribute to interpretate the basis of cell growth. We use CHO cells (MT58), a temperature-sensitive mutant defective in CTα, as a model. After MT58 cells are shifted to the restrictive temperature, we found that CTα is inactivated leading to cell die. The question we wish to address is why PC deficiency is essential for cell growth? To answer this question we measured changes in cell´s morphology, viability and DNA synthesis. The most important change we found was the cessation of DNA synthesis. Therefore, we investigated whether exogenous added phospholipids were able to reestablish DNA replication and if the nuclear localization of CTα is essential to induce growth.
Since the described results indicate that cell cycle progression is sensitive to PC content, we used transcriptional approaches to study the regulatory mechanism involved in Ctα expression during the cell cycle. The results indicate that this process is regulated by EF2. Since a similar mechanism regulates the expression of genes involved in DNA synthesis, we propose that both PC and DNA replication are coordinately regulated during cell growth.

LI-P18.
LIPID DROPLETS IN FRACTIONS ENRICHED IN SPERMATOCYTES, SPERMATIDS AND RESIDUAL BODIES

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In seminiferous tubules, Sertoli cells play a chief role in providing structural and functional support to spermatogenesis. Those germ cells that cannot be supported are led into apoptosis and rapidly eliminated by phagocytosis. At their luminal pole, Sertoli cells also support spermiation: as spermatids are released from the seminiferous epithelium, residual bodies are rapidly engulfed and degraded in Sertoli cells. In this work we isolated cell fractions enriched in pachytene spermatocytes, spermatids, and residual bodies to study their lipids and fatty acids. None of the germ cell fractions contained cholesterol esters, confirming that within seminiferous tubules these lipids are exclusive of Sertoli cell droplets. Glycerophospholipids rich in 22:5n-6 and sphingomyelin with 28:4n-6 and 32:5n-6 were more abundant in spermatocytes and especially in spermatids than in residual bodies. In contrast, the latter contained the highest proportion of 22:5-rich triglycerides of the three fractions. When Nile Red was used as a marker of oil droplets, spermatocytes and spermatids stained faintly and residual bodies fluoresced strongly and punctuated, indicating that 22:5-rich triglycerides were the main components of lipid droplets located within these structures. In vivo, recycling of residual body constituents including lipids and fatty acids may be one of the duties of Sertoli cells.

LI-P19.
CHOLESTEROL REMOVAL BY HUMAN APOLIPOPROTEIN A-I DEPENDS ON MEMBRANE LIPID ORGANIZATION

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We previously showed that overexpression of rat Stearoyl CoA Desaturase gene (SCD) in CHO-K1 cells induced a decrease in cholesterol (Chol) removal mediated by human apolipoproteinA-I, compared to control cells (SAIB 2006). Here we observed a differential activity of cellular acyl-CoA cholesterol acyl-transferase (ACAT) both in SCD and control cells. Furthermore, we enlarged our studies on Chol solubilization by measuring Laurdan General Polarization (GP) under two-Photon Fluorescence Microscopy. The GP value of SCD cells was similar to that of control cells, suggesting that the increase in membrane fluidity due to higher 16:1/16:0 and 18:1/18:0 ratios in phospholipids fatty acids composition, might be compensated by higher contents of membrane-ordering lipids, probably Chol. However, when Chol is removed from the membrane the GP of control cells increases, while it decreases in SCD cells. In order to interpret these results, we analyzed GP changes when Chol is removed from Giant Unilamellar Vesicles showing lipid coexistence. In this case, GP decreased when Chol was removed from more fluid domains, but it increased when Chol was removed from ordered domains. Data suggest that Chol could be solubilized preferentially from liquid-ordered domains in control cells, and from more fluid domains in SCD-cells. Results are discussed in terms of lateral phase separation.

LI-P20.
LIPID RAFTS OF ERYTHROCYTES ARE INVOLVED IN THE ACTION MECHANISM OF E. COLI ALPHA-HEMOLYSIN

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Alfa-Hemolysin (HlyA) is an extracellular protein toxin (107 kDa) secreted by E. coli that acts at the level of plasma membranes of target eukaryotic cells. Considering that certain bacterial toxins utilize lipid rafts as a site for high affinity binding and oligomerization on the surface of host cells our objective was to study the role of these microdomains in the action mechanism of HlyA.
Using FRET technique we demonstrated that HlyA forms an oligomer on erythrocytes membranes and that FRET efficiency decreases when ghost erythrocytes were cholesterol depleted. The cholesterol depletion was quantified by HPTLC and [^{14}C]cholesterol incorporation. We determined whether HlyA physically associates with lipid rafts. Ghost erythrocytes were incubated with HlyA. Detergent resistant membranes (DRMs) were obtained by incubation with Triton X-100 and density gradient ultracentrifugation. Inmunoblot analysis revealed that a substantial proportion of cell-associated toxin was associated with DRMs. Instead, sheep erythrocytes treated with beta-methylcyclodextrin show a mark decrease in the HlyA association with DRMs. Finally, the hemolytic activity of the toxin diminished when erythrocytes were cholesterol depleted using egg Small Unilamellar Vesicles. These results suggest the implication of lipid rafts in the oligomerization of the toxin on the sheep erythrocyte membranes.

LI-P21.
TL(III) INTERACTION WITH CARDIOLIPIN ALTERS MEMBRANE PHYSICAL PROPERTIES AND CYTOCHROME C BINDING

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Trivalent thallium (Tl(III)) is highly toxic to humans through not well understood mechanisms. In PC12 cells, we demonstrated that Tl(III) causes mitochondrial dysfunction and cell apoptosis. In the present study, we evaluated *in vitro* the effects of Tl(III) (5-75 μM) on cardiolipin (CL)-containing membranes physical properties, and how cytochrome c binding to CL is affected. Working with PC:CL liposomes (80:20 molar ratio) we found that after 1 h of incubation at 37ºC, no Tl(III) was bound to membranes, since it was reduced to Tl(I). Accordingly, a significant oxidative damage to both fatty acids and CL polar headgroup was observed. Immediately after Tl(III) addition, liposome membrane surface potential, fluidity and hydration were significantly decreased, and CL pKa decreased from 10.1 to 8.6. The magnitude of Tl(III)-mediated membrane rigidification and dehydration was even higher after 1 h of incubation. Cytochrome c binding to CL was significantly impaired in liposomes incubated for 1 h in the presence of Tl(III). Present results indicate that Tl(III) significantly affect the physical properties of CL-containing membranes, altering cytochrome c binding to CL. These deleterious effects of Tl(III) could be partially involved in mitochondrial dysfunction in cells exposed to this metal.
This work was supported with grants from UBA (B072) and CONICET (PIP 5536), Argentina.

LI-P22.
PREPARATION AND CHARACTERISATION OF ULTRADEFORMABLE ARCHAEOSOMES

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Ultradeformable archaeosomes (UD ARC) are controlled released nano-systems which have unique properties such as high chemical-physics resistance and are capable of acting as adjuvants, increasing the cellular, humoral and memory immune response. In this work, we prepared UD ARC using Argentinean Halorubrum archaeas. Colonies isolated from upper supernatant (US), grey crystals (GC), red crystals (RC) and black mood (BM) strata of the salt pound. From total polar lipids (TPL) isolated from each colony, UD ARC and combined TPL- phospholipon 90 ARC were prepared (using Sodium Cholate as a border activator), by hydrating the thin lipid film, sonication and extrusion. Ultradeformability was tested by submitting UD under pressure through a 50nm-pore filter. Bovine seroalbumine (BSA) was incorporated into UD ARC. The protein / lipid ratio was 20 μg mg^{-1} and the encapsulation efficiency between 3-4%. The effective diameters in average were about 100 nm. Finally, BSA incorporated into UD ARC didn't alter their ultradeformability.

LI-P23.
ULTRADEFORMABLE LIPOSOMES: TOWARDS A PRESERVING FORMULATION FOR NANOMEDICAL USE

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Ultradeformable liposomes (UL) are matrices made of phospholipid bilayers and surfactants. Their special properties enable them to change shape keeping integrity and to experience locomotion across hydration gradients, allowing them for crossing nanochannels at the stratum corneum. While their use on nanomedicine grows, there is no data on how UL could be preserved dried. We explored different strategies to preserve UL's integrity when reconstituted after drying by lyophilization or air-dry. UL were prepared as described in bibliography, adding sucrose or trehalose (5 to 20% w/v in buffer) to get suspensions of unilamellar vesicles (100 nm of diameter). For lyophilization studies, suspensions were divided in aliquots of 450 μl and pre-freezed at -18ºC or -120ºC, (different nucleation/ice formation conditions). For air-dry studies, 50 μl aliquots were placed into desiccators for 96 hr. After reconstitution, size change was evaluated by light scattering, aggregation by turbidity measurement, ultradeformability by passing through 50 nm pores, and sugar-lipid matrix interactions by FTIR and DSC.
While trehalose 10% on controls (conventional liposomes) was sufficient for reconstitution upon lyophilization (but not for UL), only the air-dry method at sucrose or trehalose ratio of 20% allowed to reconstitute UL keeping size and ultradeformability.

LI-P24.
LIPID INTERCHANGE BETWEEN LIPOPROTEINS AND HEPATOPANCREAS IN ARACHNIDS

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Three lipoproteins, HDL-1, HDL-2 and VHDL, were isolated from hemolymph of the arachnid Polybetes pythagoricus. Phospholipids, free fatty acids and triglycerides were the major lipids. Our aim was to study the lipid uptake through lipoproteins and their interchange with hepatopancreas (HP - similar to liver in mammals).
HP and hemolymph (HL) were labeled by ammonium palmitate C14 injection. At different times of incubation in vivo, HP were dissected, HL extracted and lipoproteins isolated by ultracentrifugation. Labeling was counted in a liquid scintillation counter. Lipids were separated by TLC, and radioactivity was quantified using a scanner.
In HL labeling was incorporated mainly in HDL-1 and VHDL. Throughout time, labeling proportion in HL decreased whereas it increased in HP. At short times, most labeling was found in free fatty acids, but at longer times of incubation the incorporation was observed in phospholipids as well as in triacylglycerols which were mainly present in HP.
In conclusion, hemolymphatic lipoproteins of P. pythagoricus are able to incorporate palmitic acid and to transfer it to tissues. The synthesis of phospholipids and acylglycerides was demonstrated, consequently HP would be an important organ for the lipid metabolism.

LI-P25.
MITOCHONDRIAL GPAT IS ESSENTIAL FOR DE NOVO TRIACYLGLYCEROL SYNTHESIS IN CRUSTACEAN HEPATOPANCREAS

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In mammals, four isoforms of glycerol-3-phosphate acyltransferase (GPAT) have been described: Although ER-GPATs account for the highest specific activity in mammalian cells, it appears that mitochondrial GPAT1 may be the first-appearing acyltransferase in evolution. Bacterial GPAT has a molecular mass similar to GPAT1 and 30% aa identity. We report that triacylglycerol (TAG) synthesis in Macrobrachium borellii hepatopancreas depends solely on a mitochondrial GPAT.
Hepatopancreas has a high-TAG biosynthetic activity. In mitochondria, we identified both GPAT activity and protein similar to mammalian GPAT1. The activity was resistant to inactivation by SH-reactive substances, it was activated by polymixin-B, and its substrate preference was for palmitoyl-CoA. We also visualized a 67-kDa protein band reactive to anti-rat liver GPAT1 antibody. Surprisingly, we did not detect GPAT activity in microsomes, even though they can synthesize TAG from [^{14}C]palmitate. When we used [^{14}C]glycerol-3-phosphate as substrate, no [^{14}C]TAG was produced in microsomes, and in mitochondria the mainly biosynthetic product was [^{14}C]lysophosphatidate. We conclude that this crustacean model is unique in that the first step in the de novo biosynthetic pathways for glycerolipids is carried out exclusively in mitochondria.

LI-P26.
CHOLESTEROL DEPLETION BY METHYL-BETA-CYCLODEXTRIN INHIBITS *BUFO ARENARUM* OOCYTE MATURATION

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Caveolae are small plasma membrane invaginations enriched in sphingolipids and cholesterol involved in cell signaling regulation. Cholesterol-enriched low-density membranes containing an immunodetectable caveolin-like protein from *Bufo arenarum* oocytes have been isolated. To further characterize these membranes, a distinctive band was identified as a non-muscle myosin heavy chain by mass spectrometry (ESI-MS/MS). In addition, a signal molecule such as c-Src associated to this membrane fraction, was detected by immunoblot. In order to explore the involvement of cholesterol in the maturation process, oocytes arrested in G2 of meiosis I were treated with 5-50 mM methyl-β-cyclodextrin (MβCD) at room temperature for 60 minutes. The cholesterol content of treated oocytes decreased when compared with control oocytes. When MβCD-treated oocytes were incubated with progesterone, the *in vitro* maturation was inhibited in a dose-dependent manner. MβCD alone didn´t induce germinal vesicle breakdown. Repletion of cholesterol showed a recovery of the maturation ability of MβCD-treated oocytes, particularly at the 25 mM concentration in which the reversibility was near the controls level. Results suggest that oocyte *caveolae*-like structures play a role in signaling mechanisms operating in *Bufo* oocytes maturation.

LI-P27.
TRYPANOSOMA CRUZI Δ9 DESATURASE: MOLECULAR AND BIOCHEMICAL CHARACTERIZATION

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Lipids have been considered important chemotherapeutics target in *T. cruzi*. The presence of Δ^9 and Δ^{12} desaturase was previously demonstrated by us. Later on, we proved that Δ^9 desaturase from microsomal fraction can use stearic, palmitic and phosphatidylcholine radioactive substrates. In this work we provide some molecular and biochemical characteristics of this enzyme. We used no labeled substrate and identified reaction products by GC. Enzyme molecular mass was determined using native SDS-page gel and charge characteristics by means of cationic gel electrophoresis, identified with immunoblot analysis. Total RNA was extracted and cDNA was prepared by RT-PCR. Δ^9 desaturase activity with 16:0 as substrate was 12% higher at 5% of fetal bovine serum (FBS) than at 10%. When the substrate was 18:0 the desaturation was 8% higher at 10% than 5% of FBS. The enzyme was inhibited by ketoconazole. Δ^9 desaturase molecular mass was estimated in 40 kDa. We also found that this enzyme is a basic protein. The probe generated of 1649 bp, was coincident with a gene with putative characteristic of desaturase using databases from *T. cruzi* genome. Δ^9 desaturase is a promising therapeutic target as it was inhibited by ketoconazole. Since it is considered a key enzyme, research on it might contribute to the knowledge of *T. cruzi* metabolism.

LI-P28.
PHOSPHATIDYLCHOLINE FUNCTION IN RHIZOBIAL STRAINS NODULATING PEANUT: GENES AND ENZYMATIC ACTIVITIES

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Phosphatidylcholine (PC), the major membrane phospholipid in eukaryotes, is also found in rhizobacteria and other bacteria interacting with eukaryotic hosts. In the case of alfalfa and soybean, rhizobial PC is required for a successful interaction of the bacteria with the legume host plants. We have previously suggested that PC may be involved in the rhizobial response to adverse conditions. Our aim was to study the role of bacterial PC in the rhizobium-peanut symbiosis. Using in vitro assays we have detected PC synthase (Pcs) and phospholipid N-methyl transferase (Pmt) activities in crude extracts of *Bradyrhizobium* SEMIA 6144 (slow growing strain) and in *Rhizobium* TAL 1000 (fast growing strain). Southern blot analysis using pmt- and pcs-probes of *Bradyrhizobium japonicum* USDA110 and of *Sinorhizobium meliloti* 1021 revealed pmt and pcs homolog genes of *B. japonicum* USDA 110 in *Bradyrhizobium* SEMIA 6144 and of *S. meliloti* in *Rhizobium* TAL 1000. We have constructed a pmtA knock-out mutant in *Bradyrhizobium* SEMIA 6144 and preliminary results indicate that it is severely affected in its symbiosis with peanut. These results suggest that PC formation in the peanut-nodulating strains *Bradyrhizobium* SEMIA 6144 and *Rhizobium* TAL 1000 is due to both methylation- and Pcs- mediated biosynthetic pathways and that wild type levels of PC are required for the peanut-

LI-P29.
FATTY ACIDS PATTERN IN RHIZOBIUM PEANUT NODULANT: ROLE IN HYPERTERMIA AND SALINITY

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Most microorganisms adapt their membrane lipid composition to environmental changes in order to stabilize the fluidity of the membranes, using *cis-trans* isomerization on unsatured fatty acids (FA) or modifying the saturated to unsatured FA ratio. We have studied in this work the effect of temperature and salt concentration on the viability and FA composition of *Rhizobium* TAL 1000.
The strain was grown under control conditions (28ºC), hypersalinity (0.3 M NaCl), hyperthermia (37ºC) and hyperthermia-hypersalinity (37ºC+0.3 M NaCl). Cells were harvested, total lipids were extracted and FA methyl esters were prepared using BF3 in methanol. FA analysis was performed using GC.
Rhizobium TAL 1000 was able to grow in the presence of 0,3 M NaCl or at 37ºC. The viability decreased under salinity. However, there were not differences when the strain was exposed to the combination of both stresses. The detected FA were octadecenoic (18:1), octadecanoic (18:0), hexadecanoic (16:0) and a FA of twenty carbon atoms, probably eicosatrienoic, (20:3) as major FA. All FA were affected by temperature, being the most relevant fact a decrease of 18:1 (87%) and an increase of saturated FA (100%).
The FA composition of *Rhizobium* TAL 1000 resembles to that described for other rhizobia. FA of this strain were modified in response to temperature changes in order to survive under adverse conditions.

BT-P01.
ISOLATION AND PURIFICATION OF MCCP136, A COLD ACTIVE BACTERIOCIN PRODUCED BY A SERRATIA sp

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Antimicrobial peptides are molecules widespread in life forms to mediate competition, and their industrial production could be important for the potential use as preservative in food, cosmetical and pharmaceutical industries. Cold environments could be a suitable source of microorganisms with ability to produce cold-active antimicrobials of biotechnological interest. Objectives: The aim of the present study was to lab-scale produce and purify MccP136, a widespread-pathogen inhibition spectrum molecule by using a psychrotrophic *Serratia sp*. Methods: Batch fermentations were carried out in a BIOFLO III bioreactor under pH, temperature and DO controlled conditions. The growth curve and MccP136 production were determined during the fermentation process. The MccP136 was collected during the process in the foam phase and centrifuged, cell-free supernatant was subjected to a two step purification procedure: Solid Phase extraction in a C18 RP-Cartridge followed by HPLC. Results: The MccP136 was produced on 5L batch culture at 8ºC and the cell-free supernatant was used in order to develop an efficient purification procedure.
Conclusions: We have evaluated and developed a cost-efficient production and purification process for MccP136, an antimicrobial compound produced at low temperatures with a broad pH range of activity against a broad spectrum of widely known pathogenic bacteria.

