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PABMB

10th Congress
X Congreso
-Panamerican Association for Biochemistry and Molecular Biology-

SAIB

41st Annual Meeting
XLI Reunión Anual
-Argentine Society for Biochemistry and Molecular Biology-
-Sociedad Argentina de Investigación Bioquímica y Biología Molecular-

SAN

20th Annual Meeting
XX Reunión Anual
-Argentine Society for Neurochemistry-
-Sociedad Argentina de Neuroquímica-

December 3-6, 2005
Pinamar, Buenos Aires
- República Argentina -

Acknowledgments

The following Institutions supported the organization of this Meeting

- **Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)**
- **Agencia Nacional de Promoción Científica y Tecnológica**
- **Universidad Nacional de Buenos Aires**
- **Fundación Instituto Leloir**
- **International Union of Biochemistry and Molecular Biology (IUBMB)**
- **European Molecular Biology Organization (EMBO)**
- **Federation of European Biochemical Societies (FEBS)**
- **International Brain Research Organization (LARC-IBRO)**
- **International Society for Neurochemistry (ISN)**
- **International Collaboration on Repair Discoveries (ICORD)**
- **Sociedad Española de Bioquímica y Biología Molecular**
- **Sociedad Mexicana de Bioquímica**
- **Sociedade Brasileira de Bioquímica e Biologia Molecular**
- **Sociedad de Bioquimica y Biología Molecular de Chile**
- **Sociedad de Bioquimica y Biología Molecular de Uruguay**

Members of the PABMB Board

Members of the SAIB Board

CONGRESS OVERVIEW

SATURDAY 03-12-05

10:00 - 15:00	**REGISTRATION**		
15:00 - 16:00	**Room B** **"SAN" YOUNG INVESTIGATORS LECTURES**		
16:00 -16:30	**Room A** **OPENING CEREMONY**		
16:30 - 17:30	**Room A** **OPENING LECTURE** ***Lorenzo Lamattina*** ***"Nitric oxide builds its own history in plants"***		
17:30 - 19:30	**Room A** **"Fundación Inst. Leloir"** **SYMPOSIUM** ***"Glycobiology I "***	**Room B** **"SBBq" (Brasil)** **SYMPOSIUM** ***"Chimerical Proteins and Peptides"***	**Room C** **"IBRO"** **SYMPOSIUM** ***"Neurodegenerative Diseases From Protein Structures to Neurobiology"***
19:30 - 20:00	*Coffee break*		
20:00 - 21:00	**Room A** **"EDUARDO DE ROBERTIS" LECTURE** ***Enrico Stefani*** ***"Heart Estrogen Receptors"***		

SUNDAY 04-12-05

	Room A		
8:15 – 9:15	**"Ranwel Caputto" LECTURE** ***Ramón Latorre*** ***"Too hot or too cold? TRP channels as temperature sensors"***		
	Room A		
9:15 – 10:15	**"EMBO" LECTURE** ***Jean Marc Egly*** ***"Xeroderma Pigmentosum and Trichothiodystrophy: From Transcription to Clinic"***		
10:15 – 10:45	*Coffee break*		
	Room A	**Room B**	**Room C**
10:45 – 12:45	**SYMPOSIUM** ***"Recent Advances In Cellular Calcium Signaling"***	**"SBBM" (Uruguay) SYMPOSIUM** ***"Systems Biology and Integrative Biochemistry"***	**SYMPOSIUM** ***"Signal Transduction In Plant Development"***
			Room C
12:45 – 14:30	*Lunch*		***Technical Microconferences "Lovob Cientifica"***
	Room A	**Room B**	**Room C**
14:30 – 16:30	**ORAL COMMUNICATIONS** • ***Cell Biology (CB) 1-5*** • ***Proteomics (PT) 1-3***	**ORAL COMMUNICATIONS** • ***Microbiology (MI) 1-4*** • ***Lipids (LP) 1-4***	**ORAL COMMUNICATIONS** •***Enzymology and Structural Biology (EN) 1-8***
16:30 – 17:00	*Coffee break*		
	Room A	**Room B**	**Room C**
17:00 – 18:00	**LECTURE** ***Jean Lud Cadet*** ***"Participation of multiple apoptotic pathways in methamphetamine (METH)-induced cell death in the brain"***	17:00 – 19:00 **"Fundación Inst. Leloir" SYMPOSIUM** ***"Glycobiology II"***	17:00 – 19:00 **SYMPOSIUM** ***"Lipids"***
18:00 – 19:00	**LECTURE** ***Ligia Toro*** ***"MaxiK channel signaling domains"***		
19:00 – 21:00	**POSTERS** • ***Biotechnology (BT) 1-60*** • ***Cell Biology, (CB) 1-74*** • ***Enzymology and Structuctural Biology (EN) 1-25*** • ***Behavior, Learning and Memory, (BL) 1-28*** • ***Biochemistry of the Nervous System, (NC) 1-20***		
21:30	***Welcome cocktail***		

MONDAY 05-12-05

8:15 – 9:15	**Room A** **"IUBMB" LECTURE** ***John S. Parkinson*** ***"Bacterial chemotaxis: dissecting the three-protein brain of E. coli"***		
9:15 – 10:15	**Room A** **"PABMB" PLENARY LECTURE AWARD** ***George Kenyon*** ***"Creatine Kinase: Structure and Function of an Energetic Enzyme"***		
10:15 – 10:45	***Coffee break***		
10:45 – 12:45	**Room A** **SYMPOSIUM** ***"From Signal-Transduction to Virulence In Bacteria"***	**Room B** **"IUBMB" SYMPOSIUM** ***"Pathways for Protein Degradation"***	**Room C** **SYMPOSIUM** ***"Regulation of Behavior and Hormone Secretion by Brain Peptides and Neurotransmitters"***
12:45 – 14:15	***Lunch***		**Room C** ***Medias Exhibitions in Biochemistry***
14:15 – 16:45	**Room A** **ORAL COMMUNICATIONS** • ***Plant Biochem and Mol Biol (PL) 1-7*** • ***Enzymol. and Struct. Biol. (EN) 9-11***	**Room B** **ORAL COMMUNICATIONS** • ***Biotechnology (BT) 1-6*** • ***Gene expression (GE) 7-9***	**Room C** **ORAL COMMUNICATIONS** • ***Signal transduction (ST) 1-10***
16:45 – 17:00	***Coffee break***		
17:00 – 19:00	**Room A** **"SBBq-RTPD Network" SYMPOSIUM** ***"Proteomics"***	**Room B** **"ISN" SYMPOSIUM** ***"Signaling Pathways In Development, Plasticity and Diseases"***	**Room C** **SYMPOSIUM** ***"Eukaryotic Gene Expression, Impact In Cell Physiology and Disease"***
19:00 – 21:00	**POSTERS** • ***Enzymology and Structural Biology, (EN) 26-65*** • ***Gene Expression, (GE) 1-43*** • ***Genomics, (GN) 1-2*** • ***Lipids, (LP) 1-21*** • ***Microbiology (MI) 1-65*** • ***Chronobiology, (CH) 1-2*** • ***Development Neurobiology, (DN) 1-15*** • ***Neurotransmitters, Neurorreceptors, Neuromodulators, (NR) 1-24***		***PABMB General Assembly***
21:00	***SAN General Assembly***		

PROGRAM

SATURDAY, December 3, 2005

10:00 - 15:00 **REGISTRATION**

15:00 - 16:00 **Room B**
"SAN" YOUNG INVESTIGATOR LECTURES

16:00 - 16:30 **Room A**
OPENING CEREMONY

16:30 - 17:30 **Room A**
OPENING LECTURE
Lorenzo Lamattina
Instituto de Investigaciones Biológicas, Universidad Nacional de Mar del Plata, Buenos Aires. Argentina
"Nitric oxide builds its own history in plants"
Chair: Nestor Carrillo

17:30 - 19:30 **Room A**
"Fundación Instituto Leloir" SYMPOSIUM
"Glycobiology I"
Chairperson: Armando J. Parodi, Fundación Instituto Leloir, Buenos Aires, Buenos Aires, Argentina

17:30 - 18:10
S1
UNIQUE SORTING AND SUGAR CHAIN PROCESSING IN ER QUALITY CONTROL
Gerardo Z. Lederkremer.
Dept. of Cell Research and Immunology, Tel Aviv University, Israel.

18:10 - 18:50
S2
MONITORING ACQUISITION OF TERTIARY AND QUATERNARY STRUCTURES IN ER QUALITY CONTROL
Caramelo Julio Javier.
Fundación Instituto Leloir, Buenos Aires, Argentina.

18:50 - 19:30
S3
THE STRUCTURE OF GLYCOGEN SYNTHASE FROM *Pyrococcus abyssi*. CLUES FOR THE REGULATION OF THE EUKARYOTIC ENZYMES
Joan J. Guinovart.
Departament de Bioquimica i Biologia Molecular, Universitat de Barcelona, Spain.

17:30 - 19:30 **Room B**
SBBq (Brasil) SYMPOSIUM
"Chimerical Proteins and Peptides"
Chairperson: Leila Maria Beltramini, Universidade de São Paulo, São Carlos, SP, Brasil.

17:30 - 18:00
S4
RECONSTRUCTING PULCHELLIN *IN VITRO*: TOWARDS BIOTECHNOLOGICAL APPLICATIONS
Ana Paula Ulian de Araújo
Instituto de Física de São Carlos, Universidade de São Paulo, Brasil.

18:00 - 18:30
S5
ENZYMES INVOLVED IN AMINO ACID CATABOLISM IN TRYPANOSOMATIDS: FROM SEQUENCE TO FUNCTION
Nowicki Cristina.
Depto. de Química Biológica, Fac. de Farmacia y Bioquímica, Buenos Aires, Argentina.

18:30 - 19:00
S6
IMPROVING THE ACTIVITY OF SUGARCANE CYSTATINS BY DIRECTED EVOLUTION
Flavio Henrique Silva.
Department of Genetics and Evolution Federal University of São Carlos, São Carlos, Brasil.

19:00 - 19:30
S7
HYBRID AND MODIFIED ANALOGS OF SYNTHETIC ANTIMICROBIAL AND ANTIGENIC PEPTIDES
Georgina Tonarelli.
Facultad de Bioquímica y Cs. Biológicas, UNL, Santa Fé, Argentina.

17:30 - 19:30 **Room C**

"IBRO" SYMPOSIUM

"Neurodegenerative Diseases: From Protein Structures to Neurobiology"

Chairperson: Claudio O. Fernández, Instituto de Biología Molecular y Celular de Rosario, Argentina.

17:30 - 18:00
S8
THE ROLE OF OXIDATIVE METABOLISM OF DOPAMINE IN NIGRO-STRIATAL NEURODEGENERATION IN PARKINSON´S DISEASE (PD)
Paris, I.; Fuentes-Bravo, P.; Cardenas, S.; Graumann, R.; Lozano, J.; Riveros, P.; Perez, C.; Caviedes, P.; and Segura-Aguilar, J.
Mol. Clin. Pharmacol., Faculty of Medicine, University of Chile.

18:00 - 18:30
S9
MODULATION OF NEURONAL APOPTOSIS BY p75 NEUROTROPHIN RECEPTOR
Luis Barbeito.
Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay.

18:30 - 19:00
S10
CONFORMATIONAL BASIS AND NOVEL TARGETS FOR ß-AMYLOID-INDUCED NEURONAL DYSFUNCTION IN ALZHEIMER'S DISEASE

Sergio T. Ferreira.
Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

19:00 - 19:30
S11
STRUCTURAL BIOLOGY AND PATHOGENESIS OF PARKINSON DISEASE: TARGETING α-SYNUCLEIN
Bertoncini, C.W. , Rasia, R.M. , Binolfi, A., Hoyer, W. , Jovin, T.M , Zweckstetter, M. , Griesinger, C., Fernández, C.O.
IBR, CONICET, UNR, Argentina and Max Planck Institute for Biophysical Chemistry, Germany.

19:30 - 20:00 ***Coffee break***

20:00 - 21:00 **Room A**
"Eduardo de Robertis" LECTURE
Enrico Stefani
Department of Anesthesiology, UCLA School of Medicine, Los Angeles, USA
"Heart Estrogen Receptors"
Chair: Osvaldo Uchitel

SUNDAY, December 4, 2005

8:15 - 9:15 **Room A**
"Ranwel Caputto" LECTURE
Ramón Latorre
Dpto Biofisica y Fisiología Molecular, Centro de Estudios Científicos (CECS), Valdivia, Chile.
"Too hot or too cold? TRP channels as temperature sensors"
Chair: Hugo J.F. Maccioni

9:15 - 10:15 **Room A**
"EMBO" LECTURE
Jean Marc Egly
Institut de Génétique et de Biologie Moléculaire et Cellulaire, Cedex, France.
"Xeroderma Pigmentosum and Trichothiodystrophy: From Transcription to Clinic"
Chair: Alberto Kornblihtt

10:15 - 10:45 ***Coffee break***

10:45 - 12:45 **Room A**
SYMPOSIUM
"Recent Advances in Cellular Calcium Signaling"
Chairperson: Ana Russo de Boland, Universidad Nacional del Sur, Bahía Blanca, Buenos Aires.

10:45 - 11:15
S12
SIGNAL TRANSDUCTION AND GENE EXPRESSION REGULATED BY CALCIUM RELEASE FROM INTERNAL STORES IN EXCITABLE CELLS
Enrique Jaimovich, José M. Eltit and César Cárdenas.
Centro de Estudios Moleculares de la Célula, ICBM, Facultad de Medicina, Universidad de Chile.

11:15 - 11:45
S13
THE CONTRIBUTION OF CALCIUM INFLUX TO MITOCHONDRIA-TRIGGERED CELL DEATH
Javier García-Sancho.
Instituto de Bioquímica y Genética Molecular (IBGM), Universidad de Valladolid y CSIC, Facultad de Medicina -Valladolid, España.

11:45 - 12:15
S14
NICOTINIC ACID ADENINE DINUCLEOTIDE PHOSPHATE (NAADP) A NEW INTRACELLULAR SECOND MESSENGER
Eduardo Nunes Chini.
Department of Anesthesiology, Mayo Clinic and Foundation, Rochester, USA.

12:15 - 12:45
S15
Ca^{2+}-ATPase: MECHANISM OF ACTION AND ITS RELEVANCE IN PATHOLOGICAL STATES
Juan Pablo F.C. Rossi.
IQUIFIB, Facultad de Farmacia y Bioquímica, Buenos Aires, Argentina.

10:45 - 12:45 **Room B**

SBBM (Uruguay) SYMPOSIUM

"Systems Biology and Integrative Biochemistry"

Chairperson: Luis Acerenza, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay.

10:45 - 11:05
S16
WHAT IS SYSTEMS BIOLOGY?
Acerenza, Luis.
Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay.

11:05 - 11:30
S17
GLUCOKINASE SUBCELLULAR LOCATION
Loranne Agius, Victoria Payne, Catherine Arden, Mohammed Mukhtar.
School of Clinical Medical Sciences-Diabetes, The University of Newcaste upon Tyne, UK.

11:30 - 11:55
S18
LOOKING FOR NEW TARGETS IN CANCER THERAPY FROM A METABOLOMIC APPROACH
Marta Cascante, A. Ramos, P. Vizan, .J. Boren, P. de Atauri, J.J. Centelles, L. G. Boros, P. WN. Lee , V. Selivanov.
Dept. Bioquímica i Biol. Mol., CeRQT-Parc Cientific Barcelona, Associated Unit to CSIC, Univ. Barcelona. España.

11:55 - 12:20
S19
STANDARDS, SOFTWARE, MOTIFS AND SYSTEMS BIOLOGY
Herbert Sauro.
Keck Graduate Institute, Claremont, USA.

12:20 - 12:45
S20
HOW CAN WE MAKE SYSTEMS BIOLOGY WORK?
Athel Cornish-Bowden, María Luz Cárdenas, Juan-Carlos Letelier, Jorge Soto-Andrade and Flavio Guíñez Abarzúa.
CNRS-BIP, Marseille, France; and Biología and Matemáticas, Facultad de Ciencias, Santiago, Chile.

10:45 - 12:45 **Room C**

SYMPOSIUM

"Signal Transduction in Plant Development"

Chairperson: Elena G. Orellano, IBR, Universidad Nacional de Rosario. Rosario, Argentina.

10:45 - 11:15
S21
SALICYLIC ACID AND THE ACTIVATION OF CELLULAR STRESS PROTECTION GENES IN ARABIDOPSIS THALIANA
Loreto Holuigue.
Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile. Chile

11:15 - 11:45
S22
STRESS-ACTIVATED PHOSPHOLIPID SIGNALLING PATHWAYS IN PLANT
Teun Munnik.
Swammerdam Institute for Life Sciences, University of Amsterdam, The Netherlands.

11:45 - 12:15
S23
REGULATION OF GENE EXPRESSION BY MICRO RNA FAMILIES IN PLANTS
Javier Palatnik.
Instituto de Biología Molecular y Celular de Rosario, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Argentina.

12:15 - 12:45
S24
DEVELOPMENTAL AND ENVIRONMENTAL INTER-ACTIONS IN PLANT RESPONSES TO STRESS
Montserrat Pages.
IBMB-CSIC, Barcelona, Spain.

12:45 - 14:30 ***Lunch***

12:45 - 14:30 **Room C**

Technical Micro Conferences (Lovob Científica)

14:30 - 16:30 **Room A**

ORAL COMMUNICATIONS

Cell Biology (CB) 1-5

Proteomics (PT) 1-3

Chairpersons: Carlos O. Arregui, Universidad de San Martín.
Mirtha Biscoglio de Jiménez Bonino, IQUIFIB (UBA-CONICET).

14:30 - 14:45
CB-C1
c-FOS TYR PHOSPHORYLATION REGULATES c-FOS/ ER ASSOCIATION AND c-FOS DEPENDENT PHOSPHOLIPID SYNTHESIS ACTIVATION
Portal M.M., Ferrero G.O. and Caputto B.L.
CIQUIBIC-CONICET, Dpto. de Química Biológica, Facultad de Ciencias Químicas, Univ. Nacional de Córdoba, Argentina.

14:45 - 15:00
CB-C2
ENDOPLASMIC RETICULUM-BOUND PTP1B IS TARGETED TO NEWLY FORMING CELL-MATRIX ADHESIONS
Davies Sala, Maria G., Hernández, Mariana V., Balsamo, Janne, Lilien, Jack, and Arregui, Carlos O.
Instituto de Investigaciones Biotecnológicas, IIB-INTECH, Universidad de San Martín, Buenos Aires, Argentina and Department of Biological Sciences, University of Iowa, USA.

15:00 - 15:15
CB-C3
CK2 ECTOKINASE ACTIVITY OF CELLS TRANSFECTED WITH CK2 SUBUNITS
Rodríguez, Fernando A., Allende, Catherine C. and Allende, Jorge E.
ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile.

15:15 - 15:30
CB-C4
DOWN-REGULATION OF LRP-1 EXPRESSION BY INSULIN IN J774 MURINE MACROPHAGES CELL LINE
Ceschin, DG., Cáceres, LC, Sánchez, MC; Chiabrando, GA.
Dpto. de Bioquímica Clínica - CIBICI (CONICET), Facultad de Ciencias Químicas, Univ. Nacional de Córdoba, Córdoba, Argentina.

15:30 - 15:45
CB-C5
USE OF *Chorella kessleri* EXTRACTS FOR THE PROTECTION OF HEPATOCYTE PRIMARY CULTURES FROM PIG AND HUMAN LIVERS
Ithuralde, Esteban, Lorenti, Alicia, Rogati, Sebastián, Barbich, Mariana, Chaufan, Gabriela, Juárez, Angela, Ríos de Molina, M. C.
Departamento de Química Biológica. FCEyN-CONICET, Ciudad Universitaria, Bs. As, Argentina. And Instituto de Ciencias Básicas y Medicina Experimental. Hospital Italiano de Buenos Aires. Buenos Aires, Argentina.

15:45 - 16:00
PT-C1
USING PROTEOMICS TO IDENTIFY POTENTIAL VACCINES COMPONENTS AGAINST *Bordetella pertussis*
Perez Vidakovics, M. Laura, Paba Martinez, Jaime, Lamberti, Yanina, Serra, Diego, Yantorno, Osvaldo, Ricart,, Carlos André, Del Valle Sousa, Marcelo and Rodriguez, M. Eugenia.
CINDEFI, Fac. Cs. Exactas-UNLP, La Plata , Argentina and CBSP, Universidad de Brasilia, Brasil.

16:00 - 16:15
PT-C2
A PROTEOMIC APPROACH TO DEFINE THE CONOTOXIN BINDING SITES IN THE NICOTINIC ACETYLCHOLINE RECEPTOR

Cortez, Leonardo, Hellman, Ulf, Marino-Buslje, Cristina, Biscoglio de Jiménez Bonino, Mirtha.
IQUIFIB (UBA-CONICET). Facultad de Farmacia y Bioquímica. UBA Buenos Aires, Argentina and Ludwig Institute for Cancer Research, Uppsala, Sweden.

16:15 - 16:30
PT-C3
IDENTIFICATION OF COMPONENTS OF THE POLYADENYLATION COMPLEX IN *Trypanosoma cruzi*: ANALYSIS OF THE INTERACTION INTERFACE BETWEEN TCCPSF30 AND TCFIP1 FACTORS AND ITS POTENTIAL USE AS DRUG TARGET
Natalia Bercovich, Mariano Levin and Martin Vazquez.
Laboratory of Molecular Biology of Chagas Desease , INGEBI (CONICET) - FBMC, Faculty Sciences, University of Buenos Aires, Argentina.

14:30 - 16:30 **Room B**

ORAL COMMUNICATIONS

Microbiology (MI) 1-4

Lipids (LP) 1-4

Chairpersons: Teresita A. Lisa, Universidad Nacional de Rio IV, Córdoba, Argentina.
Claudia Banchio, IBR, Universidad Nacional de Rosario, Argentina.

14:30 - 14:45
MI-C1
CHEMORECEPTOR TRIMERS OF DIMERS AND COLLABORATIVE SIGNALING TEAMS
Studdert, Claudia A., Massazza, Diego A. and Parkinson, John S.
Instituto de Investigaciones Biológicas, Universidad Nacional de Mar del Plata, Argentina and Biology Department, University of Utah, USA.

14:45 - 15:00
MI-C2
STUDY OF pchP PROMOTER ACTIVITY IN *P. aeruginosa* THROUGH TRANSCRIPTIONAL FUSION
Massimelli, M. Julia; Beassoni, Paola R.; Domenech, Carlos E.; Lisa, A. Teresita.
Dpto. de Biología Molecular, FCEFQyN, UNRC., Río Cuarto Córdoba, Argentina.

15:00 - 15:15
MI-C3
ROLES OF A TolC HOMOLOGUE IN *B. suis*
Posadas, D.M.; Martín, F.A.; Campos, E. and Zorreguieta, A.
Fundación Instituto Leloir, CONICET, FCEyN, UBA. Argentina.

15:15 - 15:30
MI-C4
ISOLATION AND CHARACTERIZATION OF UNSATURATED FATTY ACIDS AUXOTROPHS OF *Streptococcus pneumoniae*
Altabe, S. and de Mendoza, D.
IBR-CONICET. Area Microbiología. Fac. Cs. Bioq. y Farm. UNR. Rosario. Argentina.

15:30 - 15:45
LP-C1
A NOVEL ACYLTRANSFERASE INVOLVED IN CONJUGATION OF BILE ACIDS AND FATTY ACIDS IN PEROXISOMES

Sarah-Jayne Reilly, Ethna O'Shea, James O'Byrne, Stefan E. H. Alexson and Mary Hunt.
Karolinska Institutet, Department of Laboratory Medicine, Division of Clinical Chemistry, Stockholm, Sweden.

15:45 - 16:00
LP-C2
ACYL-COA THIOESTERASES - CENTRAL MEDIATORS OF LIPID METABOLISM
Stefan E.H. Alexson, Maria A.K. Westin and Mary C. Hunt.
Department of Laboratory Medicine, Division of Clinical Chemistry, Karolinska Institutet, Stockholm, Sweden.

16:00 - 16:15
LP-C3
EFFECT OF ARACHIDONIC ACID ON CHOLESTEROL TRANSPORT IN MITOCHONDRIA
Castillo, Fernanda, Converso, Daniela, Duarte, Alejandra, Poderoso, Juan J., Podestá, Ernesto J.
Department of Biochemistry, School of Medicine, Laboratory of Oxygen Metabolism, University Hospital, and University of Buenos Aires, Argentina.

16:15 - 16:30
LP-C4
CTP: PHOSPHOCHOLINE CYTIDLYLTRANSFERASE ALPHA EXPRESSION IN QUIESCENT CELLS
Banchio, Claudia and Vance, Dennis E.
División Biología del Desarrollo, IBR CONICET, Facultad de Cs Bioq. y Farm, Universidad Nacional de Rosario, Argentina.

14:30 - 16:30 **Room C**

ORAL COMMUNICATIONS

Enzymology and Structural Biology (ES) 1-8

Chairpersons: Alejandro Vila, IBR, Rosario. Argentina.
María T Tellez de Iñon, INGEBI. Buenos Aires. Argentina.

14:30 - 14:45
ES-C1
THE EFFECT OF ACIDIC LIPIDS ON THE Ca^{2+}-INDEPENDENT PHOSPHATASE ACTIVITY OF THE PLASMA MEMBRANE Ca^{2+} PUMP
Luciana R. Mazzitelli and Hugo P. Adamo.
IQUIFIB-Facultad de Farmacia y Bioquímica UBA-CONICET, Buenos Aires. Argentina.

14:45 - 15:00
ES-C2
EFFECTS OF THE SUBSTITUTION OF ASN879 BY ASP ON THE pNPPase ACTIVITY OF THE Ca^{2+} PUMP FROM HUMAN PLASMA MEMBRANE
Rinaldi, Débora E.; Adamo, Hugo P.
IQUIFIB- Facultad de Farmacia y Bioquímica UBA-CONICET, Buenos Aires. Argentina.

15:00 - 15:15
ES-C3
RIBOSE 5-P-ISOMERASE OF *Trypanosoma cruzi*: SITE-DIRECTED MUTAGENESIS AND REACTION MECHANISM
Stern, Ana Laura and Juan José Cazzulo.
Inst. Investigaciones Biotecnológicas IIB-INTECH. Buenos Aires, Argentina.

15:15 - 15:30
ES-C4
***Pseudomonas aeruginosa* PHOSPHORYLCHOLINE PHOSPHATASE IS A NEW MEMBER FOR THE BACTERIAL HALOACID DEHALOGENASES HYDROLASE SUPERFAMILY**
Beassoni, Paola R., Massimelli, M. Julia, Lisa, A. Teresita, and Domenech, Carlos E.
Dpto Biol. Molecular - Universidad Nacional de Río Cuarto. Río IV, Córdoba, Argentina.

15:30 - 15:45
ES-C5
MALONYL-CoA - FapR INTERACTION REGULATES LIPID HOMEOSTASIS IN BACTERIA AND IS A NOVEL TARGET FOR ANTIBIOTICS
Schujman, Gustavo E., Guerin, Marcelo, Buschiazzo, Alejandro, Llarrull, Leticia1, Vila, Alejandro, Alzari, Pedro and de Mendoza, Diego.
IBR-CONICET; Facultad de Cs. Bioq. y Farm., UNR, Rosario, Argentina, and Institut Pasteur, Paris, France.

15:45 - 16:00
ES-C6
NMR CHARACTERIZATION OF β-SYNUCLEIN, A PROTEIN IMPLICATED IN PARKINSON DISEASE
Andres Binolfi, Carlos W. Bertoncini , Rodolfo M. Rasia , Thomas M. Jovin and Claudio O. Fernandez
IBR, CONICET, UNR, Argentina and Max Planck Institute for Biophysical Chemistry, Germany.

16:00 - 16:15
ES-C7
MODULATION OF CHLOROPLAST 2-CYS PEROXIREDOXIN ACTIVITIES BY NUCLEOTIDES
Aran, Martin, Etchegoren, Juan I., Caporaletti, Daniel; Senn, Alejandro; Tellez de Iñon, María T. and Wolosiuk, Ricardo A.
Instituto Leloir, Buenos Aires, and INGEBI, Buenos Aires, Argentina.

16:15 - 16:30
ES-C8
THE SUBSTRATE BINDING SITE OF THE *Escherichia coli* GLYCOGEN SYNTHASE
Yep, Alejandra; Ballicora, Miguel A. and Preiss, Jack.
Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, USA

16:30 - 17:00 ***Coffee break***

17:00 - 18:00 **Room A**
LECTURE
Jean Lud Cadet
"Participation of multiple apoptotic pathways in methamphetamine (METH)-induced cell death in the brain"
Molecular Neuropsychiatry Branch, NIH, National Institute on Drug Abuse, Baltimore, USA.
Chair: Marta Antonelli

17:00 - 19:00 **Room B**
"Fundación Instituto Leloir" SYMPOSIUM
"Glycobiology II"
Chairperson: Hugo J.F. Maccioni, CIQUIBIC, Universidad Nacional de Córdoba, Córdoba, Argentina.

17:00 - 17:40
S25
REGULATION OF TGF-β SIGNALING DURING MYOGENESIS
Rebeca Droguett, Claudio Cabello, Claudia Hurtado and Enrique Brandan.
CRCP, MIFAB and Facultad de Ciencias Biológicas, P. Universidad Católica de Chile, Santiago, Chile.

17:40 - 18:20
S26
THE GOLGI APPARATUS NUCLEOTIDE SUGARTRANSPORT/ANTIPORT CYCLE: FROM BASIC SCIENCE TO DISEASE
Carlos B. Hirschberg.
Boston University, USA.