BT-P02.
METABOLIC MODIFICATION TO ENHANCE RESISTANCE TO *PHYTOPHTORA INFESTANS*

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It is well known that pathogen infection triggers the induction of defense-related reactions such as the natural accumulation of phenolic compounds like chlorogenic acid, lignins, flavonoids with phytoalexin activity and salicylic acid (responsible of the "systemic acquired resistance" or SAR). These metabolites are products of the phenylpropanoid pathway and are accumulated in infected tissues as a defense response to pathogen attack. There is a good correlation in plants between higher levels of phenolic compounds and enhanced resistance to pathogenic fungy. The intermediates and products of the phenylpropanoid pathway are excellent substrates for the polyphenol oxidase (PPO). This enzyme modifies these phenol containing molecules by hydroxylation and oxidation affecting their chemical properties.
In order to enhance resistance to pathogenic fungy infection via indirect modifications of the phenylpropanoid metabolic pathway, PPO gene family was downregulated applying RNAi gene silencing.
The transgenic potato plants with reduced PPO activity show a remarkable enhanced tolerance against *P. Infestans* when compared to WT potato plants and increased phenolic compounds accumulation colocalizing with the pathogen growth. As far as we know, this is the first report of a second generation transgenic functional food which also presents an enhanced resistance to plant pathogens.

BT-P03.
BIOCHEMICAL AND FUNCTIONAL STUDIES OF BIOINSECTICIDE MOLECULES AGAINST HAEMATOBIA IRRITANS LARVAE

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We are interested in the study of the *H. irritans* larvicide activity of two groups of bioinsecticides; the *B. thuringiensis* Cry proteins and the xanthene photoactivable Phloxine B. Respect to the Cry proteins, we previously reported the detection of a *B. thuringiensis* strain toxic against *H. irritans* larvae, the characterization of two Cry proteins present in this strain, and the cloning and characterization of the respective genes. In this work we constructed *E. coli* strains overexpressing each of the cry genes in order to determine the toxicity of these strains against *H. irritans* larvae. The results obtained indicated that each gene confers similar toxicity to the recombinant *E. coli* strains. Respect to Phloxine B, we previously reported that the toxicity of this compound against *H. irritans* was approximately 100 times higher than that determined against related flies (*Ceratitis capitata, Drosophila melanogaster*). With the aim to characterize the observed toxicity and taking into account that the *H. irritans* larvae are fed with bacteria present in the bovine feces, the toxicity of Phloxine B against these bacteria was determined. It was found that this compound behaves as a potent bactericide leaving a minor population of resistant cells. We are at present analyzing the effect of *B. thuringiensis* and Phloxine B on related fly plague such as *C. capitata and D. Melanogaster*.

BT-P04.
DESIGN OF A LOW-COST PROCESS FOR PEROXIDASE PRODUCTION IN INSECT LARVAE

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Baculovirus-insect cell system is a well-known choice for expressing foreign genes. We describe a technology to scale up horseradish peroxidase isozyme C (HRPC) production using insect larvae as biofactories.
Two strategies were applied in order to obtain polyhedra, the natural viral infective structure, containing rAcMNPV: 1-Mixed polyhedra (MP) were obtained by co-infection of wild type AcMNPV and AcMNPV HRP+/occ- at different multiplicities of infection (MOI) of both virus and ratios between them. 2- AcMNPV HRP+/occ+ construction by co-transfection with transfer vector pAcGP67-HRP and bAcGOZA.
Four instar starved larvae of R. nu and S. frugiperda were fed with artificial diet contaminated with 1x106 pol/100 mg of both polyhedra suspensions and HRP activity was measured in haemolymph at days 4 and 8 post-infection respectively. R. nu larvae infected with MP containing 98.9% rAcMNPV (as determined by real time PCR) expressed 31.1 mg HRPC/l. When the same larvae were infected with AcMNPV HRP+/occ+ polyhedra, HRPC production was approximately 50% higher (46.8 mg/l). Similar results were obtained with S. frugiperda larvae but HRPC production was 137 lower. Taking these results into account, R. nu larva is the best choice for HRPC production by oral infection with AcMNPV HRP+/occ+. This low-cost strategy is a practical way for infecting a high number of insects easily.

BT-P05.
AQUEOUS TWO-PHASE SYSTEMS FOR PURIFICATION OF RECOMBINANT PROTEINS EXPRESSED IN PLANTS

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Bovine viral diarrhea virus (BVDV) and bovine rotavirus (BRV) cause significant economic losses in Argentina. Proteins E2T and VP8 from BVDV and BRV are being expressed in alphalpha to develop vaccines. In this way, aqueous two-phase partition systems (ATPS) are eligible to purify them because of their low cost and easy scale-up. In these systems, experiments have to be performed to find differential partition (the protein of interest is in one phase while the contaminants partition mainly in the other phase). The effect of PEG molecular weight, pH and added salts in PEG/phosphate ATPS was assessed.
In the case of E2T, the higher the PEG molecular weight, the higher was the partition constant (18 for PEG 4000). Salts added and pH variations failed to change significantly the partition behavior of the protein. In the case of VP8, the partition constant was higher than 11 for PEG 600/phosphate and PEG 1500/phosphate ATPS. The addition of 0.5 M NaCl to PEG 1500/phosphate ATPS increased the partition constant by 13% while the partition constant of the proteins in the PEG 600/phosphate ATPS remained essentially unchanged under the same conditions. pH variation did not bring about any effect on the VP8 partition. Results obtained allow us to establish the conditions for recuperation of both protein with high yield, low cost and the possibility to carry out the scale-up easily.

BT-P06.
A GENOMIC SEQUENCE SURVEY OF THE RUMINAL BACTERIA *BUTYRIVIBRIO FIBRISOLVENS*

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Microbial degradation of forage material in the rumen is the main source of energy for the ruminant animal. Complex plant cellulose and hemicelluloses constitute a significant proportion of the energy in forage. The anaerobe *Butyrivibrio fibrisolvens* is a low G+C content bacteria that inhabit bovine and ovine rumen and presents high activity of endoglucanases and hemicellulases. *B. fibrisolvens* can metabolize a wide variety of carbohydrates, producing butyric acid as the major final fermentation product. The molecular genetic knowledge of the biochemistry of *B.fibrisolvens* is largely unknown. To contribute to understand the physiology of *B. fibrisolvens* we started with a genomic sequence survey (GSS) strategy. Genomic DNA from a native strain of *B. fibrisolvens* was mechanically fragmented, repaired and cloned in a replicative vector in *Escherichia coli*. Recombinant plasmidic DNA were sequenced and the results analyzed in GenBank database using BLASTX. A high number of sequences shown homologies with bacteria of the phylum Firmicutes (like *Clostridium* spp and *Ruminococcus* spp). We identified genes that encoding for putative proteins with high homologies with glycoside hydrolases, proteases and transposases. These results open the opportunity for gene discovery and to identify genes encoding for hydrolytic enzymes with bioindustrial applications.

BT-P07.
STAPS INTERACTS WITH DAMAGED SPERM PLASMA MEMBRANES
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The use of antimicrobial agents from natural origin in contraception has been widespread due to their potential dual benefits. Plant-derived compounds exert spermicidal activity due to an interaction with the sperm plasma membrane (SPM). We have previously isolated potato aspartic proteases (StAPs) that are able to interact with membrane of frozen spermatozoa. The aim of this work was to study the effect of membrane alteration degree (induced by cryopreservation or by Triton) in the interaction StAPs-SPM. Thereby, fresh or frozen human sperm were incubated with FITC-StAPs. The results showed that StAPs were able to interact with frozen human sperm but not with fresh human sperm. As a model of membrane alteration, bovine sperm were treated with different concentrations of Triton-X100 and then incubated with FITC-StAP1. Triton reduced both total and progressive motility in a dose-dependent manner. It is concluded that the StAP1-SPM interaction differs according to the membrane damage degree suggesting that binding is affected by the SPM structure and/or organization. These results could be used to evaluate the StAP1 use as a marker in the analysis of the insemination sample quality.
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BT-P08.
SCALE UP OF A LOW MOI PROCESS FOR THE PRODUCTION OF AN INSECTICIDE BACULOVIRUS IN CELL CULTURES
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The *Anticarsia gemmatalis* multicapsid nucleopolyhedrovirus (AgMNPV) is currently used as an efficient biological pesticide for the control of the velvetbean cartepillar (*Anticarsia gemmatalis*), an important pest in soybean crops. In vitro infection of insect cells with the baculovirus is increasingly considered as a feasible means for the production of this bioinsecticide. Batch fermentation processes traditionally employ intermediate to high multiplicities of infection (MOI), needing two parallel scale-up processes, one for cells and one for virus. In this work, we considerate the possibility to scale up a batch process to produce AgMNPV in serum-free suspension cultures of the UFL-AG-286 cell line infected at low initial cell density (ICD) and low multiplicity of infection (MOI). These parameters were previously optimized in a small scale following a full factorial experimental design with a center point. The polyhedral inclusion bodies (PIBs, the insecticide phenotype) productivity obtained in a 2 liter stirred reactor was 2,2x106 PIBs.ml-1.h-1. This productivity was larger than the productivities reached in the low scale optimization experiments (1 - 1,6x106 PIBs.ml-1.h-1). These results demonstrate that it is possible to obtain an optimal yield of AgMNPV PIBs when the process of infection is scaled up preserving the conditions of infection optimized on a smaller scale.

BT-P09.
EFFECT OF SELECTED PARAMETERS IN HYBRIDOMA CELLS IMMOBILIZED IN SOL-GEL SILICA MATRICES
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The sol-gel process is an inorganic polymerization taking place in mild conditions, allowing the association of mineral phases with organic or biological systems. Optimization of the technological parameters affecting the mechanical properties and permeability of sol-gel matrices is essential to develop matrices with improved properties for cell immobilization. In this work, the effect of different parameters such as the concentration of different sol-gel precursors and immobilized cell density, on the function of hybridoma cells producing anti-peptidoglycan receptor protein (PGRP) I-α.monoclonal antibody (mAb) has been investigated. Cell morphology was conserved as it was observed by optical and fluorescence microscopy. Cellular activity of immobilized hybridoma cells was determined by the tetrazolium assay (MTT assay) and a LIVE/DEAD cell vitality fluorescent assay. Viability was 89.5% in tetrakis (2-hydroxyethyl) ortosilitate-derived matrices and 60.5% in tetraethoxisilane- derived matrices. No differences were observed between cell densities employed in this work ($5x10^5$ - $3x10^6$ cells/ml). MAb production from immobilized cells was detected by ELISA even after 10 days post immobilization.
The design of such novel devices with significant added value is a key factor when foreseeing industrial developments of sol-gel materials in medicinal science.

BT-P10.
ANTIBODIES DETECTION EMPLOYING SOL-GEL IMMOBILIZED CELLS
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Immunoflourescence (IFA) and Enzyme Immuno Assays (EIA) are useful diagnosis techniques for different diseases through specific antibodies (Abs) detection. Both techniques involve several fixation alternatives for immobilization of microorganisms, parasites or cellular fragments. The objective of this work was to generate a versatile system for antigen covalent attachment for the detection of serum Abs from mice infected with different pathogens. For this purpose the attachment of *Trypanosoma cruzi* and *Leishmania guyanensis* over a silicon oxide polymer covered surface was developed. The films were prepared using the sol-gel method. Standard microscope slides were coated with TEOS and APTES as polymeric precursors. These slides were used for indirect IFA and EIA analysis and both compared with the heat fixation technique. When acetone was used as the coating solvent IFA analysis, employing mouse infected sera, showed the fluorescence of attached parasites without matrix background interference. Similar results were observed when EIA techniques were evaluated. Thus, EIA and IFA are suitable methods for Abs detection from sera of infected mice when the antigen was covalent attached in soft conditions. Finally, the present immobilization method was able to maintain antigenic capability of attached cells allowing leading to homogeneous, ready to use and long lasting time coated slides.

BT-P11.
UREASE REUTILIZATION AFTER IMMOBILIZATION ONTO MAGNETIC CHITOSAN BEADS

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The use of enzymes in clinical applications is often limited due to their high cost, instability and low availability. These enzymes are also soluble in aqueous media, being its recovery difficult and expensive. This restricts the use of soluble enzymes to batch operations, followed by disposal of the enzyme-containing solvent. This lead to an intense research in the field of immobilized biocatalysis. Even when immobilized enzymes often shows lower catalytic activity than free ones, they are reusable, more stable and, consequently, less costly. Our aim was to develop a versatile enzyme immobilization system for its recovery after a biological assay and its further reutilization. For this purpose urease was immobilized onto magnetic chitosan beads.
Magnetic beads were obtained by coprecipitation of a chitosan, $FeCl_3$ and $FeCl_2$ mixture with NaOH. The beads thus generated were treated with glutaraldehyde, a bifunctional reagent that attaches to both chitosan and urease free NH_2 groups. These enzyme-particle complexes are insoluble in aqueous solution and, therefore, recoverable by means of a magnet after an assay.
The optimum enzyme/bead ratio was found to be 10 μg/mg. The immobilized enzyme maintains its activity during five consecutive reaction-recovery cycles. The reutilization of this system after one week storage at 4ºC without losing its activity demonstrated its stability.

BT-P12.
EVALUATION OF RHIZOBIA IMMOBILIZED IN SILICATE MATRICES AS AN ALTERNATIVE INOCULANT FORMULATION

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Symbiotic nitrogen fixation results from the interaction of a legume host with rhizobia, leading to root nodulation of the host plant. One of the main problems of the inoculation industry is to keep rhizobial cells viable in large numbers in the inoculants. Furthermore, soil is a heterogeneous, unpredictable environment, where the inoculated bacteria find it often difficult to survive amongst the competitors, resulting in a progressive decline in the bacterial density.
Immobilization of microbial cells into polymer matrices has proved to be advantageous over direct soil inoculation.
A inoculant formulation consisting of *Mesorhizobium* loti immobilized in a porous silicate matrix is proposed. Bacteria were immobilized using sodium silicate as the sol precursor and citric acid as the polymerization catalyst. The results obtained demonstrate the long time preservation of entrapped cells, at room temperature, for periods exceeding 10 months and the ability of rhizobia to effectively nodulate roots once they are freed from the polymer, confirming their presence in the nodules by means of PCR.
Their viability at different pHs, from 3 to 7, and their survival in sterile soil were also evaluated showing better results than liquid inoculants.
Further studies are being performed to use rhizobia immobilized in silicate gels as alternative inoculant formulations in real field conditions.

BT-P13.
HYBRID ORGANIC-INORGANIC POLYMERS FOR BACTERIA IMMOBILIZATION

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Silica matrices made by the hydrolysis and condensation of silicon alkoxides are mechanically stable and conserve a high level of moisture. However, they are less biocompatible than silicate-derived matrices, obtained by the aqueous route. Therefore, silicate gels are preferred for bacteria immobilization. Despite of this advantage, inorganic silicate gels, tend to shrink and collapse with time, loosing mechanical strength and water-holding capacity. Furthermore, the stress induced by gel aging dramatically affects bacteria viability.
Hybrid materials can be defined as nanocomposites with organic and inorganic components, intimately mixed. The aim of this work was to combine organic and inorganic components allowing the construction of a new material that could find a compromise between different properties (mechanics, permeability) and protect bacteria against gel aging. For this reason, we used sodium silicate as the inorganic component and glycerol or carboxymethylcellulose (CMC) as the organic part. Different glycerol and CMC concentrations were evaluated and better results were obtained when using CMC than with glycerol. CMC gels retained approximately 80% of water, after their aging in a controlled atmosphere, and showed favorable physical properties. E. coli was immobilized as a model microorganism into these hybrid materials with high viability even after gel aging.

BT-P14.
IE1 REGULATION IN THE AGMNPV GENOME

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In the baculovirus, the homologous regions (hrs) have been implicated both as transcriptional enhancers and origins of DNA replication. Hrs and IBMs (IE1 binding motif) sequences has been shown to bind IE1 protein in gel mobility shift assays. IE1 is an important transcriptional transactivator of the baculovirus. Several studies showed that the IE1 protein has an essential role in the transcriptional regulation of early and late genes and in the viral replication.
The goals of this work were to find homologous motif to hrs and IBMs in the AgMNPV genome, and analyze the role in the transcription regulation in presence of the IE1. Computational analysis of the complete AgMNPV genome was used to identified hrs and IBMs motifs similar to that found in AcMNPV. We have identified several putative sequences located upstream to the different ORFs.
In order to evaluate if these motifs were involved in the interaction with IE1, we performed gel shift assays, using a DNA fragment containing a putative IBM motif found in the 5´ UTR of ie1 gene. To confirm if these genes were transactivated by IE1, we construct different reporter plasmids with the 5´ -galactosidase ORFβUTR region containing the putative hrs or IBMs driving galactosidase-βtranscription. Through transient assays, we were able to measure activity in presence or absence of the IE1 and confirm the transactivation.

BT-P15.
CLONING AND EXPRESSION IN SACCHAROMYCES CEREVISIAE OF POLYGALACTURONASE 1 FROM ASPERGILLUS KAWACHII

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Pectinases catalyze the hydrolysis of pectin and/or pectic acid. Among pectinases, endopolygalacturonases (PGase) (E.C. 3.2.1.15.) are, probably, the most important biocatalysts. *Aspergillus Kawachii* express at least 4 PGases, one of them, namely PG1 is active at acidic pH values. Low expression levels of wild type enzymes require cloning and over expression of these enzymes for their industrial applications. PG1 gene from the filamentous fungus *A. kawachii* was amplified by PCR using specific primers and cloned (BamH1-EcoR1) into the pYES2 expression vector (INVITROGEN). *E. coli* TOP10F´ (INVITROGEN) cells were then transformed. About 50 bacterial clones were tested by colony PCR using specific primers for Pg1 gene sequences, obtaining 14 positive clones (named G1-G14). Two positive clones (G11 and G12) showed correct open reading frame. The pYES2 vector containing the Pg1 gene was obtained from these clones and used to transform *Saccharomyces cerevisiae* INVSc1 (INVITROGEN). Transformed yeast clones were analyzed by colony PCR. Two positive clones were obtained and tested in terms of Pg1 expression. Both clones show an active PGA expressed after 24 hours of induction with galactose. Recombinant protein expressed in these *S. cerevisiae* clones was identified as a polygalacturonase by hydrolysis of polygalacturonic acid measured as an increment in reducing power at pH 4.

BT-P16.
PREDICTED STRUCTURAL AND FUNCTIONAL DOMAINS OF BACULOVIRUS P74 PROTEIN

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Baculoviridae is a virus family specific of insects like Lepidoptera, that infect only closely related species, and are good candidates as bioinsecticides. These viruses contain a large cccdsDNA genome, associated to proteins and enveloped. During the virus cycle exist two phenotypes: BVs (Budded Viruses), responsible to systemic infection; and OBs (Occluded Bodies), containing a protective protein matrix and responsible of per os infection. Each phenotype has a different membrane protein involved in specific cell recognition, BVs: GP64 or F, OBs: P74. P74 is a polypeptide presents in all baculoviruses and its deletion causes a non-per os infection virus phenotype. Experimental and bioinformatic studies predict that P74 could have at least two domains, a functional amino end and a structural carboxy end. More evidences are required to establish a model of structure and function. Therefore, in order to associate the role of domains, we work with P74 of four baculoviruses and with *Spodoptera exigua* and *Rachiplusia nu* larvae's.
To reach these objectives, we realized complement trans assays by in vitro cell cotransfection of an AcMNPV(Dp74) genome with different constructs expressing each P74, the amino end domains or chimerical P74s. The infectivity of OBs was studied in 2nd stage larvae of both hosts. The results confirmed previous predictions about P74 structure and function.

BT-P17.
CHARACTERIZATION OF ATRAZINE DEGRADING BACTERIA ISOLATED FROM ARGENTINEAN AGRICULTURAL SOILS

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The herbicide atrazine is widely employed in agriculture. Its physicochemical properties and extensive use increase the risk of soil and water contamination. The purpose of this work was the enrichment, isolation and characterization of microorganisms able to degrade atrazine, from agricultural fields (20 years of atrazine use) of Argentina Humid Pampa. Enrichments in liquid media were established, using atrazine as sole N and/or C source. 30 microbial strains were purified and genetically characterized by sequencing the 16S rDNA gene. Characterization of catabolic microbial associations was also carried out. Growth and catabolic performance were determined in liquid media with different atrazine concentrations, and kinetic parameters were calculated. Atrazine and its main metabolites were detected by HPLC. Results showed a great degrading microflora diversity: 63% of the isolates belonged to the phylum Proteobacteria (mainly Alphaproteobacteria), 30% were Actinobacteria and 7% Firmicutes. Three different bacteria associations were the more efficient in degradation, with 100% removal at concentrations between 25 and 500 ppm, although with different degradation rates. Herbicide degradation was facilitated by the combined activities of consortia members, due to their synergistic enzymatic activities. These cultivable microorganisms become potential candidates for bioremediation trials.

BT-P18.
POTENTIAL ROLE OF HYOSCYAMINE 6-β-HYDROXYLASE PLANT ENZYME IN PHARMACEUTICAL INDUSTRY

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Plants produce compounds of interest in medicine. The South American native plant, *Brugmansia candida*, produces tropane alkaloids including hyoscyamine and scopolamine. The anticholinergic properties of them made both alkaloids the most important of the group. Scopolamine is widely used due to the fewer side effects generated. Hyoscyamine 6β-hydroxylase (H6H, EC 1.14.11.11) is the enzyme responsible for the conversion of hyoscyamine into scopolamine. In this work we report the expression of H6H enzyme in *Saccharomyces cerevisiae* as a potential tool for the industrial production of scopolamine. The *h6h* cDNA was cloned into the pYES2 and the pYES2.1-TOPO TA vectors. The constructions were introduced by chemical transformation in *S. cerevisiae* CEN PK2. The enzyme was expressed 4 hs after induction with galactose as it was observed in Western blot analysis. The enzyme in vitro activity assay is being carried out according to the bibliography described methods using crude and IMAC semi-purified enzyme preparations. For the assay analysis it was developed an HPLC method able to detect and quantify low concentrations of alkaloids. It was possible to detect less than 2 ppm of both alkaloids with a 2.6 resolution factor.

BT-P19.
INDUSTRY AND ACADEMY BIOTECHNOLOGICAL INTERACTION: KEY ENDEAVOUR
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Environmental pollution has stimulated the application of biotechnologies and decontamination processes that tend to reduce the ecological and social impact over communities and the ecosystem. The most promising approach is to biotreat and bioremediate the waste and the polluted environment by exploiting the tremendous catabolic capability of microorganisms. Here we report the isolation and selection of natural microbial isolates and their use for bioremediation of contaminated environments. Among the natural isolates that degraded a wide range of PAH we identified members of the genus Pseudomonas. The presence of plasmids, the production of biosurfactants (rhamnolipids, Rhl) and swarming motility were characterized for some of the isolates. These PAH-degrading microorganisms were able to grow in synthetic media using PAH as the unique energy and carbon source. Furthermore, they were able to bioremediate complex samples of petrochemical-derived hydrocarbons (more than a hundred of different PAH) in pure cultures or in presence of the indigenous effluent flora. We also found that some of these isolates are able to degrade BTX and Styrene, and form good biofilms, properties which allow them to be used in polluted gas treatment processes. These results acquire biotechnological importance for their use in the resolution of environmental pollution.

BT-P20.
EXPRESSION AND PURIFICATION OF UV-SPECIFIC DNA REPAIRING ENZIME WITH POTENTIAL TRANSDUCING ACTIVITY
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UV radiation produces mutations that correlate with the onset of actinic keratoses and basal and squamous cell carcinomas. *Xeroderma pigmentosum* (XP) is a heritable disease characterized by an extreme sensitivity to UV radiation. Previously, we have described the production of a stable UV-specific DNA recombinant endonuclease (αUveA), from *Micrococcus luteus*. XP fibroblasts transfected with αUveA gene increased the resistance to UV radiation. Since an efficient intracellular delivery system is required for the development of a topical enzymatic formulation to repair DNA damage, αUveA was fused to Tat transducing peptide (αTatUveA). Thus, the purpose of this work was to obtain an active form of αTatUveA. With this aim αTatUveA was purified and refolded from inclusion bodies (IBs). Cultures were carried out using small erlenmeyers and a bioreactor, rendering 130 and 700 mg/l of protein, respectively. Purified IBs were used to test several solubilization and folding conditions. The best DNA repairing activity was obtained when IBs were solubilized in reducing conditions and folded in oxidative buffer using L-Arginine. As a second strategy the protein was fused to a His tag to directly obtain a soluble enzyme, and higher yields of αTatUveA were obtained. Further works will include the analysis of both αTatUveA transducing ability and its *in vivo* repairing activity.

BT-P21.
CONSTITUTIVE EXPRESSION OF ACC DEAMINASE IN *MESORHIZOBIUM LOTI* PROMOTES NODULATION
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Ethylene inhibits the establishment of symbiosis between rhizobia and legumes. Several rhizobia species express the enzyme ACC deaminase, which degrades ACC (precursor of ethylene) leading to the reduction of the amount of ethylene evolved by the plant.
M. loti has a gene that encodes ACC deaminase but it is under the activity of a NifA-rpoN-dependent promoter thus, it is only expressed in the bacteroid form, inside nodule. We wanted to know if the presence of ACC deaminase activity in the first steps of the *M. loti*-Lotus interaction could promote the efficiency of the nodulation process.
M. loti ACC deaminase structural gene (*acdS*) was introduced into *M. loti* MAFF303099 and *M. loti* Ayac 1BII in a plasmid vector, replicative in rhizobia, upon the constitutive promoter activity of P*lac*. The resulting *M. loti* strains show high ACC deaminase activity in the free-living cells.
Nodulation assays were carried out with the wild-type as well as with the constitutive ACC deaminase-producing strains. Data showed that the recombinant strains induced more nodules (2-2,8 /plant) than the wild-type ones (1-1,5 /plant). This result indicates that there could be a good possibility to increase the nodulation efficiency in field by the use as inocula these *M. loti* genetically engineering strains. Conforte and Echeverría contributed equally to this work.
Supported by CIC and UNSAM.

BT-P22.
A TRYPSIN INHIBITOR FROM *MACLURA POMIFERA* (RAF.) SCHNEID. SEEDS
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Plant proteinase inhibitors (PIs) play a central role in the diversification of both the insect herbivores as well as the host plants. These proteins are fundamental in the control and/or the protection against proteolytic action of the digestive enzymes of seed predators. One way to obtain better inhibitors of insect proteinases is, to search among plant species unrelated to host plant for PI families that do not exist in the host plants. Crude extract of *M. pomifera* seeds was prepared by grinding of twenty gram of seeds, previously washed and dried, with 100 ml of saline Tris buffer (50 mM Tris, pH 7.2; 0.15 M NaCl) in a mixer. The homogenate was filtered through a two-folded piece of gauze to remove plant debris, and then centrifuged for 20 min at 10000 x g. The crude extract was partially purified by precipitation with 5 volumes of acetone. The precipitate was redissolved with 2.5 volumes of saline Tris buffer and applied to a gel filtration column (Sephadex G-50 Fine). The fraction with antitrypsin activity was submitted to an anion exchanger column (Q-Sepharose FF) pre-equilibrated with 50 mM Tris buffer, pH 7.2 and eluted using a liner gradient of NaCl. The purified inhibitor was homogeneous by SDS-PAGE and IEF, with pI 5.2 and Mr 14 kDa, common features of Kunitz inhibitors.
1: CONICET Fellow, 2: CONICET Researcher Career.

BT-P23.
CHARACTERIZATION OF A NEW CYSTEINE PROTEASE FROM LATEX OF *VASCONCELLEA QUERCIFOLIA (CARICACEAE)*

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Latex of all *Vasconcellea* species analyzed to date exhibits higher proteolytic amidase activities, generally attributed to cysteine proteinases, than the latex of *Carica papaya*. The crude extract of *Vasconcellea quercifolia* was characterized by SDS-PAGE and isoelectrofocusing-zimogram revealing the presence of several basic proteins with high proteolytic activity and also other acid fractions without proteolytic activity. The most basic component (quercifoliain II) present in latex extracts was purified by cation exchange chromatography (SP-Sepharose HR, Tris-HCl 50mM, pH 7.5 and sodium tetrathionate 1mM) using FPLC system. Homogeneity was evaluated by 2D-PAGE and mass spectroscopy. Molecular mass of the enzyme was 23.984 Da (MALDI-TOF TOF/MS) and its isoelectric point was > 10.25. The N-terminal sequence of quercifoliain II (IPASIDWRQKGAVTPIRLQG) by BLAST analysis showed a great deal of sequence similarity to the other cysteine endopeptidases belong to *Vasconcellea* species, being 90% identical to proteases from *Vasconcellea heilbornii* and *Vasconcellea stipulate.*
The Peptide Mass Fingerprinting (PMF) characteristic of the enzyme was achieved by using a trypsin (0,4 µg/µl) for 12 h at 37ºC and analyzed by MALDI-TOF TOF/MS.

BT-P24.
CLONING AND SEQUENCING OF A CYSTEINE PROTEASE FROM FRUITS OF *BROMELIA HIERONYMI* MEZ

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Total RNA was extracted from fruits of *Bromelia hieronymi* and the 3'RACE-PCR method was applied to obtain protease cDNA. The PCR products were analyzed by agarose gel electrophoresis and showed the presence of bands around 900 pb, corresponding to cDNA fragments of cysteinproteases. The cDNA was cloned in *E. coli* (XL1-Blue) using the pGEM-T Easy Promega vector. Clones containing the plasmidic cDNA with the insert were sequenced.
The consensus sequence of the cysteine peptidase was obtained by analysis of the sequencing information (ProtParam Tool). This cysteine peptidase, named Bh-CP, is constituted by 875 pb, of which the 690 first ones codify for a polypeptid chain. The mature peptidase has 230 amino acids and possesses seven cysteine residues. Some physicochemical properties of the polypeptide were calculated: molecular mass, 24.773; pI, 8.6; extinction molar coefficient, 58.705 M^{-1} cm^{-1}. Bh-CP sequence shows high percentage of identity with the sequences of other cystein plant proteases as fastuosain, ananain, macrodontain and stem bromelain. The presence of highly preserved residues is observed, among them those belonging to the catalytic site (Gln19, Cys25, His159 and Asn175, papain numeration) that define its biological function, as well as six Cys residues, that realize a major contribution to the three-dimensional structure for formation of disulfide bounds.

BT-P25.
NOVEL ANTIMICROBIAL PEPTIDE DERIVED FROM THE FUSION OF BACTERIOCINS FROM GRAM (+) AND GRAM (-) BACTERIA

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Enterocin CRL35 is a class IIa bacteriocin produced by *Enterococcus mundtii*, a Gram(+) bacterium, while colicin V is a microcin produced by the Gram(-) *E. coli*. Both antimicrobial peptides are active against their closely-related microorganisms. The aim of this work was to fuse the structural gene of enterocin CRL35 (*munA*) and colicin V (*cvaC*) in order to obtain recombinant fusion proteins, namely MunA-CvaC and CvaC-MunA with antimicrobial activity of wider antimicrobial spectrum. Since these peptides do not have postranslational modifications, the construction of a hybrid antimicrobial peptide was possible. The structural genes of Enterocin CRL35 and Colicin V were amplified by PCR. Then, they were fused using the megaprimer technique. The amplicons were cloned in the vector pET28b and expressed in *Escherichia coli* BL21[DE3]. The transforming clones were cultivated in LB media and gene expression was induced with IPTG. Polyacrilamide gel electrophoresis both in native and denaturalizing conditions were performed in order to confirm the correct expression of the hybrids. Antimicrobial activity on the native gel was determined with sensitive strains. A Gram (+) and Gram (-) active fusion peptide Mun35-CvaC was obtained that could be visualized by polyacrilamide gel electrophoresis. These results showed a promising technique for obtaining novel antimicrobial peptides.

BT-P26.
BIODEGRADATION OF HYDROCARBONS AND A NEW OPTIC ANALYSIS FOR CHEMOTACTIC RESPONSE IN BACTERIA

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Bioremediation takes advantage of some microorganism capacities to transform xenobiotics into harmless compounds. The accessibility to the polluting agents has been identified like one of the greater limitations for an efficient bioremediation of contaminated places. This availability could be facilitated by bacterial chemotaxis. In this work one strain identified as P. aeruginosa was able to degrade different hydrocarbons of high purity, and displayed chemotaxis towards them. The assays were carrying out in discontinuous flask cultures at 25ºC, and cellular growth (OD600) and hydrocarbon degradation (GC) as the only source of carbon and energy were registered. The traditional methods for chemotactic studies require high cellular concentrations and specific time to be able to detect chemotactic response; also show difficulty to differentiate bacterial accumulation from viability and motility levels in the same sample. In this work, bacterial chemotactic response towards hydrocarbons was evaluated using an analysis of recorded videos with one digital camera added to an optical microscope. After that, the image sequences were processed using the entropy concept. This technique offers an alternative of greater sensitivity than traditional methods, presenting a complementary analysis to these traditional techniques and allowing a more exhaustive analysis of the chemotactic process.

BT-P27.

PHB PRODUCTION FROM GLYCEROL IN RECOMBINANT *E. COLI* EXPRESSING A GRANULE-ASSOCIATED PROTEIN

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Poly(3-hydroxybutyrate) (PHB) is a biodegradable plastic, which is accumulated by many bacteria under unfavorable growth conditions. PHB granules in natural producers are surrounded mainly by a protein called PhaP, which affects the size of PHB granules and the amount of polymer accumulated. PHB biosynthetic genes from *Azotobacter* sp. FA8 have been cloned in our laboratory and expressed in *E. coli*, resulting in strain K24K, which is able to accumulate PHB from several carbon sources.

The aim of this work is to analyze the effect of PhaP on growth and PHB accumulation patterns in *E. coli* in different growth conditions and substrates, in particular glycerol. Glycerol has become a very interesting substrate for bacterial fermentations, because it is a major byproduct in the synthesis of biodiesel.

A new *E. coli* strain, K24KP, which accumulates PHB and expresses PhaP, was constructed. Strains K24K and K24KP were grown in bioreactor batch cultures in a semisynthetic medium with glycerol as carbon source. Maximal growth rates were similar for both strains. However, higher biomass concentration and PHB accumulation was attained for K24KP when compared to K24K. These results indicate that the PhaP expressing strain has a much higher PHB production ability, making it suitable for the production of this biodegradable polymer from glycerol.

BT-P28.

***IN VITRO* CULTURE OF PSEUDANANAS SAGENARIUS (ARRUDA) CAMARGO TO OBTAIN ENDOPEPTIDASES**

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Plants of *Pseudananas sagenarius* were culture in MS (Murashige & Skoog) to obtain the protease from the medium. A comparison between this protease and the endopeptidases isolated from fruits of the same plants were made. After 40 days in culture different biochemical assays of the medium were made. The cultures were maintained at 25 +/- 2 ºC; with 10.000 lux and 8 h photoperiod. Milk clotting activity was carried out at 37° C during 5 hours using unfatted powder milk in 10 mM $CaCl_2$. The results confirm the presence of enzymes with milk clotting activity. The reaction mixture of caseinolytic activity contained casein 1% in 0.1 M Tris-HCl buffer, pH 8.0, containing 12 mM cysteine. The reaction was carried out at 37° C and stopped by the addition of trichloroacetic acid at different times. The activity was measure as change in absorbance at 280 nm. The activity increased slowly with time. The final activity was 0.003 Ucas/ml of the medium. In the SDS-PAGE a protein band was observed whit the molecular weight expected to cysteine endopeptidases and only a band with proteolytic activity was detected by isoelectric focusing. The results confirm the presence of cysteine proteolytic enzymes and a protein profile similar to that obtain from fruits of *P. sagenarius* previously studied in our lab.

A: CONICET Fellowship; b: CONICET Researcher Career c: CIC Researcher Career.

BT-P29.

A HEAVILY N-GLYCOSYLATED INTERFERON ALPHA ANALOG WITH ENHANCED ANTITUMOR ACTIVITY

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Interferons play a central role in the regulation of cell growth and differentiation. Due to its antiviral, antiproliferative and immunomodulatory effects, IFN-α has become a therapeutic agent for the treatment of a wide range of malignant and viral diseases. Yet, rapid systemic clearance of the cytokine demands high and frequent doses, which in turn bring about significant side effects. Besides, as therapeutic levels are difficult to sustain, the effectiveness of IFN-α in the therapy of solid tumors is severely compromised.