18:20 - 19:00
S27
UNDERSTANDING THE ORGANIZATION OF GLYCOLIPID SYNTHESIS IN THE GOLGI COMPLEX
Maccioni, H.J.F.
CIQUIBIC, Departamento de Química Biológica, Facultad de Ciencias Químicas, UNC, Córdoba, Argentina.

17:00 - 19:00 **Room C**

SYMPOSIUM

"Lipids"

Chairperson: Maria C. Fernández Tome, IQUIFIB, Universidad Nacional de Buenos Aires. Argentina.
Claudia N. Tomes, IHEM, Universidad Nacional de Cuyo. Mendoza. Argentina.

17:00 - 17:40
S28
ROLE OF SEC14 DOMAINS IN THE REGULATION OF LIPID HOMEOSTASIS AND VESICULAR TRANSPORT
Christopher R McMaster.
Departments of Pediatrics and Biochemistry and Molecular Biology, Atlantic Research Centre, Dalhousie University, Halifax, Nova Scotia, Canada.

17:40 - 18:20
S29
CONTROL OF CANCER CELL SURVIVAL BY MEMBRANE MICRODOMAIN ASSOCIATED CAVEOLIN-1
Andrew F.G. Quest.
Centro FONDAP de Estudios Moleculares de la Célula (CEMC), Laboratorio de Comunicaciones Celulares, Facultad de Medicina, Universidad de Chile, Santiago, Chile.

18:20 - 19:00
S30
ISOPRENOID BIOSYNTHESIS IN TRYPANOSOMATIDS: ENZYMES, INHIBITORS AND INTRACELLULAR LOCALISATION
Dolores Gonzalez Pacanowska.
Instituto de Parasitología y Biomedicina "López-Neyra", Consejo Superior de Investigaciones Científicas, Granada, Spain.

18:00 - 19:00 **LECTURE**

Ligia Toro

Department of Anesthesiology UCLA School of Medicine, Los Angeles, USA.

"MaxiK channel signaling domains"

Chair: Cecilia Bouzat

19:00 - 21:00 **POSTERS**

Biotechnology (BT) P1 - P60

Cell Biology, (CB) P1 - 74

Enzymology and Structural Biology (EN) P1 - P25

Behavior, Learning and Memory, (BL) P1 - P28

Biochemistry of the Nervous System, (NC) P1 - P20

21:30 ***Welcome cocktail***

MONDAY, December 5, 2005

8:15 - 9:15 **Room A**

"IUBMB" LECTURE

John S. Parkinson

Department of Biology, College of Science, University of Utah, USA

"Bacterial chemotaxis:dissecting the three-protein brain of *E. coli*"

Chair: Diego de Mendoza

9:15 - 10:15 **Room A**

"PABMB" PLENARY LECTURE AWARD

George Kenyon

College of Pharmacy, University of Michigan, Ann Arbor, USA.

"Creatine Kinase: Structure and Function of an Energetic Enzyme"

Chair: Juan J. Cazzulo

10:15 - 10:45 ***Coffee break***

10:45 - 12:45 **Room A**

SYMPOSIUM

"From Signal-Transduction to Virulence in Bacteria"

Chairperson: Fernando Soncini, IBR, Universidad Nacional de Rosario, Rosario, Argentina.

10:45 - 11:15

S31

A NOVEL BACTERIAL METAL-ION SENSING AND DETOXIFICATION SYSTEM

Fernando C. Soncini; Susana K. Checa; Martín Espariz; and María E. Perez Audero

IBR-CONICET, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Argentina.

11:15 - 11:45

S32

MOLECULAR MECHANISM OF FtsZ RING FORMATION IN *Bacillus subtilis*

Federico Gueiros Filho.

Instituto de Quimica, Universidade de Sao Paulo, Brasil.

11:45 - 12:15
S33
***Mycobacterium tuberculosis* VIRULENCE FACTORS**
Brigitte Gicquel.
Unité de Génétique Mycobactérienne, Institut Pasteur, Paris, France.

12:15 - 12:45
S34
***Brucella abortus* VIRULENCE. SIGNALS AND DELIVERY SYSTEMS REQUIRED FOR AN INTRACELLULAR JOURNEY**
Rodolfo A Ugalde, N. Iñón, D. Comerci, R. Sieira, A Ciocchini, M. Roset, I. Marchesini, G. Briones, E. Moreno, I. Moriyón and J. Gorvel.
Instituto de Investigaciones Biotecnológicas, Universidad Nacional de General San Martín/CONICET. Provincia de Buenos Aires, Argentina.

10:45 - 12:45 **Room B**

"IUBMB" SYMPOSIUM

"Pathways for Protein Degradation"

Chairperson: Juan J. Cazzulo, INTECH, Universidad Nacional de General San Martín, Bs As, Argentina.

10:45 - 11:10
S35
HUMAN CYSTEINE CATHEPSINS: STRUCTURE AND FUNCTION
Turk, Vito; Stoka, Veronika and Turk, Boris
Dept. of Biochemistry & Molecular Biology, J. Stefan Institute, Ljubljana, Slovenia.

11:10 - 11:35
S36
EFFECTIVE INHIBITION OF VIRAL AND MICROBIAL PROTEASES
Ernesto Freire.
Department of Biology, The Johns Hopkins University, Baltimore.

11:35 - 12:00
S37
MEPRIN METALLOPROTEINASES: STRUCTURES AND FUNCTIONS
Bond, Judith S.
Department of Biochemistry and Molecular Biology, Penn State University, Hershey Pennsylvania , USA.

12:00 - 12:25
S38
SUBSTRATE SPECIFICITY OF HUMAN KALLIKREIN 6: SALT AND GLYCOSAMINOGLYCAN ACTIVATION EFFECTS
Pedro Francisco Ângelo, Aurelio Resende Lima , Maria Aparecida Juliano, Isobel A. Scarisbrick, Michael Blaber and Luiz Juliano.
Department of Biophysics, Escola Paulista de Medicina, Universidade Federal de São Paulo. Brazil; Department of Biomedical Sciences, College of Medicine, Florida State University, Tallahassee, USA; and Departments of Neurology and Immunology, Mayo Medical and Graduate School, Mayo Clinic Rochester, Rochester, USA.

12:25 - 12:45
S39
A NEW LANDSCAPE FOR PROTEINACEOUS INHIBITORS OF CARBOXYPEPTIDASES.
Francesc Xavier Avilés.
Institut de Biotecnologia i de Biomedicina, Universitat Autónoma de Barcelona, Bellaterra, Barcelona, Spain.

10:45 - 12:45 **Room C**

SYMPOSIUM

"Regulation of Behavior and Hormone Secretion by Brain Peptides and Neurotransmitters"

Chairpersons: Graciela Jahn, LARLAC- IMBECU - CONICET, Mendoza. Argentina.
Alicia Seltzer, IHEM. Universidad Nacional de Cuyo. Mendoza.

10:45 - 11:15
S40
CATECHOLAMINERGIC SYSTEMS IN STRESS: MOLECULAR GENETIC APPROACHES
Kvetnansky Richard.
Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia.

11:15 - 11:45
S41
5-HT FEMINIZES THE RAT BRAIN BY REDUCING ANDROGEN RECEPTORS
C.A. Wilson, C.L. Dakin, I. Kalló, C.W. Coen and D.C. Davies.
Division of Basic Medical Sciences, St George's University of London. ; Institute of Experimental Medicine, Budapest.; and Centre for Neuroscience, Kings College, London.

11:45 - 12:15
S42
POSSIBLE MECHANISM FOR NEUROPEPTIDE-GLUTAMIC ACID-ISOLEUCINE ACTION IN LH RELEASE
María Ester Celis, Andrés Attademo, Ana De Paul, Alicia Torres, Graciela Jahn, Carol Elias, Jackson Bittencourt.
Lab. de Fisiol; Centro de Microscopía Electrónica; Fac. de Med., UNC, LARLAC, Arg. and Inst. Biomed. Sci. USP, Brasil.

12:15 - 12:45
S43
SYNAPSE NUMBER IN THE RAT AVPVN
D.C. Davies, L. Woods, C.L. Dakin and C.A. Wilson.
Division of Basic Medical Sciences, St George's University of London.

12:45 - 14:15 ***Lunch***

12:45 - 14:15 **Room C**

Media Exhibitions in Biochemistry

14:15 - 16:45 **Room A**

ORAL COMMUNICATIONS

Plant Biochemistry and Molecular Biology (PL) 1-7
Enzymology and Structural Biology (EN) 9-11

Chairpersons: Alberto A Iglesias, Universidad Nacional del Litoral, Santa Fe; Argentina.
Gabriela Amodeo, Facultad de Medicina, Universidad de Buenos Aires, Argentina.

14:15 - 14:30
PL-C1
ARABIDOPSIS MUTANTS LACKING GLYCERALDEHYDE-3-P DEHYDROGENASE (NON-PHOSPHORYLATING): EFFECTS ON GLYCOLYSIS, PHOTOSYNTHESIS AND OXIDATIVE STRESS
Rius, Sebastián P. , Casati, Paula, Iglesias, Alberto A. and Gomez-Casati, Diego F.
IIB-INTECH, UNSAM-CONICET, Chascomús; CEFOBI, UNRosario; UNLitoral, Santa Fe; Argentina.

14:30 - 14:45
PL-C2
REVERSIBLE GLYCOSYLATED POLYPEPTIDE IS ASSOCIATED TO GOLGI MEMBRANES AS A PROTEIN COMPLEX
De Pino, V., Gonzalez M., Norambuena,L., Azúa, A., Orellana, A. and Moreno, S.
Núcleo Milenio en Biología Celular Vegetal, Centro de Biotecnología Vegetal, Universidad Andres Bello, Santiago, Chile; and Fundación Instituto Leloir, Instituto de Investigaciones Bioquímicas, Argentina.

14:45 - 15:00
PL-C3
MITOCHONDRIAL LOCALIZATION OF TWO FUNCTIONAL ARGINASES IN ARABIDOPSIS
CD Todd, ME Hoyos, RT Mullen, P Dhanoa, EE Jarvis, L Lamattina, JC Polacco.
Dept. Biology, University of Saskatchewan, Canada; Dept. Biochemistry, University of Missouri-Columbia, USA; Dept. Molecular and Cellular Biology, University of Guelph, Canada; and IIB- UNMdP, Argentina

15:00 - 15:15
PL-C4
IDENTIFICATION OF AQUAPORINS IN *Fragaria x ananassa* AND ANALYSIS OF THEIR EXPRESSION PATTERN DURING FRUIT RIPENING
Bustamante, Claudia, Mut, Paula, Alleva, Karina, Sutka, Moira, Martinez, Gustavo, Civello, Pedro and Amodeo, Gabriela.
IIB-INTECH, UNSAM-CONICET, Chascomús, Argentina; and Laboratorio de Biomembranas, Facultad de Medicina, Universidad de Buenos Aires, Argentina.

15:15 - 15:30
PL-C5
SALICYLIC ACID IS INVOLVED IN ARABIDOPSIS DEFENSE AGAINST Cd-INDUCED OXIDATIVE STRESS
M. Zawoznik, C. Azpilicueta and M.P. Benavides.
Dto de Química Biológica, Facultad de Farmacia y Bioquímica, UBA, Bs.As, Argentina.

15:30 - 15:45
PL-C6
TRANSGENIC TOBACCO PLANTS EXPRESSING A BACTERIAL FLAVODOXIN EXHIBIT ENHANCED TOLERANCE TOWARDS INFECTION WITH THE PATHOGEN *Xanthomonas campestris*
Zurbriggen, M., Tognetti, V., Hajirezaei, M., Fillat, M., Valle, E. and Carrillo, N.
Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET-UNR), Rosario, Argentina; Institut of Plant Genetic and Crop Plant Research (IPK), Gatersleben, Germany; and Facultad de Ciencias, Universidad de Zaragoza, Spain.

15:45 - 16:00
PL-C7
POLYSACCHARIDES FROM THE GREEN SEAWEEDS CODIUM FRAGILE AND C. VERMILARA. STRUCTURE, LOCALIZATION AND ANTIVIRAL ACTIVITY
Kasulin, Luciana, De Dios Agustina, Estevez, José M., Pujol, Carlos A., Damonte, Elsa B., Ciancia, Marina, and Cerezo, Alberto S.
Cátedra de Química Orgánica, Departamento de Biología Aplicada y Alimentos (CIHIDECAR-CONICET), Facultad de Agronomía, Universidad de Buenos Aires, Argentina; Departamento de Química Orgánica (CIHIDECAR-CONICET) and Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina.

16:00 - 16:15
ES-C9
SUBSTRATE SPECIFICITY OF HUMAN KALLIKREIN 6: SALT AND GLYCOSAMINOGLYCAN ACTIVATION EFFECTS
Pedro Francisco Ângelo, Aurelio Resende Lima , Maria Aparecida Juliano, Isobel A. Scarisbrick, Michael Blaber and Luiz Juliano.
Department Biophysics, Escola Paulista Medicina, Universidade Federal São Paulo, Brazil; Department of Biomedical Sciences, College of Medicine, Florida State University, USA; and Departments of Neurology and Immunology, Mayo Medical and Graduate School, Mayo Clinic Rochester, Rochester.

16:15 - 16:30
ES-C10
NOVEL NUCLEOTIDE-LIKE BINDING SITES IN THE GLUCOSE TRANSPORTER GLUT1.
Ormazábal, Valeska; Salas, Alexis; Zúñiga, Felipe; Rivas, Coralia I.; Reyes, Alejandro; Vera, Juan Carlos.
Departamento de Fisiopatología, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile, and Instituto de Bioquímica, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile.

16:30 - 16:45
ES-C11
FLUX CONTROL IN METABOLIC PATHWAYS: IN VIVO STUDIES ON GLYCOGEN SYNTHESIS.
Preller, A; Quiroga, D. and Ureta, T.
Departamento de Biología, Facultad de Ciencias, Universidad de Chile. Santiago, Chile.

14:15 - 16:45 **Room B**

ORAL COMMUNICATIONS

Biotechnology (BT) 1-6

Gene expression (GE) 7-9

Chairpersons: Beatriz S Méndez, Universidad de Buenos Aires, Buenos Aires. Argentina.
Rodolfo A. Ugalde, Universidad Nacional de Grl San Martin, Buenos Aires, Argentina.

14:15 - 14:30
BT-C1
PHYTOCHEMICALS AS NATURAL ANTIMICROBIAL AGENTS
Silvia Moreno, Catalina Romano, Tamara Scheyer, Adrián Vojnov.
Fundación Instituto Leloir, I.I.B.B.A - CONICET.

14:30 - 14:45
BT-C2
NUTRACEUTICAL PRODUCTION BY LACTIC ACID BACTERIA: EVALUATION OF RIBOFLAVIN, FOLATES AND α-GALACTOSIDASE ENZYME
LeBlanc, Jean Guy; Sesma, Fernando and Savoy de Giori, Graciela.
CERELA-CONICET, Tucumán, Argentina.

14:45 - 15:00
BT-C3
MICRO-AEROBIC POLY(3-HYDROXYBUTYRATE) ACCUMULATION IN *Escherichia coli*
Nikel, Pablo I., Galvagno, Miguel A., Pettinari, M. Julia and Méndez, Beatriz S.
IIB, UNSAM-CONICET, Dpto. Química Biológica, FCEyN, UBA; and Dpto. Ingeniería Química, FI, UBA, Buenos Aires, Argentina.

15:00 - 15:15
BT-C4
PROPERTIES OF A RECOMBINANT *Pseudomonas fluorescens* STRAIN WITH ACQUIERED CAPACITY TO DEGRADE 2,4-DINITROTOLUENE
Monti M. R., Fabro G., Álvarez M. E., Smania A. M. and Argaraña C. E.
CIQUIBIC-CONICET, Dpto. de Química Biológica. Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina.

15:15 - 15:30
BT-C5
IDENTIFICATION OF DOMINANT BACTERIAL POPULATIONS IN NONYLPHENOL POLYETHOXYLATE DEGRADING ACTIVATED SLUDGE
Lozada, Mariana, Figuerola, Eva L. M., Itria, Raúl F. y Erijman, Leonardo.
INGEBI-CONICET-UBA, CIIA-INTI, Argentina.

15:30 - 15:45
BT-C6
STRUCTURAL HOMOLOGY WITH UBIQUITIN IN THE HALOALKALIPHILIC ARCHAEA *Natrialba magadii*
Nercessian, Débora, Marino-Buslje, Cristina, De Castro, Rosana E. and Conde, Rubén D.
Instituto de Investigaciones Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata; and Instituto de Química y Fisicoquímica Biológica, Facultad de Farmacia y Bioquímica, Universidad Nacional de Buenos Aires.

15:45 - 16:00
GE-C7
ISOLATION AND IDENTIFICATION OF THREE NOVEL PUTATIVE TRANSCRIPTIONAL REGULATORS OF THE *Brucella abortus* virB OPERON
Rodrigo Sieira, Diego J. Comerci, and Rodolfo A. Ugalde.
Instituto de Investigaciones Biotecnologicas, Universidad Nacional de General San Martin, Buenos Aires, Argentina.

16:00 - 16:15
GE-C8
THE EFFECT OF DISRUPTION OF RNA-POLYMERASE B TRANSCRIPTION FACTOR 3 (BTF3) ON THE EXPRESSION OF INDUCIBLE GENES IN THE FILAMENTOUS FUNGUS *Trichoderma reesei*
Saenz, Erik C., Ramos Augusto P. & El-Dorry Hamza.
Department of Biochemistry - Institute of Chemistry, USP, São Paulo. Brasil.

16:15 - 16:45
GE-C9
TRANSCRIPTOME PROFILING INDUCED IN *Lotus japonicus* ROOTS IN RESPONSE TO INOCULATION WITH DIFFERENT *Mesorhizobium loti* STRAINS
D' Antuono, Alejandra; Ott, Thomas; Udvardi, Michael; Ugalde, Rodolfo; and Lepek, Viviana.
Instituto de Investigaciones Biotecnológicas, IIB-UNSAM, Buenos Aires, Argentina.

14:15 - 16:45 **Room C**

ORAL COMMUNICATIONS

Signal transduction (ST) 1-10

Chairpersons: Pablo Wappner, Fundación Instituto Leloir, Buenos Aires, Argentina.
Sebastián Katz, Universidad Nacional del Sur, Bahía Blanca, Argentina.

14:15 - 14:30
ST-C1
COORDINATED ACTION OF PKA AND ERK IN CHOLESTEROL TRANSPORT ACTIVATION
Cecilia Poderoso, Soledad Galli, Paula Maloberti, Juan José Poderoso and Ernesto Podestá.
Department of Biochemistry. School of Medicine. Laboratory of Oxygen Metabolism. University Hospital; and University of Buenos Aires, Buenos Aires, Argentina.

14:30 - 14:45
ST-C2
INTRACELLULAR SIGNALING FOR ESTRADIOL INDUCED AXOGENESIS
Gorosito, Silvana; Carrer, Hugo and Cambiasso, María J.
INIMEC-CONICET. Córdoba, Argentina.

14:45 - 15:00
ST-C3
DUAL ROLE FOR MAPK'S IN GLIAL CELLS SURVIVAL AND DEATH
Nahuel Villegas, Elina Francisco, Mirta Reynaldo, Marcos Dreon, Horacio Heras, and Néstor Gabriel Carri.
IMBICE and INIBIOLP, Fac. Cs. Médicas, CONICET-UNLP.

15:00 - 15:15
ST-C4
MODULATION OF ERK1/2 AND P38 MAPK SIGNALING PATHWAYS BY ATP IN OSTEOBLASTS
Katz Sebastián, Santillán Graciela and Boland Ricardo.
Departamento de Biología, Bioquímica y Farmacia. Universidad Nacional del Sur, Bahía Blanca, Argentina.

15:15 - 15:30
ST-C5
ROLE OF $P2Y_2$ RECEPTOR AND MECHANICAL STRESS-ACTIVATED Ca^{2+} INFLUX (SACI) IN ERK1/2 AND P38 MAPK STIMULATION BY ATP IN OSTEOBLASTS
Santillán Graciela, Katz Sebastián and Boland Ricardo.
Departamento de Biología, Bioquímica y Farmacia. Universidad Nacional del Sur, Bahía Blanca, Argentina.

15:30 - 15:45
ST-C6
IP3 STIMULATES *IN VITRO* TRANSCRIPTIONAL ACTIVITY IN C2C12 AND HELA CELL PROTEIN EXTRACTS
Bustamante, Mario; Cárdenas, J. César; Maldonado, Edio and Jaimovich, Enrique.
Centro de Estudios Moleculares de la Célula. ICBM, Facultad de Medicina, Universidad de Chile. Santiago, Chile.

15:45 - 16:00
ST-C7
A FATAL AFFAIR: BDNF LEAVES TRKB FOR P75NTR AFTER SEIZURES
Unsain, Nicolás; Mascó, Daniel H.
Laboratorio de Neurobiología, Centro de Biología Celular y Molecular, FCEFyN, UNC, Córdoba, Argentina.

16:00 - 16:15
ST-C8
GENOME WIDE RNAi SCREEN FOR GENES INVOLVED IN THE TRANSCRIPTIONAL RESPONSE TO HYPOXIA
Andrés Dekanty and Pablo Wappner.
Fundación Instituto Leloir, Buenos Aires, Argentina.

16:15 - 16:30
ST-C9
THE PMRA/PMRB AND RCSC/YOJN/RCSB SYSTEMS CONTROL EXPRESSION OF THE SALMONELLA O-ANTIGEN CHAIN LENGTH DETERMINANT
Mónica A. Delgado, Chakib Mouslim and Eduardo A. Groisman.
Department of Molecular Microbiology, Washington University School of Medicine, USA.

16:30 - 16:45
ST-C10
INFILTRATION OF INFLAMMATORY CELLS PLAYS AN IMPORTANT ROLE IN MATRIX METALLOPROTEINASE EXPRESSION AND ACTIVATION IN THE HEART DURING SEPSIS
Cuenca Jimena, Boscá Lisardo and Goren Nora.
Centro Nacional de Investigaciones Cardiovasculares, CNIC, Madrid, España.

16:45 - 17:00 ***Coffee break***

17:00 - 19:00 **Room A**

"SBBq-RTPD Network" SYMPOSIUM

"Proteomics"

Chairperson: Silvia Moreno, Universidad de Buenos Aires, Buenos Aires, Argentina.

17:00 - 17:30
S44
FROM VENOMICS TO PATHOLOGY: DISULPHIDE BONDS IN THE DIVERSIFICATION OF PROTEIN STRUCTURE AND FUNCTION
Calvete, Juan J.
Instituto de Biomedicina de Valencia, Spain.

17:30 - 18:00
S45
USING PROTEOMICS TO STUDY THE MOLECULAR EVENTS ASSOCIATED TO THE ROLE OF SPARC IN TUMOR PROGRESSION
Andrea S. Llera, M. Romina Girotti, M. Soledad Sosa and Osvaldo Podhajcer.
Fundación Instituto Leloir, Buenos Aires, Argentina.

18:00 - 18:30
S46
SER/THR PROTEIN KINASES IN MYCOBACTERIA: AUTOPHOSPHORYLATION AND SUBSTRATE IDENTIFICATION BY PROTEOMIC APPROACHES
Carlos Cerveñansky.
Instituto de Investigaciones Biológicas Clemente Estable/ Facultad de Ciencias/ Institut Pasteur Montevideo, Uruguay.

18:30 - 19:00
S47
NEW INSIGHTS INTO LEPROSY PATHOGENESIS BY DEFINING THE PROTEOME OF *Mycobacterium leprae*
M C.V. Pessolani, M.A.M .Marques, E.K.Xavier da Silveira, A. Chapeaurouge, Y-S. Cho, A.G. N. Ferreira, R.H. Valente, R.B. Silva Filho, B.J. Espinosa, J. Perales, K.M. Dobos, J.T. Belisle, J.S. Spencer and P.J. Brennan.
Lab.Cel.Microbiol & ; Toxicol.Lab, Inst.Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil; and Dep.Microbiol., Immunol. & Pathol., Colorado State Univ., Fort Collins, USA.

17:00 - 19:00 **Room B**

"ISN" SYMPOSIUM

"Signaling Pathways in Development, Plasticity and Diseases"

Chairperson: Santiago Quiroga, CIQUIBIC, Universidad Nacional de Córdoba, Córdoba, Argentina.

17:00 - 17:25

S48

REGULATING FILOPODIAL DYNAMICS THROUGH ACTIN-DEPOLYMERIZING FACTOR/ COFILIN

James R. Bamburg.

Department of Biochemistry, Colorado State University, Fort Collins, Colorado, U.S.A.

17:25 - 17:50

S49

DIFFERENTIAL ROLE OF WNT LIGANDS ON SYNAPTIC STRUCTURE AND FUNCTION: ROLE OF THE ALZHEIMER´S AB-PEPTIDE

Nibaldo Inestroza.

Centro FONDAP de Regulación Celular y Patología "Joaquín V. Luco", Universidad Católica de Chile. Chile

17:50 - 18:15

S50

SIGNALING PATHWAYS AND GENE EXPRESSION IN MEMORY FORMATION. COMMON MECHANISMS FROM CRUSTACEANS TO MAMMALS

Arturo Romano.

Laboratorio de Neurobiologia de la Memoria, Departamento de Fisiología, Biologia Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, IFIByNE, CONICET, Buenos Aires, Argentina.

18:15 - 18:35

S51

REGULATION OF MEMBRANE EXPANSION AT THE NERVE GROWTH CONE

Santiago Quiroga.

Departamento de Química Biológica, Facultad. de Ciencias Químicas y CIQUIBIC, Universidad Nacional de Córdoba y CONICET, Ciudad Universitaria, Córdoba, Argentina.

18:35 - 19:00

S52

SIGNALLING IN DEVELOPING NEURONS: THE CASE OF MARCKS PHOSPHORYLATION OUTSIDE THE EFFECTOR DOMAIN

Cristina Arruti.

Laboratorio de Cultivo de Tejidos, Seccion Biologia Celular, DBCM, Facultad de Ciencias, Universidad de la Republica, Montevideo, Uruguay.

17:00 - 19:00 **Room C**

SYMPOSIUM

"Eukaryotic Gene Expression, Impact in Cell Physiology and Disease"

Chairperson: José L. Bocco, CIBICI, Universidad Nacional de Córdoba, Córdoba, Argentina.

17:00 - 17:30

S53

SP1/KLF TRANSCRIPTION FACTORS: THE CHANGE OF A PARADIGM

Urrutia, Raul.

GI Research Unit, St Mary's Hospital, Mayo Clinic, Rochester, USA.

17:30 - 18:00
S54
A FUNCTIONAL INTERACTION BETWEEN ATF7 AND TAF12 THAT IS MODULATED BY TAF4 AND SUMOYLATION
Hamard Pierre-Jacques, Dalbies-Tran Rozenn, Guittaut Michael, Hauss Charlotte, Dujardin Denis, Davidson Irwin, Oegelschlager Thomas, Kédinger Claude and Chatton Bruno.
Ecole Supérieure de Biotechnologie de Strasbourg, Strasbourg Illkirch, France.

18:00 - 18:30
S55
A POLAR MECHANISM COORDINATES DIFFERENT REGIONS OF ALTERNATIVE SPLICING WITHIN A GENE
Fededa, Juan P., Petrillo, Ezequiel, Nogués, Guadalupe, and Kornblihtt, Alberto R..
LFBM, Depto. de Fisiol., Biol. Mol. y Cel., IFIBYNE-CONICET, FCEN-UBA, Buenos Aires, Argentina.

18:30 - 19:00
S56
SUBCODES WITHIN THE CONTEXT OF THE HISTONE CODE
Lomberk, Gwen; Bensi, Debora; Fernandez-Zapico, Martin; and Urrutia, Raul.
GI Research Unit, St Mary's Hospital, Mayo Clinic, Rochester, USA.

19:00 - 21:00 **POSTERS**
Enzymology and Structural Biology, (EN) P26 - P65
Gene Expression, (GE) P1 - P43
Genomics, (GN) P1 - P2
Lipids, (LP) P1 - P21
Microbiology (MI) P1 - P65
Chronobiology, (CH) P1 - P2
Development Neurobiology, (DN) P1 - P15
Neurotransmitters, Neurorreceptors, Neuromodulators, (NR) P1 - P24

19:00 **PABMB General Assembly**

21:00 **SAN General Assembly**

TUESDAY, December 6, 2005

8:15 - 9:15 **Room A**
"SEBMB" (Spain) LECTURE
César de Haro
Centro de Biología Molecular (CSIC-UAM), Universidad Autónoma, Madrid, Spain
"Control of translation in Sindbis virus-infected cells"
Chair: Ricardo Boland

9:15 - 11:15 **Room A**
SBBq-RTPD Network SYMPOSIUM
"Genomics"
Chairperson: Arnaldo Zaha, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.

9:15 - 9:45
S57
UNDERSTANDING ALKALINE PH STRESS RESPONSE IN S. CEREVISIAE: A GENOMIC APPROACH
Joaquín Ariño.
Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Spain.

9:45 - 10:15
S58
GENE EXPRESSION PROFILE ANALYSIS IN HUMAN TUMORS USING CDNA MICROARRAYS
Luiz Fernando Reis.
Instituto Ludwig de Pesquisa sobre o Câncer, Brazil.

10:15 - 10:45
S59
FUNCTIONAL GENOMIC ANALYSIS OF *Trypanosoma cruzi* DIFFERENTIATION
Samuel Goldenberg.
Instituto de Biologia Molecular do Paraná (IBMP), Brazil.

10:45 - 11:15
S60
COMPARATIVE ANALYSIS OF MYCOPLASMAS GENOMES
Arnaldo Zaha.
Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.

9:15 - 11:15 Room B

SYMPOSIUM

"Transgenic and Mutant Mice for the Study of Molecular Mechanisms"

Chairperson: Marcelo Rubinstein, INGEBI, Buenos Aires, Argentina.

9:15 - 9:45
S61
USING KNOCKOUT MICE TO UNDERSTAND EPITHELIAL ION TRANSPORT
C. A. Flores, M. Catalán, J. E. Melvin, L.P. Cid and F.V. Sepúlveda.
Centro de Estudios Científicos (CECS), Valdivia, Chile.

9:45 - 10:15
S62
CONDITIONAL RESTORATION OF WILD TYPE MECP2 IN A MOUSE MODEL OF RETT SYNDROME
Young, Juan I.
Centro de Estudios Científicos, CECS, Valdivia, Chile.

10:15 - 10:45
S63
BASAL MELANOCORTIN RECEPTOR FUNCTION AND POTENTIAL INVERSE AGONISM OF AGOUTI OR AGOUTI RELATED PEPTIDE (AGRP) IN PROOPIOMELANOCORTIN (POMC) DEFICIENT MICE
Low, Malcolm J. and Tolle, Virginie Vollum.
Institute and Center for the Study of Weight Regulation, Oregon Health & Science University, Portland, USA.

10:45 - 11:15
S64
NEURAL-SPECIFIC EXPRESSION AND MODULAR ENHANCER STRUCTURE OF THE POMC GENE
de Souza, Flávio; Bumaschny, Viviana, Santangelo, Andrea; Low, Malcolm and Rubinstein, Marcelo.
INGEBI-CONICET. Bs. As., Argentina; and Vollum Institute-OHSU. Portland, OR, USA.

9:15 - 11:15 **Room C**

SYMPOSIUM

"Biochemical and Molecular Biology Education"

Chairperson: Leila Beltramini, Education Committee of PABM, Universidade de Sao Paulo, Brazil.

9:15 - 9:45
S65
SCIENCE FOR SOCIETY: THE SCIENTIST'S SOCIAL RESPONSIBILITY
Joan J. Guinovart.
Departament de Bioquímica i Biologia Molecular and Institut de Recerca Biomèdica (IRB), Parc Científic de Barcelona, Universitat de Barcelona

9:45 - 10:15
S66
THE PATENTING OF KNEW KNOWLEDGE AS A WAY TO TEACH SCIENCE
Manuel Krauskopf.
Universidad Andrés Bello, Instituto Milenio de Biología Fundamental y Aplicada, Santiago, Chile.

10:15 - 10:45
S67
SCIENCE AND ART - DIDACTIC MATERIAL THAT MAY ARISE EMOTION AMONG STUDENTS
Leopoldo de Meis, Diucênio Afonso Rangel do Carmo, Alexandro Machado de Freitas and Luis Antonio Dourado Júnior.
Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

10:45 - 11:15
S68
MOLECULES WITHIN REACH OF THE HANDS: OUR EXPERIENCE FOR COMMUNITY OF PABMB
Leila M. Beltramini.
Instituto de Física de São Carlos, USP, São Carlos, Brasil.

11:15 - 11:30 **Room A**

Micro-Conference

"Institute Pasteur at Montevideo, Uruguay: an opportunity for regional young scientists"

Carlos Cerveñansky
Inst. Investigaciones Biológicas Clemente Estable, Fac. de Ciencias, Institut Pasteur Montevideo, Uruguay.

11:30 - 13:30 **POSTERS**

Microbiology (MI) P66 - P100
Molecular Genetics (MG) P1 - P11
Plant Biochemistry and Molecular Biology, (PL) P1 - P79
Proteomics (PT) P1 - P6
Signal Transduction (ST) P1 - P38
Pathologies of the Nervous System (NP) P1 - P27
Toxicology (TX) P1 - P15

13:30 - 14:30 ***Lunch***

14:30 - 15:15 **Room A**

"SBBMC" (Chile) LECTURE

Luis O. Burzio

Fundación Ciencia para la Vida, Santiago de Chile, Chile.

"Novel Mitochondrial chimeric RNAs: Target for diagnosis and therapy of cancer"

Chair: Beatriz Caputto

14:30 - 15:15 **Room B**

"SMB" (México) LECTURE

Salvador Uribe-Carvajal

Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Mexico.

"Compatible solute (trehalose)-mediated stabilization of proteins. Studies on the plasma membrane H^+-ATPase from Kluyveromyces lactis"

Chair: Norma Sterin de Speziale

14:30 - 15:00 **Room C**

Short LECTURE

Francisco J. Urbano

Instituto de Neurociencias de Alicante; CSIC-Univ. Miguel Hernández,. San Juan de Alicante, Spain.

"Tonic activation of presynaptic kainate receptors in mice thalamocortical synapses"

Chair: Carlota Gonzalez Inchauspe

15:15 - 16:00 **Room A**

LECTURE

Mario Amzel

Johns Hopkins University, Baltimore, USA.

"Structure and mechanism of farnesyl diphosphate synthase from *Trypanosoma cruzi*: implications for drug design"

Chair: María T. Tellez de Iñon

15:15 - 16:00 **Room B**

"SBBq" (Brasil) LECTURE

Anibal Vercesi

Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil.

"Mitochondrial Dysfunction in Genetically Hyperlipidemic Mice"

Chair: Rodolfo Brenner

15:00 - 15:30 **Room C**

Short LECTURE

Carmen Aragón

Centro de Biologia Mol Severo Ochoa, Facultad de Ciencias, Univ Autonoma de Madrid, CSIC, Madrid, Spain.

"Trafficking of the neuronal glycine transporter, GLYT2"

Chair: Marta E. Hallak

16:00 - 18:30 **Room A**

ORAL COMMUNICATIONS

Cell Biology (CB) 6-13

Chairpersons: María I. Colombo, IHEM, Universidad Nacional de Cuyo, Mendoza, Argentina.
Jose L. Daniotti, CIQUIBIC, Universidad Nacional de Córdoba, Argentina.

16:00 - 16:15

CB-C6

VITAMIN C METABOLISM IN CANCER CELLS

Rivas, Coralia I.; Sotomayor, Kirsty; Muñoz, Carola; Quilodrán, Jessica; Maurin, Michelle; Barra, Valeria; Henríquez, Esther; Maldonado, Mafalda; Vera, Juan Carlos.
Departamento de Fisiopatología, Facultad de Ciencias Biológicas, Universidad de Concepción. Chile.

16:15 - 16:30

CB-C7

FUNCTIONAL REDUNDANCY OF *Caenorhabditis elegans* NUCLEOTIDE-SUGAR TRANSPORTERS

Caffaro CE, Hirschberg CB, Berninsone P.
Department of Molecular and Cell Biology.Boston University Goldman School of Dental Medicine, USA.

16:30 - 16:45

CB-C8

MACROPINOCYTOSIS IS THE NOVEL MECHANISM OF ENDOCYTOSIS FOR THE GPI ANCHORED PROTEIN UPAR

Sahores, M. Macarena, Madsen, Chris D., Chiabrando, Gustavo A., and Blasi, Francesco.
Università Vita Salute San Raffaele, Milano, Italy; FIRC Institute of Molecular Oncology, Milano, Italy and Departamento de Bioquímica Clínica, CIBICI-CONICET, U.NC., Córdoba, Argentina.

16:45 - 17:00

CB-C9

PROTECTIVE ROLE OF AUTOPHAGY AGAINST EL TOR HEMOLYSIN, A BACTERIAL EXOTOXIN FROM *Vibrio cholerae*

Gutierrez MG, Saka HA, Bocco JL and Colombo María I.
Lab. de Biología Celular y Molecular, IHEM-CONICET, Facultad de Ciencias Médicas, UNCuyo, Mendoza, Argentina; and Departamento de Bioq. Clínica, Facultad de Ciencias Químicas, UNC, Córdoba, Argentina.

17:00 - 17:15

CB-C10

CHOLESTEROL CONTENT REGULATES ACROSOMAL EXOCYTOSIS BY ENHANCING RAB3A PLASMA MEMBRANE ASSOCIATION

Belmonte,Silvia; López,Cecilia; Roggero,Carlos; De Blas, Gerardo; Tomes, Claudia; Mayorga, Luis.
Lab. de Biología Celular y Molecular, IHEM-CONICET, Facultad Ccias. Médicas, U. N. de Cuyo, Mendoza, Argentina.

17:15 - 17:30
CB-C11
MEMBRANE PERMEANT RAB3A TRIGGERS ACROSOMAL EXOCYTOSIS IN THE ABSENCE OF EXTRACELLULAR CALCIUM
Lopez Cecilia I., Belmonte Silvia A., De Blas Gerardo A. and Mayorga Luis S.
Instituto de Histología y Embriología, Facultad de Ciencias Médicas, U. N. de Cuyo Mendoza Argentina.

17:30 - 18:00
CB-C12
CHARACTERIZATION OF SPERM SERINE PROTEASE BSp66 IN MOUSE
Cesari, Andreina, Brown Paula & Eddy E Mitch.
Instituto de Investigaciones Biológicas, UNMdP, Argentina; and NIEHS/NIH, Department of Health and Human Services, Gamete Biology Section, LRDT, USA.

18:00 - 18:30
CB-C13
MECHANICAL STRESS LEADS TO CHANGES IN MEMBRANE BIOPHYSICS, TUBULIN ORGANIZATION AND CELLULAR SIGNALING
Verstraeten, Sandra V., Mackenzie, Gerardo G., and Oteiza, Patricia I.
Depto. Química Biológica, IQUIFIB (UBA-CONICET), School of Pharmacy and Biochemistry, UBA. Buenos Aires, Argentina.

16:00 - 18:30 **Room B**

ORAL COMMUNICATIONS

Microbiology (MI) 5-12

Chairpersons: Eleonora García Véscovi, IBR, Universidad Nacional de Rosario, Argentina.
Gladys Mori, Universidad Nacional de Río Cuarto, Córdoba, Argentina.

16:00 - 16:15
MI-C5
CLONING OF P. mirabilis mrpA AND EXPRESSION OF THE FIMBRIAL PROTEIN IN *Lactococcus lactis*
Paola Scavone, Anderson Miyoshi, Vasco Azevedo, Pablo Zunino.
Laboratorio de Microbiología, IIBCE, Montevideo, Uruguay; and Laboratorio de Genetica Celular e Molecular, ICB, UFMG, Minas Gerais, Brasil.

16:15 - 16:30
MI-C6
A B-CELL MITOGEN OF *Brucella abortus* IS AN IMMUNOMODULATOR
Spera, Juan M., Comerci, D. J., Ugalde, J. E., Iñon, N. and Ugalde, R.A.
Instituto de Investigaciones Biotecnológicas (IIB-UNSAM). Buenos Aires, Argentina.

16:30 - 16:45
MI-C7
2-THIOPHEN CARBOXYLIC ACID HYDRAZIDE (TCH): AN ISONIAZID ANALOG THAT DOES NOT INHIBIT MYCOLIC ACID BIOSYNTHESIS IN *Mycobacterium bovis* AND *Mycobacterium tuberculosis*
de la Iglesia, Agustina I., Emma J. Stella and Héctor R. Morbidoni.
Cátedra de Microbiología, Facultad de Ciencias Médicas, Universidad Nacional de Rosario.

16:45 - 17:00
MI-C8
PROMOTER ACTIVITY OF GENES RELATED TO HYPOXIA IN AEROBIC AND ANAEROBIC CONDITIONS
Alito, Alicia; Farber, Marisa; Bigi, Fabiana; Elizondo, Ana; Garbaccio, Sergio and Cataldi, Angel.
Instituto de Biotecnología, INTA Castelar, Buenos Aires, Argentina.

17:00 - 17:15
MI-C9
POLYSACCHARIDES FROM THE GREEN SEAWEEDS CODIUM FRAGILE AND C. VERMILARA. STRUCTURE, LOCALIZATION AND ANTIVIRAL ACTIVITY
Kasulin, Luciana, De Dios Agustina, Estevez, José M., Pujol, Carlos A., Damonte, Elsa B., Ciancia, Marina, and Cerezo, Alberto S.
Cátedra de Química Orgánica, Departamento de Biología Aplicada y Alimentos (CIHIDECAR-CONICET), Facultad de Agronomía, Universidad de Buenos Aires, Argentina; Departamento de Química Orgánica (CIHIDECAR-CONICET) and Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina.

17:15 - 17:30
MI-C10
GLUCOSYLCERAMIDE SYNTHASE, A KEY ENZYME IN THE GLYCOSPHINGOLIPID PATHWAY OF *Plasmodium falciparum*
Malena Landoni, Vilma Duschak, Valnice Peres, Alejandro M. Katzin and Alicia S. Couto.
CIHIDECAR-Depto. Q. Orgánica, FCEyN, UBA; Inst. Nac. de Parasitología, Dr. M. Fatala Chabén, ANLIS-Malbrán,, Min. de Salud y Ambiente; and Depto. de Parasitologia, Inst. de Cs. Biomédicas, USP, Brazil.

17:30 - 17:45
MI-C11
METACASPASES AND APOPTOSIS IN *Trypanosoma cruzi*
Alvarez, Vanina, Kosec, Gregor, Agüero, Fernán, Sánchez, Daniel, Dolinar, Marko, Turk, Boris, Turk, Vito, and Cazzulo, Juan J.
IIB-INTECH, UNSAM-CONICET, Buenos Aires, Argentina and Jozef Stefan Institute, Ljubljana, Slovenia.

17:45 - 18:00
MI-C12
IDENTIFICATION OF AN ATPASE ACTIVITY ASSOCIATED WITH THE ROTAVIRUS PHOSPHOPROTEIN NSP5
Bar-Magen, Tamara, Taraporewala, Zenobia F., González-Nilo, Fernando, Spencer, Eugenio, Patton, John T.
Laboratorio de Virologia, Facultad de Química y Biología, Universidad de Santiago, Chile; Centro de Bioinformática y Simulación Molecular (CBSM), Universidad de Talca, Chile; and Laboratory of Infectious Diseases, NIAID, National Institutes of Health, Bethesda.

16:00 - 18:30 **Room C**

ORAL COMMUNICATIONS

Gene Expression (GE) 1-6

Genomics (GN) 1

Molecular Genetics (MG) 1-2

Chairpersons: Anabella Srebrow, Laboratorio de Fisiol. y Biol. Mol, IFIBYNE-CONICET, Argentina.
José L. Bocco, CIBICI, Universidad Nacional de Córdoba, Argentina.

16:00 - 16:15
GE-C1
MDCK EPITHELIAL CELLS EXPRESS mRNAs FOR Kv1.4, Kv1.6 AND Kv1.7 POTASSIUM CHANNELS
García-Villegas, M. R., Escamilla, J., Fiorentino, R., Cuéllar,F., and Cereijido, M.
Departamento de Fisiología, Biofísica y Neurociencias, México.

16:15 - 16:30
GE-C2
RATS BRED AS HEAVY ALCOHOL DRINKERS REDUCE THEIR CONSUMPTION WHEN TREATED WITH AN ADENOVIRAL VECTOR THAT EXPRESSES AN ANTI-ALDEHYDE DEHYDROGENASE ANTISENSE GENE
Paula Ocaranza, María Elena Quintanilla, Lutske Tampier, Amalia Sapag, Eduardo Karahanian, Yedy Israel.
Programa Doctorado en Bioquímica, Facultad de Ciencias Químicas y Farmacéuticas; Facultad de Medicina, Universidad de Chile y Facultad de las Ciencias de la Salud, Universidad Diego Portales. Santiago, Chile.

16:30 - 16:45
GE-C3
SILENCING ENDOGENOUS KLF6 REVEALS A NEW FUNCTION AS A POSITIVE REGULATOR OF CELL PROLIFERATION
D'Astolfo, D.; Gehrau, R.; Bocco, J.L. and Koritschoner, N.
Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET). Facultad de Ciencias Químicas, UNC, Córdoba, Argentina.

16:45 - 17:00
GE-C4
TRANSCRIPTIONAL CONTROL OF THE HUMAN KLF6 GENE
Gehrau, R., D'Astolfo, D.; Bocco, J.L.; and Koritschoner, N.P.
CIBICI-CONICET-Facultad de Ciencias Químicas, UNC, Córdoba, Argentina.

17:00 - 17:15
GE-C5
THE *Trypanosoma cruzi* CYCLOPHILIN GENE FAMILY AS TARGET OF THE TRYPANOCIDAL ACTIVITY OF CYCLOSPORIN A ANALOGS"
Búa, Jacqueline; Galat, Andrej; Potenza, Mariana, Migliori, María Laura; López, Andrés M. and Ruiz, Andrés M.
Instituto Nacional De Parasitología "Dr. Mario Fatala Chabén", Buenos Aires Argentina ; and Departement D'ingenierie Et D'etudes Des Proteines, Dsv/Cea, Gif-Sur-Yvette Cedex, France.

17:15 - 17:30
GE-C6
CONCERTED REGULATION OF ALTERNATIVE SPLICING AND TRANSLATION BY EXTRACELLULAR CUES
Blaustein, Matías; Pelisch, Federico; Quadrana, Leandro and Srebrow, Anabella.
Laboratorio de Fisiología y Biología Molecular, IFIBYNE-CONICET, Argentina.

17:30 - 17:45
GN-C1
POTATO ROUGH DWARF VIRUS (PRDV) AND POTATO VIRUS P (PVP) ARE STRAINS OF THE SAME CARLAVIRUS
Massa, Gabriela A., Segretin, M E, Riero, M F , Colavita, M L, Bravo-Almonacid, F , & Feingold, S.
Lab. de Biotecnología Agrícola, Ed. Propapa. INTA-Balcarce, Argentina, INGEBI-CONICET, Argentina and Cát. de Fitopatología, Fac. de Cs Ag, UNMdP, Argentina.

17:45 - 18:00
MG-C1
SEARCHING FOR NEW GLYCOSYLTRANSFERASES IN COSMOMICYN BIOSINTHETIC PATHWAY FROM *Streptomyces olindensis*
Borda, Charlotte C.; Garrido, Leandro, M. and Padilla, Gabriel.
Departamento de Microbiologia. Instituto de Ciências Biológicas, Universidade de São Paulo, Brasil.

18:00 - 18:15
MG-C2
HIF1α/SIMA SUBCELLULAR LOCALIZATION DEPENDS ON NOVEL NUCLEAR EXPORT SIGNALS LOCALIZED IN THE bHLH-DOMAIN
Nuria Romero and Pablo Wappner.
Fundación Instituto Leloir, FCEyN-UBA, Buenos Aires, Argentina.

18:30 - 19:00 **Coffee break**

19:00 - 20:00 **Room A**
"Alberto Sols" LECTURE
Lisardo Boscá
Instituto de Bioquímica, Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain.
"Nuclear receptors, inflammation y atherosclerosis"
Chair: Ernesto Podestá

20:00 - 21:00 **Room A**
"FEBS" LECTURE
Peter Csermely
Semmelweis University, School of Medicine, Department of Medical Chemistry, Budapest, Hungary.
"Molecular Chaperones and multi-Target Therapy"
Chair: Ricardo Wolosiuk

21:00 - 21:30 **CLOSING CEREMONY**

Fundación Instituto Leloir Symposium
Glycobiology I

S1.
UNIQUE SORTING AND SUGAR CHAIN PROCESSING IN ER QUALITY CONTROL
Lederkremer GZ.
Dept. of Cell Research and Immunology, Tel Aviv University, Tel Aviv 69978, Israel. E-mail: gerardo@post.tau.ac.il

Sugar chain processing is traditionally associated with Golgi sorting of glycoproteins towards their final destinations. Recent evidence from our lab and others suggests that glycan processing also determines pre-Golgi sorting events. In mammalian cells, sugar-chain trimming of N-linked glycoprotein precursors to $Man_{9-8}GlcNAc_2$ is followed by cycles of reglucosylation by UDPGlc:glycoprotein glucosyltransferase (UGGT) and binding to the chaperone/lectin calnexin until correct folding allows release to ER exit sites. Other lectins, like ERGIC-53 then help deliver the glycoprotein to the Golgi. We found that misfolded glycoproteins that are substrates for endoplasmic reticulum-associated degradation (ERAD) are further processed to $Man_{5-6}GlcNAc_2$. By eliminating the mannose acceptor for UGGT this processing removes them from the calnexin binding cycles without requiring folding. The ERAD substrates are then delivered to a distinct pericentriolar compartment, which we termed the ER-derived quality control compartment (ERQC). Another lectin, EDEM, then participates in their targeting to the cytosol. Interestingly, also in the cytosolic side other lectins, Fbs1/2, which are components of E3 ubiquitin ligases, tag the glycoprotein for delivery to the proteasomes. Thus, glycan processing and lectin binding events guide a glycoprotein from its birth, through folding, quality control, refolding and final sorting towards the Golgi if it is properly folded and to proteasomal degradation if it is not.

S2.
MONITORING ACQUISITION OF TERTIARY AND QUATERNARY STRUCTURES IN ER QUALITY CONTROL
Caramelo JJ.
Fundación Instituto Leloir, Buenos Aires, Argentina.

Folding of glycoproteins entering the secretory pathway is strictly surveyed in the endoplasmic reticulum by a quality control system. Folding intermediates and proteins irreparably misfolded are marked via glucosylation by the UDPglucose:glycoprotein glucosyltransferase, an enzyme that acts as a folding sensor by exclusively labeling glycoproteins not displaying their native structures. We explore the structural determinants for GT recognition by using a family of chemically glycosylated proteins derived from chymotrypsin inhibitor-2 as GT substrates. Structural characterization of species showing higher glucose acceptor capacity suggests that GT recognizes solvent accessible hydrophobic patches in molten globule-like conformers mimicking intermediate folding stages of nascent glycoproteins. We show that this sensing mechanism also applies to the oligomerization of protein complexes, as the glucosyltransferase appeared to be able to glucosylate folded complex subunits lacking the full complement of oligomer components.

S3.
THE STRUCTURE OF GLYCOGEN SYNTHASE FROM *Pyrococcus abyssi*. CLUES FOR THE REGULATION OF THE EUKARYOTIC ENZYMES
Horcajada C, Fita I, Ferrer JC, Guinovart JJ.
Dep. Boquímica i Biologia Molecular, Univ. Barcelona, Inst. de Recerca Biomèdica (IRB), & Inst. de Biologia Molecular de Barcelona (IBMB-CSIC), Parc Científic Barcelona.
Glycogen and starch synthases are retaining glycosyltransferases that catalyze the successive transfer of glucosyl residues to the non-reducing end of a glowing α-1,4-glucan chain, a central process in the carbon/energy metabolism and the primary storage form of glucose in almost all living organisms. Here we report the recombinant expression and the crystal structure of the archaeal glycogen synthase from *Pyrococcus abyssi* (*Pa*GS), an enzyme that can use both ADP- and UDP-glucose as glucosyl donors. The overall topology of subunits of the archaeal synthase, a pair of β/α/β Rossmann-fold type domains with the catalytic site at their interface, is similar to that found for the recently reported bacterial glycogen synthase from *Agrobacterium tumefaciens*. Nevertheless, the molecular organization of *Pa*GS presents striking differences. The archaeal enzyme is a homotrimer both in solution, as determined by analytical ultracentrifugation, and in the crystal. The C-domains are not involved in intersubunit interactions of the trimeric molecule, thus allowing for movements, likely required for catalysis, across the narrow hinge that connects the N- and C-domains. The radial disposition of subunits confers the molecule a distinct triangular shape, clearly visible with negative staining electron microscopy, in which the upper and lower faces present a sharp asymmetry. Comparison of the archaeal synthase with the ADP-glucose dependent bacterial enzyme suggests the basis for the specificity of the glucosyl donors. These and other structural and functional features make the archaeal enzyme a suitable model to study the UDP-glucose specific and highly regulated, both by reversible phosphorylation and by allosteric effectors, animal/fungal glycogen synthases.

SBBq (Brasil) Symposium
Chimerical Proteins and Peptides

S4.
RECONSTRUCTING PULCHELLIN *IN VITRO*: TOWARDS BIOTECHNOLOGICAL APPLICATIONS
Grupo de Biofísica Molecular Sérgio Mascarenhas, Instituto de Física de São Carlos, Universidade de São Paulo, São Carlos, SP, Brasil. E-mail: anapaula@if.sc.usp.br

Pulchellin is a type 2 ribosome-inactivating protein (RIP) found in seeds of the *Abrus pulchellus tenuiflorus* plant. This work aims the obtaining of active and homogeneous the toxic chain of pulchellin for structural studies and biotechnological applications. The DNA fragment encoding Pulchellin A-chain (PAC) was cloned and inserted in pGEX-5X to express the recombinant pulchellin A-chain (rPAC) as a fusion protein in *Escherichia coli*. The ability of the rPAC to depurinate rRNA in yeast ribosome was also demonstrated *in vitro*. In order to verify the toxic activity of the rPAC *in vivo*, a protocol was used to obtain a functional heterodimer (named rPAB) *in vitro*, by the association of the two pulchellin subunits (expressed separately). Both chains were incubated in the presence of a reduced/oxidized system, yielding an active heterodimer (rPAB). The toxic activities of the rPAB and native pulchellin were compared by intraperitoneal injection in mice and the results indicated that the heterodimer presented toxic activity and a conformational pattern similar to pulchellin. rPAC produced in this heterologous system is currently used in the preparation of immunoconjugates and also bound with another lectin that recognizes specifically cancer cells, with great potential as a therapeutic agent in both cases.

S5.
ENZYMES INVOLVED IN AMINO ACID CATABOLISM IN TRYPANOSOMATIDS: FROM SEQUENCE TO FUNCTION
Nowicki C.
Departamento de Química Biológica, Facultad de Farmacia y Bioquímica (UBA-CONICET), Buenos Aires, Argentina. E-mail: cnowicki@criba.edu.ar

One of the challenges in the post-genomic era is that of understanding the structural features responsible of the substrate specificity of the new enzymes. In *Trypanosoma cruzi*, a tyrosine aminotransferase (TAT) and an aromatic L-α-hydroxyacid dehydrogenase (AHADH) are involved in the aromatic amino acids catabolism. *T. cruzi* AHADH possesses a high sequence identity (over 50%) with most of the cytosolic malate dehydrogenases (cMDH), yet has no MDH activity. Mutagenesis studies based on molecular modeling showed that this enzyme shares with the MDHs most of the essential residues for catalysis. The double substitution A102R/Y237G created a chimeric AHADH, which is able to reduce oxaloacetate without detrimental effects towards its natural substrates. On the other hand, the amino acid sequence of *T. cruzi* TAT resembles that of mammalian TATs (40% identity) however; *T. cruzi* enzyme has different substrate specificity, including a high alanine aminotransferase activity. Crystallographic as well as comparative kinetic and mutagenesis studies on *T. cruzi* and rat TATs evidenced that in both enzymes the N-terminal region is implicated in the catalytic mechanism. The rat TAT shortened by the first sixty residues becomes an inactive enzyme, although able to bind natively the coenzyme in the active site. It is likely that upon substrate binding, the active site of TATs is closed by the N-terminal "arm motif" which may play the role of a lid.

S6.
IMPROVING THE ACTIVITY OF SUGARCANE CYSTATINS BY DIRECTED EVOLUTION
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Cystatins are natural inhibitors of cysteine proteinases. Their inhibitory activity is explained by the presence of three contact points with the proteinase targets. The first point is an N-terminal glycyl-containing segment that interacts with the S1 and S2 enzyme sub-sites; the second is a hairpin loop containing a QVV motif highly conserved in cystatin superfamily, and the third one is a hairpin loop formed by a tryptophan-containing segment. Because plant cystatins present some structural peculiarities, they are called phytocystatins. These proteins, which expression can be induced by wound and methyl jasmonate, play a defensive role in plants because of their effects on exogenous proteinases such as those produced by fungi, insects and nematodes. We have described and characterized the first sugarcane cystatin (which we dubbed canecystatins). This protein is able to inhibit phytopathogenic fungi, as well several cysteine proteinases such as human cathepsins (e.g. cathepsin K, L, V and others from parasites). We have performed *in vitro* evolution experiments using different sugarcane cystatins aiming at obtaining chimeric cystatins with selectivity, and improved inhibitory activities. Some chimeric clones obtained after *DNA shuffling* were analyzed concerning their activity, and they have shown distinct activity and selectivity compared with the wild type genes used in the experiment. These experiments are giving us insights about the relationship between structure and function of cystatins.

S7.
HYBRID AND MODIFIED ANALOGS OF SYNTHETIC ANTIMICROBIAL AND ANTIGENIC PEPTIDES
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Lactic acid bacteria secrete bacteriocins, antimicrobial polypeptides attractive to be used as preservatives in food industry. The *Lactobacillus plantarum* NRIC 149 strain produce a bacteriocin named Plantaricin 149 (Pln 149) and *Pediococcus parvulus*, Pediocin (PA-1). Our group has synthesized C-terminal carboxamide peptides from Pln149 (Pln149a), its modified analogs and N-terminal acyl conjugates (Pln149ac), by n-octanoic acid, and a hybrid peptide formed by Pln 149 and PA-1. Besides, peptide sequences of proteins from HIV-1 constitute a strategic target for new antiviral drug design and development of diagnostic reagents and vaccines. In this sense, native and modified peptide sequences of the capside protein (p-24) from HIV-1 have also been prepared for structural and biological studies. Pln149a showed inhibitory activity against *Staphylococcus aureus* coagulase (+) and four strains of *Listeria*. CD spectra of Pln149a, in aqueous solution, showed an unstructured shape, however, in presence of TFE and reversed micelles of AOT, an α-helix shape was observed. The high cationicity combined with an amphipathic α-helical structure may be related to Pln149a activity. All the other modified synthetic bacteriocins inhibited the growth of *Listeria* strains including Pln149ac, this last also enlarged the antimicrobial activity spectrum towards other pathogenic bacteria. Modified peptides from p-24 HIV-1 had the α-helix content increased and this was correlated with an improvement of the recognition by antibodies in ELISA and conformational stability. Increase of α-helix content was also observed by interactions between the peptides with monolayers, vesicles and micelles. These strategies can be used to design bioactive peptides with potential therapeutic and biotechnological applications.