In order to increase the in vivo biological activity of IFN-α, a heavily glycosylated derivative, 4N-IFN, was constructed by introducing four N-glycosylation sites via site-directed mutagenesis. Immunoblots of the mutein expressed in CHO cells showed a broad band between 27 and 44 kDa consistent with a great heterogeneity of glycoforms with increased size and charge. Purified 4N-IFN was found to have a 25-fold longer plasma half-life and a 20-fold reduced systemic clearance, in comparison with non-glycosylated IFN-α after subcutaneous inoculation in rats. Moreover, in spite of its lower *in vitro* activity, 4N-IFN showed a markedly enhanced *in vivo* antitumor activity in human prostate carcinoma implanted in nude mice.

This remarkable improvement of 4N-IFN properties caused by glycosylation may confer clinical advantages over conventional IFN therapies.

BT-P30.

ANALYSIS OF PARAMETERS THAT AFFECT THE CUPRIC ION BIOACCUMULATION-BIOSORPTION BY *ESCHERICHIA COLI*

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Copper, one of the most toxic heavy metals responsible for contamination of rivers and deep waters, is attracting the attention from environmentalists, since mining activities increased. The potential of using microorganisms in the treatment of heavy metal-contaminated wastewaters has become important. The aim of the present work was to investigate the removal of Cu^{2+} ions from water via bioaccumulation using *Escherichia coli* AN387. Several parameters were studied (pH, temperature and competitive metal ions) on the Cu^{2+} bioaccumulation-biosorption. The assays were carried out in distilled water containing 10^9 cells with different copper salts concentrations (0 to 10 mM) and incubated for 30 min. *pH effect:* the removal of Cu^{2+} from aqueous solution was significantly reduced by the pH decreased. *Temperature effect:* no variation was observed in the accumulation of copper when the temperature was adjusted in the range from 27ºC to 42ºC. *Competitive metals effect:* different concentrations of Co^{2+}, Cd^{2+}, Mn^{2+}, and Fe^{3+} were assayed within a Cu^{2+} concentration. Only when the level of these ions was similar to the Cu^{2+} concentration used, a decrease in the copper accumulation was observed. Further studies should be done in order to use the microorganism on wastewater treatments before rejecting liquid residues from pollution sources.

BT-P31.
CHARACTERIZATION OF NEW POTENTIAL PERTUSSIS VACCINE CANDIDATES INDENTIFIED BY IMMUNE PROTEOMICS

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The low efficacy in preventing colonization of pertussis vaccines in use have led to disease re-emergence. Opsonins and antibodies capable of blocking initial attachment are critical to prevent colonization. Antigens included in vaccines able to induce this kind of antibodies have varied along the years leading to circulating bacteria with different variants than vaccine strains. On top of this, since *Bordetella pertussis* (Bp) seems not to be transmitted in virulent phase antigens included in vaccines might not be present in the infective phenotype. Using a proteomic approach we screened for new immunogens that should be expressed under physiological conditions. Four proteins induced under iron limitation, the main nutritional stress in vivo, not included in any of the current vaccines though present in the infective phenotype, were differentially recognized by sera from infected individuals. Two of them with predicted bacterial surface location, proved antigenic in mice. Antibodies against these proteins recognized surface exposed antigens that were overexpressed under iron limitation as determined by whole cell ELISA. Furthermore, they showed aggluttinin and opsonic activity as detected by microscopy and flow cytometry using iron straved Bp. Since these are key biological activities to prevent colonization these proteins may prepresent attractive vaccines candidates.

BT-P32.
***A. TUMEFACIENS* AND BIOLISTIC MEDIATED TRANSFORMATION OF MORINDA CITRIFOLLIA CELL SUSPENSIONS**

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Morinda citrifollia accumulate anthraquinones (AQs) which are secondary metabolites with therapeutic potential. AQs are formed from the methylerythritol 4-phosphate (MEP) pathway. 1-deoxy-D-Xylulose-5-phosphate synthase (DXS) the first enzyme in the MEP pathway catalyzes the transketolase reaction converting pyruvate and glyceraldehyde 3-phosphate into 1-deoxy-D-Xylulose-5-phosphate. Also it has been shown that Octadecanoid-Derivative Responsive Catharanthus Apetala 2-domain Protein transcription factors (ORCA) induce the synthesis of specific enzymes from secondary metabolism as well as another kind of response defenses against pathogens. In the present work we used to different methods to obtain transgenic cell lines of *M. citrifolia*. *Agrobacterium* mediated transformation and biolistic. Two binary vectors were used, in the first one the cDNA of *dxs* obtained from *Catharanthus roseus*, was cloned between the Cauliflower mosaic virus 35S-promoter (CaMV-35S) and the potato proteinase inhibitor terminator (Pit). The cassette expression was inserted into the binary vector pMOG22-GUS, containing the GUS reporter gene. In the second one, the ORCA gene was cloned into the pCAMBIA 1300binary vector containing GFP as a reporter gene. *M. citrifollia* cell suspensions were transformed by *A. tumefaciens* and biolistic. Transformations were checked by expression of the reporter genes, GUS and GFP.

BT-P33.
CONSTRUCTION OF A GENETIC CASSETTE TO EXPRESS IMMUNOGENIC DENGUE VIRUS PROTEIN IN A PLANT SYSTEM

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Production of recombinant proteins in plant systems has received great attention because of the advantages compared with others production systems. Plant cells can glycosilate and do the post-transcriptional arrangements need for complex glycoproteins. Dengue virus genome encodes three structural (C, prM, E) and seven non-structural proteins. E is an immunogenic glycoprotein and needs to be directed to the secretory pathway through the N-terminus signal peptide (SP), to be N-glycosilated. The presence of an ER retention signal in the C-terminus (KDEL) of some proteins increases their stability and yield in plant systems. The aim of this work is to obtain a genetic construct carrying E and CprME protein with a SP and KDEL cloned in a binary plasmid. E protein was amplified from plasmid pMT/V5-HisA given by Dr. Gamarnik and PS was obtained from pCAMBIA1305.2 plasmid by PCR. CprME was obtained from pMT vector by enzyme restriction. Promoter (35S) and terminator (T) were obtained by PCR from pMOG843 plasmid. The intermediate constructs were cloned into pGEM T Vector and sequenced. We obtained the genetic constructions: 35S-PS-E-KDEL-T, 35S-PS-CprME-KDEL-T, 35S-PS-CprME-T and 35S-GRP-E-Term. We cloned these constructions into a pCAMBIA1305.2 binary vector and we are currently doing the Agrobacterium-mediated plant transformation.

BT-P34.
NANO-CONTROLLED RELEASED SYSTEM (NANOCRS) AGAINST TOXOPLASMA GONDII FOR ORAL ADMINISTRATION

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Immunosuppressed patients in which reactivation of latent T.gondii infection is often fatal, are conventionally treated with high doses of sulfadiazine (SDZ) together with pyrimetamine, a drug of high toxicity. Dendrimers (D) exhibit unique properties that make them suitable candidates as nanoCRS. As part of our search for a nanoCRS capable of entering the host cells in order to increase the efficiency of SDZ delivery, we have previously shown that D can be used as nanoCRS of SDZ; complexes D-SDZ presented high antiparasitic activity on infected cells with T. gondii even at ultra-low nanomolar doses. In this work we performed two sets of studies: in vitro set of studies was aimed to determine the D-SDZ membrane damage, and the intracellular uptake of D-SDZ labelled with FITC, on Vero (endocytosis) and J774 (phagocytosis) cells; in vivo studies consisted of determining biodistribution and pharmacokinetic parameters of free and D-SDZ after i.v. and oral administration to healthy rats. Our results indicated that D-SDZ behaved strongly different from free SDZ, resulting in a high retention of the SDZ in blood circulation upon both administrations. Taken together those in vivo data with the high in vitro activity, we could expect that further administration of D-SDZ to infected animals could be employed to reduce therapeutic SDZ doses, avoiding the use of the highly toxic pyrimetamine.

BT-P35.
NON-TOXICITY NANO-CONTROLLED RELEASE SYSTEM OF SIRNA
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PAMAM dendrimers could be potential nano-Controlled Release System (nano-CRS) for nucleic acids because of their nearly perfect monodispersity and controlled size in the nanorange. In this work, complexes were prepared by combining generation 4 (G4) PAMAM dendrimers with anti-green fluorescein protein (GFP) siRNA. Interaction between G4 and siRNA was determined by measuring the ability of G4 to displace the intercalating dye ethidium bromide from siRNA. Analysis of the formation of complexes was made evident by detecting retarded migration of siRNA in a polyacrilamide 20% gel electrophoresis, as the stability of the complex in the presence of RNAsa and human serum. Cytotoxicity after 24 hs incubation of the complexes G4-siRNA and lipofectamine (L-siRNA), at different concentrations, was determined by methyl thiazolyl tetrazolium (MTT) assay, on Vero cells (endocytic fibroblast) and J774 cells (macrophages). Viability of both cell lines decresed only in the presence of the higher concentration of G4 and siRNA. On the contrary, L-siRNA complex decreased viability at minor concentrations. Finally, the efficiency of gene silencing was analyzed for G4-siRNA complexes, that showed to be more efficient for silencing GFP than the commercial reagent Lipofectamine 2000.

BT-P36.
PMCA SUBCLONING AND EXPRESSION IN AN INSECT STABLE CELL LINE
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The mammalian plasma membrane Ca2+pump (PMCA) is a P-ATPase which extrudes Ca2+ from the cell using energy obtained from the hydrolysis of ATP. PMCA is encoded by four different genes, that can be alternatively spliced, which yield a wide variety of different PMCA isoforms.
The aim of this work is to express PMCA isoform h4b in an insect stable cell line. In this way, high amount of biologically active PMCA could be obtained, which will be used to study variations in its expression in comparison with that transiently expressed. To carry out this study, PMCA h4b was subcloned in a vector containing an early and hard promoter (OpiE2) from Baculovirus, specific for insect cells. The plasmid pSG5 containing the PMCA h4b sequence was digested to release the PMCA DNA sequence, which was then sub-cloned in the plasmid pIB/V5 His Topo, previously digested. Ultra-competent cells were transformed with the plasmid pIB/V5 His Topo containing the PMCA isoform. The plasmids were purified from the colonies and digested with the enzymes used to clone the isoform. The products of the enzyme digestion showed a molecular weight according to the isoform h4b.
We are carrying out transfections of Sf9 insect cells with this plasmid using liposome. PMCA expression will be confirmed by Western-blot. The enzyme will be purified from these cells and then characterized by kinetic and structural studies.

BT-P37.
SOLID-PHASE PEPTIDE SYNTHESIS OF DESMOPRESSIN AND NOVEL PEPTIDE ANALOGS WITH ANGIOSTATIC ACTIVITY
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Desmopressin is a safe hemostatic compound that acts as a selective agonist for the vasopressin V2 membrane receptor, which is expressed in endothelium and also in some tumor cells. It has been used during surgery to prevent bleeding in patients with coagulation defects and recently to minimize spread of residual malignant cells after cancer surgery. Desmopressin and two analogs were synthesized using the Fmoc chemistry in the SPPS strategy. Desmopressin was called QN and its analogs were designated AQ and VQ, depending on the substitutions in position 4 and 5. The resin used was Rink-amide MBHA, thus resulting in amide peptides. Deprotection of thiol groups was performed at 2% trifluoroacetic acid with 5% triisopropylsilane as a scavenger. Oxidation of thiols to disulphides was performed with I2 under high dilution conditions. Intravenous injection of desmopressin (2 μg/kg) significantly reduced tumor-induced angiogenesis in syngeneic Balb/c mice bearing F3II mammary carcinoma cells. We explored in vitro the angiostatic activity of desmopressin and its analogs using the MCF-7 human breast carcinoma, a cell line that expresses the V2 receptor. Treatment of MCF-7 monolayers with desmopressin (100 nM) in the presence of plasminogen induced the formation of angiostatin. Interestingly, an enhanced production of angiostatin was obtained with the novel peptide analogs.

BT-P38.
CHARACTERIZATION OF AFFINITY ADSORBENTS FOR PROTEINS SEPARATION
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Adsorbents or particles of affinity for protein separation were prepared. Yeast cells were modified by chemicals and the Cibacron blue 3GA ligand molecule immobilized to the wall cell by covalent bond. This adsorbent was characterized by determination of maximum adsorption capacity (qm) and dissociation constant (Kd) by means of adsorption isotherms using bovine seroalbumin (BSA) as target protein. Adsorption experiments were carried out in batch. The BSA concentration in liquid phase (c*) was obtained by spectrofhotometric determination and the concentration in solid phase (q*) was calculated by mass balance. Values of adsorption such as Kd = 4x10-5 M and qm = 270 mg BSA/gad. were calculated by linear transformation of the Langmuir equation and using the Origin Pro 7.5 program with graphics and regression analysis. Preparation of immobilized metal ion affinity adsorbents constituted by the complex yeast cell-Cibacron blue-metal (Zn++) were performed to improve the adsorption of BSA. The concentrations of the metal ion in aqueous phase were measured with an atomic absorption spectrophotometer. The amounts of chelated metal ions were calculated by mass balance. Significant amounts of Zn were immobilized on adsorbent (up to 90 mg Zn/g.ad). Dye-ligand affinity adsorbents play an important role in the separation and purification of proteins.

BT-P39.
BIODEGRADATION AND MECHANICAL PROPERTIES OF A BIO-PLASTIC STERILIZED WITH ETHYLENE OXIDE

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Ethylene oxide gas (EtO) is widely used as sterilizing agent for industrial applications because of its excellent diffusion capacity and adsorption. A plastic material produced with biopolymers materials as protein and starch from vegetal resources was submitted to EtO sterilization process. The behavior of this material was investigated in reference to the mechanical properties and the biodegradation capacity. To demonstrate the biodegradability degree, a methodology according to international standards, that quantifies the CO2 production, was used; complemented by electronic microscopy. The influence of compost was evaluated in only-soil microcosms. And three controls were run in parallel studies: positive, negative and incidental inhibition. Ethylene oxide treatment increased the ultimate elongation by about 65% without any significant loss of tensile strength. Comparing the sterilized and non-sterilized material we conclude that both were degraded in similar periods of time and thus sterilization process not altered the biodegradation capacity of the bio-plastic material. The presence of compost made a significant difference on the degradation degree. After 60 days of incubation a 70% were reach in presence of compost, while the microcosm without compost showed only a 40%. The microscopy studies demonstrated the gradual assimilation of the plastic material in the microcosm.

BT-P40.
EFFECT OF HETEROLOGUE SEMINAL PLASMA OVER CRYOPRESERVED RAM SPERM

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Sperm cryopreservation produces membrane alterations resembling physiological capacitation. Ram sperm are vulnerable to freezing, producing low fertility rates and the addition of seminal plasma (SP) to frozen/thawed semen reverts this effect. When sperm are incubated with homologue SP, some proteins are attached to the sperm surface and sperm motility is improved. SP from different breeds have similar protein concentration but differ in their electrophoretic pattern. The aim of this work was to evaluate if heterologue SP can improve cryopreserved semen from a particular breed. In order to investigate the effect of SP from other breeds over Frison sperm, a cell suspension was incubated ± SP from Corriedale or Texel and motility was assayed. No differences in total motility but an improvement in progressive motility was observed. To detect proteins that interact with the sperm surface, sperm membrane proteins from Frison were immobilized and incubated with heterologue SP. The retained proteins were analyzed by SDS-PAGE. Four proteins from Corriedale's SP and five from Texel's SP interacted with Frison sperm surface proteins. We concluded that heterologue SP improve frozen/thawed sperm motility and that SP proteins of other breeds interact with heterologue sperm. Whether these proteins are responsible of the improvement in motility requires further research.

EN-P01.
FRUCTOSE OVERLOAD AND ENZYMES INVOLVED IN GSH AND HYDROGEN PEROXIDE METABOLISM IN RAT ADIPOSE TISSUE

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Glutathione peroxidase (GPx) and hydrogen peroxide cellular levels have been associated to insulin resistance (IR). This work examines the enzymatic activities involved in glutathione (GSH) metabolism in epididymal white adipose tissue (EWAT) from rats subjected to FO, a model of IR. Catalase (CAT) and superoxide dismutase (SOD), enzymes involved in the degradation and production of hydrogen peroxide respectively, were also evaluated. Two groups of male Sprague-Dawley rats were studied: C (n=6, tap water to drink) and F (n=6, 10 % fructose solution) by 7 weeks. Enzymatic activities were measured spectrophotometrically: GPx by the GR-coupled method, GR monitoring NAPDH loss in the presence of GSSG, CAT following hydrogen peroxide loss and SOD by the cytochrome c method. GPx activity decreased in F (52±5 nmoles/min/mg prot) vs C (87±10 nmoles/min/mg prot) ($P<0.05$). GR did not show differences: F: 17±2 and C: 21±2 nmoles/min/mg prot. CAT and SOD activities were unchanged by the treatment (CAT = F: 21±3 and C: 21±2 nmoles/min/mg prot and SOD= F: 7±1 and C: 7.4±0.9 U/mg prot). In conclusion, FO decreased GPx activity in EWAT suggesting an impaired capacity to eliminate potential excess in peroxides production. Further experiments should be necessary to evaluate the impact of this effect on GSH steady state levels.

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EN-P02.
A HMG-COA LYASE ISOFORM CODIFICATED BY HMGCLL1 GENE INTERACTS WITH TUBULIN

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Acetoacetyl-CoA thiolase and 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase are enzymes that participate in the HMG-CoA-generating system in hepatic cholesterogenesis and ketogenesis, they exhibit dual mitochondrial and cytoplasmic localization. In contrast, HMG-CoA lyase (HL), an enzyme proper of ketogenesis, appears to be localized in mitochondrion. The identity and regulation of these enzymes remain unclear. Recently in our laboratory, a HMG-CoA lyase new gene (HMGCLL1) has been cloned. Kinetic analysis showed that the product of HMGCLL1, HL-like, has a similar behavior to HMG-CoA lyase activity found in the cytosolic fraction of mouse testicle. Confocal images of cultured cells transfected with the HMGCLL1 gene shows that HL-like co-localized with cytoskeleton and endoplasmic reticulum proteins, indicating that cytoplasmic ketogenesis could occur. In this work, biochemical and immunofluorescent experiments show that: a) HL-like is a peripheral protein of endoplasmic reticulum, b) In vitro, HL-like interacts with tubulin, c) In vitro, tubulin activates HL-like approximately 50 times and d) the cytosolic lyase activity was affected by taxol and nocodazol in cultured cells. These results suggest that tubulin can be involved in the regulation of the cytosolic HMGCoA lyase activity, which is carried out by the HMGCLL1 gene product.