IBRO Symposium
Neurodegenerative Diseases: from Protein Structures to Neurobiology

S8.
THE ROLE OF OXIDATIVE METABOLISM OF DOPAMINE IN NIGRO-STRIATAL NEURODEGENERATION IN PARKINSON´S DISEASE (PD)
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We have strong evidences supporting a possible neuroprotective role of DT-diaphorase in dopaminergic neurons: (a) DT-diaphorase prevents Cu-neurotoxicity in RCSN-3 cells. The mechanism of Cu-neurotoxicity seems to involve (i) formation of a Cu-DA complex; (ii) uptake via DAT; (iii) oxidation of DA to aminochrome; (iv) one-electron reduction of aminochrome to leukoaminochrome o-semiquinone radical by inhibition of DT-diaphorase; (b) DT-diaphorase prevents Mn-neurotoxicity. Administration of intracerebral Mn^{3+} together with dicoumarol into the left medial forebrain bundle produced a behavioural pattern characterized by contralateral rotational behaviour when the rats were stimulated with apomorphine, in a manner similar to that observed in animals with unilateral 6-hydroxy-dopamine-induced lesions; (c) DT-diaphorase prevents dopamine dependent iron neurotoxicity in RCSN-3 cells. The mechanism of iron-neurotoxicity seems to involve: (i) formation of Fe-dopamine complex; (ii) specific uptake of Fe-dopamine complex via monoaminergic transporter; (iii) dopamine oxidation to aminochrome; (iv) one-electron reduction of aminochrome when DT-diaphorase is inhibited with dicoumarol; (d) DT-diaphorase prevent neurotoxicity in RCSN-3 cells when VMAT is inhibited.
Supported by FONDECYT 1020672.

S9.
MODULATION OF NEURONAL APOPTOSIS BY p75 NEUROTROPHIN RECEPTOR
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A toxic "gain-of-function" of mutant Cu-Zn superoxide dismutase 1 (SOD1) has been involved in the pathogenesis of familial Amyotrophic Lateral Sclerosis (ALS). Expression of mutant forms of the human SOD1 gene causes astrocyte activation and degeneration of motor neurons (MNs). Damaged motor neurons release fibroblast growth factor-1 (FGF-1) in response to damage linked to the expression of mutated SOD-1. Although FGF-1 is neuroprotective, we found that it can activate spinal cord astrocytes, which in turn can induce apoptosis in p75-expressing MNs. p75NTR-dependent MNs apoptosis was mediated by nitric oxide (NO) and astrocytic secretion of nerve growth factor (NGF). FGF-1 also induced Nrf2, a redox-sensitive transcription factor that regulates several ARE-containing genes, including HO-1 and enzymes involved in glutathion synthesis. Induction of ARE/Nrf2 pathway in astrocytes completely prevented $p75^{NTR}$-dependent MN apoptosis. Thus, p75NTR-dependent MN apoptosis seems to be largely modulated by the phenotype of adjacent astrocytes.

S10.
CONFORMATIONAL BASIS AND NOVEL TARGETS FOR ß-AMYLOID-INDUCED NEURONAL DYSFUNCTION IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the most common form of dementia in the elderly, affecting 10% of the individuals older than 65. The precise causes of dementia are not known and there are no effective therapeutics available. Two hallmarks of the neuropathology are the extracellular senile plaques, consisting of aggregated ß-amyloid peptide (Aß), and the neurofibrillary tangles, intraneuronal inclusions formed by hyperphosphorylated microtubule-associated protein tau. Recent evidence indicates that soluble Aß oligomers (rather than the amyloid fibrils that were first identified in senile plaques) are the main neurotoxins that cause early synaptic dysfunction and may trigger other deleterious neuronal effects in AD. However, the mechanisms involved in neuronal impact of Aß oligomers are still largely unknown. Our group is interested in investigating the molecular/conformational basis of neuronal dysfunction induced by amyloid oligomers and in the identification of neuronal receptors of Aß. Recent results in these two research lines will be presented and possible implications of these findings to our understanding of the mechanisms of neurotoxicity in AD will be discussed.
Supported by CNPq, FAPERJ, FINEP and Pronex (Brazil) and HHMI (USA).

S11.
STRUCTURAL BIOLOGY AND PATHOGENESIS OF PARKINSON DISEASE: TARGETING α-SYNUCLEIN

Bertoncini CW[2], Rasia RM[2], Binolfi A[1,2], Hoyer W[2], Jovin TM[2], Zweckstetter M[2], Griesinger C[2], Fernández CO[1,2].
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The aggregation of α-synuclein (αS) is characteristic of Parkinson disease (PD). To understand the structural determinants of αS fibrillation we have used a multidisciplinary approach to characterize the conformation adopted by native αS in solution,[1] the effects induced by the missense mutations A30P and A53T,[2] and the interaction with metal ions.[3,4] We found that long-range intramolecular interactions are responsible for maintaining αS in an auto-inhibitory conformation, whereas the Parkinsonism-linked mutants of αS showed a destabilization of specific tertiary interactions essential for the native state of αS. From the interaction of αS with metal ions we showed conclusively that Cu(II) is the only studied metal ion that binds specifically to the N-terminal of αS and triggers its aggregation under conditions that might be relevant for the development of PD. Our findings support a tighter link between PD and other disorders such as Alzheimer and prion diseases, suggesting that perturbations in metal homeostasis may constitute a more widespread element in neurodegenerative disorders than recognized previously.
1.Bertoncini *et al*., PNAS (2005) 102: 1430-35; 2.Bertoncini *et al*., J. Biol. Chem. (2005) 280: 30649-52; 3.Rasia *et al.*, PNAS (2005) 102: 4294-99; 4.Fernández *et al*., EMBO J (2004) 23: 2039-46.

Recent Advances in Cellular Calcium Signaling

S12.
SIGNAL TRANSDUCTION AND GENE EXPRESSION REGULATED BY CALCIUM RELEASE FROM INTERNAL STORES IN EXCITABLE CELLS

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In the past few years, we have gathered important information studying fluorescent calcium signals from cultured skeletal muscle cells. Both K^+ depolarization and tetanic electrical stimulation were shown to produce IP_3 dependent slow Ca^{2+} signals, unrelated to contraction and associated to regulation in gene expression. Treatment of cells with a G protein inhibitor blocks both calcium signals and IP_3 transients. Viral transduction of a G-beta-gama scavenger (Ad-betaARK) also inhibits the slow calcium response. Immunocytochemical studies using an anti PLC antibodies show changes in both PLC beta3 and gama1 30 to 50 s post electrical stimulation. A sensor for PI3K translocation shows involvement of this enzyme in the transduction process. We characterized a fraction of purified nuclei from rat skeletal myotubes. Immunofluorescence and immuno-electron microscopy studies localized IP_3 receptors type 1 and type 3 in the nuclear region, type 2 was confined to cytoplasm. Isolated nuclei responded to IP_3 with rapid and transient Ca^{2+} elevations, which were inhibited by blockers of IP3 signals. Nuclear Ca^{2+} increase triggered by IP_3 evoked CREB phosphorylation, with kinetics compatible with sequential activation. These results support the idea that nuclear Ca^{2+} signals, triggered by electrical activity in muscle cells, are likely to participate in gene regulation mediated by CREB.
Financed by FONDAP 15010006.

S13.

THE CONTRIBUTION OF CALCIUM INFLUX TO MITOCHONDRIA-TRIGGERED CELL DEATH

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Targeted protein probes allow selective measurements of $[Ca^{2+}]$ inside organella. Using mitochondria-targeted aequorins we find that this organelle can take up very large amounts of calcium through the mitochondrial uniporter when the local cytosolic $[Ca^{2+}]$ ($[Ca^{2+}]_c$) increases above 2-4 μM. These high $[Ca^{2+}]_c$ microdomains are generated at the vicinity of open plasma membrane Ca^{2+} channels of excitable cells. Excessive Ca^{2+} entry and the resulting mitochondrial Ca^{2+} overload has been suggested to induce apoptotic neuronal dead. We test here whether expression of plasma membrane Ca^{2+} channels would render T lymphocytes susceptible to death by Ca^{2+} overload. Massive influx through through expressed vanilloid receptor type 1 (VR1) channels, but not capacitative Ca^{2+} influx stimulated by the muscarinic type 1 receptor, induced sustained $[Ca^{2+}]_c$ rises, exposure of phosphatidylserine, and cell death. Ca^{2+} influx was necessary and sufficient to induce mitochondrial damage, as assessed by opening of the permeability transition pore and collapse of the mitochondrial membrane potential. Ca^{2+}-induced cell death was inhibited by ruthenium red, protonophores or cyclosporin A. Thus, Ca^{2+} influx triggers a distinct program of mitochondrial dysfunction leading to paraptotic cell death.

S14.

NICOTINIC ACID ADENINE DINUCLEOTIDE PHOSPHATE (NAADP) A NEW INTRACELLULAR SECOND MESSENGER

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Nicotinic acid adenine dinucleotide phosphate (NAADP) is a potent activator of intracellular Ca^{2+} release in several vertebrate and invertebrate systems. The role of the NAADP system in physiological processes is being extensively investigated at the present time. The NAADP receptor and its associated Ca^{2+} pool have been hypothesized to be important in several physiological processes including fertilization, T cell activation, pancreatic secretion,smooth muscle contraction and neural functions. Recent research has determined the role of NAADP as an intracellular second messenger for several cells. The *in vivo* metabolism of NAADP and the molecular nature of its cellular target(s) have not been fully characterized. Many fundamental questions remain open. Does NAADP activates a specific intracellular Ca channel? Where is the NAADP target of action? Is NAADP metabolized by the enzyme CD38? How is the metabolism of NAADP regulated? On going research from several laboratories are providing some important clues to this questions. In this presentation I will discuss some of these new findings that are helping us answer these important questions.Nicotinic acid adenine dinucleotide phosphate (NAADP) is a potent activator of intracellular Ca^{2+} release in several vertebrate and invertebrate systems. The role of the NAADP system in physiological processes is being extensively investigated at the present time. The NAADP receptor and its associated Ca^{2+} pool have been hypothesized to be important in several physiological processes including fertilization, T cell activation, pancreatic secretion,smooth muscle contraction and neural functions. Recent research has determined the role of NAADP as an intracellular second messenger for several cells. The *in vivo* metabolism of NAADP and the molecular nature of its cellular target(s) have not been fully characterized. Many fundamental questions remain open. Does NAADP activates a specific intracellular Ca channel? Where is the NAADP target of action? Is NAADP metabolized by the enzyme CD38? How is the metabolism of NAADP regulated? On going research from several laboratories are providing some important clues to this questions. In this presentation I will discuss some of these new findings that are helping us answer these important questions.

S15.

Ca^{2+}-ATPase: MECHANISM OF ACTION AND ITS RELEVANCE IN PATHOLOGICAL STATES

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The basic function of the plasma membrane calcium pumps (PMCAs) is to maintain the 10,000-fold calcium gradient across the plasma membrane via the highly regulated active expulsion of calcium from the cell. Although, the role of the complex isoform diversity and especially the contribution of specific isoforms to pathological conditions is less well understood. There are more than 30 splice variants formed from the four PMCA isoforms, each differing in its affinity for calcium and calmodulin, with some isoforms showing tissue-specific expression. PMCA isoforms are differentially regulated by protein kinases (PKA, PKC), by proteases (calpain), by effector caspases, and by interaction with phospholipids like phosphatidylserine and phosphatidylinositol, which act to shape the time course of the calcium signals. PMCA isoforms can also have tissue-specific roles, such as the regulation of the rate of clot retraction in platelets and the secretion of insulin in pancreatic islets. A human disease has been linked recently to a defect in PMCA2. This defect cannot be overcome by other present isoforms, as shown by the indispensable role of PMCA2 demonstrated in transgenic mice (deaf-waddler variant). Changes of PMCA function are often detected in cell types different from the specific type involved in the pathology, pointing to more general defects, i.e. the erythrocytes in diabetes and blood platelets in hypertension. These changes suggest the significance of PMCA in Ca^{2+} homeostasis both in excitable and non-excitable cells.

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SBBM (Uruguay) Symposium)
Systems Biology and Integrative Biochemistry

S16.
WHAT IS SYSTEMS BIOLOGY?
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The reductionist method has been successful to describe simple systems, but shows an important drawback when applied to study properties of complex systems. The problem is that it is a priori difficult to decide what are the components of the system which are necessary to describe a process of interest, without losing significant interactions. Systemic approaches are mainly concerned with tackling this problem.
Systems Biology could be defined as the integrated study of complex biological processes. For this aim it uses two different types of analysis: modular and comprehensive. Modular analysis conceptually divides the intact system in modules, lumping all what is irrelevant to the question that we want to answer, including all what we ignore and whose knowledge is not required to obtain the answer. In contrast, comprehensive (genome-scale) analysis aims to deal with all the complexity, trying to describe, simultaneously, what happens to all the components of the system.
In the presentation we shall give a brief introduction to the emergent field of Systems Biology and describe some strategies of modular and genome-scale analyses for metabolic systems using examples developed in our laboratory.
This work is supported by CSIC,Universidad de la República, Montevideo and PEDECIBA, Montevideo.

S17.
GLUCOKINASE SUBCELLULAR LOCATION
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The glucokinase gene (hexokinase IV/D) is expressed in liver (hepatocytes) and in pancreatic β-cells. Usage of different glucokinase promoters in the two cell types enables differential control of glucokinase gene expression in response to hormonal and other stimuli. The sub-cellular location of glucokinase differs in the liver and pancreatic β-cells because of expression of different glucokinase binding proteins in the two cell types. Recent evidence has revealed a remarkable complexity of sub-cellular location of glucokinase in both liver and pancreatic β-cells that is attributed to distinct roles of various glucokinase binding proteins. Some of these proteins are specific for glucokinase and others multi-functional. Glucokinase binding proteins control the enzyme activity, sub-cellular location, and protein stability and expression by post-transcriptional mechanisms. Glucokinase regulatory protein and the bifunctional enzyme phosphofructokinase-2 / fructose bisphos -phatase-2 acts as nuclear and cytoplasmic receptors, respectively for glucokinase in the hepatocyte. Other proteins are involved in regulating binding to mitochondria and insulin granules.

S18.
LOOKING FOR NEW TARGETS IN CANCER THERAPY FROM A METABOLOMIC APPROACH
Cascante M, Ramos A, Vizan P, Boren J, Atauri P de, Centelles JJ, Boros LG, Lee PWN, Selivanov V.
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Metabolic profile is the end point of the signalling events, where changes caused by diseases like cancer may be reflected. We characterized the metabolic pathways (utilizing gas chromatography coupled to mass spectrometry) implied in glucose metabolism and ribose synthesis in tumoral and non tumoral cells and we characterize some metabolic adaptations accompanying tumor cell proliferation. We integrated the experimental data in mathematical models and we used Metabolic Control Analysis (MCA) to identify the main enzymes controlling ribose-5-P synthesis and to plan combined target strategies. Finally, we validated the obtained strategies using specific inhibitors and we studied the effects produced in cell proliferation as well as in the proteomic profile.

S19.
STANDARDS, SOFTWARE, MOTIFS AND SYSTEMS BIOLOGY
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The last five years of more has seen one of the most significant shifts in thinking by mainstream biologists since the discovery of the structure of DNA. That change is the realization that a deeper understanding of cellular function will require a more quantitative and systems approach. This field has been called systems biology.
In this talk I will discuss three areas of interest to me. The first involves the development of standards to allow different researches to exchange computational models; I will in particular emphasize the role of SBML (Systems Biology Markup Language) as one of the primary modes for exchanging models. The second area I will discuss is the development of extensible software tools (SBW - Systems Biology Workbench) which permit computational models to be tested and studied. Finally I will present an application we have been working on which is the modeling of the p53/Mdn2 pulse train which arises when cells are exposed to ionizing radiation. An understanding of this model requires an appreciation of functional network motifs, and how modularity permits us to understand a complex network.
This work is supported by grants from the DOE, DARPA and NSF.

S20.
HOW CAN WE MAKE SYSTEMS BIOLOGY WORK?
Cornish-Bowden A[1], Cárdenas ML[1], Letelier JC[2], Soto-Andrade J[3], Guíñez Abarzúa F[3].
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Efforts to apply systems biology began in the middle of the 20th century, but it has suddenly become fashionable, with many new publications using the two words, not always with the same meaning. Reductionism remains dominant, however, and systems biology is often seen as no more than integration of diverse data into models of systems. The emphasis ought, however, to be on the needs of the system as a whole for understanding the components, not the converse. Metabolism tends to be viewed as static, although enzymes are continuously synthesized and degraded. They are themselves therefore metabolites: this fact introduces great complexity to metabolism, with an implication of infinite regress; understanding how to escape from this is essential for understanding life. The concept of metabolism-repair systems provide a major step in this direction, but it has remained very obscure for most biologists.

Signal Transduction in Plant Development

S21.
SALICYLIC ACID AND TEH ACTIVATION OF CELLULAR STRESS PROTECTION GENES IN *Arabidopsis thaliana*
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It has been clearly established that salicylic acid (SA) is accumulated in plants subjected to different stressful conditions, and that this accumulation is crucial for development of a successful defense response. In this response, the increase in SA concentration is always associated to the increase in the cellular levels of reactive oxygen species (ROS). During the last years, increasing evidence supports the idea of a mechanistic interplay between SA and ROS in the defense response. In our group we are interested to study this interplay, particularly associated to the early events of transcriptional activation activated by SA. Analyses of transcript profiles in Arabidopsis thaliana, by using cDNA-Amplified Fragment Length Polymorphism (cDNA-AFLP) and cDNA microarrays, allowed us to identify a group of 232 genes which are early and transiently activated by SA. Interestingly, an important group of these genes have antioxidant activity. Furthermore, evidence obtained from molecular genetics approaches indicate that redox-mediated signals are involved in the activation by SA of defense genes. Interestingly, oxidative species and redox controlled activators, have been shown to mediate the transcriptional activation of defense genes by SA. These lines of evidence support the idea that SA plays a role in controlling the cellular redox balance in the onset of the defense response.
This work was supported by research grant 1020593 from Fondecyt-Conicyt, Chile.

S22.
STRESS-ACTIVATED PHOSPHOLIPID SIGNALLING PATHWAYS IN PLANT
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It is becoming increasingly clear that plants, just like animals, contain a variety of phospholipid-based signal transduction systems. These include phospholipase C (PLC), PLD, PLA_2, but also lipid kinases such as diacylglycerol kinase (DGK), which produces the lipid second messenger phosphatidic acid (PA), and phosphoinositide (PPI) kinases, which produce all kinds of PPI isomers, e.g. PI3P, PI5P and $PI(3,5)P_2$. There are also differences compared to animals. For example, plants lack PIP_3 and contain PA kinase (PAK), a recently discovered enzyme that phosphorylates PA into the novel lipid diacylglycerol pyrophosphate (DGPP). For review see Meijer and Munnik (2003; Annu. Rev. Plant Physiol).
Over the last few years, we have shown that a number of these pathways are differentially activated in response to a wide variety of biotic- and abiotic plant stresses. In general, activations are rapid (within seconds to minutes of stress application) and transient. However, still very little is known of 'how' pathways become activated (receptor coupling), 'where' in the cell or plant this takes place, and 'what' the functional significance of the activation is. To start answering some of these questions, several new opportunities are being explored. These include, i) the use of *Arabidopsis* KO mutants, ii) expression of GFP-based lipid biosensors to visualize lipid signalling *in vivo*, and iii) lipid-protein proteomics to identify, isolate and characterize targets for the new lipid second messenger, PA.

S23.
REGULATION OF GENE EXPRESSION BY MICRORNA FAMILIES IN PLANTS
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MicroRNAs (miRNAs) are small RNAs of 18 to 20 nucleotides in length that regulate gene expression in multicellular organisms. They are transcribed as larger precursors with extensive secondary structure that are processed by RNAse III enzymes such as DICER LIKE 1 in plants, which release the mature miRNA. They are then incorporated into RISC complexes (RNA Induced Silencing Complexes) where they provide for sequence specificity. MiRNAs recognize partially complementary sequences in target mRNAs and guide them to cleavage or translational arrest. In plants, miRNAs regulate different kinds of genes and pathways, including transcription factors, hormone signaling and metabolism.
Generally, miRNAs in plants and animals are encoded by small gene families of up to 14 members. A typical miRNA family usually pools miRNAs of similar sequences, however it is still not known if slight changes in the miRNA sequences can have any biological significance. We will present data showing the role of miRNAs during plant development and the functional specialization of miRNA variants.

S24.
DEVELOPMENTAL AND ENVIRONMENTAL INTER-ACTIONS IN PLANT RESPONSES TO STRESS
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The maize Abscisic acid (ABA) responsive protein Rab17 is a late embryogenesis abundant protein involved in plant responses to stress. Here we provide evidence of the importance of Rab17 phosphorylation by protein kinase CK2 in growth related processes under stress conditions. Transgenic plants overexpressing Rab17 but not the unphosphorylated Rab17, arrest the process of seed germination under stress conditions. Thus, the role of Rab17 in growth processes is mediated through its phosphorylation by protein kinase CK2. Transcriptional regulation of rab17: using yeast one-hybrid screening we previously isolated, two new DRE-binding proteins DBF1 and DBF2. The ZmDBFs genes are members of the AP2/ERF transcription factors family. Analysis of mRNA accumulation profiles showed that DBF1 is induced during maize embryogenesis and after desiccation. Moreover, ABA plays an important role in the regulation of DBF1 activity, and suggested the existence of an ABA-dependent pathway for the regulation of genes through the C-repeat/DRE.Transgenic plants overexpressing DBF1 are more tolerant to osmotic stress than control plants.

Fundación Instituto Leloir Symposium
Glycobiology II

S25.
REGULATION OF TGF-β SIGNALING DURING MYOGENESIS
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Molecular mechanisms that control skeletal muscle differentiation are partially known. Growth factors, such as transforming growth factor β ((TGF-β), are strong repressors of this process *in vitro* and depletion of them from myoblasts culture media trigger muscle formation. However it is not know how this process is controlled. We have evaluated the expression of the molecules involved in the signaling pathway dependent of TGF-β during skeletal muscle differentiation. We found that the signaling decrease significantly during differentiation albeit the expression of the transducing receptors increase. The decrease in signaling is explained, in part, by a decrease in the expression of the smad-2 and smad-4 proteins, key molecules that are translocated to the nucleus in response to TGF-β.The increase in transducing receptors seems to be required during all the differentiation process, because dominant negative forms of them inhibit myogenesis. Another mechanisms that reduce TGF-β signaling during skeletal muscle formation is the binding of? TGF-β to the extracellular matrix molecules. Decorin null and biglycan null myoblasts show a significant increase in the binding of TGF-β to the transducing receptors. This binding can be reverted by *de novo* synthesis of the TGF-β binding proteoglycans. These results indicate that decorin and biglycan could acts as an important component for TGF-β binding and signaling through its receptors. Therefore during myogenesis TGF-β dependent signaling can be modulated by at least two different mechanisms at the intracellular and extracellular level.
Supported by MDA3790, FONDAP-CRCP, HHMI and MIFAB.

S26.

THE GOLGI APPARATUS NUCLEOTIDE SUGARTRANSPORT/ANTIPORT CYCLE: FROM BASIC SCIENCE TO DISEASE

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In eukaryotes half of cellular proteins are secreted or membrane bound. Both groups of proteins are synthesized on membrane bound polysomes, translocated into the lumen of the endoplasmic reticulum (ER), transported first to the Golgi apparatus (GA) and thereafter to their final destination within or outside the cell. In the lumens of the ER and GA eighty percent of these proteins become glycosylated, sulfated and phosphorylated by enzyme catalyzed reactions using as substrates nucleotide-sugars, nucleotide-sulfate and ATP. These nucleotide derivatives must be transported into the lumens of the above organelles from the cytosol, the site where most are synthesized. Transport of these substrates into the lumen of the Golgi apparatus is mediated by specific transporters, which have been found to occur in every eukaryote studied to date. Transporters are antiporters with the corresponding nucleoside monophosphate; this enables nucleotide sugars to be concentrated in the lumen of the GA relative to their concentration in the cytosol. While the initially characterized transporters were specific for one substrate recent studies have shown that others may be multisubstrate. Mutants in these transporters have been described in uni- and multi-cellular eukaryotes such as yeast, *Leishmania, Entamoeba, Drosophila*, nematodes, plants and mammals. In many of these organisms mutations of transporter proteins result in striking developmental phenotypes including diseases such as Leukocyte Adhesion Deficiency Syndrome II which affects growth and brain development. Very recent studies with *C. elegans* suggest tissue functional redundancy of these transporters.

S27.

UNDERSTANDING THE ORGANIZATION OF GLYCOLIPID SYNTHESIS IN THE GOLGI COMPLEX

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Glycolipid glycosyltransferases are synthesized in the endoplasmic reticulum (ER) and transported towards their site of residence, the Golgi complex. They form different physical associations which overlap along the proximo-distal axis of the Golgi, although those involved in the synthesis of simple glycolipid species concentrate in the proximal-while those for complex species in the distal- (trans Golgi and trans Golgi network or TGN) aspects of the organelle. The N-terminal domain [comprising the **c**ytoplasmic **t**ail (ct), the **t**rans**m**embrane **r**egion (tmr) and few amino acids of the stem region] bears information for the journey from the ER to the Golgi and for the formation of associations as do the full length forms of these enzymes. Selective concentration at ER exiting sites depends on interactions of a [RK](X)[RK] motif in the ct with the small GTPase Sar1 involved in COPII vesicle formation, while complex formation seems to depend on interactions among the tmrs. Currently we are investigating whether the N-terminal domain of SialT2, concentrated in proximal Golgi compartments, and the one of GalNAcT, a more distally concentrated Golgi enzyme, are able to concentrate reporter proteins in the corresponding sub-Golgi compartments. Double color fluorescence microscopy in single CHO-K1 cells, and sub-cellular fractionation showed that SialT2 N-terminal domains concentrate spectral variants of the GFP in a proximal- and that of GalNAcT in a distal- sub-Golgi compartment. Exchanging the cytoplasmic tails of SialT2 and GalNAcT indicate that information for proximal or distal localization associates to the ct of these N-terminal domains.

Lipids

S28.

ROLE OF SEC14 DOMAINS IN THE REGULATION OF LIPID HOMEOSTASIS AND VESICULAR TRANSPORT

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The regulated transport of vesicles within cells is an essential process for the maintenance of organelle integrity as well as for specialized processes such as neurotransmitter release and lipoprotein secretion. The CRAL/TRIO domain binds lipids and is found in several mammalian proteins as well as yeast Sec14. Yeast Sec14 has been observed to be ssential for vesicular fission from the Golgi and it appears to link lipid metabolism with the regulated release of vesicles from the Golgi. However, a direct link between lipid metabolism and specific components of the vesicular trafficking apparatus is still missing. We will describe high throughput synthetic genetic array analysis and its use to discover the first set of proteins that link Sec14 mediated regulation of lipid metabolism with known components of the vesicular transport apparatus. This strategy has also allowed us to classify proteins as either positive or negative regulators of Sec14 mediated vesicular transport and increased the number of proteins that regulate this process from 6 to over 30. Based on this new data, models on how lipids regulate vesicular transport will be presented.

This work was supported by the Canadian Institutes of Health Research and the Canada Research Chairs fund.

S29.

CONTROL OF CANCER CELL SURVIVAL BY MEMBRANE MICRODOMAIN ASSOCIATED CAVEOLIN-1

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Caveolin association with cholesterol-rich membrane microdomains, often referred to as "rafts", is required for the formation of morphologically distinguishable structures called caveolae. These structures are implicated in a variety of cellular functions including transcytosis, cholesterol transport and as cellular sites of cross-talk in signal transduction. A large body of available data, including results from this laboratory, implicate a member of the caveolin family, caveolin-1, as a functional tumor suppressor protein in cancer cells. Also, data in the literature suggest that caveolin-1 may do so by blocking cell proliferation and/or promoting cell death. Here I will discuss our recent results linking caveolin-1-mediated inhibition of β-catenin/Tcf-Lef dependent transcription and the resulting decrease in expression of the IAP survivin to cell cycle changes and increased apoptosis.

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S30.

ISOPRENOID BIOSYNTHESIS IN TRYPANOSOMATIDS: ENZYMES, INHIBITORS AND INTRACELLULAR LOCALIZATION

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Isoprenoid biosynthesis is an essential process for viability and an attractive route for chemotherapeutic intervention in the Trypanosomatidae. Several enzymes involved in this metabolic pathway exhibit potential for inhibitor development. 3-Hydroxy methyl-3 glutaryl Coenzyme A reductase, farnesyl pyrophosphate synthase, squalene synthase and sterol methyl transferase have been characterized in *Leishmania major* and *Trypanosoma cruzi* in order to identify characteristics exploitable en in a drug design process. Thus, we have established that some of these enzymes exhibit unique structural features, kinetic properties and inhibition profiles. Recombinant soluble proteins have been used in the screening of different classes of inhibitors and promising results have been achieved with regard to antiprotozoal activity and selectivity. We also have analyzed the intracellular localization of isoprenoid biosynthetic enzymes in *Leishmania* by means of permeabilization analysis, indirect immunofluorescence and inmunoelectron microscopy. While early steps have a major mitochondrial localization, farnesyl diphosphate synthase is a cytosolic enzyme and later steps involved in ergosterol formation are associated with specific membrane structures. Hence, isoprenoid biosynthesis exhibits particular characteristics in the Trypanosomatidae regarding its intracellular location and the steps involved take place in multiple subcellular compartments.

From Signal-Transduction to Virulence in Bacteria

S31.

A NOVEL BACTERIAL METAL-ION SENSING AND DETOXIFICATION SYSTEM

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The MerR family is a group of transcriptional regulators with similar N-terminal DNA binding regions and C-terminal effector binding domains that respond specifically to certain environmental stimuli, such as heavy metals, oxidative stress or antibiotics. Among the heavy metal responsive regulators, different subfamilies can be distinguished based on key residues in their sequences, including a group that recognizes Cu(I); a separate group that specifically senses divalent metals such as Zn(II), Co(II), Cd(II), and Pb(II); and the group of Hg(II) responsive regulators. My group has characterized a three-gene *Salmonella*-specific locus coding for a protein with high homology to the MerR-like copper-responsive regulators, a metal-transporting P-type ATPase, and a polypeptide with homology to copper chaperones. Activation of this locus depends on both the intactness of the regulator and micromolar concentration of Au^{3+} salts in the culture medium, and it is not affected by addition of other mono or divalent heavy metal cations. In addition, a strain deleted in the locus was highly susceptible to gold salts, highlighting its role in gold detoxification. This is the first gold sensing and detoxification system identified in bacteria. This horizontally acquired system allows *Salmonella* to monitor and to withstand environments with high concentration of gold salts.

S32.
MOLECULAR MECHANISM OF FtsZ RING FORMATION IN *Bacillus subtilis*
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Cytokinesis in rod-shaped bacteria entails the invagination of cell membranes and cell wall remodeling resulting in the formation of the so-called division septum. The first step in septum formation is the polymerization of FtsZ, the prokaryotic homolog of tubulin, into a ring-like structure associated with the inner face of the cell membrane. This cytoskeletal structure, known as the Z-ring, serves as a scaffold for the assembly of a large macromolecular machine, composed of about a dozen proteins and whose activity leads to the construction of the new septum. Our lab is interested in understanding the requirements for Z-ring formation and how this event is regulated in time and space during the bacterial cell cycle. Z-ring formation seems to be regulated by the interplay between proteins that promote FtsZ polymerization and proteins that inhibit it. To search for proteins capable of promoting Z-ring formation, we carried out a screen for genes whose overexpression restored division in the presence of an inhibitor of FtsZ polymerization. This screen identified ZapA, a 10 kDa protein widely conserved in bacteria whose function was previously unknown. Here, we will describe cytological and biochemical experiments, as well as structure-function studies, aimed at elucidating the mechanism by which ZapA promotes Z-ring formation.

S33.
***Mycobacterium tuberculosis* VIRULENCE FACTORS**
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Mycobacterium tuberculosis developed various procedures to survive inside professional phagocytes and avoid the different defence mechanisms of the host. After interacting with receptors at the phagocyte surface, *M. tuberculosis* is phagocytosed and remains in a phagosome that does not fuse with lysosomes and does not acidify, thus allowing the bacilli to multiply. The route of entry of the bacterium into the cell might be important. Interactions of *M. tuberculosis* with dendritic cells occur through the specific binding of LAM with DC-SIGN. This leads to an absence of bacilli multiplication. This is in contrast to the behaviour of the TB bacilli in macrophages that do not express DC-SIGN. Alveolar macrophages express DC-SIGN after infection. This might contribute to the spread of infection and development of pulmonary TB.
In addition to the study of LAM and other ligands of DC-SIGN, a search for virulence factors involved in interactions of bacilli with phagocytes was undertaken through the isolation of mutants impaired in *in vivo* growth. Genetic tools were developed enabling the transfer of DNA into mycobacteria and the inactivation of genes. The construction of mutant libraries after Signature Transposon Mutagenesis (STM) allowed the screening of avirulent mutants in a mouse model. Using this technique thirteen different virulence loci were identified. Three of them are located in a cluster of genes that are responsible for the synthesis of dimycoserosates (DIM) or their transport to the bacterial cell surface. DIM protects *M. tuberculosis* from the cidal activity of reactive nitrogen intermediates produced by macrophages and modulates the early immune responses to infection by inhibiting the synthesis of TNF alpha and IL6.

S34.
***Brucella abortus* VIRULENCE. SIGNALS AND DELIVERY SYSTEMS REQUIRED FOR AN INTRACELLULAR JOURNEY**
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Brucella spp. the pathogens that cause brucellosis, are intracellular bacteria that persist and multiply in the macrophages. In terms of virulence brucellae are characterized by an active adaptation to the intracellular environment and resisting the innate and acquired immune system. Brucellae multiply in a ER-like vacuole compartment named the brucellosome. To reach the brucellosome *B. abortus* alters the intracellular trafficking escaping from the endocytic pathway by preventing the fusion of the brucellae containing vacuole (BCV) with the lysosome. This process requires a functional type IV secretion system (the *virB* operon,) and the synthesis and secretion of cyclic β (1-2) glucans (CβG). We postulate that *virB* exerts its action delivering effectors to the BCV that alter its intracellular trafficking preventing fusion with the lysosome. The intracellular *virB* expression must be tightly regulated to exert this action, being turned on during the initial stages of infection and turned off after reaching the brucellosome. This precise regulation requires the action of the bacterial histone integration host factor. CβG also circumvents host cell defenses preventing BCV lysosome fusion through a novel mechanism that disrupts phagosome lipid raft organization.

Pathways for Protein Degradation

S35.
HUMAN CYSTEINE CATHEPSINS: STRUCTURE AND FUNCTION
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There are 11 human cathepsins (B, C, F, L, K, V, S, X, H, W and O) present in human genome in papain-like cysteine proteases. They are monomeric proteins with the only exception of the tetrameric cathepsin C. Most of cathepsins exhibit predominantly endopeptidase activity, while the cathepsins B, C, H and X are exopeptidases. Their rather short active/site cleft comprises three well defined substrate binding subsites S2, S1 and S1', as confirmed by the X-ray analysis. The cathepsins are regulated by their endogenous protein inhibitors cystatins and thyropins. Although cathepsins are involved in many physiological and/or pathological processes there is recent evidence, that these enzymes also play an important role in apoptosis. We found that proapoptotic Bcl-2 family member Bid is cleaved by lysosomal extracts and several cysteine cathepsins. Thus, Bid may be an important mediator of apoptosis induced by lysosomal disruption.
Stoka *et al*. (2005) IUBMB Life 57: 347-353

S36.
EFFECTIVE INHIBITION OF VIRAL AND MICROBIAL PROTEASES
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Protease targets of viral or microbial origin display different levels of heterogeneity due to: 1) genetic diversity; 2) drug resistant mutations; 3) binding site dynamics. Structure-based drug design against heterogeneous targets requires a departure from the classic "lock and key" paradigm that leads to the development of conformationally constrained molecules unable to adapt to target variations. Heterogeneous targets need adaptive drug molecules, characterized by the presence of flexible elements and/or asymmetric chemical functionalities that allow them to maintain a viable binding affinity against existing or expected polymorphisms. Adaptive ligands have characteristic structural and thermodynamic signatures that distinguish them from their rigid counterparts. This realization has lead to new design guidelines involving: 1) a new representation of binding sites that include a structural mapping of genetic polymorphisms and conformational dynamics; 2) a different way of optimizing binding affinity; 3) a different way of achieving specificity and selectivity; and, 4) specific criteria to achieve adaptability to target heterogeneities.
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S37.
MEPRIN METALLOPROTEINASES: STRUCTURES AND FUNCTIONS
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Meprins are oligomeric, brush border membrane or secreted zinc proteases that have unique and complex structures. They are highly expressed in kidney and intestine, and in leukocytes and cancer cells under some conditions. Meprins are composed of multidomain, highly glycosylated, evolutionarily-related α and β subunits that form disulfide-linked homo- or heterooligomeric dimers. The meprin ß subunits are type I transmembrane proteins that form only homodimers or heterotetramers (α_2,$ß_2$; α_3,$ß_1$). The mature meprin α subunits form homodimers, lose their transmembrane domains in the endoplasmic reticulum during intracellular transport, tend to self-associate, and are secreted through the apical side of polarized epithelial cells as very high molecular mass multimers of 1,000,000 to 6,000,000 Da, among the largest extracellular proteolytic complexes known. Meprins are capable of cleaving cytokines, growth factors, bioactive peptides, hormones, and extracellular matrix proteins. Recent studies have established that meprins interact with other membrane-bound proteins. Studies of ENaCs (epithelial sodium channels) show that the gamma subunit of this channel interacts with the cytoplasmic tail of meprin β. In addition, the ENaC protein is cleaved by meprin β in an extracellular loop, and this results in increased ion transport.
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S38.

SUBSTRATE SPECIFICITY OF HUMAN KALLIKREIN 6: SALT AND GLYCOSAMINOGLYCAN ACTIVATION EFFECTS

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Human kallikrein 6 (hK6) has been proposed as the homologue to rat myelencephalon-specific protease (MSP), an arginine-specific degradative-type protease abundantly expressed in the central nervous system (CNS) and implicated in demyelinating disease. The present study provides biochemical data about the substrate specificity and activation of hK6 by glycosaminoglycans (GAGs) and by kosmotropic salts, which followed the Hofmeister series. The screening of fluorescence resonance energy transfer (FRET) peptide families derived from Abz-KLRSSKQ-EDDnp resulted in the finding that Abz-AFRFSQ-EDDnp is the best synthetic substrate so far described for hK6 (k_{cat}/K_m = 38,667 $mM^{-1}s^{-1}$). It is noteworthy that the AFRFS sequence was found as a motif in the amino-terminal domain (ATD) of seven human ionotropic glutamate receptor subunits. We also examined the hK6 hydrolytic activity on FRET peptides derived from human myelin basic protein (MBP), precursor of the Aβ amyloid peptide, reactive center loop of α_1-antichymotrypsin, plasminogen, and maturation and inactivation cleavage sites of hK6, which were earlier described as natural substrates for hK6. The best substrates derived from MBP. The hK6 maturation cleavage site was poorly hydrolyzed, and no evidence was found to support a previously reported two-step self-activation process. Finally, we assayed FRET peptides derived from sequences that span the cleavage sites for activation of protease activated receptors PAR 1 to 4, and only the substrate with the PAR 2 sequence was hydrolyzed. These results further support the hypothesis that hK6 expressed in the CNS is involved in further support the hypothesis that hK6 expressed in the CNS is involved in normal myelin turnover/demyelination processes, but is unlikely to self-activate. The present report also suggests the possible modulation of ionotropic glutamate receptors and activation of PAR 2 by hK6.

S39.

A NEW LANDSCAPE FOR PROTEINACEOUS INHIBITORS OF METALLOCARBOXYPEPTIDASES

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Recently, several novel proteins showing strong inhibitory capability against metallocarboxypeptidases have been found or characterized by our groups: i.e. from ticks, from humans, and from a marine worm (see below). The former (TCI) is a 76 residues protein, with two small structural domains related to crotamine snake venoms. The middle one (ECI), is much larger (222 residues), and is folded in two subdomains consisting of an α-helix enveloped by a curved b-sheet, reminiscent of cystatins. Finally, the last one, from a marine annelida, is composed of 173 residues folded in three disulfide-rich domains.
In most cases, the protein carboxypeptidase inhibitors display a "reactive" C-tail which docks into the active center of the enzyme. However, ECI acts differently, stablishing inhibitory interactions between an edge of its incomplete β-barrel and the entrance of the active site funnel of the enzyme; this is more reminiscent of the inhibitory mechanisms in procarboxy-peptidases and in complexes of cystatins with cysteine proteases. The new findings might change the current views on the evolutionary generation and distribution of carboxypeptidase inhibitors, and on their biological roles. *Supported by MEC, CeRBa & CYTED grants.*

Regulation of Behavior and Hormone Secretion by Brain Peptides and Neurotransmitters

S40.

CATECHOLAMINERGIC SYSTEMS IN STRESS: MOLECULAR GENETIC APPROACHES

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The specific activation of two components of the sympathoadrenal system (adrenomedullary and sympathoneural) by various stressors has been shown. The aim of the present work was to investigate changes in enzymes involved in catecholamine synthesis - tyrosine hydroxylase (TH) and phenylethanolamine N-methyltransferase (PNMT)- their gene expression, immunoprotein levels and activities in the adrenal medulla (AM), sympathetic ganglia and brain nuclei of rats and mice after a single or repeated exposure to various stressors. Immobilization for 2h (IMO), cold 4ºC (COLD), administration of insulin 5IU (INS) or 2-deoxyglucose 500 mg/kg (2DG) were used. A single exposure to IMO, COLD, INS or 2DG was found to induce increases in both TH and PNMT mRNA levels in the studied organs. Increased transcription rate is responsible for stress-induced TH and PNMT gene expression. Repeated exposure to these stressors elevated enzyme mRNA and protein levels. PNMT gene expression is mainly regulated by HPA axis and in corticoliberin gene knock-out mice is basically reduced especially after stress exposure. Cold-adapted rats responded to heterotypic novel stressors (IMO, INS, or 2DG) by exaggerated responses.

Thus, our data suggest an adaptation of TH and PNMT gene expression during long-term exposure to stressors. An exposure of adapted rats to novel stressors induces exaggerated responses. The described stress-induced changes in gene mechanisms involved in catecholamine production play an important role in adaptation of organisms to repeated stress exposure. *Grant SP-51/028 08 00/028 08.*

S41.

5-HT FEMINIZES THE RAT BRAIN BY REDUCING ANDROGEN RECEPTORS

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Masculinization of rat brain structure is principally induced by oestradiol derived from perinatal testosterone secretion, although aspects of masculinization require the action of testosterone itself on androgen receptors (ARs). Hypothalamic 5-HT turnover is transiently decreased on day 14 *post partum* (*pp*) in male rats. If this decrease is counteracted by administration of the 5-HT_2 receptor agonist ((-) DOI); (-) [2,5 dimethoxy-4-iodophenyl]-2-aminopropane HCl), the structure of the male brain is feminized. Thus, 5-HT antagonises the masculinizing action of the perinatal rise in steroids. In order to elucidate the mechanisms underlying this antagonism, the effect of (-) DOI treatment (0.25 mg/kg on days 8-16 *pp*) on the number of steroid receptor immunopositive cells in the sexually dimorphic AvPVN and SDN-POA was investigated on day 18 *pp*. (-) DOI treatment feminized the size of these two nuclei in males and concurrently reduced the number of AR immunopositive cells in the AvPVN by 36% ($P<0.05$) and in the SDN-POA by 50% ($P<0.001$). (-) DOI treatment had no effect on the number of oestrogen receptor α, oestrogen receptor β, or progesterone receptor immunoreactive cells in either the AvPVN or SDN-POA. Therefore, a critical density of ARs appears to be required for brain masculinization and this is associated with a transient fall in 5-HT activity on day 14 *pp*.

S42.

POSSIBLE MECHANISM FOR NEUROPEPTIDE-GLUT-AMIC ACID-ISOLEUCINE ACTION IN LH RELEASE

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The neuropeptide-glutamic acid-isoleucine (NEI) encoded from ppMCH is expressed in the perikarya of the lateral hypothalamus and the zona incerta (ZI) and projects broadly through the CNS. The possibility of an interaction between NEI-LH and GnRH was studied, using icv injections of NEI in male and ovariectomized (OVX) rats treated with estradiol benzoate (EB) or EB plus progesterone (P), in pituitary cell culture; immunohistochemistry (IHC) and in situ hybridization. The results have shown that: NEI, administered icv in male or OVX rats, increased LH levels compared to the controls. 2µg of NEI into the pituitary cultured cells in different time points (1 to 36h), induced LH release following the treatment. Using quantitative hybridization and IHC demonstrated that both EB or EB plus P given to OVX rats modified ppMCH mRNA in the ZI but not in the lateral hypothalamus. We observed that NEI-immunoreactive neurons do not express estrogen receptor α, but receive apparent terminals from tyrosine hydroxilase fibers located in the ZI. Moreover, we found NEI terminals in close apposition with GnRH cells, and in the blood vessels of the median eminence (ME). Our findings suggest that NEI is indirectly controlled by ovarian hormones and might regulate the release of LH through projections to the ME as well as to GnRH neurones, in the preoptic area.

S43.

SYNAPSE NUMBER IN THE RAT AVPVN

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The rat AvPVN controls the cyclical release of gonadotrophin and is involved in the transduction of hormonal feedback on LH secretion. It is sexually dimorphic, being larger in females than in males. Masculinization of the AvPVN is dependent upon the perinatal secretion of testicular testosterone and a transient fall in hypothalamic 5-HT turnover on day 14 *post partum*. Since sexual differentiation of brain function may involve changes in neural connectivity, the effect of sex and neonatal treatment with the 5-HT_2 agonist (-) [2,5 dimethoxy-4-iodophenyl]-2-amino propane hydrochloride ((-) DOI) on synaptic density within the young adult rat AvPVN was investigated, using quantitative electron microscopy. There was no significant difference between males and females in the density of asymmetric (putative excitatory) or symmetric (putative inhibitory) synapses within the AvPVN. However, (-) DOI treatment significantly ($P = 0.026$) increased the density of asymmetric synapses irrespective of sex. Estimating total synapse number from the synapse densities and size of the AvPVN revealed that there are significantly more synapses in the female compared to the male AvPVN ($P < 0.01$). Furthermore, (-) DOI treatment abolished this sex difference, by significantly increasing and thus feminizing the number of synapses in the male AvPVN, with the greatest effect on asymmetric synapses ($p < 0.05$). The precise way in which the sexual differentiation of AvPVN size and synaptic connectivity leads to functional differentiation, remains to be elucidated.

Proteomics

S44.

FROM VENOMICS TO PATHOLOGY: DISULPHIDE BONDS IN THE DIVERSIFICATION OF PROTEIN STRUCTURE AND FUNCTION

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Many eukaryotic proteins that pass through or reside in the cell secretory pathway undergo posttranslational disulfide bond (SS) formation. SS linkages contribute to the attainment of the correctly folded structure of proteins and may therefore represent a modulatory mechanism of their biological activities. However, determination of disulfide bonds is far from being a trivial task, specially in SS-rich proteins, and non-conventional mass spectrometry-based techniques are needed. The strategy employed in our laboratory for SS bond determination will be briefly outlined. As examples, the evolutionary divergence of the disintegrin family (snake venom antagonists of the function of integrin receptors) by disulfide bond engineering will be discussed, and the identification by proteomic analysis of bovine and human collagen NC1 a3(IV) conformers, whose redox state appears to be involved in the physiopathology of the autoimmune Goodpasture disease, will be reported.

S45.

USING PROTEOMICS TO STUDY THE MOLECULAR EVENTS ASSOCIATED TO THE ROLE OF SPARC IN TUMOR PROGRESSION

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SPARC is a glycoprotein from the extracellular matrix that elicits changes in cell shape and proliferation. SPARC is overexpressed in different tumors, in association with tumor progression. Our previous results showed that stable transfection of tumor cells with antisense SPARC DNA abolished tumorigenicity in an *in vivo* melanoma murine model, through still not clear molecular mechanisms. We have started a proteomic analysis of proteins expressed by human melanoma cells with modulated SPARC expression, in order to identify putative mediators of SPARC activity. We have used 2D-electrophoresis to compare conditioned media from human melanoma MEL-LES cells with antisense-mediated downregulation of SPARC expression (clone L-1D) and its control cell line L-CMV. We found 25 differential spots, 17 of which were identified by peptide fingerprinting analysis. A selected group of three of these proteins was chosen for validation by immunoblotting, real time PCR and other techniques. Differences in those proteins were confirmed not only in the aforementioned cells but also using other transient (i.e. adenoviral) and stable (i.e.shRNAi) methods of SPARC down-regulation on MEL-LES cells. Furthermore, L-1D cells transduced with SPARC-expressing adenovirus reverted levels of differential proteins to those in L-CMV. This is the first evidence that SPARC and these three proteins may participate in a single molecular network that leads to tumor progression.

S46.

SER/THR PROTEIN KINASES IN MYCOBACTERIA: AUTOPHOSPHORYLATION AND SUBSTRATE IDENTIFICATION BY PROTEOMIC APPROACHES

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Ser/Thr-specific protein phosphorylation was considered a fundamental regulatory mechanism unique to eukaryotes but more recent evidences revealed that some prokaryotes capable of differentiating in complex developmental stages, sporulation or secondary metabolite production also contain the necessary kinases and phosphatases enzyme homologs. The sequencing of an important human pathogen genome, *Mycobacterium tuberculosis*, was completed few years ago and led to the identification of 11 presumptive Ser/Thr protein kinases and only one protein phosphatase. These kinases (PknA, B, D, E, F and G) and the phosphatase (PstP) have already been produced as recombinant proteins and showed the expected activity as well as biochemical properties similar to the eukaryotic counterparts, including autophosphorylation as a presumptive mechanism of activity modulation. Different mass spectrometry and analytical strategies to identify phosphorylation sites are presented. We attempt to characterize, using proteomic approaches, the physiological roles of these protein kinases-phosphatase systems by deciphering how phosphorylation pattern in the activation loops relates to protein kinase activity regulation, which is the autophosphorylation pattern prevailing and which may be the natural substrates for the phosphorylating activities. The final goal is to establish how these signaling systems may be of any relevance to the physiopatology of mycobacteria in relation to the host cells.

S47.

NEW INSIGHTS INTO LEPROSY PATHOGENESIS BY DEFINING THE PROTEOME OF *MYCOBACTERIUM LEPRAE*

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Recently the sequence of the *Mycobacterium leprae* chromosome, the only known obligate intracellular mycobacterium, was completed, showing a dramatic reduction in functional genes, with a coding capacity of only 49.5%, the lowest one so far observed among bacterial genomes. The leprosy bacillus seems to preserve a minimal set of genes that allows its survival in the host and the identification of genes that are actually expressed by the bacterium is of high significance in the context of mycobacterial pathogenesis. In this current study, a proteomic approach was undertaken to identify the proteins present in the cell wall, soluble/cytosol and membrane subcellular fractions obtained from armadillo-derived *M. leprae*. 1-DE, 2-DE and 2D-LC-MS/MS were combined and a total of 256 *M. leprae* proteins were so far identified constituting 16% of predicted gene products. Based on the information in the *M. leprae* genome database (http:www.sanger.ac.uk/Projetcts/ M_leprae), most of the proteins were functionally distributed into intermediary metabolism and respiration, cell wall and cell processes, information pathways, lipid metabolism, and conserved hypotheticals. Of importance, two of these proteins constitute major adhesins involved in the attachment of the bacteria to host cells. The data generated constitute an appreciable contribution to *M. leprae* proteome definition and represent the first application of proteomics to a host-derived *Mycobacterium*.

ISN Symposium
Signaling Pathways in Development, Plasticity and Diseases

S48.
REGULATING FILOPODIAL DYNAMICS THROUGH ACTIN-DEPOLYMERIZING FACTOR/COFILIN
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S49.
DIFFERENTIAL ROLE OF WNT LIGANDS ON SYNAPTIC STRUCTURE AND FUNCTION: ROLE OF THE ALZHEIMER´S AB-PEPTIDE
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S50.
SIGNALING PATHWAYS AND GENE EXPRESSION IN MEMORY FORMATION. COMMON MECHANISMS FROM CRUSTACEANS TO MAMMALS
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S51.
REGULATION OF MEMBRANE EXPANSION AT THE NERVE GROWTH CONE
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S52.
SIGNALLING IN DEVELOPING NEURONS: THE CASE OF MARCKS PHOSPHORYLATION OUTSIDE THE EFFECTOR DOMAIN
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Eukaryotic Gene Expression, Impact in Cell Physiology and Disease

S53.
SP1/KLF TRANSCRIPTION FACTORS: THE CHANGE OF A PARADIGM
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Sp1 is one of the best characterized transcriptional activators. The biological importance of Sp1 is underscored by the fact that several hundreds of genes are thought to be regulated by this protein. However, during the last five years, a more extended family of Sp1-like transcription factors has been identified and characterized by the presence of a conserved DNA-binding domain consisting of three Krüppel-like zinc fingers. Each distinct family member differs in their ability to regulate transcription, and as a consequence, to influence cellular processes. Specific activation and repression domains located within the N-terminal regions of these proteins are responsible for these differences by facilitating interactions with various coactivators and corepressors. The discovery of Sp1-like transcriptional repressors represents a significant step, in the transcriptional field, toward changing the early paradigm of "*Sp1 activates all GC-rich sites*". The new, more accurate paradigm emphasizes that GC-rich sites are not necessarily the target of Sp1, but instead may be activated or repressed depending on the family member by which it is recognized. Accumulating evidence in this field has highlighted the complex nature of the biological effects generated by the existence of various KLF proteins, thereby justifying active investigations on the role of these transcription factors in biochemistry and cell biology. Primarily focusing on discussing the structural, biochemical, and biological functions of the repressor members of this family of transcription factors, the existence of these proteins provides a tightly regulated mechanism for silencing a large number of genes that are already known to be activated by Sp1.

S54.
A FUNCTIONAL INTERACTION BETWEEN ATF7 AND TAF12 THAT IS MODULATED BY TAF4 AND SUMOYLATION
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The ATF7 transcription factors are members of the CREB/ATF family and display quite versatile properties: they interact with the adenovirus E1a oncoprotein, heterodimerize with the Jun, Fos or related transcription factors, likely modulating their DNA-binding specificity; they also recruit to the promoter a stress-induced protein kinase (JNK2). In this study, we investigate the functional relationships of ATF7 with hsTAF12, a component of the general transcription factor TFIID. We show that overexpression of hsTAF12 potentiates ATF7-induced transcriptional activation through direct interaction with ATF7, and Chromatin Immunoprecipitation (ChIP) experiments confirm the interaction on an ATF7-responsive promoter. We also show that the TAF12-dependent transcriptional activation is competitively inhibited by TAF4. Although both TAF12 isoforms (TAF12-1 and 2, formerly $TAF_{II}20$ and $TAF_{II}15$) interact with the ATF7 activation region through their histone-fold domain, only the largest, hsTAF12-1, mediates transcriptional activation through its N-terminal region. We also demonstrate that sumoylation of ATF7 modifies its nuclear localization and impairs the transactivation process.

S55.
A POLAR MECHANISM COORDINATES DIFFERENT REGIONS OF ALTERNATIVE SPLICING WITHIN A GENE
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Soon after the discovery of splicing it became evident that many genes contain more than one region of alternative splicing (AS). The fibronectin (FN) gene is a paradigmatic example as it has three regions of AS that display cell type- and development-specific regulation. From 5' to 3' these regions are EDII, EDI and IIICS. This complex pattern can eventually give rise to up to 20 mRNA isoforms. Although other genes with multiple regions of AS have been found, the prevalence of this phenomenon and a putative coordination between regions within the same gene have not been studied systematically. We provide here bioinformatic evidence that approximately 25% of human genes contain multiple AS regions. Analysis of expressed sequence tags (ESTs) with two alternative regions revealed several genes with some exon combinations more frequent than others, suggesting a coordinating mechanism. This was investigated by transfecting human cells with minigenes carrying two alternative EDI regions in tandem, separated by 3,400 bp spanning three constitutive exons and their introns. Mutations that either inhibit or enhance the inclusion of the upstream alternative EDI exon deeply affect inclusion of the downstream one. However, similar mutations introduced in the downstream alternative exon have little effect on the inclusion of the upstream one. This polar effect is promoter-specific and is affected by inhibition of P-TEF-b kinase in a way that implicates transcriptional elongation. Consistently, cells from mutant mice with either constitutive or null inclusion of the EDI alternative exon revealed coordination with the IIICS region, located far downstream. Using heteroallelic mice we demonstrate that this coordination occurs in cis.

S56.
SUBCODES WITHIN THE CONTEXT OF THE HISTONE CODE
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Refining the histone code paradigm by learning how proteins binding to histone marks are switched on and off will clarify how this code is further modulated. According to the current understanding of the histone code, the mammalian heterochromatic HP1α and HP1β as well as the pan-nuclear HP1γ are "gatekeepers" of methyl-K9-H3-mediated silencing. Here, we report that all three HP1 isoforms can undergo extensive modifications similar to histones, suggesting that silencing may be further regulated beyond the histone code. To document the potential impact of these modifications, we chose $P\text{-}Ser^{83}$-HP1γ as a model, demonstrating that this modified HP1γ adopts an exclusively euchromatic localization, interacts with Ku70, and displays impaired silencing activity. Combined, these observations predict that regulation of silencing by methyl-K9-H3 *via* modification of mammalian HP1 proteins may be more complex than previously appreciated and suggest the existence of an HP1-mediated "silencing subcode" underlying the instruction of the histone code.

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Genomics

S57.
UNDERSTANDING ALKALINE pH STRESS RESPONSE IN S. cerevisiae: A GENOMIC APPROACH
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Alkalinization of extracellular medium represents a stress situation that drastically alters homeostatic mechanisms in the yeast *S. cerevisiae*. This organism react in different forms, including remodeling of its gene expression program. We have characterized this response by combining genomic technologies, as well as classical genetic and biochemical approaches. Our findings indicates that, among other effects, alkaline stress may alter the availability of key nutrients, such as glucose, phosphate, copper or iron and that this fact is reflected in its adaptive transcriptional response.

S58.
GENE EXPRESSION PROFILE ANALYSIS IN HUMAN TUMORS USING CDNA MICROARRAYS
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S59.
FUNCTIONAL GENOMIC ANALYSIS OF *TRYPANOSOMA CRUZI* DIFFERENTIATION
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The transformation of epimastigotes into metacyclic trypomastigotes (metacyclogenesis) is a good model for studies of *Trypanosoma cruzi* differentiation because it can be mimicked *in vitro*, and *bona fide* metacyclic trypomastigotes obtained. The expression of most, if not all trypanosomatid protein-coding genes is regulated post-transcriptionally. Several regulatory mechanisms have been proposed. Studies of genes developmentally regulated during *T. cruzi* metacyclogenesis have shown that the transcripts of these genes are present in total cytoplasmic RNA from stages in which these genes are not usually expressed, suggesting that mRNA is selectively mobilised to the polysomes. We constructed a *T. cruzi* microarray comprising more than 5,200 different sequences spotted in triplicate on each slide. These microarrays were hybridised with total and polysomal RNA from several developmental stages of the parasite. We found that total RNA populations from the various stages differed in complexity and that many genes displayed differential expression in polysomal RNA populations.
Financial support from CNPq, PRONEX (Fundação Araucaria-CNPq), Fiocruz, NIH.