EN-P03.
MEMBRANE TUBULIN AND NA+,K+-ATPASE ACTIVITY OF HYPERTENSIVE PATIENTS ERYTHROCYTES
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We previously showed that, in several cell types, acetylated tubulin associates with plasma membrane Na+,K+-ATPase and inhibits the enzyme activity. This also occurs in normal human erythrocytes. Since Na+ accumulates in erythrocytes of hypertensive patients due to an inhibited state of Na+,K+-ATPase, we considered the possibility that Na+,K+-ATPase was inhibited by interaction with tubulin. We analyzed the amount of tubulin and Na+,K+-ATPase activity in erythrocytes membranes from hypertensive patients (HP) as compared with normal individuals. Our results show that: 1) The amount of total tubulin in membranes of HP erythrocytes is higher than those of controls; 2) Na+,K+-ATPase activity in HP erythrocytes is 50% lower than in controls; 3) The relative proportions of the different isotypes of tubulin (tyrosinated, detyrosinated and acetylated) were the same in membranes erythrocytes of HP and controls; 4) Tubulin coprecipitates with a 110 kDa protein when detergent-solubilized HP's erythrocyte membranes were treated with antibody to total tubulin linked to Sepharose beads; 5) HP`s erythrocytes contain higher quantity of taxol-sedimentable tubulin. According with these results, it seems that the sodium pump in erythrocytes from hypertensive patients is forming part of a complex with a 110KD protein, presumably Na+,K+-ATPase, resulting in inhibition of its enzyme activity.

EN-P04.
REGULATION OF P-TYPE ATPASES ACTIVITIES BY INTERACTION WITH ACETYLATED TUBULIN
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We previously reported that three P-type ATPases (Na+, K+-, H+- and Ca2+-ATPase) are regulated by a common effector: acetylated tubulin. Interaction of tubulin with these three plasma membrane ATPases, while leading to the formation of a relatively stable protein complex, produces inhibition of their catalytic activities. In this communication, we show that: a) In yeast, tubulin/H+-ATPase complex dissociation is dependent on microtubule dynamics; treatment with nocodazole and/or taxol influence stability of the complex and hence ATPase activity; b) in isolated yeast membranes, tubulin is degraded in a pH-dependent manner; c) Calmodulin, which activates plasma membrane Ca2+-ATPase (PMCA), provokes dissociation of the tubulin/Ca2+-ATPase complex and activates enzyme activity in plasma membrane vesicles from synaptosomes; d) PMCA is activated also by ethanol treatment of the membrane vesicles and this occurs also in cultured cells. These results reinforce the idea that ATPases of the P-type are regulated by their interaction with tubulin and that this is modulated by internal factors such as microtubule dynamics, calmodulin and pH, or external factor such as ethanol.

EN-P05.
INCREASED AMOUNT OF MEMBRANE TUBULIN IN ERYTHROCYTES FROM DIABETIC PATIENTS
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Part of tubulin in the cell associates with plasma membrane Na+,K+-ATPase forming a tubulin/ATPase complex that results in inhibition of enzyme activity. We previously demonstrated that treatment of cells with L-glutamate dissociates the complex and stimulates of enzyme activity, and re-formation of the complex requires the presence of glucose. It is known that in erythrocytes (RBC) of diabetics patients (DP), Na+,K+-ATPase activity is partially inhibited. We suspected that high glucose induced association of tubulin with Na+,K+-ATPase with resulting inhibition of the enzyme. Our preliminary studies showed that: a) Plasma membrane from DP's RBC contains 120% more tubulin than controls. b) DP`s RBC contain higher quantity of taxol-sedimentable tubulin. c) The characteristic marginal band of microtubules present in normal RBC seems to be absent in DP's RBC. Results in a), b) and c) were similar to those obtained when normal RBC were treated with 20 mM glucose. A tentative conclusion is that high glucose induces displacement of tubulin to the membrane and interact with Na+,K+-ATPase. In agreement with this hypothesis, treatment of COS and CAD cells with high glucose increased the amount of microtubules and of membrane tubulin/ATPase complex. Our results support the idea that Na+,K+-ATPase is regulated by membrane tubulin which, in turn, is influenced by glucose levels.

EN-P06.
CHANGING THE SUBSTRATE SPECIFICITIES OF M32 PEPTIDASES OF *T. CRUZI* BY SITE-DIRECT MUTAGENESIS
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Trypanosoma cruzi, the causative agent of Chagas Disease, encodes two metallocarboxypeptidases (*Tc*MCPs) of the M32 family which are 64% identical at amino acid sequence level. Despite having very similar tertiary structures, *Tc*MCPs substrate specificity differ markedly. Whereas *Tc*MCP-1 cleaves in vitro the C-terminal Arg or Lys residue from peptides and synthetic substrates, *Tc*MCP-2 prefers aromatic and aliphatic residues at P1' position. Sequence alignments and homology models, based on the crystal structure of *Pyrococcus furiousus* carboxypeptidase, led us to map four sites (Met/Arg-304, Gln/Met-305, Asp/Thr-350, Ala/Ser-351; *Tc*MCP-1 residues listed first) in *Tc*MCPs substrate-binding channels that appeared to be positioned to account for the differences in specificity. To examine the role that these residues might play in determining P1' preference, site-directed mutagenesis was undertaken replacing the *Tc*MCP-1-specific residues by those present in *Tc*MCP-2. The substitution of Met304, Gln305 by Arg304, Met305 swapped the activity of *Tc*MCP-1 from furylacryloyl(FA)-Ala-Lys dipeptide towards FA-Phe-Phe substrate, thus showing that those residues are indeed involved in specificity determination.

EN-P07.
A HIGHLY EFFICIENT FERREDOXIN-NADP(H) REDUCTASE IN THE REDOX METABOLISM OF *LEPTOSPIRA INTERROGANS*

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Ferredoxin-NADP(H) reductases (FNR) are flavoenzymes that deliver NADPH or low potential one-electron donors (ferredoxin, flavodoxin) to redox metabolisms in plastids, mitochondria and bacteria. There are differences in catalytic efficiencies among the members of the FNR family. Whereas plant FNRs display turnover numbers related to the needs of the photosynthesis, bacterial reductases are much less active. It is not known how this catalytic improvement was accomplished but probably was obtained by subtle changes in the protein structure and FAD conformation. We found that FNR from *Leptospira interrogans* (LepFNR), a parasitic bacterium of animals and humans, belongs to the plastidic class of FNRs at variance of all other bacterial enzymes. The structural and biochemical characteristics of plant FNRs revealed for LepFNR support the notion of a putative lateral gene transfer offering *L. interrogans* evolutionary advantages. Looking for the physiological substrate of this reductase we cloned two ferredoxin genes (LFd1 and LFd2) from the bacterium genome. We found that LFd1 displays sequence and spectral similarities with [2Fe-2S] ferredoxins with tiorredoxin-like folding whereas LFd2 is a [4Fe-4S] ferredoxin. Our previous studies indicate that these Fds may be implicated in the non-mevalonate dependent isopronoid biosynthesis pathway, a possible target for antibiotic development.

EN-P08.
STRUCTURAL INTERCONVERSION BETWEEN PLANT AND BACTERIAL FERREDOXIN-NADP(H) REDUCTASES

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FNRs are flavoenzymes that participate in a broad range of redox metabolic pathways. An aromatic residue (Y308 in pea FNR) occupies the NADP(H) binding site; which has to be displaced for the substrate to interact with the active site. In bacterial FNRs, the Y residue is followed by a W which may restricts the Y displacement. Moreover, in plastidic FNRs, a loop maintains the FAD in an extended conformation. The absence of this loop in bacterial FNRs, combined with the lack of movement of the Y residue, could be responsible for the low catalytic efficiency of this type of reductases. In order to shed light into this mechanism, we engineered a plastidic FNR variant. By means of site-directed mutagenesis we deleted the loop and/or added a W residue next to Y308 in pea FNR. Interestingly, the deletion of the loop did not produce a soluble FNR. Nevertheless, the addition of a W residue completely suppressed this effect. This mutant FNR was purified, characterized and their kinetic parameters determined. Surprisingly, these parameters did not differ significantly from those of the wild type pea FNR. Taken together, these observations led us to conclude that the modifications introduced in pea FNR may not be essential for catalytic efficiency and that the terminal aromatic amino acid in bacterial FNRs are mainly involved in the structural integrity of the enzyme.

EN-P09.
MG2+, ZN2+ AND CU2+ ARE ACTIVATORS AND ANTIINHIBITORS OF P. AERUGINOSA PHOSPHORYLCHOLINE PHOSPHATASE

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Phosphorylcholine phosphatase (PchP) from *P. aeruginosa* PAO1 belongs to the HAD superfamily and contains the motifs I, II and III, that are involved in phosphate moiety of the substrate and the Mg^{2+} binding. With phosphorylcholine (Pch) or *p*-NPP as substrates, PchP is also activated by Zn^{2+} or Cu^{2+}. To know the activation mechanism produced by these divalent cations we studied their effect with Pch. Saturation curves of recombinant PchP (expressed in *E. coli*) by different Pch concentrations against variable metal ion concentrations indicated that PchP contains two sites for Pch and two sites for the cation. The affinity of the enzyme for Pch was dependent on the quality of the metal ion. The K_{M1} values for Pch in the presence of Zn^{2+}, Cu^{2+} or Mg^{2+} was 0.03 mM, 0.06 mM or 0.2 mM, respectively. The K_{M2} values, ranged between 0.5 and 1.0 mM. Under assay conditions adapted for each metal ion, the K_{A1} values for Zn^{2+}, Cu^{2+} and Mg^{2+} were 0.06 μM, 1.8 μM and 15 μM, respectively. The K_{A2} values for Zn^{2+}, Cu^{2+} and Mg^{2+} were 10 μM, 20 μM and 1500 μM, respectively. High concentrations of these cations decreased the enzyme inhibition produced by high Pch concentrations. Therefore, the divalent cations besides to act as activators may also act as anti-inhibitors of PchP activity. These results led us to search for a new molecular model for PchP that involves more than the motifs I, II and III.

EN-P10.
A METHOD FOR THE SCREENING OF NADP MALIC ENZYME ACTIVITY IN BACTERIA COLONIES

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Random replacement of residues in protein sequences and selective studies of new variants, which is commonly known as *in vitro* evolution, represent a useful strategy for the identification of residues that confer special kinetic and/or structural properties. The key point in this approach is the screening of a large number of clones in order to identify those that have a special feature such as higher catalytic efficiency, resistance to high temperatures or denaturing agents, among others. The aim of this work was to develop an assay to detect catalytic activity of NADP dependent Malic Enzyme (NADP-ME) in a large number of *E. coli* colonies within a reasonable period of time.
The strategy consists of fixing the proteins in a solid support to facilitate the detection of activity *in vivo*. To reach this purpose, the colonies transformed were absorbed by a nitrocellulose filter and transferred to LB plates. After overnight incubation, when the colonies had reached the same size, the cell walls were lysed and the proteins fixed to the membrane to evaluate enzymatic activities. This assay allows us to recognize recombinant proteins with different levels of activity minimizing the interference of background endogenous activity. To resume, it is remarkable to notice that this technique is an essential tool for the screening of new versions of malic enzymes.

EN-P11.
KINETIC STUDY OF CYCLODEXTRIN GLYCOSYLTRANSFERASE INHIBITION BY REACTION PRODUCTS

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Cyclodextrin glycosyltransferase (CGTase) is used for industrial cyclodextrin (CD) production. The cyclization reaction with starch as substrate proceeds according to Michaelis-Menten kinetic. The enzyme kinetic behavior is important in the design of the reaction process. In previous work, we have observed that β- cyclization activity from *Bacillus circulans* DF 9R CGTase was inhibited by α, β and γ -CD and the inhibition kinetic appeared to be competitive. Here, we study the CGTase inhibition kinetic using maltose and maltotriose as inhibitors.
In order to analyze the effect of the small linear oligosaccharides on the β- cyclization reaction, the previously purified CGTase was incubated with different concentrations of potato soluble starch as substrate in presence or absence of maltose or maltotriose. The reaction conditions were 25 mM phosphates buffer pH 6.4 and 55ºC. β–CD production was determined by the Phenolphthalein Method. Kinetic analysis of the results was performed by the representation of the Lineweaver - Burk plot and the kinetic parameters were determined. The reaction was inhibited by maltose with an acompetitive mechanism that could result from interference with the role of the CGTase E-domain on starch binding. A mixed type of inhibition by maltotriose was observed: competitive inhibition, which take place at the active site and acompetitive inhibition as the observed with maltose.

EN-P12.
MOLECULAR STUDIES OF GLYCEROL-3-PHOSPHATE DEHYDROGENASE FROM TRIATOMA INFESTANS

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Triatoma infestans (*T. infestans*), Chagas' disease vector, acquires wings and the ability to fly after the last molt from fifth instar nymph to adult. The ability to fly is important for insect dispersion. In previous studies, in our laboratory, it was demonstrated that glycerol-3-phosphate dehydrogenase (GPDH), which is involved at glycerophosphate shuttle, increase its activity 30 fold in adults thoracic muscles. Adults muscles should have higher glycolitic and respiratory capacity to support fly activity. Electrophoretic studies from thoracic muscles showed two GPDH isoenzymes. Nymphs predominant isoform has less mobility. The aim of this work is to begin GPDH molecular studies. Using cDNA pools with degenerated primers, we amplified a 200 bp RT-PCR product from 1 to 3 days old *T. infestans* adults thoracic muscles. Upon sequencing and database alignments the fragment shows to be part of GPDH cDNA. Based on the sequence obtained, specific primers were designed for RACE experiment. We successfully amplified completes cDNA of two GPDH isoforms. Sequencing results allow identity, homology and conserved domains comparative studies with others species. GPDH isoform expression,studied by RT-PCR showed stage and tissue specific pattern.

EN-P13.
OXIDATIVE STRESS, NITRIC OXID AND ACTIVITY OF PARAOXONASA IN ACUTE INFARCT OF MYOCARDIUM

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Oxidative stress is a process of cellular damage triggered by free radicals. This process is associated to pathogenic mechanisms of different diseases and physiological processes like the aging. The ischemica cardiopathy and the acute infarct of myocardium (IAM), are processes in which the free radicals are present. The IAM is the acute myocardial necrosis of schemic origin, secondary, to the thrombotic occlusion of a coronary artery. Our objective was to determine in patients with IAM, parameters of oxidative stress and to relate them to marker enzymes of IAM, in order to attach them with the intensity of clinical picture, not communicated previously. In 20 samples of patients with IAM and 15 healthy controls (CO), were determined: markers of oxidative stress (TBARS), nitric oxide (NO) and like marker of antioxidant defense: paraoxonasa (PON). The TBARS were determined according to Jentzsch 1969; PON in agreement Beltowski 2002; NO by reaction of Gries: Schulz 1999 and the enzymes cardiac by automatization. The data were analyzed by program PRISM. We observed a significant increase in TBARS and NO ($P<0001$) but not in the activity of the PON in patients with IAM respect to the CO. We only observed a positive correlation between TBARS and PON ($P<0.01$) and between the activities of CPK and GOT ($P<0.005$) in CO. PON is not a significant antioxidant protection marker in IAM.

EN-P14.
KINETIC AND COMPLEMENTATION STUDIES ON NUCLEOTIDE-BINDING MUTANTS OF GLYCOSYLTRANSFERASE GUMK

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Prokaryotic glycosyltransferases are enzymes involved in the synthesis of polysaccharides. The bacterial glucuronosyltransferase GumK is involved in the transfer of a glucuronic acid residue from UDP-glucuronic acid to mannose-α-1,3-glucose-β-1,4-glucose-P-P-polyisoprenyl, an intermediate step in the synthesis of xanthan, an exopolysaccharide produced by Xanthomonas campestris. Based on previous structural data from our laboratory we studied the molecular contacts that bind the donor substrate to the enzyme, performing site-directed mutagenesis on the residues involved. Besides, based on the proposed catalytic mechanism for inverting glycosyltransferases, we mutated individual acidic residues whose positions in the crystal structure make them candidates to be the catalytic residue. Mutated proteins were purified and the kinetic parameters for each mutant were analyzed trough an enzymatic assay using radioactive donor substrate. Also, the mutant ORFs were cloned in the wide-host plasmid pBBRprom and the effect of mutations on the activity of the enzyme was assessed in vivo trough the quantification of xanthan production in complementation assays on gumK- mutant strain XcK. Finally, the gathering of biochemical and structural data has allowed us to postulate aspartate 157 as the catalytic residue, responsible for the nucleophilic attack on the acceptor substrate.

EN-P15.
ADPGLCPPASE AND UDPGLCPPASE: UNDERSTANDING CARBON PARTITIONING IN *MYCOBACTERIUM TUBERCULOSIS*
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UDP-glucose pyrophosphorylase (EC 2.7.7.9; UDPGlcPPase) catalyzes the reaction Glc1P + UTP <=> UDPGlc + PPi, in the presence of Mg^{2+}. In bacteria, UDPGlc is the main glucosil donor for the biosynthesis of structure polysaccharides, glycolipids, glycoproteins and UDPGlc derived nucleotide-sugars. On the other hand, ADPGlc, synthesized via ADPGlcPPase (EC 2.7.7.27) is the glucosil donor for the pathway leading to glycogen. Characterization of both NDPGlcPPases is critical for the better understanding of Glc partitioning into reserve and structure carbohydrates. We have solved the expression of *M. tuberculosis* ADPGlcPPase. The enzyme was characterized and found to be regulated by PEP and Glc6P. The latter, increase the enzyme affinity for Glc1P and interestingly, was not described as an effector for other ADPGlcPPases. The same scheme was used to obtain UDPGlcPPase. The *M. tuberculosis gal*U gene was cloned into pMIP12 and competent *M. smegmatis* mc^2155 were transformed with the construct [pMIP12/*Mtgal*U]. The protein was expressed with a C-Term HisTag and purified chromatographically. Functional characterization was performed in both UDPGlc synthesis and pyrophosphorolysis. The analysis of the kinetic and regulatory properties of both NDPGlcPPases is useful to propose a regulatory scenario for Glc1P partitioning into different metabolic pathways for carbon and energy in mycobacteria.