S60.
COMPARATIVE ANALYSIS OF MYCOPLASMAS GENOMES
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Mycoplasmas comprise a group of wall-less bacteria that include more than 180 described species. They are strictly parasites of a wide range of organisms including humans, plants, animals, and insects. The primary habitats of human and animal mycoplasmas are the mucosal membranes of the urogenital, respiratory, and gastrointestinal tracts, as well as the eyes, mammary glands, and joints. This work reports the sequences of three mycoplasma genomes: a pathogenic and a non-pathogenic strain of the swine pathogen *Mycoplasma hyopneumoniae*, and a single strain of the avian pathogen *Mycoplasma synoviae*. These genome sequences and others reported in the literature have been compared. Strain-specific regions, including integrative and conjugative elements, were observed in the *M. hyopneumoniae* strains, together with genome rearrangements and alterations in adhesin sequences. Genomic comparisons revealed that reduction in genome size implied in loss of redundant metabolic pathways with maintenance of alternative routes in different species. Horizontal gene transfer was consistently observed between *M. synoviae* and *Mycoplasma gallisepticum*. Preliminary analysis using immunoproteomic approaches allowed the identification of immunogenic proteins from *M. hyopneumoniae*.
Suported by: MCT/CNPq.

Transgenic and Mutant Mice for the Study of Molecular Mechanisms

S61.
USING KNOCKOUT MICE TO UNDERSTAND EPITHELIAL ION TRANSPORT
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Our understanding of the function of ion channels and transporters in epithelial physiology and pathophysiology has been greatly advanced through the study of mouse models. We shall provide examples of this work based on our own studies of two null mice models, the *Kcnn4* and the *Clcn2* KO animals. *Kcnn4* encodes for the Ca^{2+}-activated K^+ channel of intermediate conductance IK1. Ca^{2+}-dependent anion secretion requires the simultaneous activity of apical CFTR Cl^- channels modulated by cAMP and basolateral (BL) K^+ channels activated by Ca^{2+}, of which there are four candidates in intestinal epithelium. Epithelial transport experiments demonstrate that the IK1 channel is essential for this secretory process in small and large intestine. Ca^{2+}-dependent K^+ secretion, on the other hand, proceeds normally in *Kcnn4*-null mouse tissues suggesting separate Ca^{2+}-activated K^+ secretory channels. *Clcn2* encodes for an inwardly rectifying, voltage-gated Cl^- channel expressed in transporting epithelia, where it has been proposed as an alternative route for Cl^- efflux that might compensate for the malfunction of CFTR in cystic fibrosis (CF). We have resolved a controversy concerning the cellular and membrane location of ClC-2 in intestinal tissue by immunolocalization studies using tissues from ClC-2 KO animals as control. ClC-2 is exclusively localized at the BL membranes of surface colonic cells or villus duodenal enterocytes, consistent with an absorptive role for ClC-2 and putting an end to speculation that activation of ClC-2 might ameliorate the CF phenotype.

S62.
CONDITIONAL RESTORATION OF WILD TYPE MECP2 IN A MOUSE MODEL OF RETT SYNDROME
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Mouse models of Rett syndrome exhibit all major aspects of this severe neurodevelopmental disorder. To establish whether the disease is reversible and the window of time in which a possible replacement therapy should be applied, we have generated transgenic mice that are capable of expressing a wild type copy of MeCP2 on demand. A tetracycline repressible promoter provides temporal regulation. In addition, tissue specific promoters driving the required tetracycline transactivator provide regional specificity. Different from what was observed in mice overexpressing MeCP2 in all neurons, transgenic mice expressing MeCP2 only in the forebrain (CamKII promoter-directed expression) or in the cerebellum and striatum (Eno2 promoter-directed expression) do not exhibit any overt somatic or behavioral abnormality. Expression of the transgenic wild type MeCP2 partially rescues some of the motor and behavioral impairments of the *Mecp2*-mutant mice when expressed under the CamKII promoter. Expression of the same transgene under the control of the Eno2 promoter did not result in any detectable improvement in the *Mecp2*-mutant mice. These preliminary data suggest that those phenotypes may originate in the forebrain. The data also suggest a need for a more inclusive driver to enhance the rescuing effects.

S63.
BASAL MELANOCORTIN RECEPTOR FUNCTION AND POTENTIAL INVERSE AGONISM OF AGOUTI OR AGOUTI RELATED PEPTIDE (AGRP) IN PROOPIOMELANOCORTIN (POMC) DEFICIENT MICE
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We used mutant mice devoid of all melanocortin (MC) agonists encoded by the *Pomc* gene and homozygous recessive for the agouti gene (*a/a*) to analyze the potential physiological role of constitutive MC-R activity *in vivo*. Hair follicle melanocytes synthesized wild-type levels of black pigment (eumelanin) indicating that the MC1-R in skin has high basal activity in the absence of its native agonist. Introduction of the dominant A allele resulted in significant accumulation of yellow pigment (phaeomelanin) suggesting that agouti can function as an inverse agonist at the MC1-R to decrease its high basal signaling through the intracellular cAMP/PKA pathway. Next we analyzed the parallel neurochemical system in the brain consisting of the MC4-R, MC peptides, and AGRP. POMC deficient mice were hyperphagic and obese but more sensitive than wildtype mice to the anorexigenic action of the MC4-R agonist MT-II. Despite this indication of increased MC4-R expression and the ability of i.c.v. AGRP co-administration to competitively antagonize the MT-II response in POMC deficient mice, AGRP by itself had no effect in contrast to its potent and prolonged stimulation of feeding in wildtype mice. We conclude that unlike agouti at the peripheral MC1-R, AGRP does not appear to act as an inverse agonist at the central MC4-R *in vivo*.

S64.
NEURAL-SPECIFIC EXPRESSION AND MODULAR ENHANCER STRUCTURE OF THE POMC GENE
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The proopiomelanocortin (POMC) gene is expressed in the pituitary and arcuate neurons of the hypothalamus. To identify functional cis-acting elements that control neuronal POMC-expression in the hypothalamus we used a combination of phylogenetic footprinting and transgenic mouse analysis and identified two conserved neuronal POMC enhancers designated nPE1 (600 bp) and nPE2 (150 bp). Our data show that (1) a distal genomic region containing nPE1 and nPE2 is necessary and sufficient to direct authentic neuron-specific expression of reporter genes to POMC arcuate neurons; (2) either nPE1 or nPE2 assures proper reporter expression in POMC arcuate neurons whereas simultaneous deletion of these two enhancers completely eliminates expression in POMC neurons; (3) nPE1 and nPE2 nucleotide sequences and genomic organization are both highly conserved among mammals but not between mammals and birds, amphibians or fish; (4) the enhancer activity of mouse and human genomic fragments containing nPE1 and nPE2 is functionally conserved; (5) POMC expression in the brain and pituitary is controlled by different and independent sets of enhancers; and (6)conserved and aligned putative transcription factor binding sites are present in these enhancers. Our study advances the under-standing of the molecular nature of hypothalamic POMC neurons and will be useful to determine whether polymorphisms in POMC regulatory regions participate in the predisposition to obesity.

Biochemical and Molecular Biology Education

S65.
SCIENCE FOR SOCIETY: THE SCIENTIST'S SOCIAL RESPONSIBILITY
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Science is an intellectual adventure in which the ideas of creativity and progress are inherent. It is an essential part of modern culture and has revolutionised our conception of the world and of ourselves. The main function of science is to construct verifiable knowledge that is constantly open to confirmation or rejection. When scientists transmit this knowledge to society they contribute to generating ideas and concepts. These help people to live in the current changeable global society with greater rationality, security and freedom. In addition, the applications of science have profoundly transformed daily life, to the extent that science and technology are now key factors in a country's economic development. For these reasons, *the scientific community cannot remain indifferent to society's view of science and its level of acquisition of scientific culture*.
It is usually taken for granted that the experimental sciences' characteristic knowledge is ethically and morally neutral. However, some aspects of it go beyond the strictly cognitive (theories, empirical evidence); and involve elements related to values and even ethics. In advanced democratic societies, some activities can either directly or indirectly affect to varying degrees: collective ideas, values, interests, preferences, needs and opportunities. These activities are: the selection of priority areas of research, the way research is undertaken, and the technological developments arising from it. In developed societies most scientific research is carried out in public institutions or using public funds (universities, Public Research Organisations, technological centres, health system centres, etc.). Therefore, scientific researchers have an additional commitment to the social environment they belong to and depend on. At present, the attitude of the scientific community does not *generally* appear to recognise that its work is conditioned by the preferences and needs of society. It is therefore important to focus on incorporating an attitude of recognition into research culture. Scientists must be motivated to accept this social commitment.

S66.
THE PATENTING OF KNEW KNOWLEDGE AS A WAY TO TEACH SCIENCE
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Science and technology are accepted to be power engines of development in our global world. Thus, in addition to the cultural value of knowledge *per se*, science education requires additional strategies to motivate and deliver new consciousness of its social impact. Current studies have shown that one of the most quantitative approaches to illustrate the impact of science on technology is to analyze the citations that mainstream articles receive from patents. In collaboration with ipIQ (Haddon Heights, NJ) we have identified the Chilean authored mainstream articles linked to technologies via US patent citations to those publications. The concentration of patent citations to Chilean authored articles is in the biomedical field, being Biochemistry & Molecular Biology the most relevant sub field. The patents, companies, and full bibliographic information were assessed. US, not Chilean companies are the holders of patents most often citing. The Chilean most cited articles in patents were not necessarily highly cited by other scientific articles.
Our pioneering study in Latin America provides new grounds for public policy decisions regarding the unquestionable relevance of basic science in technology and innovation. In a region where allocation of funds for basic science is poor the findings are clearly determinant for strategies concerning social development.
In the context of education in Biochemistry and Molecular Biology, our results provide a suitable amount of study cases to show that local basic research in this field is linked to relevant patents and products (mainly from the US). Furthermore, to emphasize that no local companies had the culture expressed in industrial countries to use the unprotected knowledge created in Chile.

S67.
SCIENCE AND ART - DIDACTIC MATERIAL THAT MAY ARISE EMOTION AMONG STUDENTS
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Undergraduates, independent of their field of studies, tented to associate art to abstract concepts such as sensitivity, inspiration, imagination and freedom, leading to innovation and originality whereas science was seen as a cold, methodic subject, where reason prevails, lacking and kind of emotional component. Scientific methodology was perceived as a limitation to creativity. The dichotomy was not a result of local cultural influences because the same idea were expressed whether the students were from Brazil of from the USA. The students view is in contrast with what imminent scientists expressed when talking about science. They thought that logic and methodology, as well as intuition, creativity and emotion, were all ingredients needed for the making of science. This suggests that preconceived ideas in relation to science may be inhibiting creative young students from selecting science as a career.
On the basis of these observations we started a program where artist and biochemist interact for the preparation of didactic material that may arose emotion. These include illustrated books for school students and a serie of DVD's "Teaching Science with Art". Two volumes were completed, Vol. 1 deals with the mitochondria, Krebs Cycle and A Brief Story of Knowledge". Vol 2 deals with Muscle Contraction. Samples of the two DVD's will be shown.

S68.

MOLECULES WITHIN REACH OF THE HANDS: OUR EXPERIENCE FOR COMMUNITY OF PABMB

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Researchers from Center for Structural Molecular Biotechnology (CBME) developed and evaluated a set of teaching tools and strategies organized to promote the dissemination and modernization of concepts related to Biotechnology and Structural Molecular Biology. Some of the material produced were: Building DNA and RNA Molecules, plastic pieces to building 3D molecules of DNA and RNA; Building Topological Models of Proteins, plastic pieces to building models for proteins; Virtual Cell, interactive media of 3D cells and their organelles, addressed to high school students; Protein Synthesis Game, addressed to under graduation and high school students level; Amino Acid Disc and Nucleotides Polygons gyratory discs containing chemical and structural properties of the amino acids, nucleic acids and the genetic code; CBME inFORMAÇÃO, a scientific dissemination newspaper with notices related to molecular biology and biotechnology (http://cbme.if.sc.usp.br). These materials were analyzed by researchers and also evaluated as educational tools. Students from High schools, under graduation and graduation courses were evaluated through questionnaires containing basic concepts on the theme applied before (pre-test) and after (post-test) the set of activities in order to measure, respectively, the previous and acquired knowledge. The Analysis of the pre- and post-tests revealed that there was an increase of the average percentage of correct answers and an improvement on the elaboration of the answers. These results assure the efficiency of the teaching tools, program and the methodology used.

BT-C1.
PHYTOCHEMICALS AS NATURAL ANTIMICROBIAL AGENTS

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The development of drug resistance in bacteria and the accumulation of chemicals in the environment led to strict regulations that limit the use of antibiotics and other chemicals. Thus, the use of natural antimicrobial compounds seems to be important in the preservation of foods and also in the control of human diseases of microbial origin. Antimicrobial activity of chemically characterized rosemary extracts was evaluated by disk diffusion assay and by broth dilution technique. Our research showed a high radical scavenging activity of all rosemary extracts and a different efficacy as antimicrobial agent, linked to their particular polyphenol compositions. The effect of selected concentrations of the plant extract alone or in combination with other natural and synthetic compounds on microorganism growth, was evaluated. The antimicrobial action against common human pathogens and/or food microbes depends on microorganism type, because large differences in the minimal inhibitory concentrations values were observed. A high antimicrobial activity of organic rosemary extracts against Gram-positive bacteria (coccus and bacillus) and yeast was found. In addition, a moderate effect was observed against Gram-negative bacteria. By contrast, water extract showed a narrowly activity. The minimal inhibitory concentration value of the extracts is in a good correlation with the values obtained with the main polyphenols isolated from the extracts. Therefore, Argentinean rosemary extract may be a good candidate for pharmaceutical plant-based products as well as for functional foods.

BT-C2.
NUTRACEUTICAL PRODUCTION BY LACTIC ACID BACTERIA: EVALUATION OF RIBOFLAVIN, FOLATES AND á-GALACTOSIDASE ENZYME.

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The consumption of functional foods containing nutraceuticals should be encouraged since these foods provide more than simple nutrition; they supply additional physiological benefit to the consumer. Lactic acid bacteria (LAB) are ideal candidates for the production of these beneficial compounds because of their history of safe use in the food industry. Native and engineered LAB were studied because of their capacity to produce B vitamins (folates and riboflavin) and the enzyme á-galactosidase (á-Gal). Using animal models, *Lactococcus lactis* was shown to be able to reverse megaloblastic anemia, increase biological levels of folates and riboflavin in blood and organs, and eliminate most physiological manifestations associated with deficiencies of these vitamins. *Lactobacillus fermentum* CRL 722, a high á-Gal producing strain, was used to reduce the non-digestible sugars (á-GOS) found in legumes such as soybeans. This strain was capable of eliminating the flatulence associated with these á-GOS. These results should open the door to many applications in the development of both new food products with enhanced nutritional value and probiotic preparations with well-demonstrated *in vivo* activity.

BT-C3.
MICRO-AEROBIC POLY(3-HYDROXYBUTYRATE) ACCUMULATION IN *Escherichia coli*

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Many bacterial species synthesize polyhydroxyalkanoic acids (PHAs), which accumulate in the cytoplasm as hydrophobic granules and function as a carbon reservoir and electron sink. These thermoplastic polymers have drawn great interest since their discovery due to their degradability and the potential to produce them from renewable carbon and nitrogen sources. Poly (3-hydroxybutyrate) (PHB) is the best-characterized PHA. The use of recombinant *Escherichia coli* has many advantages over the use of natural PHA producers in fermentation processes. Physiology, biochemistry and genetics of *E. coli* have been studied in great detail. High-cell-density cultivation strategies for *E. coli* are well established; it grows fast, and offers a well-defined physiological environment for the construction and manipulation of various metabolic pathways. PHB production in low oxygen conditions represents an interesting strategy for large-scale biopolymer production because it will permit a simpler reactor design, control strategies, and operating conditions. Totally anaerobic systems could also have the added benefit of potentially co-producing other valuable by-products like ethanol, lactate, succinate or hydrogen. The genes responsible for PHB biosynthesis in *Azotobacter* sp. strain FA8 have been cloned and expressed in *Escherichia coli* in our laboratory. One recombinant strain which contains a plasmid that allows the expression of these genes and is able to accumulate PHB was used to analyze the effect of low oxygen concentrations on PHB synthesis in bioreactor cultures.

BT-C4.
PROPERTIES OF A RECOMBINANT *PSEUDOMONAS FLUORESCENS* STRAIN WITH ACQUIERED CAPACITY TO DEGRADE 2,4-DINITROTOLUENE

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We previously reported the construction of a recombinant *Pseudomonas fluorescens* strain for degradation of 2,4-dinitrotoluene (2,4-DNT). This strain, named RE, was obtained by the chromosomal insertion of the *dnt* catabolic genes from the megaplasmids present in *Burkholderia* DNT and R34 strains. We here analyze different features of the recombinant strain relevant for its potential application in bioremediation processes. It is shown that, in contrast to the plasmid borne *Burkholderia* DNT, *P. fluorescens* RE presents a long-stable degradative phenotype. Moreover, the recombinant strain was capable of completely degrading 2,4-DNT at low temperatures whereas *Burkholderia* DNT was inefficient in the same conditions. *P. fluorescens* RE was also able to counteract 2,4-DNT toxicity on *Nicotiana tabacum* and *Arabidopsis thaliana* development in synthetic medium containing the nitroaromatic compound. Interestingly, *P. fluorescens* RE was found to be non- toxic for *A. thaliana* and *Nicotiana tabacum*, whereas *Burkholderia* DNT inhibited *A. thaliana* seed germination and produced plant lethality in such *in vitro* assays. Finally, the presence of *P. fluorescens* RE in soil microcosms containing plant lethal levels of 2,4-DNT significantly decreased the toxic effects of this nitrocompound on *A. thaliana* growth. These results reveal the advantages of *P. fluorescens* RE and reinforce its potentiality as vehicle for the cleaning up of 2,4-DNT contaminated environments.

BT-C5.

IDENTIFICATION OF DOMINANT BACTERIAL POPULATIONS IN NONYLPHENOL POLYETHOXYLATE DEGRADING ACTIVATED SLUDGE

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We have analyzed bacterial communities associated with nonylphenol polyethoxylate (NPE) biodegradation in laboratory scale activated sludge. Four replicate lab-scale reactors were built and operated in a fill-and-draw mode, showing stable performance for a period of 21 months. All four reactors were fed with synthetic sewage, and two of them received additionally 1% NPE. The marked differences observed between treated and control reactors in NPE degradation capabilities suggested the existence of a microbial community specialized in surfactant degradation.
The dynamics of the bacterial community in the reactors sludge was analyzed using nonparametric multivariate statistical analysis and diversity analysis. The clustering of NPE treated replicate reactors at similar times and the decrease in diversity in NPE treated reactors suggested the community changes were driven by a succession process consisting of the selection and establishment of dominant populations specialized in NPE degradation.
Using culture independent molecular methods (ribosomal RNA genes amplification, DGGE, library construction, cloning with specific primers, membrane rRNA hybridization,fluorescence *in situ* hybridization and real time PCR) we identified and quantified these dominant bacterial populations associated with long term exposure to NPE in the treated reactors.

BT-C6.

STRUCTURAL HOMOLOGY WITH UBIQUITIN IN THE HALOALKALIPHILIC ARCHAEA *Natrialba magadii*

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**These authors had similar contribution to the paper.*

Ubiquitin, a small protein that remains highly conserved among eukaryotes, has been not found in prokaryotes. Recently, several reports have been presented that associate the tertiary structure of ubiquitin with those of other proteins from eukaryotes and prokaryotes. The amino acid sequences of these proteins share very low identity. Indeed, they exhibit both fold and function similar to that of ubiquitin.
We have previously found that several haloalkaliphilic archaea contain proteins that react with antibodies against ubiquitin. Following these studies we have obtained in Natrialba magadii a PCR product of 400bp, which has an open reading frame homologous with the ubiquitin related proteins of the ThiS family, ThiS, MoaD and Urm1. The polypeptide encoded by this sequence, denoted as P400, displayed similar structural features to those of ubiquitin and related proteins. The alignment of these proteins indicates the preservation of several structurally and functionally significant amino acid residues.
Supported by UNMdP, CONICET and Fundación Antorchas.

CB-C1.

c-FOS TYR PHOSPHORYLATION REGULATES c-FOS/ ER ASSOCIATION AND c-FOS DEPENDENT PHOSPHOLIPID SYNTHESIS ACTIVATION

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c-Fos, a component of the AP-1 transcription factor family, participates in a variety of cellular processes ranging from gene transactivation in the nucleus to phospholipid synthesis activation in the cytoplasm. For this latter activity, c-Fos associates with ER membranes. Herein, the regulation of c-Fos/ER association and consequently c-Fos dependent phospholipid synthesis activation were examined in T98G cells. The small amounts of c-Fos present in quiescent cells were found phosphorylated on tyrosine (Y) residues, whereas re entry of cells to cell cycle results in Y-dephosphorylation. Y-dephosphorylated-c-Fos is now capable of associating to the ER and activating phospholipid synthesis. If Y-dephosphorylation is impaired by a phosphatase inhibitor, no c-Fos/ER association is observed and no phospholipid synthesis activation takes place. Furthermore, purified Y-phosphorylated-c-Fos failed to activate phospholipid synthesis *in vitro*. Bioinformatic analysis showed 4 Y residues in c-Fos predicted as phosphorylatable. Single point mutation over Y residues (Y/F) was performed to determine which phosphorylated Y participates in regulating c-Fos/ER association. Only phosphorylation over Y10 and Y30 impaired c-Fos/ER dissociation whereas that on Y106 and Y337 had no effect. We postulate c-Fos Y phosphorylation as a regulatory mechanism to determine the rate of synthesis of phospholipids by regulating c-Fos/ER interaction.

CB-C2.

ENDOPLASMIC RETICULUM-BOUND PTP1B IS TARGETED TO NEWLY FORMING CELL-MATRIX ADHESIONS

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Here we define the mechanism through which PTP1B is targeted to cell-matrix adhesion sites. GFP-labeled PTP1B bearing the substrate trapping mutation D181A is seen in punctate structures in lamellae. The puncta co-localize with FAK and Src, and define the distal tips of cell-matrix adhesion sites as revealed by paxillin and vinculin. PTP1B is largely associated with the external face of the ER and the puncta develop from ER projections over cell-matrix adhesion sites, a process dependent on microtubules. Deletion of the ER-targeting sequence results in cytosolic localization and alters the distribution of PTP1B at cell-matrix foci, while mutations disrupting interactions with SH3 domains, and the insulin and cadherin receptors have no effect. PTP1B recognizes substrates within forming adhesion foci as revealed by its preferential association with paxillin as opposed to zyxin-containing foci. Our results suggest that PTP1B targets to immature cell-matrix foci in newly forming lamellae by dynamic extensions of the ER and contributes to the maturation of these sites.
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CB-C3.
CK2 ECTOKINASE ACTIVITY OF CELLS TRANSFECTED WITH CK2 SUBUNITS

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CK2 is an ectokinase that can phosphorylate extracellular proteins. We have studied the requirements for CK2 export using cultured HEK293 cells transfected with pCEFL-HA constructs of CK2α, α' and/or β subunits. Shedding into extracellular media was achieved upon addition of substrate to the media. CK2 subunits were recovered by immunoprecipitation using anti-HA antibody. CK2 activity assays of immunoprecipitates from extracellular media and total cell extracts employed CK2-specific peptide as substrate. Holoenzyme HA-CK2$\alpha_2\beta_2$ is exported to the extracellular medium whereas the free α/α' subunits are not significantly exported. Export of free HA-CK2β was estimated by its capacity to stimulate added free recombinant CK2α activity and by Western blot and again negligible export is detected. Cells cotransfected with HA-CK2β and a HA-CK2α mutant resistant to heparin inhibition, exported a holoenzyme activity also resistant to heparin. A catalytically inactive mutant of CK2 holoenzyme is also exported. Comparison of the appearance of HA-CK2 holoenzyme activity in whole cell extracts and extracellular media shows that export is delayed to about 10 hours after transfection whereas the CK2 activity in the total cell extract appears much earlier. Immunofluorescence of cells transfected with the CK2 subunits allowed visualization of CK2 on the cell membrane.

CB-C4.
DOWN-REGULATION OF LRP-1 EXPRESSION BY INSULIN IN J774 MURINE MACROPHAGES CELL LINE

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The association of different factors such as dyslipidemias, hypertension, glucose intolerance and insulin resistance increase the risk of atherosclerosis. Atherosclerosis is characterized by plaque formation and intimal hyperplasia of smooth muscle cells contributing to the risk of coronary heart disease. It is generally accepted that atherosclerotic lesions are initiated by an enhancement of LDL uptake by macrophages via specific LDL receptors and by scavenger receptor system. It has been suggested that LRP-1 (by low density lipoprotein receptor-related protein) could play key role in the development of atherosclerotic lesions by contributing to the formation of foam cells. However, how these processes are regulated remains unknown. In this work, we demonstrated that insulin down-regulates both LRP-1 protein and mRNA levels in J774 murine macrophages cell line. Moreover, insulin changes the intracellular distribution of LRP-1 by modifying the vesicular traffic, which depends on this hormone. In addition, we showed that these effects were mediated by the insulin receptor involving both PKB and MEK/MAPK intracellular signalling pathways. Hence, we propose that insulin promotes LRP-1 down regulation with consequent increased lipoprotein up-takes by scavenger receptors favouring to the formation of atherosclerotic lesions.

CB-C5.
USE OF *Chorella kessleri* EXTRACTS FOR THE PROTECTION OF HEPATOCYTE PRIMARY CULTURES FROM PIG AND HUMAN LIVERS

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The antioxidant capability of extracts from *C. kessleri* was assayed in this work. Primary cultures of pig hepatocytes were protected during "ex vivo" oxidative stress (OS) processes. This protection reached 100% even when the lowest concentrations of extracts were tested, against both endogen and exogenous oxidant agents. Preliminary results with human hepatocytes cultures (1 month cultures) showed different morphologic patterns with the different concentrations of the extracts used (from 1:400 to 1:20000). Extraction and characterization of active principles were carried out, in order to scream the subcellular localization. The highest antioxidant activity was found on the particulate fraction. The UV/vis spectrum analysis from aqueous and methanol extracts showed the presence of different substances, some of them being characteristic algae pigments. These results show that the antioxidant compound (present in great amounts in *C kessleri*) obtained by this methodology allow us to protect hepatocyte primary cultures.

CB-C6.
VITAMIN C METABOLISM IN CANCER CELLS

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Although much is known about the role of vitamin C in the maintenance of the normal physiology, less is known about its role in the phatophysiology of cancer cells, with conflicting reports on cytotoxicity versus cytoprotection. Using as models human tumor cells (leukemia, breast, prostate and colon cancer cells) and human tumor samples, we analyzed the expression and function of the molecular components involved in the acquisition, accumulation and recycling of vitamin C in cancer. Our data indicate that the metabolism of vitamin C in cancer cells is determined by changes, qualitative as well as quantitative in the expression and function of vitamin C transporters and reductases, that together with changes in the content of glutathione affect the antioxidant capacity of the cells. Overall, the increased content of vitamin C in the tumor cells is associated to an elevated resistance to oxidative stress, which is consistent with a protective role of vitamin C in the phatophysiology of the cancer cells.

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CB-C7.
FUNCTIONAL REDUNDANCY OF *Caenorhabditis elegans* NUCLEOTIDE-SUGAR TRANSPORTERS
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The physiological roles of NSTs may be hampered by redundant gene function. *C.elegans* is a genetically and developmentally well characterized organism for which only 2 out of 16 NST-like proteins have been described. In the present work, C03H5.2 –a cDNA encoding a putative NST- was functionally expressed in *S.cerevisiae.* The corresponding Golgi vesicle enriched fraction transported UDP-GalNAc and UDP-GlcNAc in a temperature-dependent and saturable manner. UDP-GlcNAc transport activity was also demonstrated *in vivo* by complementation of a *Kluyveromyces lactis* mutant defective in this transport. Inactivation of C03H5.2 in wild type worms by RNAi didn't show any visual phenotype. Srf-3, a previously described NST in *C.elegans* also has UDP-GlcNAc transport activity and *srf-3* mutants have a very mild visual phenotype. Therefore, a possible biochemical redundancy in this activity was investigated. Inactivation of C03H5.2 by RNAi in *srf-3* mutants showed a striking phenotype characterized by abnormal positions of eggs, oocyte accumulation and body constriction. Moreover, transgenic animals for C03H5.2 promoter::GFP fusion were examined and compared to the expression pattern of srf-3, a partial overlapping localization was revealed. This provides the first evidence for biochemical redundancy of NSTs in any organism and constitutes a starting point to understand regulation of these transporters in multicellular organisms.

CB-C8.
MACROPINOCYTOSIS IS THE NOVEL MECHANISM OF ENDOCYTOSIS FOR THE GPI ANCHORED PROTEIN UPAR
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uPAR is internalized via an uPA:PAI-1 complex- clathrin- and LRP-dependent pathway. We have shown that uPAR was internalized by a constitutive mechanism in an uPA:PAI-1- clathrin- LRP-, raft-, and Rac1/RhoA/cdc42- independent fashion. Here, we used a novel protocol for flow cytometry in order to study the endocytosis of uPAR in HEK293-uPAR cells. Amiloride, a drug known to inhibit macropinocytosis, decreased the rate of internalized uPAR in a dose-dependent manner. Amiloride also inhibited the endocytosis of 70kDa-Dextran (a marker for macropinocytosis), CD59 (another GPI-anchored protein) and ATF (the amino terminal fragment of uPA that binds to uPAR). Interestingly, amiloride was unable to inhibit the endocytosis of Transferrin, LRP or uPA:PAI-1, known to be internalized via clathrin-coated pits. Thus, at least two endocytic routes for uPAR internalization might exist. One is induced by uPA:PAI-1, and mediated by LRP and clathrin-coated pits. The second one does not require ligands, even though seems to internalize uPAR alone and uPAR-uPA complexes as well, it is constitutive and occurs via macropinocytosis, as a common mechanism for other GPI anchored proteins.