EN-P16.
CHARACTERIZATION OF A NEW THIOREDOXIN SYSTEM FROM *ENTAMOEBA HISTOLYTICA*
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The thioredoxin system, composed by thioredoxin and thioredoxin reductase, serves as a reduction equivalents donor in the reduction of disulfides. *Entamoeba histolytica*, a unicellular parasite, usually lives and multiplies within the human gut, under reduced oxygen pressure. During tissue invasion, it is exposed to increased amounts of reactive oxygen species, which are highly toxic for the parasite. The metabolic pathways used by this organism to cope with such environmental changes and redox homeostasis are a matter of our work. Recently, we characterized in *E. histolytica* its functional thioredoxin system (*Eh*TRXR/*Eh*TRX41). In this work, we present the cloning, expression and characterization of a new thioredoxin from *E. histolytica* (*Eh*TRX8). *Eh*TRX8 was evaluated in its ability to catalyse the NADPH dependent reduction of cystine (k = 124,7 $M^{-1}s^{-1}$), GSSG (k = 55.23 $M^{-1}s^{-1}$), TS_2 (k = 118,56 $M^{-1}s^{-1}$) and insulin (k = 2435.13 $M^{-1}s^{-1}$). In addition, the *Eh*TRXR/*Eh*TRX8 system was able to work together to *Eh*P29 (a typical 2CysPrx) in the NADPH dependent reduction of hydroperoxides. The standard redox potential of *Eh*TRX8 (-0.282 V) was estimated by equilibrium with NADPH/NADP at pH 7.0. This work strongly supports the occurrence in *E. histolytica* of a new thioredoxin, which was not previously described in the parasite.
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EN-P17.
KINETIC CHARACTERIZATION AND HOMOLOGY MODELING OF UDPGLUCOSE PYROPHOSPHORYLASE FROM *XANTHOMONAS*
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UDPglucose pyrophosphorylase (EC 2.7.7.9; UDPGlcPPase) is of central importance for the synthesis of polysaccharides, since UDPGlc is the essential precursor in the pathway. We cloned, expressed in a recombinant manner and purified the enzyme from two *Xanthomonas* species: *X. campestris* and *X. axonopodis* pv. *citri*. Both enzymes exhibited identical properties. *Xanthomonas* UDPGlcPPase was characterized as a homodimer, after native- and SDS-PAGE of oligomeric structures stabilized by cross-linking with bisuberate. One particular behavior, not described for other UDPGlcPPases is that at dilutions utilized for activity assay the enzyme undergoes inactivation. The latter is prevented by UTP, which also stabilized the dimeric form of the enzyme in experiments of subunits cross-linking. Kinetics of the enzyme inactivation performed at different UTP concentrations allowed to calculate a dissociation constant for the substrate of 0.010 mM. Studies of homology modeling were performed using the structures of the recently crystallized UDPGlcPPases from *Escherichia coli* and *Corynebacterium glutamicum*. This analysis shows the relevance of the C-terminal alpha-helix, and the helixloop-helix motif around K84 for a tight interaction between subunits. Results suggest that the dimer is the active form of the Xanthomonas UDPGlcPPase and that its dissociation accounts for the enzyme inactivation.

EN-P18.
GLUTATHIONE REDUCTASE FROM *PHAEODACTYLUM TRICORNUTUM*: PURIFICATION AND CHARACTERIZATION
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The cellular redox state is a crucial mediator of multiple metabolic, signalling and transcriptional processes in the cells. Glutathione (GSH) is a widely distributed low molecular weight thiol, which provides reducing equivalents to the cell under conditions of oxidative stress, being oxidized to glutathione disulfide (GSSG) in the process. The enzyme glutathione reductase, a member of pyridine nucleotide-disulfide oxidereductase family, uses the reducing power of NADPH to regenerate GSH from GSSG. Diatoms, brown unicellular algae, are important components of marine phytoplankton, being particularly relevant for geochemical cycling of minerals, and global carbon fixation. As a consequence of their importance in the global ecosystem, their ecology and physiology have been the focus of research. In this work, a putative glutathione reductase was purified from cellular extracts of *P. tricornutum* and functional characterized. The *K*m value for NADPH and GSSG were 14 and 32 μM, respectively. The enzyme was specific for NADPH but not for NADH as electron donor. The enzyme activity was markedly inhibited by metal ions such as Cu^{2+}, Zn^{2+}, Co^{2+} and Ni^{2+}. The activity was maximum at pH 8.0 and 32ºC. Our results strongly support the occurrence of the GSH system in *P. tricornutum*, which was poorly described in this diatom.
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EN-P19.
UNDERSTANDING ALLOSTERIC REGULATION OF ADPGLUCOSE PYROPHOSPHORYLASE FROM *ESCHERICHIA COLI*
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ADPglucose pyrophosphorylase (ADPGlcPPase) from *E. coli* is allosterically activated by fructose-1,6-bisphosphate (FBP), and inhibited by AMP. To elucidate structure to function relationships, we constructed the double mutant enzymes: Q74A/G336D, W113A/G336D, N-δ15/Q74A and N-δ15/W113A. Structural characteristics of these mutants combine changes in protein domains identified with regulatory properties. Single mutants Q74A and W113A have similar specific activity than the wild type enzyme, but are insensitive to FBP activation. Mutants G336D and N-δ15 are pre-activated enzymes, as they exhibit specific activity, in the absence of activator, comparable to that of the wild type in the presence of FBP. The introduction of the second mutation (W113A or Q74A) decreased sensitivity to FBP activation and AMP inhibition of the pre-activated mutants, suggesting that these residues are critical for the conformational change induced by the activator. Also, introduction of the second mutation decreased the specific activity of N-δ15 in the absence of FBP, but scarcely affected G336D kinetic behavior. It is suggested that the activated conformation arises from two different changes in protein structure at the N- and C-term domains, respectively. Results reinforce the idea that AMP inhibition in the mutant proteins, as in the wild type enzyme, mainly modifies the activation produced by FBP.

EN-P20.
CONSTRUCTION AND CHARACTERIZATION OF A CHIMERIC NDPGLUCOSE PYROPHOSPHORYLASE
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Nucleoside-diphospho-sugar pyrophosphorylases (NDPSugPPases) are enzymes involved in carbohydrate metabolism, catalyzing the synthesis of different NDPSug derivatives. All NDPSugPPases from bacteria have a similar 3D structure, except for ADPGlcPPases that are longer proteins mainly because of an additional C-term domain. Most of the ADPGlcPPases are allosterically regulated; with the C-term domain playing a key role in this characteristic, distinctive respect to other NDPSugPPases. We constructed a chimeric gene codifying for a hybrid combining the whole UDPGlcPPase (EC 2.7.7.9) from *Streptococcus mutans* plus the C-term (amino acids 295 to 432) of the ADPGlcPPase (EC 2.7.7.27) from *Escherichia coli*. The former is an unregulated enzyme exhibiting specificity toward UTP, whereas the ADPGlcPPase is specific for ATP and is allosterically regulated by fructose-1,6-bisP (activator) and AMP (inhibitor). The hybrid gene was expressed in *E. coli* as a recombinant-His-tag protein and then purified. The chimeric protein was active as UDPGlcPPase and it was sensitive to activation by 3P-glycerate. Thus, the hybrid enzyme exhibited regulatory properties similar to the ADPGlcPPase from cyanobacteria. Results agree with the view that the C-term domain is critical for allosteric regulation of PPases, being its interaction with the N-term determinant for the specificity to the regulatory molecule.

EN-P21.
STUDYING CARBON METABOLISM IN *PHAEODACTYLUM TRICORNUTUM*
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Diatoms are key constituents of aquatic phytoplankton communities. Their contribution to marine primary productivity was estimated between 30-40%. They can grow auto-, hetero-, and mixotrophically. Because of its small genome (less than 30 Mb) and the possibility of being routinely transformed, *Phaeodactylum tricornutum* has emerged as a model species for dissecting diatom biology. Our interest is to characterize changes in enzymes levels and properties associated with different growing conditions of the algae. We optimized the diatom auto- and mixotrophic growth using diverse substrates in batch mode. The highest biomass concentration for mixotrophic growth was obtained using acid casein hydrolysate (1.5 g/l) and glycerol (46.05 g/l) as nutrients. Distinct activities of enzymes involved in carbon metabolism were assayed in extracts of the diatom samples. Also, the genes coding for fructose1,6bisP aldolase (FBA), three glyceraldehyde3P dehydrogenases, two P-glycerate kinases (PGK) and trioseP isomerase were cloned. Expression of the genes in *Escherichia coli* rendered the proteins in inclusion bodies, except for FBA and PGK1 that were obtained as soluble recombinant enzymes. Characterization of the kinetic, regulatory and structural properties of the enzymes is being helpful to better understand metabolic pathways and their regulation in the diatom.

EN-P22.
PATHOLOGICAL DAMAGE INDUCED BY A BOTHROPS ALTERNATUS HEMORRHAGIC METALLOPROTEINASE
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Snake Venom Metalloproteinases are multiple domain enzymes whose principal toxic effects are due to disruption of the hemostatic system. In a previous study we evaluated a wide variety of local damage induced by a hemorrhagic metalloproteinase (class P-III) isolated from *B. alternatus* venom from Argentinean northeast.
In this work, systemic alterations produced by this metalloproteinase were studied in mice. Histological and lethality assays were carried out. Various i.v. doses of hemorrhagin were evaluated. One hour after injection, mice were sacrificed and lungs, heart, kidneys and liver were dissected out for light microscopic observation. Lethality was determined by the i.p. route. Histological observations in lungs showed mainly hemorrhagic areas in alveolar spaces, congestion and enhancement of alveolar septum due polymorphonuclear infiltrate and mononuclear cells. Kidney examination revealed congestion, subcapsular hemorrhage with local capsule detachment, inflammatory infiltrate and low degeneration of tubular cells. Blood vessels congestion and turbid degeneration of hepatocytes was observed in liver only at high doses. No alterations were evidenced in the myocardium at doses assayed. Besides, 250 μg/g of enzyme induced 100% lethality. It is concluded that *B. alternatus* metalloproteinase play a relevant role in systemic damage characteristics of bothropic envenomation.

EN-P23.
DISRUPTION OF CARBOHYDRATE METABOLISM SECONDARY TO AN ACUTE TOXIC PORPHYRIA
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As it has been reported by our group, key enzymes of gluconeogenesis and glycogenolysis are impaired in liver both in acute and chronic porphyrias; such alterations leading to a diminished availability of glucose. The aim of the present work was to study the behavior of key enzymes of glycolysis such as phosphofructokinase (PFK) and pentose pathway such as glucose 6-phosphate dehydrogenase (G6PDH) in an acute porphyria model. Famale Wistar rats were treated with 2-allyl-2-isopropylacetamide (AIA, 100, 300 or 500 mg/kg.bw) and 3,5-diethoxycarbonyl 1,4-dihydrocollidine (DDC, 50 mg/kg.bw). As porphyric parameter, hepatic 5-aminolevulinic-synthase (ALA-S) was measured. Enzymatic activities were monitored spectrophotometrically, measuring the decrease of NADH in a coupled reaction for PFK and the increase of NADPH for G6PDH. The results obtained provide clear evidence of a diminution of PFK depending on the dose of AIA. This decrease was about 60% respect to the control for the highest dose of AIA assayed. G6PDH showed a decrease for minor and medium doses but an increase for the highest one. These results suggest that in this intoxication, PFK responds in such a way not to reduce even more the availability of glucose already diminished by the gluconeogenic blockade, whereas G6PDH exhibits a protective behavior against oxidative stress promoted by drugs.

EN-P24.
HYDROLYSIS OF BOVINE MILK CASEIN BY ASPARTIC PEPTIDASES FROM SALPICHROA ORIGANIFOLIA FRUITS
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The use of enzymes in the food industry is becoming more and more interesting both for the production and for the conservation of food as an alternative or in addition to physico-chemical processes. Among them, aspartic peptidases are extensively used in food biotechnology, especially in cheese making. Calf rennet is the conventional milk clotting enzyme used in cheese making around the world. However, in many countries, the short life and the high price of calf rennet have encouraged the search for substitutes from sources such as microorganisms, fish and plants. In this work, aspartic proteinases from *Salpichroa origanifolia* (Lam) Thell fruits were investigated as a source of enzymes to be used in cheese making as an alternative or in addition to calf rennet. Milk clotting activity was measured on bovine skim milk at 37ºC and pH 6.2. Whole casein was incubated with plant extract and some breakdown products were characterized by electrophoresis. Urea-PAGE was employed to analyze the profile of hydrolysis of casein fractions. Characteristic and differential proteolytic patterns on the main casein components were obtained. αs1- casein was gradual and partially degraded during 5 hours of hydrolysis and β-casein was rapidly and extensively degraded after 3 hours of incubation. New peptides appeared in all cases after 60 min. The patterns could be correlated with the modification of functional properties. Further studies are now in process to identify biologically active peptides upon enzymatic digestion.

EN-P25.
SUCROSE HYDROLYSIS IN A FILAMENTOUS CYANOBACTERIUM UNDER SALT STRESS AND DIAZOTROPHIC GROWTH
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Cyanobacteria are prokaryotic organisms that perform oxygenic photosynthesis. Some strains also carry out atmospheric N_2 fixation. The sucrose (Suc) metabolic pathway in cyanobacteria, similar to that of plants, has been associated mainly with osmoprotection during environmental stresses (salt and osmotic stresses). Recently, it was demonstrated that Suc has a key role in *Anabaena* sp. PCC 7120 during diazotrophic growth in both vegetative and heterocyst cells. We described and characterised two alkaline/neutral invertases (A-Inv-A and N-Inv-B), responsible for Suc hydrolysis. Then we studied the function of both isoforms in filaments under salt stress and during N_2 fixation by comparing their enzymatic activities, polypeptide and mRNA levels and determining the transcription start points. Also we constructed transcriptional fusions of their promoter regions with *gfp* to study their localisation and expression. We also generated and characterized A/N-Inv "knock-out" mutants and studied their ability to grow in diazotrophic conditions and salt stress response. The results highlighted the relevant function of a differential regulation of A-Inv-A and N-Inv-B in both conditions. Furthermore, besides its role in osmoprotection, we found a high Suc turnover during salt stress, probably supplying energy to overcome the stressful situation.

EN-P26.
OSCILLATORY STATES OF HEXACHLOROBENZENE (HCB) INTOXICATED RAT MITOCHONDRIA
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Mitochondria in vitro manifest a phenomena generally behave as damped harmonic oscillators. Oscillatory changes in mitochondrial volume are followed by continuous measurements of A_{520} nm. Damping factors of peaks and troughs were used to compare the different experimental conditions; peaks correspond to mitochondrial contraction and troughs to expansion. These parameters indicate the grade of elasticity in the membranes when their volume changes, higher values correspond to a smaller damped oscillatory response of mitochondria usually due to lesser elasticity in the membranes.
Normal and HCB intoxicated female Wistar rats (150-180 g body weight) were used. HCB was administered (1 g/kg bw) daily by stomach tube. Liver mitochondria from both groups were used. The data obtained in both groups show very similar mean damping factor values. They would indicate that HCB treatment does not have marked influence on mitochondria membrane properties. It has been reported that oxidative phosphorylation uncouples as 2,4-dinitrophenol, can inhibit rat mitochondria oscillation. At low concentration they increase the damping factor by increasing the passive permeability of membrane to protons. Our data are not in agreement with these results, may be due to a weaker uncoupling effect of the HCB metabolite (pentachlorophenol) at the concentration levels in the experiments.

EN-P27.
GLYCOGEN PRIMER: GLYCOGENIN GLUCOSYLATION MECHANISM AND ITS SELF-MODULATION
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The rate and mechanism of incorporation of the first sugar molecule into the tyrosine residue of glycogenin (Gn) was studied for the first time by autoxylosylation from UDP-xylose. The molecular aggregation of the enzyme and the kinetics of the subsequent autoglycosylation was also studied, using non-glucosylated Gn (apo-31) and partially glucosylated Gn (wt-31) truncated at residue 270. Gn eluted as dimer when loaded at high protein concentration and monomer loaded at low concentration. The first sugar was incorporated into the tyrosine residue by an intramolecular reaction, with slightly higher catalytic efficiency by monomeric than dimeric Gn. The intermediate growth was best achieved by intramolecular transglucosylation between the subunits of dimeric Gn. The final growth of the Gn-bound maltosaccharide occurred at a high specific reaction rate by intramolecular glucosylation of monomeric Gn. The whole autoglucosylation of apo-31 showed high specific glucosylations at the low concentrations at which the enzyme existed as monomer. Higher concentrations resulted in formation of an immature Gn-bound maltosaccharide, reported to be unable to serve as primer. This concentration dependent self-modulation of the full autoglucosylation is a heretofore not described property by which Gn might be involved in the regulation of the *de novo* biosynthesis of glycogen.

EN-P28.
INSIGHT INTO FERREDOXIN-NADP(H)-REDUCTASE CATALYSIS INVOLVING THE INVARIANT GLU IN THE ACTIVE SITE
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Ferredoxin-$NADP^+$ reductases are enzymes harbouring one molecule of non-covalently bound FAD. These flavoproteins (FNR/FPR) catalyse reversible reactions between obligatory one- and two-electron carriers. Even though members of the FNR/FPR superfamily (plant-type) exhibit a conserved structure, sequence analysis reveals two groups: plastidic and bacterial FNR. The first one, characterized by an extended FAD conformation and high catalytic efficiency, is involved in photosynthesis transferring e^- to $NADP^+$ from reduced Fd. The second one displays a folded FAD molecule and low turnover rates, and catalyses the inverse reaction, reduction of oxidized Fd from NADPH.
Analysis of protonation behaviour of FNR titrable residues was done for all crystallographic structures available up to date, as well as many FNR-substrate complexes. To compute the protonation probabilities Poisson-Boltzmann electrostatic calculations and Metropolis Monte Carlo titration calculations have been performed. A highly conserved Glu in the active site showed a differential titration behaviour in plastidic and bacterial FNRs. This difference could be related to the direction of the physiological reaction they catalyze. Also, its protonation probability proved to be sensitive to the substrate bound, suggesting that the Glu might be involved in FNR catalytic mechanism as proton donor/acceptor.

EN-P29.
RAT MITOCHONDRIAL RESPIRATION PARAMETERS IN HEXACHLOROBENZENE (HCB) EXPERIMENTAL PORPHYRIA
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The toxic effect of HCB in mammals closely resembles human Porphyria Cutanea Tarda (PCT), both clinically and biochemically. Normal (N) and HCB intoxicated female Wistar rats (150-180 g body weight) were utilized for this study. HCB was administered (1 g/kg bw) daily by stomach tube. Liver mitochondria from both groups were used. Effects on electron transport and oxidative phosphorylation were compared in both groups. The respiratory control ratio (RCR), indicator of mitochondrial membrane integrity and the capacity of phosphorylation, is defined as the ratio of the ADP stimulated velocity (state3) to the velocity after exhaustion of ADP (state 4). There was not significant difference in the mitochondrial respiratory velocities during active metabolism with the substrates malate-glutamate or succinate. The efficiency of oxidative phosphorylation process was further tested in HCB treated mitochondria: a sizable increase in the respiratory velocities at rest (state 4) and a significant decrease of the means of RCR and ADP/O ratio occurred with these substrates used. The presence of albumin entirely restores both RCR and ADP/O ratio and the respiratory velocity (state 4), that is, fully reverse the uncoupling of the oxidative phosphorylation process of HCB mitochondria. The complete restoration by albumin indicates that no irreversible damage occurs in the inner mitochondrial membrane.

SB-P01.
IMMUNOCYTOLOCALIZATION OF *RHIZOCTONIA* ALPHA-GLUCANS AND POTATO ALPHA-GLUCANASE ACTIVITY
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The wall interface between plants and pathogens plays an important role in their interaction. Studies about fungal cell walls are scarce and the results show the existence of α-1, 3-glucans in addition to ß-glucans. α-1, 3-glucans are not present in plant cell walls and α-glucanase activity in plants has not been described before. In a previous work, we purified an α-1, 3-glucan from a binucleated non-pathogenic *Rhizoctonia* (BNR) isolate, which induces plant defense responses. In order to study the architecture of the fungal cell wall, as well as the accessibility and localization of the α-glucan, we prepared an antibody against the α-1, 3-glucan and analyzed its localization by TEM. Immunolocalization showed the presence of the α-1, 3-glucan in the intercellular spaces and along the cell walls, mainly on the inner layers. This result and the presence of the α-glucan in liquid culture, where BNR was grown, confirmed that the glucan had been secreted. In addition, α-glucanase activity in potato sprouts was detected using cell wall glucans from the pathogenic isolate *R. solani* AG-3 as substrates. Our results suggest that the presence of α-1, 3-glucans could be related with the formation and integrity of cell wall and with plant-fungi interactions. This is the first report that describes α-glucanolytic activity in plants.

SB-P02.
CRYSTALLOGRAPHIC AND RMN STRUCTURAL STUDY OF 5' GCG-AGA-GC 3' AND 5' CGC-GAG-AGC-G 3' OLIGONUCLEOTID

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In order to compare the structural differences observed in solution and in solid state, both oligonucleotides were studied by RMN and X-ray diffraction. These two oligonucleotides differ only in a GC base-pair added in the stem. Crystals were obtained in the presence of hexamine Co, diffraction spectra were collected on beamline ID14-1 at European Synchrotron Radiation Facility and on Rigaku MicroMax-O7 generator, MAR345 detector respectively. The 5' GCG-AGA-GC 3' sequence crystallized in the space group $P6_322$ with unit cell parameters a = 36.619(2) b = 36.619(2) Å c = 65.090 Å , $\alpha = \beta = 90°$, $\gamma = 120°$ and showed ordered diffraction at 1.5 Å resolution; the 5' CGC-GAG-AGC-G 3' oligonucleotide crystallized in the space group C2 with unit cell parameters of a = 64.461 (3) b = 24.589 (2) c = 43.279 (3) Å $\alpha = \gamma = 90°$, $\beta = 126,9(3)°$ and showed ordered diffraction at 2.7 Å resolution. Molecular-replacement trials are currently in progress. The solution study was performed using a 500 MHz Varian Inova NMR spectrometer. Measurements of the Diffusion coefficient were used in order to assess if these oligonucleotides are in the hairpin or the duplex conformation. Molecular modelling under NMR restrains are in progress.

SB-P03.
ESSENTIAL RESIDUES FOR 14-3-3 ANAAT STABLE COMPLEX FORMATION

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The importance of 14-3-3 protein family has lately risen to a key position in cell biology because these proteins contribute to a wide range of regulatory processes in the cell. These are small proteins (~30 kD) that form both homo- and heterodimers and can bind more than 200 target proteins. These partner proteins are very specific and can bind 14-3-3 with higher affinity in a phosphorylation dependent manner. Therefore specificity and affinity are concepts that require special care when regards to 14-3-3 complexes. To get further understanding of the h14-3-3/oANAAT complex *in vivo* we performed Bimolecular Fluorescence Complementation (BiFC) assay. For this purpose, 4 different ANAAT mutants were created by site directed mutagenesis: glutamic acids 50 and 87, defined as anchor residues, and arginines 53 and 89, defined as hot spot, were replaced with alanines. Mutants were cloned in mammalian expression vectors fused to truncated YFP, as well as human wild type h14-3-3z. HeLa cells were visualized 24 hours after transfection with these constructs. Results indicate that these residues are essential for stable complex formation. oANAAT anchor and hot spot mutants could not interact in a stable manner with h14-3-3. The correlation between in silico predictions and in vivo observations, in cellular physiological conditions, was corroborated.

SB-P04.
3-D STRUCTURE OF *T. CRUZI* BROMODOMAIN PROTEINS AS POTENTIAL DRUG DESIGN TARGETS FOR CHAGAS

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Introduction: *Trypanosoma cruzi* is the etiologic agent of Chagas' disease. The proteins implicated in *T. cruzi* bromodomains are potential protein targets for drug design. The objective of our work is the resolution by X-ray crystallography of the three-dimensional structure of these proteins as a first step for rational drug design based on the structure. Materials and Methods: The *T. cruzi* proteins under study are: TcBDF2 (bromodomain factor 2) and TcBDF3 (bromodomain factor 3). They have been overexpressed in *E. coli* as an N-terminal fusion His-tag protein. The proteins have been obtained in native form and purified by Ni-Sepharose affinity chromatography. The folding status was evaluated by Native-PAGE and Urea-PAGE. Crystallization screenings of His-TcBDF3 have been carried out by microbatch under-oil with and without N-acetyl-lysine. Results and Discussion: His-TcBDF2 y Tc-BDF3 have been overexpressed and purified up to a concentration of > 20mg/ml. Electrophoretic assays have confirmed that the proteins are in native state. Preliminary crystallization screenings are under way.

SB-P05.
3-D STRUCTURE OF *T. CRUZI* mRNA MATURATION PROTEINS AS POTENTIAL DRUG TARGETS FOR CHAGAS DISEASE

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Introduction: *Trypanosoma cruzi* is the etiological agent of Chagas' disease. In *T. cruzi*, regulation of gene expression is governed by mRNA processing and stability. The proteins implicated in mRNA maturation are potential targets for drug design. The objective of our work is the resolution by X-ray crystallography of the three-dimensional structure of these proteins as a first step for rational drug design based on the structure. Materials and Methods: The *T. cruzi* proteins studied are: TcFIP1-like (factor interacting with Pap1) and TcCPSF30 (cleavage and polyadenylation specificity factor). N-terminal His-tagged versions of these proteins have been overexpressed in *E. coli*. The proteins, obtained in inclusion bodies, have been purified by affinity chromatography. Refolding screenings have been done to obtain native protein for crystallization and other structural assays. The folding status of the protein was evaluated by electrophoretic and electroscopic analyses in each case. The crystallization screening was performed by microbatch under oil method. Results and Discussion: TcCPSF30 have been overexpressed, purified and refolded. TcCPSF30 crystals have been obtained in different conditions. Crystallization conditions will be improved to obtain optimal samples for diffraction analyses. TcFIP1 have been overexpressed and purified satisfactory. The refolding screenings are underway.

SB-P06.

3-D STRUCTURE OF *T. CRUZI* ENERGETIC METABOLISM PROTEINS AS POTENTIAL DRUG TARGETS FOR CHAGAS DISEASE

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Introduction:*Trypanosome cruzi* is the etiological agent of Chagas' disease. Proteins involved in *T cruzi* energetic metabolism are potential targets for drug design. The objective of our work is the resolution by X-ray crystallography of three-dimensional structures of these proteins as a first step for rational drug design. Materials and Methods: *T. cruzi* proteins under study are: TcAK (arginine kinase), TcNDPK1 (nucleoside diphosphate kinase 1) and TcADK1 (adenylate kinase 1). Proteins have been overexpressed in *E. coli* and purified by Ni^{+2}-Sepharose affinity chromatography. The crystallization screening has been done by micro-batch technique. Hanging drop and capillary methods have been used for the optimization step. Results and Discussion: TcNDPK1 was overexpressed, purified and crystallized. The initial crystallization condition was found using the microbatch method. Optimal crystals for X-ray diffraction analysis were obtained using capillary-batch and vapor diffusion methods. TcADK1 was overexpressed and purified in the native form. TcADK1 initial steps of crystallization screening are under development.Finally, TcAK structure has been refined up to 1.9 Å resolution.

SB-P07.

CYCLODEXTRIN GLUCANOTRANSFERASE FROM BACILLUS CIRCULANS DF 9R: STRUCTURE-FUNCTION RELATIONSHIP

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Cyclodextrin glucanotransferase (CGTase; EC 2.4.1.19) is a member of the α-amylase family and catalyses the conversion of starch and related alpha glucans (1→4) into cyclodextrins (CD) through intramolecular transglycosylation as well as intermolecular transglycosylation reactions and a weak starch hydrolysis. Three Histidine residues (H140, H233 and H327) are conserved in most α-amylases and other related enzymes and in all known CGTases and are located in conserved regions. Enzyme treatment with ^{14}C ethoxyformyc anhydride, tryptic digestion, mass spectrometry and amino acid sequence analysis allowed us to show that H233 is the most reactive His residue and plays an important role in hydrolytic and α-, β-CD formation activity. Moreover, we found that this enzyme is the only member of a 38 CGTase group having Glutamine instead of Glycine in the -6 subsite, a very conserved region involved in the substrate binding cleft. This finding supports the fact that the enzyme produces an α-CD/β-CD ratio higher than those from other *Bacillus circulans* strains which is important for industrial applications.

SB-P08.

RELATION BETWEEN A MODERATE ZINC DEFICIENCY AND APOPTOSIS IN RATS LUNGS

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Apoptosis is an important process in remodelling of tissues during injury. Zinc is a key element for maintenance of functional integrity in different tissues. Recent studies demonstrated that depletion of zinc may lead to rapid activation of apoptotic cascade in acute lung injury. However the relation between apoptosis process and zinc deficiency, is not clear yet. We analysed the expression in rat lung of Bax, Clusterina and Bad, genes involved in apoptotic pathways and if the supplementation with zinc diet (ZD refed) could lead to recuperation of normal function of lung. Wistar male rats (200 ± 20 g), were separated in three groups: (Co) with 30 mg/kg of zinc in the diet, (ZD) with 5mg/kg of zinc and (ZD refed) with 5mg/kg of zinc, this group was refed during 10 days. Total mRNA was isolated from lung tissue by using TRIzol. Aliquots of 2 μg of cDNA were used in the amplifications by PCR using specific primers. Beta actin was used as internal control.

We detected a significant increase in Bax ($p<0.05$) in ZD group, in contrast in ZD refed decreased compared to control. Bad and Clusterina expression were not significantly different in ZD and ZD refed group. These results confirm the effect of zinc deficiency in rat lung. In ZD refed it exists, at least in part, a recuperation of lung normal function and inhibition of apoptosis.

SB-P09.

MODERATE ZINC DEFICIENCY AND ANTIOXIDANT SYSTEMS IN RAT EPIDIDYMIS

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The induction of high levels of ROS subjects the cell to a state of oxidative stress, which may damage tissues. In previous studies we have analyzed the change in activities of antioxidant defense system under a moderate zinc deficiency. In all cases we obtained differences in epididymis in the Zinc deficiency (ZD) group. Here we present the effect of Zinc deficiency on level of NADPH oxidase (NOX-2), Superoxide Dismutase (SOD-2) and Catalase (CAT) expression and histology in caput epididymis. Wistar male rats (200 ± 20 g), were separated in three groups: (Co) with 30 mg/kg of zinc in the diet, (ZD) with 5mg/kg of zinc and (ZD refed) with 5mg/kg of zinc, this group was refed during 10 days (Ral). Total mRNA was isolated from tissue by using TRIzol. Aliquots of 2 ìg of cDNA were used in the amplifications by PCR using specific primers. Beta actin was used as internal control. Pieces of caput epididymis were processed for light microscopy. In epididymal caput Nox, SOD-2 and CAT increased in ZD vs Co ($p<0.05$, $p<0.01$ and $p<0.01$ respectively) and Ral vs Co increased ($p<0.01$) in SOD-2. Epididymal caput region shows clear signs of injury presumably as consequence of oxidative damage. The expression of antioxidant enzymes is correlated with their activities as adaptive responses against oxidative stress and this situation is reflected in the histoarchitecture of epididymis.

SB-P10.
NEW INSIGHTS INTO THE INTERACTION MAP OF *TRYPANOSOMA CRUZI* RIBOSOMAL P PROTEIN COMPLEX

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The large subunit of the eukaryotic ribosome possesses a long and protruding stalk formed by the ribosomal P proteins. It is involved in the translation step of protein synthesis through interaction with elongation factor 2 (EF2). The stalk, in *T.cruzi*, is composed by four proteins of about 11 KDa, Tc1α, TcP1β, TcP2α, TcP2β and a fifth TcP0 of about 30 KDa. A yeast two-hybrid based protein interaction map was generated that indicated a central role for TcP0. Using Surface Plasmon Resonance the kinetics of each of the possible interactions between the members of this protein family was tested. The assembly of ternary complexes was also assessed. TcP0 and TcP2â proteins were able to form homo dimers and also interacted with both P1 and both P2 proteins. The interaction domains of TcP0 and TcP2β were mapped using truncated proteins and synthetic peptides, respectively. All proteins, with the exception of TcP2β, interact with the EF2 but the small proteins showed a stronger affinity than TcP0. The C-terminal region of TcP2β (peptide R13) seems to be involved in the interaction with EF2, since R13 was able to inhibit the second part of the association phase. Other regions of the protein may also be involved, because R13 had no effect on the first step of the association phase. Compared to other species, *T. cruzi* clearly displays a specific pattern of ribosomal P protein interactions.

SB-P11.
CHARACTERIZATION OF A STABLE COMPLEX BETWEEN INACTIVE INSULIN-DEGRADING ENZYME WITH Aβ PEPTIDE

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Insulin degrading enzyme (IDE) is central in the turnover of amyloid β(Aβ) in the mammalian brain. Biochemical and genetic data support that IDE may play a role in late onset Alzheimer's disease (AD) by preventing Aβ aggregation, one of the characteristic process observed in AD. We have reported that the catalytic domain of natively folded recombinant wild-type IDE (wt-IDE) was capable of forming a stable complex with Aβ (IDE-AβCx) that resisted dissociation after treatment with denaturing agents. To study the nature of this interaction independent of degradation, we generated a catalytically inactive IDE mutant IDE-E111Q. Biochemical and biophysical analyses showed that this IDE mutant had identical folding to wt-IDE but lacked proteolytic activity. The kinetics of IDE-AβCx formation showed that although wt-IDE and IDE-E111Q had a similar $t_{1/2}$ (~ 30 min) the latter exhibited a 5-fold increase in the yield of IDE-AβCx. Isolation by size exclusion chromatography and time-course analysis by Western blot showed that IDE-AβCx binding was irreversible under physiological conditions. Moreover, dynamic light scattering and electron microscopy revealed that IDE-E111Q altered the kinetic and morphological characteristics of Aβ aggregation by reducing amyloid fibril formation. Our results suggest an unprecedented dead-end interaction between IDE and Aβ with implications in the pathogenesis of AD.

SB-P12.
THE CATALYTIC DOMAIN OF INSULIN-DEGRADING ENZYME FORMS A HIGHLY STABLE COMPLEX WITH AMYLOID B PEPTIDE

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Insulin-degrading enzyme (IDE) is central to the turnover of insulin and one of the amyloid β (Aβ) proteases in the mammalian brain. Here we show that a natively folded recombinant IDE was capable of forming a stable complex with Aβ that resisted dissociation after treatment with 70% formic acid, 8M urea or 6M guanidine. This interaction was also observed with rat brain IDE and detected in an SDS-soluble fraction from AD cortical tissue Aβ sequence 17-27, known to be crucial in amyloid assembly was sufficient to form a stable complex with IDE. Monomeric as opposed to aggregated Aβ was competent in complex formation following a very slow kinetics ($t_{1/2}$ ~45 min). Partial denaturation of IDE as well as pre-incubation with a 10-fold molar excess of insulin prevented complex formation, suggesting that the irreversible interaction of Aβ takes place with at least part of the substrate binding site of the protease. Limited proteolysis showed that Aβ remained bound to a ~23 kDa N-terminal fragment of IDE in an SDS-resistant manner. Mass spectrometry after in gel digestion of the IDE-Aβ17-27complex showed that peptidic fragments derived from the catalytic site of IDE were recovered with Aβ. We propose that the recognition mechanism of IDE towards amyloid forming peptides may lead to a heterologous amyloid-like interaction with novel implications in cerebral amyloidoses.

SB-P13.
CRYSTALLIZATION OF PKA REGULATORY SUBUNIT FROM *SACCHAROMYCES CEREVISIAE*

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In mammals, the PKA holoenzyme exists as a complex of two catalytic subunits and a regulatory (R) subunit dimer. R subunits have a dimerization and docking domain at the N terminus; at the C terminus, two tandem cAMP-binding domains and in between a flexible hinge region, including a substrate-like inhibitor sequence that docks to the active site cleft of the C subunit. Much effort has been put in studying structure-function relationships in mammalian PKAs. The aim of this work is to solve the structure of the R subunit from *S. cerevisiae* in order to learn which of the features of mammalian R are general and which are specific to this system. We over-expressed and purified different deletion mutants of the protein: Δ85, Δ136, Δ138, Δ140, Δ149, Δ154 and Δ168. The over-expression and stability of the proteins were assessed both in yeast and in bacterial expression systems. For setting up crystallization trials, we used sitting drop vapor diffusion, hanging drop, vapor diffusion and microbatch methods. The protein was set up with different standard crystallization conditions: Crystal Screen 110, Crystal Screen HT-130, Clear Strategy I HT, Clear Strategy II HT, Structure Screen I & II HT & PACT HT. Images of each crystal trial were manually taken at 0, 2, 7, 14, 21 and 28 days after setup. Successful crystals will be sent to the Stanford Synchrotron Radiation Laboratory.