CB-C9.
PROTECTIVE ROLE OF AUTOPHAGY AGAINST EL TOR HEMOLYSIN, A BACTERIAL EXOTOXIN FROM *Vibrio cholerae*
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Vibrio cholerae (VC) is an intestinal non-invasive pathogen that produces cholera, an acute watery diarrhea disease in humans. Besides cholera toxin, VC secretes a haemolytic toxin termed El Tor Hemolysin (ETH). This exotoxin causes extensive vacuolization in epithelial cells. However, the contribution of this phenomenon in VC pathogenesis has not still been elucidated. In this study, we explored the relationship between the vacuolization caused by ETH and the autophagic pathway. Treatment of cells with purified ETH increases the association of LC3 to membranes, a feature indicative of autophagosome formation. Thus, ETH-induced vacuoles colocalize with LC3 indicating the interaction of these vacuoles with autophagic vesicles. Moreover, culture supernatants from both wt and ETH-defective VC strains, confirmed that ETH is the only VC-exotoxin triggering authophagy. Electron microscopy analysis indicates that the vacuoles present hallmarks of autophagosomes. Furthermore, autophagy inhibition resulted in decreased survival of CHO and CaCo-2 cells upon ETH treatment. These results demonstrate for the first time that autophagy acts as a cellular defense pathway against secreted toxins such as ETH.

CB-C10.
CHOLESTEROL CONTENT REGULATES ACROSOMAL EXOCYTOSIS BY ENHANCING RAB3A PLASMA MEMBRANE ASSOCIATION
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The acrosome is an exocytic granule that overlies the spermatozoan nucleus. In response to different stimuli, it undergoes calcium regulated exocytosis. Freshly ejaculated mammalian sperm are not capable of undergoing acrosome reaction. The acquisition of this ability is called capacitation and involves a series of changes in the sperm physiology. Plasma membrane cholesterol removal is one of the sperm modifications that are associated with capacitation. However, how sterols affect acrosomal exocytosis is unknown. Here, we show that short incubations with cyclodextrin, a cholesterol removal agent, just before stimulation promote acrosomal exocytosis. Moreover, the effect was also observed in permeabilized cells stimulated with calcium, indicating that cholesterol plays a direct role in the calcium-dependent exocytosis associated with acrosome reaction. Using a photo-inhibitable calcium chelator, we show that cholesterol affects an early event of the exocytic cascade rather than the lipid bilayers mixing. Functional data indicate that one target for the cholesterol effect is Rab3A. The sterol content does not affect the Rab3A activation–deactivation cycle but regulates its membrane anchoring. Western blot analysis and immunoelectron microscopy confirmed that cholesterol efflux facilitates Rab3A association to sperm plasma membrane. Our data indicate that the cholesterol efflux occurring during capacitation optimizes the conditions for the productive assembly of the fusion machinery required for acrosome reaction.

CB-C11.

MEMBRANE PERMEANT RAB3A TRIGGERS ACROSOMAL EXOCYTOSIS IN THE ABSENCE OF EXTRACELLULAR CALCIUM

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Acrosome reaction (AR) is a regulated exocytosis in which the outer acrosomal and plasma membrane fuse and the acrosome content is released. To study the function of fusion factors during AR, we have been working in streptolysin O-permeabilized human sperm. To establish a more physiological model, we have recently developed an arginine-rich peptide that, when linked to proteins, permits the diffusion of these molecules through the plasma membrane. GST and the small GTPase Rab3A linked to the peptide RRRQRRKRRRQ (GST-R and R-Rab3A respectively) added to the extracellular medium entered into non permeabilized sperm. Moreover, R-Rab3A induced AR when loaded with GTPγS but not when loaded with GDPβS. Similar to what we have described in permeabilized sperm using Rab3A, the action of R-Rab3A was modulated by the cholesterol content of membranes. Interestingly, R-Rab3A mediated AR stimulation does not require extracellular calcium but depends on IP3 sensitive calcium channels. Our results indicate that membrane permeant proteins such as R-Rab3A will be powerful tools to study the physiology of sperm acrosomal exocytosis.

CB-C12.

CHARACTERIZATION OF SPERM SERINE PROTEASE BSp66 IN MOUSE

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BSp66 is a serine protease previously characterized in the acrosome of bovine sperm, involved in early steps of fertilization. Like other proteases, it is widespread among sperm of other mammalian species. BSp66 is not immunologically related to acrosin and its primary structure is unknown. To develop an experimental approach for the identification of BSp66 gene/s we worked on the characterization of the mouse homologue (mBSp66). Western blotting (1D and 2D) showed that mBSp66 has a Mr=50 kDa and a pI = 5.33 after deglycosidation. The treatment with PNGase was sufficient to shift the electrophoretic migration of the protein, while following sequential glycosidases did not further change its molecular mass, suggesting that mBSp66 is N-glycosilated. As spermatogenesis in postnatal mice is synchronous, total protein from a developmental series were analyzed by Western blot to study BSp66 expression. mBSp66 was detected in the round spermatids stage, consistent with the immunohistochemistry staining in sections of adult mouse testis. Expression of BSp66 in different mouse tissues showed an immunoreactive protein corresponding to glycosilated mBSp66 in testis and brain, while the deglycosilated protein was found in ovary. No signal was found in liver, heart, muscle and macrophages. This tissue-specific and delayed temporal expression is consistent with BSp66 acrosomal location and its proposed role in acrosomal reaction signaling and/or early *zona pellucida* binding.
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CB-C13.

MECHANICAL STRESS LEADS TO CHANGES IN MEMBRANE BIOPHYSICS, TUBULIN ORGANIZATION AND CELLULAR SIGNALING

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The consequences of mechanical stress (MS) on membrane physical properties and on the activation of transcription factors NF-κB, AP-1, and NFAT were investigated in Jurkat T cells. Cells were submitted to 10-passages through a 200 μm diameter gauge. The fluidity of plasma membrane both, at the water-lipid interface and in the hydrophobic region of the bilayer, significantly decreased 1 min post-MS, recovering the initial value within the next 20 min. This effect was accompanied by the rearrangement of lipids in the lateral phase of the plasma membrane. MS was associated with a rapid increase in cellular Ca^{2+} 5 min after MS and with a re-arrangement of cellular microtubules (increased soluble/polymerized β-tubulin). NFAT-, AP-1-, and NF-κB-DNA binding activities were measured by EMSA in total cell fractions. NFAT showed the highest DNA binding activity after 20 min post-MS, while NF-κB and AP-1 had their maximal binding at 30 and 60 min post-MS, respectively. Results indicate that MS affects cell membrane physical properties leading to an increase in cellular Ca^{2+} and tubulin re-arrangements. These events could be involved in the activation of NFAT, NF-κB and AP-1.
Supported by CONICET (PIP 21020), Argentina, and University of California, Davis.

ES-C1.

THE EFFECT OF ACIDIC LIPIDS ON THE Ca^{2+}-INDEPENDENT PHOSPHATASE ACTIVITY OF THE PLASMA MEMBRANE Ca^{2+} PUMP

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The purified plasma membrane Ca^{2+} pump PMCA reconstituted in phosphatidylcoline (PC) liposomes was able to hydrolyze p-nitrophenylphosphate (pNPPase) at a rate of 0.2 μmol/mg/min in the absence of Ca^{2+}, ATP and calmodulin. Reconstitution in mixture of acidic lipids (BE) increased the pNPPase 25 fold. Ca^{2+} inhibited the PC-pNPPase following a biphasic curve with K_{Ca1}= 0.52 μM and K_{Ca2}= 300 μM. In contrast the inhibition of the BE-pNPPase by Ca^{2+} exhibited only the high affinity component. The lipidic composition of the liposomes did not change the apparent affinity for pNPP. However Mg^{2+} activated the BE-pNPPase with much higher aparent affinity than the PC-pNPPase (K_{Mg} 0.59 μM and 8.44 μM respectively). The results are consistent with the idea that the E2 form (absence Ca^{2+}) of the PMCA catalyses the pNPP hydrolysis. Ca^{2+} binding to the transport site inhibit this activity. In the absence of Ca^{2+} and acidic lipids the PMCA would reside in an E2 inhibited state with low pNPPase activity and low affinity for Mg^{2+}. Under these conditions only a portion of the enzyme would bind Ca^{2+} with high affinity.
Supported by grants of CONICET and UBA.

ES-C2.

EFFECTS OF THE SUBSTITUTION OF ASN879 BY ASP ON THE pNPPase ACTIVITY OF THE Ca^{2+} PUMP FROM HUMAN PLASMA MEMBRANE

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The Asn879 of the plasma membrane Ca^{2+} pump (PMCA) would be one of the Ca^{2+} binding ligands. Previous studies have shown that the substitution of Asn879 by Ala or Asp results in an enzyme devoid of Ca^{2+} transport activity. We have constructed a mutant of the human isoform 4xb in which Asn879 was replaced by Asp. The recombinant protein was expressed in *S. cerevisiae* and purified by calmodulin chromatography. The mutant reconstituted in a mixture of acidic lipids was capable of hydrolyzing pNPP (p-nitrophenilphosphate) although at a very low rate compared with the wt enzyme, it was resistant to Ca^{2+} inhibition and the increase in the glycerol content of the reaction medium had a marginal effect on the activity of $Asn^{879}Asp$. In the absence of Ca^{2+}, vanadate inhibited the wt pNPPase activity with high apparent affinity while the pNPPase activity of the mutant remained almost unchanged up to 100 uM vanadate. As judged by the unchanged Km for pNPP the mutation did not alter directly the site of pNPP hydrolysis. Because the phosphatase activity of the PMCA is maximal in the absence of Ca^{2+} (E_2-like conformation) the efects of changing Asn 879 by Asp are consistent with the disruption of the Ca^{2+} binding site. The phosphatase activity of the $Asn^{879}Asp$ mutant was much lower than the expected for an enzyme stabilized in E_2. These results may be accounted for if the mutation $Asn^{879}Asp$ blocks Ca^{2+} binding but retains some of the properties of the Ca^{2+} bound form.

ES-C3.

RIBOSE 5-P-ISOMERASE OF *Trypanosoma cruzi*: SITE-DIRECTED MUTAGENESIS AND REACTION MECHANISM

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Ribose 5-phosphate isomerase (RPI) is an ubiquitous enzyme, essential in the pentose phosphate pathway and Calvin cycle, which interconverts ribose 5-phosphate (R5P) and ribulose 5-phosphate (Ru5P). Two unrelated enzymes are responsible of this activity: RPI A and RPI B. Searches on the *T. cruzi* Genome Project database allowed us to detect an RPI B homolog while RPI type A was not found in the genome. Moreover, selective inhibition of RPI type B completely abrogated the total enzyme activity on crude extracts of *T. cruzi* epimastigotes confirming the absence of an RPI A. RPI activty was detected in the main cellular stages of *T. cruzi* and tcRPI expression was confirmed by Western blot in the same stages. The isomerization reaction is believed to proceed via an enediolate intermediate. Further, furanose ring opening prior to isomerization is necessary when the reaction occurs from R5P to Ru5P. In order to evaluate the importance of key residues in both mechanisms His 11, His 102, His 138 and Cys 69 were mutagenizated and kinetics parameters in both senses of the reaction were obteined.
Since the Cys 69 mutant is the first inactive RPI B obtained so far, we will attempt x-ray crystallography of this protein in complex with the substrate.

ES-C4.

***Pseudomonas aeruginosa* PHOSPHORYLCHOLINE PHOSPHATASE IS A NEW MEMBER FOR THE BACTERIAL HALOACID DEHALOGENASES HYDROLASE SUPERFAMILY**

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P. aeruginosa phosphorylcholine phosphatase (PChP), product of PA5292 gene, catalyses the hydrolysis of phosphorylcholine in choline and Pi. We proposed a mechanism that would explain the pulmonary infection through the coordinated action of hemolytic phospholipase C on phosphatidylcholine or sphingomyelyn, and on phosphorylcholine. Therefore, PChP is a potential target enzyme to avoid the pathogenic action of this bacterium. Based on PChP predicted secondary structure and by comparison with the crystal structure of phosphoserine phosphatase of *Methanococcus janaschii* (PDB: 1f5s) a three-dimensional model for PChP was built. With this model, the three conserved motifs characteristics of HAD superfamily were identified in PChP. Site-directed mutagenesis of aspartyl and treonyl residues in motif I (DXDXT) plus ligand docking simulation, indicated that both aspartyl residues are essential for catalysis. The substitution of the treonyl residue by serine caused 90% depletion in the catalytic efficiency of the mutated enzyme. In addition to *P. aeruginosa* PChP, other homologous proteins found in *P. fluorescens, P. syringae*, and *P. putida* belong also to HAD superfamily.

ES-C5.

MALONYL-CoA – FapR INTERACTION REGULATES LIPID HOMEOSTASIS IN BACTERIA AND IS A NOVEL TARGET FOR ANTIBIOTICS

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Microbial fatty acid biosynthesis has emerged as an excellent target for the design of novel antibiotics. That is the reason why we have been studying the regulation of this pathway in Gram-positive bacteria. We have recently reported the isolation and characterization of FapR, a transcriptional regulator present in many Gram positive bacteria that controls the expression of most of the genes involved in fatty acid and the first steps of phospholipid biosynthesis (the fap regulon) (Dev. Cell 2003, 4:663-672). We determined later that malonyl-CoA is the signaling molecule modulating FapR activity.
Here, we present the structure of free FapR and in complex with malonyl-CoA, and a model for the malonyl-CoA-mediated conformational change of FapR. Limited proteolysis assays further supported this model. FapR mutants designed to avoid malonyl-CoA – FapR interaction were constructed and their properties analyzed in vitro and in vivo. Our results confirm the structural model and indicate that inhibition of malonyl-CoA - FapR interaction is a promising strategy to develop novel chemotherapeutic agents.

ES-C6.
NMR CHARACTERIZATION OF β-SYNUCLEIN, A PROTEIN IMPLICATED IN PARKINSON DISEASE

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Synucleins are abundant brain proteins whose physiological functions are still unknown. Two members of this family, á- and â-synuclein (áS, âS) are found co-localized in presynaptic terminals and strongly linked to the etiology of Parkinson disease (PD). áS accumulates abnormally in the form of oligomers and amyloid fibrils, causing the loss of dopaminergic neurons. Instead, âS is able to impede á-synuclein depositions. Thus, to understand the structural basis for the anti-amyloidogenic property of âS might provide a novel strategy for the treatment of PD. We report here the first structural characterization of âS by means of multidimensional Nuclear Magnetic Resonance (NMR). We have fully assigned the backbone resonances of the protein and identified specific regions with high propensities to adopt α-helical or polyproline II conformations. Pulse field gradient and paramagnetic relaxation enhancement demonstrated that âS is highly unfolded in its native state. Backbone dynamics through relaxation and residual dipolar couplings measurements reveal restricted motional properties at the C-terminus of the protein, in the nano- to microseconds range. These results represent the first step towards the understanding of the mechamism through which âS exerts its anti-amyloidogenic effect on áS.

ES-C7.
MODULATION OF CHLOROPLAST 2-CYS PEROXIREDOXIN ACTIVITIES BY NUCLEOTIDES

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Peroxiredoxins (Prx) are ubiquitous peroxidases that play an important role in controlling diverse cellular functions. Members of this protein family devoid of heme prosthetic group contain either one (1-Cys) or two (2-Cys) conserved cysteine residues for functioning in the peroxidatic activity using reduced thioredoxin and flavoprotein reductases (i.e. AhpF) as electron donors. X-ray crystal structures of human erythrocytes and *Crithidia fasciculata* 2-Cys Prx evinced the noncovalent association of five homodimers; i.e. $[(Prx)_2]_5$. We found previously that a chaperone activity is associated to the peroxidase activity in the chloroplast 2-Cys Prx from rapeseed (*Brassica napus*). In this context, we deemed it desirable to analyze whether chloroplast metabolites affect these activities. The concerted action of ATP and Mg^{2+} inhibited both the peroxidase and the chaperone activities of chloroplast 2-Cys Prx. Germane to this feature, ATP quenched partially the intrinsic emission fluorescence which, as revealed by site-directed mutagenesis studies, arose from interactions with the conserved tryptophan residue located at a C-terminal loop near the Cys175 and an internal cavity (Trp179) but not with the other placed in contact with the solvent (Trp88). Notably, as result of this interaction, ATP transfers the γ-phosphate to the oxidized protein but not to the reduced counterpart.
These results illustrated the modulation of chloroplast 2-Cys Prx functions via noncovalent interactions and covalent modifications elicited by ATP which are relevant in the regulation of the oxidative stress.

ES-C8.
THE SUBSTRATE BINDING SITE OF THE *Escherichia coli* GLYCOGEN SYNTHASE

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In this work, we report a structure-function analysis of the binding site of the ADP-glucose (ADP-Glc) in the *Escherichia coli* glycogen synthase. Bacterial glycogen synthases and starch synthases are retaining GT-B glycosyltransferases that transfer glucosyl units from ADP-Glc to the non reducing end of glycogen or starch. We modeled the *E. coli* glycogen synthase based on the known coordinates of the inactive form of the *Agrobacterium tumefaciens* glycogen synthase and the active form of the maltodextrin phosphorylase, a retaining GT-B glycosyltransferase belonging to a different family. In this model, we identified a set of conserved residues surrounding the substrate, and we replaced them for different amino acids by means of site-directed mutagenesis. Kinetic analysis of the mutants revealed the involvement of these residues in ADP-Glc binding. Replacement of Asp^{21}, Asn^{246} or Tyr^{355} for Ala decreased the apparent affinity for ADP-Glc 18-, 45- and 31-fold, respectively, without dramatic changes in the specific activity. Comparison with other crystallized retaining GT-B glycosyltransferases confirmed the striking similarities among this group of enzymes even if they use different substrates.

ES-C9.
SUBSTRATE SPECIFICITY OF HUMAN KALLIKREIN 6: SALT AND GLYCOSAMINOGLYCAN ACTIVATION EFFECTS

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Human kallikrein 6 (hK6) has been proposed as the homologue to rat myelencephalon-specific protease (MSP), an arginine-specific degradative-type protease abundantly expressed in the central nervous system (CNS) and implicated in demyelinating disease. The present study provides biochemical data about the substrate specificity and activation of hK6 by glycosaminoglycans (GAGs) and by kosmotropic salts, which followed the Hofmeister series. The screening of fluorescence resonance energy transfer (FRET) peptide families derived from Abz-KLRSSKQ-EDDnp resulted in the finding that Abz-AFRFSQ-EDDnp is the best synthetic substrate so far described for hK6 (k_{cat}/K_m= 38,667 $mM^{-1}s^{-1}$). It is noteworthy that the AFRFS sequence was found as a motif in the amino-terminal domain (ATD) of seven human ionotropic glutamate receptor subunits. We also examined the hK6 hydrolytic activity on FRET peptides derived from human myelin basic protein (MBP), precursor of the Aβ amyloid peptide, reactive center loop of α_1-antichymotrypsin, plasminogen, and maturation and inactivation cleavage sites of hK6, which were earlier described as natural substrates for hK6. The best substrates derived from MBP. The hK6 maturation cleavage site was poorly hydrolyzed, and no evidence was found to support a previously reported two-step self-activation process. Finally, we assayed FRET peptides derived from sequences that span the cleavage sites for activation of protease activated receptors PAR 1 to 4, and only the substrate with the PAR 2 sequence was hydrolyzed. These results further support the hypothesis that hK6 expressed in the CNS is involved in further support the hypothesis that hK6 expressed in the CNS is involved in normal myelin turnover/demyelination processes, but is unlikely to self-activate. The present report also suggests the possible modulation of ionotropic glutamate receptors and activation of PAR 2 by hK6.

ES-C10.
NOVEL NUCLEOTIDE-LIKE BINDING SITES IN THE GLUCOSE TRANSPORTER GLUT1
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GLUT1 is a facilitative hexose transporter whose primary structure shows several short sequences with homology to ATP binding sites (Walker motifs). Studies using tyrosine kinase inhibitors and site-directed mutagenesis suggested that a sequence with the signature of a Walker-A motif may be responsable for the inhibitory effect on GLUT1. We analyzed the mechanism by which methylxanthines, phosphodiesterase inhibitors which bind to nucleotide bnding sites, inhibit the activity of GLUT1. We performed mixed-inhibition studies (flavonoids + methylxantines) and used homology modeling for analyzing a 3-D GLUT1 model for Walker motifs. The inhibition studies confirmed the presence in GLUT1 of exofacial sites that may bind both methylxantines and flavonoids. 3-D modeling revealed the presence of an exofacial pocket containing Walker-B motif signatures that may represent the mathylxantine and flavonoid binding site in GLUT1, pocket that is different from that containing the Walker-A motif previously identified.
Support: Conicyt fellowships, grants Fondecyt 1020451 and 1040475, DIUC 03-C4-01 and Mecesup UCH0115.

ES-C11.
FLUX CONTROL IN METABOLIC PATHWAYS: *IN VIVO* STUDIES ON GLYCOGEN SYNTHESIS
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When [^{14}C]glucose is microinjected into *Caudiverbera cauidverbera* frog oocytes, around 95% of the label is incorporated into glycogen. The reminding 5% is metabolized through the pentose phosphate pathway as shown by CO_2 evolution. Glycogen synthesis occurs both through the classic direct pathway and by an indirect route which involves glycolytic degradation of glucose to pyruvate followed by gluconeogenic reconstitution of glucose-6-P and further metabolization to glycogen. The operation of one pathway or the other depends on glucose availability. Below 1 nmole injected glucose (0.33 mM intracellular), only the direct route is operative. Incorporation of glucose through the direct pathway starts above this concentration and the path will become the predominant one at 2 mM glucose (6 nmoles microinjected). Our interest is to study the enzymes involved in flux regulation in both patways. Application of metabolic control analysis allows quantitation of the influence of each enzyme of the path in terms of their control coefficients. By microinjecting into the oocytes increasing concentrations of hexokinase, values of 0.424 and 0.091 were found for the direct and indirect pathways, respectively. Microinjection of phospho-glucomutase led to negative values. Experiments to test the effects of UDP-pyrophosphoryalse and glycogen synthase are in progress. The results obtained up to now suggest that hexokinase, the first enzyme of the pathway, has an important contribution in flux control in glycogen synthesis pathway, but only in the direct route.
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GE-C1.
MDCK EPITHELIAL CELLS EXPRESS mRNAs FOR Kv1.4, Kv1.6 AND Kv1.7 POTASSIUM CHANNELS
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The kidney epithelial cell line MDCK expresses several potassium channels (KCh) in a polarized way. By using single channel electrophysiological recordings we have previously reported the presence of four apical channels of 220, 109, 89 and 31 pS and one basolateral channel of 26 pS. We also described that after tripsinization of MDCK, potassium currents (I_K) are lost and it takes about 8 h to restore the initial I_K magnitude. This phenomenon requires RNA and proteins synthesis and dependents on extracellular calcium and cell contact.
Trying to identify the molecular identity of the MDCK KCh we performed RT-PCR with primers that bind to conserved sequences of Kv1 potassium channel family. This approach helped to detect the expression of Kv1.4, Kv1.6 and Kv1.7 KCh. Kv1.7 seems to be expressed in a higher level than Kv1.4 or Kv1.6. Kv1.7 mRNA level was measured by semi-quantitative PCR finding that the mRNA increase to 130% after 4h of plating to confluence and then decrease to 50% at 8h-24h before reaching the regular level in 2-3 days. We are currently analyzing the kinetic of expression for other KCh mRNA to see if all of them behave the same or if they respond to different stimuli along the MDCK monolayer establishment and polarization.

GE-C2.
RATS BRED AS HEAVY ALCOHOL DRINKERS REDUCE THEIR CONSUMPTION WHEN TREATED WITH AN ADENOVIRAL VECTOR THAT EXPRESSES AN ANTI-ALDEHYDE DEHYDROGENASE ANTISENSE GENE
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It is well established that individuals carrying a point mutation in the *ALDH2* gene have a diminished capacity to metabolize acetaldehyde. This deficiency leads to an increase in acetaldehyde levels when they consume ethanol, which results in an aversion to alcohol.
The aim of this work was to reduce the activity of ALDH2 by transduction of the liver with an adenoviral vector carrying an anti-ALDH2 antisense gene (AdV-ALDH-AS). *In vitro* studies showed that the antisense gene is actively transcribed in rat hepatoma cells, reduces ALDH2 activity by 50-70% and increases 8-fold (10 to 80 micromolar) the levels of acetaldehyde in the presence of ethanol. *In vivo* administration of AdV-ALDH-AS (10^{12} pfu/kg) to UChB rats showed a 40-60% reduction in voluntary alcohol intake, while the consumption in rats treated with empty AdV was unaffected. The reduction in alcohol consumption in rats treated with the antisense gene was maintained for 40 days, suggesting that long-acting AdV vectors may be of value in the treatment of alcoholism.
FONDECYT 1040555, Iniciativa Científica Milenio P99-031F, Beca Posgrado Universidad de Chile PG/89/03.

GE-C3.

SILENCING ENDOGENOUS KLF6 REVEALS A NEW FUNCTION AS A POSITIVE REGULATOR OF CELL PROLIFERATION

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The Krüppel-like transcription Factor KLF6 is involved in mechanisms controlling normal cell proliferation and tumor formation. This work aimed at characterizing cell cycle control pathways that are regulated by KLF6 and thus should be responsible for the KLF6 induced phenotypes. Here we report an efficient inhibition of endogenous KLF6 expression by employing several small interfering RNAs (siRNAs) targeted to different regions of KLF6 mRNA. Up to 90% of KLF6 knock-down was achieved in COS-7 cells. BrdU incorporation analysis indicated that KLF6-defficient cells slowed their proliferation rates. This effect was seen under different experimental conditions (0.1%-4% of fetal serum). Interestingly, KLF6 knock-down increased the cyclin-dependent kinase inhibitor $p21^{cip1/waf1}$ and reduced the phosphorylation status of the retinoblastoma (Rb) protein. To test the possibility that p21 transcription might be regulated by KLF6, the promoter regions of p21 were analyzed in transfection experiments. It was determined that the knock-down of KLF6 increased the transcriptional activity of p21 gene. In conclusion, these data position the transcription factor KLF6 as a positive regulator of cell cycle progression by repressing p21 expression.

GE-C4.

TRANSCRIPTIONAL CONTROL OF THE HUMAN KLF6 GENE

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KLF6 is regulated during cell proliferation and differentiation events while its aberrant expression is associated with tumor formation. Genomic organization and cis-regulatory regions of KLF6 were analyzed to investigate its transcriptional control. Sequence alignments showed a high homology of KLF6 regulatory regions in mammals that predicts evolutionary conserved transcriptional mechanisms. Primer extension assays identified a transcription start site in the vicinity of a potential Initiator element. Reporter constructs of the 5´flanking region were used to determine KLF6 regulatory elements by transfection assays. A minimal promoter region lacking a functional TATA-box yet containing an Initiator was identified and found to be active in all cell lines analyzed. Also, two strong activating sequences (-407/-344 and -307/-207) were identified where the latter contains Sp1 sites. Ectopic expression of Sp1 increased KLF6 transcriptional activity. Additionally, DNA damaging agents provoked a significant increase of KLF6 promoter activity in p53-null cells (Hep3B), while an opposite effect was observed in p53-wt cells (HepG2). In conclusion, KLF6 gene transcription is under control of a TATA-box less initiation mechanism together with an evolutionary conserved array of positive cis-acting elements. Also, the KLF6 promoter activity was responsive, depending on the p53 status, to a variety of chemotherapeutic agents.

GE-C5.

THE *TRYPANOSOMA CRUZI* CYCLOPHILIN GENE FAMILY AS TARGET OF THE TRYPANOCIDAL ACTIVITY OF CYCLOSPORIN A ANALOGS

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The *Trypanosoma cruzi* cyclophilin gene family comprises fifteen paralogues whose nominal masses vary from 19 to 110 kDa, namely *Tc*CyP19, 20, 21, 22, 24, 25, 26, 28, 29, 30, 34, 35, 40, 42 and 110. The fifteen paralogues were aligned with 495 cyclophilins from diverse organisms. Analyses of *T. cruzi* cyclophilins clusters with others encoded in various genomes revealed that eight of them (*Tc*CyP19, 21, 22, 24, 35, and 40) belong to the established cyclophilin classes whereas the other seven display less defined patterns of their sequence attributes. Eight epimastigote cDNA clones encoding cyclophilin isoforms were studied were found transcribed in amastigotes and trypomastigotes. Four native proteins *Tc*CyP19, 22, 28 and 40 were isolated by Cyclosporin A (CsA) affinity. Recombinant proteins *Tc*CyP19, 21, 25, 28, and 40 were purified from *E. coli* extracts and their enzymatic activity and the inhibition by CsA non-immunosuppressive analogs was tested. This binding might be of importance to the mechanism of action of CsA derivatives, which proved to be potent trypanocidal drugs and therefore, of interest in the chemotherapy of Chagas´ disease.

GE-C6.