NS-P01.
RHYTHM & BLUES & STRESS: CIRCADIAN STRESS RESISTANCE IN CAENORHABDITIS ELEGANS
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Circadian rhythms in physiological patterns are ubiquitously found in nature. Recently, circadian rhythms in swimming rate and osmotic stress tolerance have been described in L1 larvae of Caenorhabditis elegans. Here we describe daily variations in adult stress responses at the behavioral and molecular level. Oxidative stress tolerance is maximal at ZT (ZeitgeberTime) 12 (0900 h, lights on) and minimal at ZT 0 (2100 h, lights off) (ANOVA, $p<0.001$). Osmotic stress tolerance was peaks at ZT 0 and decreases at ZT 12, in accordance to what was reported for L1 larvae. Since genes involved in controlling circadian behaviors are not known in C. elegans, we are studying stress-related genes and clock gene homologues. Expression of stress-related genes gpx-1 (glutathione peroxidase) and gpdh-1 (glycerol-3-phosphate dehydrogenase) shows circadian fluctuations, peaking at night.
We also studied circadian variations in mutant strains for stress-related genes. In the case of the AM1 strain, which bears a single point mutation in the gene osr-1 (a negative regulator of the gpdh-1 pathway), osmotic stress tolerance rhythms were abolished (ANOVA, $p<0.05$).
These results demonstrate the existence of daily rhythms in adult nematodes, and will allow us to employ this model for future chronobiological studies.

NS-P02.
CIRCADIAN VARIATION OF ANTIOXIDANT ENZYMES AND TBARS IN HIPPOCAMPUS OF VITAMIN A-DEFICIENT RATS
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High levels of oxidative stress and alterations in the antioxidant defense system lead to a deficit in cognitive function. Circadian expression of GPx and CAT has been reported in different tissues. Our objective was to investigate the daily variation in protein levels and activity of GPx and CAT and lipoperoxidation in the hippocampus of control, vitamin A-deficient and vitamin A-recovered rats. GPx and CAT protein levels were determined by Western blots. Their activities were measured following Flohe & Gunzler (1984) and Aebi (1984), respectively. MDA levels following Droper and Hadley (1990). We observed a rhythmic variation in the antioxidant enzymes protein levels and activities, consistent with the circadian mRNA expression. Serum and hippocampus MDA levels showed also a daily variation. Vitamin A deficiency modified the circadian pattern of GPx and CAT and phase shifted the daily variation of lipoperoxidation. Refeeding vitamin A-deficient rats with 15 days of control diet, led to a partial recovering of antioxidant enzymes activities and MDA levels. Daily variation in the activity of the antioxidant defense system suggests that its components are under biological clock-control and retinoids might have a role in the circadian regulation of cellular redox state and consequently memory and learning.
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NS-P03.
CICHLASOMA DIMERUS: A FISH MODEL FOR STUDYING STRESS AND SOCIAL CONTROL OF REPRODUCTION
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Animal behaviour has an important role in brain physiology and reproduction. The presence of dominant conspecifics may produce a suppression of reproductive maturation in non-dominant individuals. The South American freshwater fish C. dimerus represents an interesting model for studying social control of reproduction at ethological, morphological, physiological and molecular levels. Adults of both sexes (Std. lenght 9.33±1.62 cm) were placed in neutral community tanks. The experiment was repeated 3 times. Four different kinds of social status were defined: Territorial-Dominant male (TDm), Reproductive Aggressive female (RAf), T noD (TND) and noT-noD (NTND) fish. Animals were sacrificed at the most aggressive period (6 days post hatching larvae from TDm and RAf couple); weighted and measured. Cortisol and testosterone concentrations were analyzed by RIA from plasma samples. Brains were fixed for performing IHC for GnRH neurons. Pituitary homogenates were used to estimate FSH, LH and SL content. Gonads were weighted and fixed to study their morphology in different social status. NTND showed 3 fold higher levels of cortisol, lower testosterone, FSH and SL levels. There were no differences in LH levels. NTND males had their testis full of sperm though spermiation could not take place. Future studies will focus on the neuro-electrophysiological and transcriptional level.

NS-P04.
CLONING OF GTHS IN CICHLASOMA DIMERUS PITUITARY AND BRAIN: EFFECT ON PITUITARY HORMONES RELEASE
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FSH and LH play key roles in vertebrate gametogenesis and steroidogenesis. They are mainly synthesized in the pituitary gland. While investigating the ontogeny of FSH and LH cells in the cichlid fish C. dimerus by IHC, we unexpectedly found immunoreactive neurons in the preoptic area, sending their projections through different brain areas and neurohypophysis. Western blot and IHC techniques applied to adult brain confirmed these findings. To further demonstrate the extra-pituitary expression of these hormones, we performed RT-PCR detecting sequences coding for FSH-ß and LH-ß subunits in C. dimerus pituitary and brain. The expression of these transcripts in both organs was consistent with their peptide expression showing a high sequence homology when compared with other phylogenetically related fish. Pituitary explants were cultured for 2 days with different concentrations of LH or FSH. The culture media was analyzed by western blot. LH produced a dose-dependent increase in pituitary LH and SL release and an increase in FSH release. No effect was observed on GH. The effect on PRL was not consistent among treatments. FSH produced an inhibition in LH release, a dose-dependent increase FSH and SL release and no effect on PRL and GH release. This is the first work showing, in vertebrates, an effect of brain derived GtHs on different pituitary cells.

NS-P05.
DIFFERENTIAL LOCALIZATION OF ARGINYLATED CALRETICULIN IN CELLS UNDER TRANSLATIONAL OR TRANSCRIPTIONAL STRESS

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The posttranslational arginylation of proteins consists in the covalent union of an arginine into an acidic amino acid at the NH2-terminus. We demonstrated the posttranslational incorporation of arginine into calreticulin (CRT). An antibody that specifically recognizes endogenous arginylated CRT (R-CRT) was developed. By immunocytochemistry it was observed in NIH3T3 cells that R-CRT is localized in the cytosol while CRT is localized in the ER. Under environmental stress conditions (e.g. modified calcium homeostasis, heat and oxidative conditions) that activate the unfolded protein response and shuts down protein synthesis, R-CRT was found associated to stress granules that are a consequence of abortive translational initiations. On the other hand, as a response to DNA damage conditions (e.g. Doxorubicin (Dx), UV), R-CRT was found in cell nucleus. It was described that Dx and UV treatment activate transcriptional factors such as p53, which are translocated to the nucleus. We found that nuclear translocation of R-CRT is different in p53 -/- cells with respect to p53 +/+ cells, suggesting a possible relationship among both. The intranuclear redistribution found for arginylated CRT might be related to pro-apoptotic mechanisms triggered by genotoxic agents. Thus, posttranslational arginylation of CRT can regulate its intracellular localization and perhaps its cell function.
Supported by SECyT-UNC, CONICET, ANPCyT-PICT.

NS-P06.
IRON UPTAKE IN CULTURED SCHWANN CELLS. EVIDENCE FOR A NON TRANSFERRIN MEDIATED PATHWAY

Salis C, Usach V, Goitia B, Setton-Avruj P.
Cátedra de Química Biológica Patológica, FFyB, IQUFIB, UBA-CONICET, IIMHNO-UBA. E-mail: setton@qb.ffyb.uba.ar

Schwann cells (SCs) synthesize the myelin sheath in the peripheral nervous system. We have previously described that Fe^{3+} and holotransferrin (hTf) prevent SCs from dedifferentiation through the cAMP/protein kinase A pathway. A major route for the delivery of iron to the cells is the receptor mediated import although a non-Tf mediated pathway has also been described. The aim of the present work is to study the effect of serum deprivation on the kinetics of iron uptake. Cultured SCs were submitted to serum deprivation and supplemented with Fe^{3+}, aTf, hTf for 3, 6, 24 and 72 hs in the presence of ^{59}Fe ($FeCl_3$). Intracellular iron content was measured by atomic absorption. The presence of Tf receptors (TfR) was evaluated by western blot. Our results demonstrate that there is no uptake of ^{59}Fe in the cells incubated in the presence of serum or apoTf. However, in the cells supplemented with Fe^{3+} or hTf the uptake of ^{59}Fe increases from 1h to 24hs. These results agree with those referred to intracellular iron content. Deferroxamine blocks the effects promoted by Fe^{3+} or hTf. The expression of TfR increased in SCs submitted to serum deprivation. The supplementation with aTf decreases TfR levels to 66% while hTf supplementation decreases levels to 30%. Our results suggest the existence of an iron uptake mechanism independent of Tf which SCs would express when submitted to a harsh environment.

NS-P07.
BONE MARROW MONONUCLEAR CELLS CHARACTERIZATION-PARTICIPATION IN THE WALLERIAN DEGENERATION PROCESS

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We have previously described the spontaneous migration or after an intraortical injection of fresh bone marrow mononuclear cells CD34+ to the distal stump of a ligated sciatic nerve. The aim of the present work is to characterize the fresh and cultured bone marrow cells in order to know which is the population that migrates to the injured nerve during the Wallerian degeneration process. The cells were isolated from the bone marrow extruded from tibia and femur bone from adult Wistar rats. A group was immediately processed and the other was plated. After 24 and 48hs the non adherent cells were separated and the adherent cells were grown to confluence. In the suspended and plated cells the presence of a progenitor cell marker (CD34), a pan-haematopoietic cell marker (CD45) and a myelomonocytic cell marker (CD11b) were detected by western blot.
The cultured cells represent a heterogeneous population with small rounded, spindle shaped or large flattened morphology. Most cells grow and exhibit fibroblast like morphology in reaching confluence. This population is CD11b- and CD45-. After 24 and 48hs in culture the adherent and non adherent cells are CD34+ and the non adherent cells are CD45+. Our results demonstrate the existence of a progenitor cell population that spontaneously migrates to the injured nerve in order to participate in the degeneration-regeneration process.

NS-P08.
EXPRESSION OF BIOACTIVE FUSION PROTEINS OF E. COLI HEAT-LABILE TOXIN B SUBUNIT AND SYNAPSIN PEPTIDES

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Pentameric B subunit of E. coli heat-labile toxin (LTB) mediates holotoxin binding to host cell membranes and has been used as efficient mucosal carrier of chemically or genetically coupled antigens. We constructed two recombinant hybrid molecules by fusing the N-terminal of C (residues 113-308) and ABC (residues 1-308) domains of rat synapsin to the C-terminus of LTB. Both LTBSC and LTBSABC fusion proteins were inductively expressed as cytoplasmic inclusion bodies in E. coli and then purified by affinity chromatography on Ni-agarose under denaturant conditions. For in vitro refolding and oligomerization of the hybrid proteins dialysis and dilution were assayed to decrease denaturant concentration. We examined the effect of several conditions regarding redox environment and presence of L-arginine in refolding buffer. About 97 % of LTBSABC and 90% of LTBSC were recovered soluble in 0.05 M Tris buffer pH 8 containing 2 M urea. Both of them retained the ability to bind to GM1 receptor in an enzyme-linked immunosorbent assay. Also LTBSC induced oral tolerance when fed to rats before active immunization as it was shown by inhibition of the specific DTH response and in vivo and in vitro cell proliferation. These results strongly suggest that these fusion proteins are suitable for exploring mucosal adjuvant activity in autoimmune disorders involving antigens from central nervous system.

NS-P09.
VALIDATION OF A RABBIT MODEL OF NEUROPATHY INDUCED BY IMMUNIZATION WITH GANGLIOSIDES
Comín R[1], Moyano AL[1], Alaniz MA[1], Lardone RD[1], Theaux R[2], Nores GA[1]
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The induction of neurological symptoms by immunization of rabbits with gangliosides has been a controversial topic for many years. Recently, Yuki *et al*. (Ann. Neurol. (2001) 49: 712) described an immunization protocol, including keyhole limpet hemocyanin in addition to gangliosides, that induced a neurological disease resembling human Guillain-Barré syndrome. We employed this protocol in our laboratory and succeeded in reproducing the disease. Five different experiments were performed in a total of 26 rabbits. Three groups were prepared: Group 1, immunized with GM1; Group 2, with commercial bovine brain ganglioside (BBG) and Group 3; with BBG prepared in our laboratory. Rabbits in groups 2 and 3 showed clinical signs more severe than those in group 1: disease onset appears early and most of the animals developed irreversible severe weakness of the four limbs. Rabbit serum samples were screened for immunoreactivity against GM1 and structurally related glycolipids by TLC-immunostaining. All rabbits immunized showed the same pattern: IgG reactivity against GM1, GA1 and GD1b. Sciatic nerve cross sections showed pathological changes in accordance with axonal damage. Despite minor variations in onset time and severity of the induced disease, the model proved to be reproducible.

NS-P10.
GANGLIOSIDE COMPLEXES: AUTOANTIBODY TARGETS IN A RABBIT MODEL OF NEUROPATHY
Moyano AL, Comín R, Alaniz ME, Lardone RD, Nores GA.
CIQUIBIC-CONICET, Dpto Química Biológica, Fac Ciencias Químicas, Universidad Nacional de Córdoba. E-mail: almoyano@mail.fcq.unc.edu.ar

Recent studies showed that some ganglioside complexes (GSCs) are target antigens for serum antibodies in patients with GuillainBarré syndrome (GBS) and suggested that anti-GSC antibodies may be involved with particular clinical features of GBS. Conformational epitopes recognized by these antibodies may be present in neuronal plasma membranes where gangliosides reside clustered in functional microdomains. Consequently, binding of antibodies to GSCs are likely to cause nerve dysfunction. In this study, we induced an experimental neuropathy (resembling GBS) by sensitization of rabbits with bovine brain gangliosides according to the procedure of Yuki *et al*. (Ann. Neurol. (2001) 49: 712). Rabbit serum samples were obtained on the acute-phase of neurological symptoms and screened for immunoreactivity against glycolipids (GA1, GM1, GD1b, GD1a and GD1b) by thin layer chromatography immunostaining. All serum samples displayed typical IgG-antibodies against GA1, GM1 and GD1b . In addition to this reactivity, they also showed IgG-reactivity against GM1/GD1b, GM1/GD1a and GM1/GT1b complexes. These results are the first description of experimental induction of this type of antibodies and provide new evidences about the role of anti-GSCs antibodies in the development of neuropathies.

NS-P11.
INHIBITION OF CA2+ DEPENDENT GLUTAMATE RELEASE IN CEREBRAL CORTEX SYNAPTOSOMES OF RATS WITH EAE
Vilcaes AA, Furlan G, Roth GA.
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In order to better understand the mechanism leading to progressive neurological deficit in multiple sclerosis, herein we explore the contribution of glutamate release in the cerebral cortex synaptosomes isolated from experimental autoimmune encephalomyelitis (EAE) animals. Active disease was induced in rats by intradermal injection with complete Freund's adjuvant containing bovine myelin. The evoked neurotransmitter release was monitored using an enzyme-linked fluorometric assay. The total Ca^{2+} dependent glutamate release induced by KCl and 4-aminopyridine, but not by ionomycin was significantly decreased during the acute stage of the disease. When the animals were totally recovered from clinical signs, the neurotransmitter release stimulated by all the inductors were similar to controls. No significant differences were found in cytosolic Ca^{2+} measured using Fura-2. The alteration of neurotransmitter release was concomitant with an inhibition of synapsin phosphorylation. Ours results shown that the inhibition observed on the Ca^{2+} dependent neurotransmitter release from cerebral cortex synaptosomes in EAE is specific and correlates with the course of the clinical disease. Moreover, they suggest an alteration in the metabolism of proteins involved in the vesicular glutamate release more than a deregulation in the influx of cytosolic Ca^{2+}.

NS-P12.
BIOSILICIFICATION (CHALCEDONY) IN HUMAN ASTROCYTOMA AND GLIOBLASTOMA MULTIFORME FROM AGED PATIENTS
Prado Figueroa M[1], Casavilca S[2], Sánchez J[2].
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Chalcedony was observed in human brain from aged patients (Prado Figueroa *et al*., 2006). Chalcedony consists of nanoscale intergrowths of quartz and moganite. Chalcedony formation is due to the relationship between pH and Eh. Glioblastoma multiforme is the most aggressive type of primary brain tumor and it involves overexpression of the enzyme carbonic anhydrase IX (CA IX). From these evidences, we decided to document the presence of chalcedony in histological sections taken from human astrocytoma II and glioblastoma multiforme from autopsy derived and also biopsy. In those sections, naturally occurring fluorescent mineral was observed by confocal laser scanning microscopy. Our data showed that there is a green fluorescent chalcedony in astrocytoma II with a crystalline mineral phase. In glioblastoma, green fluorescent chalcedony appeared mostly as a partially polymerized fluid that is infiltrating the tissue. CA IX is involved in physiological pH regulation and may be in the depolymerization of silica in the human brain.

NS-P13.
c-FOS CONTROLS CNS AND PNS TUMOR GROWTH
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CIQUIBIC (CONICET) Dpto. Qca Biológica, Fac. Cs. Qcas, Univ. Nac.Córdoba. E-mail: ntomasini@fcq.unc.edu.ar

Neurofibromatosis Type I (NF1) is a syndrome affecting 1:3500 individuals and is characterized by the development of tumors in the peripheral (PNS) and the central (CNS) nervous systems. Many patients progress to generate glioblastomas, the most malignant brain tumors. The NPcis mice are an animal model of NF1; these mice develop CNS gliomas and PNS tumors (neurofibromas).
Previously we found that c-Fos, in addition to its AP-1 transcription factor activity, associates to the endoplasmic reticulum (ER) and activates the synthesis of phospholipids for the genesis of membrane required for cell proliferation and growth of many human brain tumors.
Herein, c-Fos expression and c-Fos dependent phospholipid synthesis activation were examined in brain tumors and neurofibromas from NPcis mice. High levels of c-Fos co-localizing with ER markers were found both in brain tumors and in neurofibromas. These levels correlate with high rates of cell proliferation. Blocking c-Fos expression in brain of NPcis mice decreased phospholipid synthesis and proliferation levels to those of non-treated animals, whereas this treatment in peripheral tumors significantly lowered tumor burden. Similar results were observed in 10 stable cell lines from neurofibromas and in another 10 from brain tumor cells. It is hypothesized that controlling c-Fos expression will enable the control of PNS and CNS tumor growth.

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