CONCERTED REGULATION OF ALTERNATIVE SPLICING AND TRANSLATION BY EXTRACELLULAR CUES

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Alternative pre-mRNA splicing is the most important source of protein diversity in vertebrates. Regulation of this process by extracellular cues represents a key event in the control of gene expression. We used the fibronectin (FN) gene as a model to study the linkage between the cellular microenvironment and the splicing machinery. We found that growth factors up-regulate the inclusion of the FN EDA alternative exon via PI 3-kinase. We also found that growth factors alter translation of reporter mRNAs containing a FN EDA exonic splicing enhancer. The effects at both splicing and translation levels are mediated by AKT and inhibited by knocking-down specific Serine/Arginine-rich (SR) splicing factors. Finally, we demonstrated that AKT phosphorylates SR proteins in vitro and that its over-expression elicits opposite effects to those evoked by the previously described SR protein kinases Clk/Sty and SRPK on SR protein localization and alternative splicing. These results show how SR protein activity is modified in response to extracellular cues leading to a concerted regulation of splicing and translation.

GE-C7.

ISOLATION AND IDENTIFICATION OF THREE NOVEL PUTATIVE TRANSCRIPTIONAL REGULATORS OF THE *Brucella abortus virB* OPERON

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The *Brucella abortus virB* operon codes for a type-IV secretion system that is essential for virulence and intracellular multiplication of the bacterium within host cells. Our previous studies showed that the expression of the *virB* operon is tightly regulated during the first stages of infection of J774 cells. It was observed that the proper regulation of the *virB* promoter (P_{virB}) is essential for the bacterium to display an effective intracellular infection. Thus, identification of transcriptional regulators and signals perceived by the bacterium to control *virB* expression is of high importance. In a previous work we analyzed the P_{virB} structure and identified a histone-like protein (IHF) that is involved in transcriptional regulation of the *virB* operon. Taking into account the architecture of the promoter and the localization of the IHF-binding site, we designed specific DNA probes in order to isolate additional transcriptional regulators of the *virB* operon. Three different proteins were isolated using an affinity purification method and subsequently identified by mass spectrometry. These factors bind specifically to different regions of the promoter that are involved in intracellular transcriptional regulation. In view of the nature and binding region of each factor, their possible role in control of *virB* transcription is discussed.

GE-C8.

THE EFFECT OF DISRUPTION OF RNA-POLYMERASE B TRANSCRIPTION FACTOR 3 (BTF3) ON THE EXPRESSION OF INDUCIBLE GENES IN THE FILAMENTOUS FUNGUS *Trichoderma reesei*

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Transcription initiation in eukaryotic organisms is a process that requires several factors that bind to the RNA polymerase II in a multiprotein complex. One of those factors is BTF3 that forms a stable complex with RNA polymerase B and represses transcription of several class II promoters. In order to understand BTF3 function in *Trichoderma reesei* we obtained transformants in which the gene was disrupted by a targeting vector containing the hygromycin B phosphotransferase gene as a selection marker. Southern blot analysis was used to verify that the cassette was integrated at the specific *Trbtf3* locus and that the transformants contained only one copy of the cassette. *T. reesei* is one of the most efficient eukaryotic microorganisms that hydrolyze cellulose to glucose. To study the effect of disruption of the *Trbtf3* gene on cellulase gene expression, we analyzed the induction of the *cbh1* gene- the major member of cellulase system of *T. reesei* - by cellulose in Δ*Trbtf3* mutants and compared it to the wild type strain. The induction of *cbh1* was monitored by Northern blot analyses of samples collected at 0, 12, 15, 18, and 21 hours after the addition of the cellulose as an inducer of *cbh1* gene expression. The results show that disruption of this gene causes a significant acceleration of *cbh1* induction by cellulose. The effect of this mutation on the expression of 2000 genes using cDNA microarrays after exposure of this microorganism to various stress conditions is currently under investigation.

Supported by FAPESP

GE-C9.

TRANSCRIPTOME PROFILING INDUCED IN *Lotus japonicus* ROOTS IN RESPONSE TO INOCULATION WITH DIFFERENT *Mesorhizobium loti* STRAINS

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Macro array technology was used to compare the transcriptome profile of roots inoculated with *M. loti* wild type and mutant strains. The mutant strains analyzed were affected in cyclic â(1-2) glucan synthesis (*M. loti cgs* mutant) and LPS O-antigen synthesis (*M. loti lpsâ2* mutant). Comparisons were carried out at 7 days and 4 weeks. We have identified a group of genes which expression was associated with nodule invasion. Real time RT-PCR assay was used at 7, 14, and 21 days post inoculation to quantitate gene expression of a set of genes involved in plant defense response, redox metabolism and some nodulins. Two different expression patterns were observed along the nodulation process. One group (resistance related protein 3, PR-10, superoxide dismutase, NtPRp27, NLS-TIR-NBS disease resistance protein, endo-1,4-beta-D-glucanase and peroxidase) corresponds to those genes that displayed maximal induction at 14 days. The other group (nodulin Nlj21, ENOD40, flavanone hydroxylase, GA 2-oxidase and snakin-1) displayed maximal expression at 21 days. Two genes (phenylalanine ammonia lyase and the transcription factor NtSubD48) displayed higher expression in roots of plant with ineffective nodules.

GN-C1.

POTATO ROUGH DWARF VIRUS (PRDV) AND POTATO VIRUS P (PVP) ARE STRAINS OF THE SAME CARLAVIRUS

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Immunological evidences indicate a high degree of similarity between PRDV (*Carlavirus*, Argentina) and PVP (*Carlavirus*, Brazil). The objective of this work is to determine the relationship between PRDV and PVP by means of biological and molecular comparison. A greenhouse experiment was carried out involving the infection on 21 differential host plants along with 7 commercial potato varieties. Symptoms we recorded visually and virus presence was assessed by DAS-ELISA. Biological results show small differences in symptoms on host plants. *N. occidentalis,* presented slightly different symptoms between both viruses. Calén INTA was not infected with PVP and Sierra Volcán INTA and Primicia INTA had differences in secondary symptoms. We have cloned and sequenced genetic regions of the replicase (RG), coat protein (CP) and 11kDa protein genes of PRDV and PVP The CP showed 100% identity, 97% identity in 11 kDa protein and 93% identity in RG. These results along with PRDV and PVP coupled in the same clade of a *Carlaviridae* phylogenetic tree, suggest that PRDV and PVP are strains of the same virus.

LP-C1.

A NOVEL ACYLTRANSFERASE INVOLVED IN CONJUGATION OF BILE ACIDS AND FATTY ACIDS IN PEROXISOMES

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Peroxisomes are organelles that are present in virtually all eukaryotic cells and are involved in the metabolism of a variety of lipids such as very long-chain fatty acids and bile acids. The importance of peroxisomal oxidation of fatty acids is underscored by the severe physiological consequences of loss of peroxisomal proteins seen in Zellweger syndrome. The final step in de-novo bile acid synthesis occurs in peroxisomes where bile acids are conjugated to taurine or glycine prior to secretion into bile. Two pathways of bile acid conjugation exist, one in peroxisomes responsible for de-novo synthesis and the other pathway in cytosol, which conjugates recycled bile acids. The cytosolic enzyme named bile acid-CoA:amino acid N-acyltransferase (BACAT) has been characterized, suggesting that a second BACAT enzyme exists in peroxisomes. Using bioinformatics, we have identified a novel enzyme, which we name acyl-CoA:N-acyltransferase (ACNAT) that shows 55% sequence identity to BACAT. Characterization of ACNAT shows that it can conjugate both bile acids and fatty acids to taurine. Our data therefore identifies the peroxisomal bile acid conjugating enzyme, which can also conjugate fatty acids to taurine and may therefore identify new peroxisomal pathways of fatty acid metabolism.

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LP-C2.

ACYL-COA THIOESTERASES – CENTRAL MEDIATORS OF LIPID METABOLISM

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Diseases related to imbalance in lipid metabolism, including obesity, type-2 diabetes and atherosclerosis is a growing world-wide problem. Fatty acids play a central role in lipid metabolism and homeostasis, not only as substrates for energy utilization, but also in cell signaling and as regulators of gene expression. Acyl-CoA thioesterases are a group of enzymes that hydrolyze acyl-CoA to the corresponding free fatty acids and CoASH, thereby regulating intracellular concentrations of these compounds. We have identified 6 homologous genes in mouse located in a gene cluster of which we have cloned, expressed and determined the cellular localization for five. The data show that this gene cluster encodes medium- and long-chain acyl-CoA thioesterases localized in cytosol, mitochondria and peroxisomes, along with a highly specific succinyl-CoA thioesterase in peroxisomes. The nuclear receptor peroxisome proliferator-activated receptor alpha (PPAR?) is a key regulator of fatty acid metabolism Interestingly, the expression of all six genes in this cluster is under control of the PPAR?, further connecting the functions of these genes to fatty acid metabolism. These functions will be discussed.

This work was supported by the FP6 European Union Project "Peroxisome" (LSHG-CT-2004-512018).

LP-C3.

EFFECT OF ARACHIDONIC ACID ON CHOLESTEROL TRANSPORT IN MITOCHONDRIA

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We have previously described a novel pathway for arachidonic acid (AA) release that involves an AA-preferring acyl-CoA synthetase (ACS4) and an acyl-CoA thioesterase (Acot2). ACS4 sequesters free AA thus forming an intracellular pool of Arachidonoyl-CoA (AA-CoA) that is delivered to Acot2 that, in its turn, releases AA in a specific compartment of the cell, the mitochondria. AA increases the expression of Steroidogenic Acute Regulatory protein (StAR) and cholesterol transport. Since AA is released into the mitochondria, a direct effect of this fatty acid on cholesterol transport can not be excluded. In order to test this hypothesis, we studied the effect of AA on cholesterol transport, determined as progesterone (P4) production in isolated mitochondria from non-stimulated MA-10 steroidogenic cells. AA elicited a stimulatory effect on cholesterol transport (0.17±0.03 vs. 0.09±0.02 ng P4/mg protein). This effect was not affected by the addition of cycloheximide, a protein synthesis inhibitor, indicating that it is independent of StAR protein induction. AA-CoA, the substrate of Acot2, increased also cholesterol transport in isolated mitochondria (0.23±0.04 vs. 0.09±0.02 ng P4/ mg protein). Our results indicate that AA *per se* stimulates cholesterol transport in mitochondria. This may be mediated by increasing membrane fluidity at the outer/inner mitochondrial membrane contact sites that facilitate the cholesterol transfer into the mitochondria.

LP-C4.

CTP: PHOSPHOCHOLINE CYTIDLYLTRANSFERASE ALPHA EXPRESSION IN QUIESCENT CELLS

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Regulation of CTP:phosphocholine cytidylyltransferase alpha (CTα) expression is critical since it is the rate-limiting enzyme in the phosphatidylcholine biosynthetic pathway. We investigated whether Sp1 acts as a negative regulator or an E2F binding site present in the CTα promoter regulates CT expression in situations where the requirement for PC decreases. Transcription of cell cycle genes is controlled by the molecular balance established between E2F/DP and repressor pocket. By ChIP assay we detected E2F, histone deacetilase (HDAC) and Rb on the CTαpromoter. By immunoprecipitation assays we demonstrated that Sp1 and E2F interact with HDAC. To determine if HDAC can affect CTα expression, we incubated fibroblasts transfected with CTα reporter construct with TSA (an HDAC inhibitor). The analysis showed that TSA induced CTα expression. Thus, HDAC may be involved in repressing CTα expression.

MI-C1.

CHEMORECEPTOR TRIMERS OF DIMERS AND COLLABORATIVE SIGNALING TEAMS

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Bacteria detect chemical gradients and respond to them with extraordinary sensitivity. In *E. coli*, four transmembrane receptors with different specificities are mainly responsible for sensing attractant and repellent compounds and for signaling control commands to the flagellar motors through a cytoplasmic phosphorelay. These receptors (known as MCPs for methyl-accepting chemotaxis proteins) possess a highly conserved cytoplasmic domain that interacts with a coupling protein (CheW) and a histidine kinase (CheA). Kinase activity is modulated by the receptors' ligand occupancy and methylation states. Although their native structure is a homodimer, the receptors form higher-order signaling complexes that cluster at the poles of the cell. Receptor-receptor interactions within the cluster have been implicated in signal amplification.

Genetic and *in vivo* crosslinking studies indicate that the receptors are organized into mixed trimers of dimers in which receptors with different specificities collaboratively control their shared kinase molecules.

We have used a cysteine-directed *in vivo* crosslinking approach to identify mutants in the serine receptor that are defective in trimer-of-dimer formation. Here, we present results on the identification and functional characterization of trimer defective mutants that suggest that trimer formation is required for signaling.

MI-C2.

STUDY OF *pchP* PROMOTER ACTIVITY IN *P. aeruginosa* THROUGH TRANSCRIPTIONAL FUSION

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Recently, we identified the ORF responsible for phosphorylcholine phosphatase (PChP) activity of *P. aeruginosa*, named *pchP*. PChP is involved together with PlcH in the obtention of choline for osmoprotection and growth during infection. Northern blot analysis suggested that *pchP* is transcribed monocistronically. Using bioinformatic tools the 0,25 kb upstream intergenic region was studied. It showed low homology with prokaryotic promoter consensus sequences. The existence of a promoter in this region, named P92, was confirmed by a *lacZ* reporter gene fusion. β-galactosidase activity of P92 increased 12 to 20-fold in bacteria grown with choline, betaine and dimethylglycine (DMG) compared with the level observed in bacteria grown with succinate and NH_4Cl. The highest P92 activity was observed with DMG as inductor. Lactate, succinate and α-ketoglutarate did not affect P92 activity, but it was inhibited by NH_4Cl, glutamine and glutamate. No P92 activity was found in hyperosmolar culture conditions with choline or betaine as osmoprotectants. We conclude that the promotor activity is included in the 0.25 kb fragment located upstream from *pchP* gene, and it is regulated by choline, its metabolites and by preferential nitrogen sources.

MI-C3.

ROLES OF A TolC HOMOLOGUE IN *B. suis*

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The ability of *Brucella* to invade and survive within cells is the cornerstone of its virulence, yet the mechanisms underlying both processes are not clear. Membrane transport systems, able to export macromolecules and toxic substances, are crucial for bacterial survival and/or pathogenesis. Proteins from the Outer Membrane Factor (OMF) or TolC family have been implicated in several transport functions. In the *Brucella suis* genome only one member of the OMF family was identified by blast search. We analyzed the biological relevance of this unique OMF of *B. suis* (Omf_{Bs}). The *E. coli* TolC has the peculiarity to participate in several transport processes such as Colicin E1 uptake, α-hemolysin secretion and drug efflux. We performed functional complementation studies in a *tolC* mutant of *E. coli* carrying the omf_{Bs} gene of *B. suis* under the control of an inducible promoter. While some of the TolC functions, such as colicin E1 uptake and efflux of acriflavine and chloramphenicol, were fully complemented by the omf_{Bs} gene of *B. suis*, protein secretion was not restored. Furthermore, an Spc(Ω) insertional mutation in the omf_{BS} strongly affected the resistance phenotype of *B. suis* to different hydrophobic chemicals like rhodamine, acriflavine, erythromycin, carbenicillin and norfloxacin. Our results demonstrate that in *B. suis* the Omf_{Bs} protein participates in efflux of toxic compounds. In addition, the Omf_{Bs} protein may be potentially involved in other transport functions.

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MI-C4.

ISOLATION AND CHARACTERIZATION OF UNSATURATED FATTY ACIDS AUXOTROPHS OF *Streptococcus pneumoniae*

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Unsaturated fatty acids (UFAs) biosynthesis is essential for the maintenance of membrane structure and function in many groups of anaerobic bacteria. Like *E. coli, St. pneumoniae*, produce the straight chain saturated (SFA) and monosaturated fatty acids. In *E. coli* UFAs synthesis requires the action of two gene products, *fabA* the essential isomerase/dehydratase and *fabB* an elongation condensing enzyme. *St. pneumoniae* lack both genes and instead employs a unique enzyme with only an isomerase function encoded by the *fabM* gene. Although the function of FabM has been deduce in vitro, no mutants in *St. pneumoniae* have been isolated. We report the isolation of such mutant, *St. pneumoniae* SH3 by gene disruption using a cloramphenicol resistance cassette. This mutant can not be grown in complex medium and the defect could be overcome by supplementing the media with oleic acid (C18:1). The *St. pneumoniae* SH3 did not have detectable levels of monosaturated fatty acids as determined by GC-MS and thin-layer chromatographic analysis of the phospholipid fatty acid composition. Therefore our experiments demonstrate that FabM is essential for growth of *St. pneumoniae* and provides the basis for developing new antibacterial strategies.

MI-C5.

CLONING OF *P. mirabilis mrpA* AND EXPRESSION OF THE FIMBRIAL PROTEIN IN *Lactococcus lactis*

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Proteus mirabilis, a common cause of urinary tract infections (UTI) in humans, can express different types of fimbriae. MR/P fimbriae contribute to bacterial colonization and MrpA, the structural protein, represents a promising candidate antigen for mucosal vaccination. So far, existing commercial complex vaccines have limited, short-lived protection and are unable to elicit a mucosal immune response. One attractive strategy for the development of mucosal live vaccines is the use of food-grade lactic acid bacteria (LAB). The use of *Lactococcus lactis*, the model LAB, as an antigen delivery vector, represents an alternative and safe vaccination strategy against UTI.

Here, we cloned the *P. mirabilis mrpA* gene and expressed MrpA protein in *L. lactis*. The *mrpA* gene was amplified by PCR and cloned into pSEC plasmid. The gene expression is under the control of a nisine inducible promoter and the protein is secreted. The induction, expression and correct protein location was checked by Western blot.

P. mirabilis MrpA was efficiently produced by recombinant *L. lactis* strains. This is the first example of a *P. mirabilis* antigen produced in a food-grade bacterium and shows a potential application of such *L. lactis* strains as possible UTI vaccines.

MI-C6.

A B-CELL MITOGEN OF *Brucella abortus* IS AN IMMUNOMODULATOR

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One of the outstanding characteristics of brucellosis is the establishment of a chronic infection that can persist over the life span of the host. The isolation and characterization of molecules that interact with host immune defenses are fundamental for understanding the pathology and for the development of rationale strategies for vaccination, immunotherapy and drug design. B. abortus Rac1 is a 35kDa protein of the proline-racemase family. These enzymes catalyze the interconvertion of L- and D-proline enantiomers and have been reported in Trypanosoma cruzi to act as B-cell polyclonal activators. It has been demonstrated that mitogens and B-cell polyclonal activators are associated in many pathogenic microorganism with immune-modulation and immunoeva-sion. We described that B. abortus Rac1 is a D-proline-isomerase rather than a proline racemase. Integrity of the catalytic site is required for in vivo and in vitro B-cell mitogenic activity. During the acute phase of infection splenocytes of mice infected with the wild type strain were unable to proliferate in response to ConA or E. coli LPS whereas those from mice infected with a rac1 mutant were competent to proliferate upon these stimulations. Moreover, persistance of rac1 mutant was severely afected in Balb/c mice experimental infections. All these results suggest that B. abortus RAC1 is a B-cell polyclonal activator and a strong immune-modulator.

MI-C7.

2-THIOPHEN CARBOXYLIC ACID HYDRAZIDE (TCH): AN ISONIAZID ANALOG THAT DOES NOT INHIBIT MYCOLIC ACID BIOSYNTHESIS IN *Mycobacterium bovis* AND *Mycobacterium tuberculosis*

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The anti-tubercular drugs Isoniazid (Isonicotinic Acid Hydrazide) and Ethionamide stop the synthesis of mycolic acid (a major structural component of the mycobacterial cell envelope) by inhibiting InhA, an Enoyl-ACP Reductase. 2- Thiophen Carboxylic Acid Hydrazide (TCH) has structural features similar to the ones of Isoniazid and Ethionamide, therefore it would be reasonable that it would also be an InhA inhibitor. To test this hyposthesis we studied the mechanism of action of TCH. Analysis of the incorporation of 1-[^{14}C] acetic acid by cultures of *M. tuberculosis* and *M. bovis* treated with this drug showed no inhibition of the synthesis of mycolic acids, suggesting a different mode of action than the one exerted by Isoniazid and Ethionamide. Ten spontaneous mutants of *M. bovis* resistent to TCH isolated during this work showed a ten-fold increase in MIC values in parallel with an increase in the MIC for Isoniazid. Determination of Catalase activity showed that several mutants were lacking this activity suggesting that TCH like Isoniazid is a pro-drug that needs Catalase activation.

MI-C8.

PROMOTER ACTIVITY OF GENES RELATED TO HYPOXIA IN AEROBIC AND ANAEROBIC CONDITIONS

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A Rv2624c protein was obtained in our lab. The Rv2624c gene belongs to a large chain of genes that are induced in hypoxia. We focalize our study on two different intergenic regions (IR) one of 309bp and other of 513 bp. We evaluate the promoter activity involved in these regions, search if there are any difference depending on the environment in a non pathogenic mycobacterium such as *M.smegmatis*. The promoter probe pJEM15 vector was used to create transcriptional fusions to lacZ. The inserted regions were not directed cloned so, we obtained for each IR two clones that were named sense and antisense. The promoter activity was measured in aerobic and anaerobic conditions. *M.smegmatis* transformant with IR309 (sense and antisense) shows potential promoter activity in aerobic and in anaerobic conditions. *M.smegmatis* transformant with IR513 (antisense) does not have promoter activity in aerobic either in anaerobic conditions. The sense one has a potential promoter activity in aerobic condition but it does not show promoter activity in anaerobic conditions. The colonies obtained in aerobic and anaerobic conditions were grown in liquid media. All of them were viable and Zhiel Nielsen positive. Inside the IR 309 there are two possible ORF(s). If these putative small proteins regulate the growth in anaerobic conditions is subject to search.

MI-C9.

POLYSACCHARIDES FROM THE GREEN SEAWEEDS *CODIUM FRAGILE* AND *C. VERMILARA*. STRUCTURE, LOCALIZATION AND ANTIVIRAL ACTIVITY

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Three different polysaccharides were present in the water extracts from both seaweeds; a water soluble sulfated arabinogalactan, a starch-type polysaccharide and a β(1→4)-d-mannan. The major product for the first species is a highly branched proteoglycan composed by a central chain of 3-linked arabinopyranose residues with long side chains of β-d-galactopyranose, partially sulfated on C-6 and also partially pyruvilated.

Fine sections of the milled seaweeds and the residues of extraction were studied by LM using different dyes. Results of these observations correlated with the chemical analysis.

All the extracts showed an important antiviral activity against herpes simplex viruses HSV-1 and HSV-2 (IC_{50}< 1μg/ml, for some of them), however this activity is lost on partially degraded derivatives and they showed no important virucidal effect.

MI-C10.

GLUCOSYLCERAMIDE SYNTHASE , A KEY ENZYME IN THE GLYCOSPHINGOLIPID PATHWAY OF *Plasmodium falciparum*

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Malaria is the most serious and widespread parasitic disease in humans. Glycosphingolipids (GSLs) are membrane lipids in most eukaryotic organisms and in a few bacteria, however little is known in parasites. The core structure of the GSLs, glucosylceramide, is synthesized by a UDP glucose: ceramide glucosyltransferase (GCS). Recently, we have shown the presence of this active enzyme in the intraerythrocytic stages of *P. falciparum*. In the present work, a metabolic incorporation of NBD ceramide and NBD-DHceramide was performed showing that *P. falciparum* has two different sphingolipid pathways: one of them, the *de novo* biosynthetic pathway for the synthesis of GSLs, and another one using ceramide from the host cell to synthesize sphingomyelin. At difference with the mammalian cells, these pathways seem not to be interrelated. When PPMP, a GCS inhibitor, was used, GSL synthesis was abolished. Taking into account that this key enzyme uses dihydroceramide as the lipidic substrate at variance with mammals, we began the study of this parasitic enzyme. We have purified by two different methods a 60 kDa protein that was recognized by a polyclonal anti-human GCS; by confocal microscopy we found it co-localized with Golgi markers. In addition, preliminar assays show it is a glycoprotein. On going studies are being carried out to determine whether GCS may constitute a new target for antimalaric drugs.

MI-C11.

METACASPASES AND APOPTOSIS IN *Trypanosoma cruzi*

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While programmed cell death (PCD) has been long considered to be present exclusively in multicellular organisms; accumulating evidence suggests that this type of cell death might also be present in unicellular organisms. However, no relatives of any classical regulator of apoptosis have been detected so far their genomes. In particular, the absence of caspases, the major executors of cell death, has been the strongest argument against a mechanistic and functional conservation of apoptosis between the multicellular and unicellular organisms. A good starting point to gain deeper insight into the mechanisms of the PCD of unicellular organisms might be the members of a novel class of proteolytic enzymes closely related to caspases called metacaspases. We have identified and characterized metacaspase genes of *T. cruzi,* as well as deduced their chromosomal organization. We have studied their expression in all parasite's life stages and evaluated the immune response of chronic Chagasic patients against the recombinant proteins. Finally, we have examined their possible involvement in *T. cruzi* PCD and their correlation with the increased caspase-like activity in this process.

MI-C12.

IDENTIFICATION OF AN ATPASE ACTIVITY ASSOCIATED WITH THE ROTAVIRUS PHOSPHOPROTEIN NSP5

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Rotavirus genome replication and the assembly of the virus particle occur in cytoplasmic inclusions termed viroplams. NSP5 and NSP2 interact to form viroplasm-like structures in the absence of other viral proteins. NSP5 is also characterized by its affinity for ssRNA and dsRNA as well as by several post translational modifications including phosphorylation, hyper-phosphorylation and NAc-O-glycosylation.

We determined by thin layer chromatography that NSP5 has an associated Mg^{2+}- dependent NTPase activity. The preferred substrate for this hydrolysis is ATP, thus defining NSP5 as an ATPase. The activity was neither affected by the presence of RNA nor the presence of NSP2. The ATPase activity of NSP5 was also not affected by mutation of serine-67, a residue of the protein that is phosphorylated by caseine kinase I. Phosphorylation of serine-67 is an activating event in the hyperphosphorylation of NSP5. Thus, the ATPase activity appears unrelated to serine-67 and to the CKI-triggered phosphorylation of the protein.

MG-C1.

SEARCHING FROM NEW GLYCOSYLTRANSFERASES IN COSMOMICYN BIOSINTHETIC PATHWAY FROM *Streptomyces olindensis*

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Many bioactive natural compounds are produced by actinomycetes, most of them by *Streptomyces* species. *Streptomyces olindensis* is a Gram-positive soil bacterium that carries out not only a complex morphological differentiation but also the production of an antitumoral antibiotic called Cosmomicyn. This molecule is a member of the polyketide group, showing a very complex glycosylation pattern. Sugars are essential for biological activity. In this way, glycosyltransferases are important biosynthetic enzymes that link sugar moieties to the aglycone core. Two glycosyltranferases genes, *cosG* and *cosK*, have been identified in the biosynthetic cluster. In order to search for new glycosyltranferases genes, degenerated primers were used to amplify new hypothetical glycosyltransferases. The PCR reaction was optimizated, and diferents fragments were obtained. This fragments were cloned in the vector plasmidial pCR4 Blunt-TOPO and pBlueScript KS. These clones were analyzed by restriction fragments and consequently by sequencing and alignment analysis being possible to identify new hypothetical glycosyltransferases.

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MG-C2.

HIF1α/SIMA SUBCELLULAR LOCALIZATION DEPENDS ON NOVEL NUCLEAR EXPORT SIGNALS LOCALIZED IN THE bHLH-DOMAIN

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The mammalian Hypoxia Inducible Factor (HIF) is an heterodimeric α/β transcription factor composed of two basic-helix-loop-helix (bHLH)-PAS proteins that induces a wide range of genes under hypoxic conditions. The mechanism of regulation by oxygen is mediated by the alpha subunit and is apparently conserved in *Drosophila*. We have previously reported that the Drosophila HIF-1 alpha homologue, Sima, is localized in the cytoplasm in normoxia and accumulates in the nucleus in hypoxia. Here we show that removal of 47aa from the bHLH domain of the protein increased Sima nuclear accumulation, consistent with the occurrence of two conserved nuclear export signal motifs (NES) in this region that are conserved in other proteins of the bHLH-PAS family. One of these NES was able to promote nuclear export of a GFP reporter construct in S2 cells, in a leptomycinB-sensitive manner. To investigate the function of these two NESs *in vivo* we over-expressed Sima protein in *Drosophila* transgenic embryos and studied subcellular localization by fluorescence microscopy. Deletion or mutation of either NES resulted in an increase of Sima steady-state nuclear localization and impairment of nuclear export upon re-oxygenation. We conclude that Sima is exported from the nucleus to the cytoplasm through the action of at least two CRM1-dependent NES localized in the bHLH domain of the protein, and that these signals are important for rapid nuclear clearance upon cessation of the hypoxic stimulus.

PL-C1.

***ARABIDOPSIS* MUTANTS LACKING GLYCERALDEHYDE-3-P DEHYDROGENASE (NON-PHOSPHORYLATING): EFFECTS ON GLYCOLYSIS, PHOTOSYNTHESIS AND OXIDATIVE STRESS**

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The role of non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase (GAPN) in plants was investigated by characterizing the effect of an insertion mutation at the *gApn* gene locus of *Arabidopsis thaliana*. *gapn* mutants showed absence in the expression of *GAPN* mRNA and lack of activity of this enzyme which cause an induction of cytosolic Ga3Pdh (*GAPC*) mRNA and an increase in its activity. Indeed, we observed higher levels of mRNA and activity of Glc6Pdh and also elevated concentrations of NADPH, but increased oxidative stress. The lack of GAPN protein decreased in the rate of CO_2 assimilation per unit leaf area in agreement with the lower levels of chlorophyll observed in gapn lines compared with the wt plants. We found that GAPN gene is expressed in leaves and roots but induced in wt flowers. Our data based on microarray analysis of *gapn* plants also indicates a down-regulation of several genes suggesting that GAPN is important for efficient glycolysis and photosynthesis in plants and could be important for plant growth and development in *Arabidopsis.*

PL-C2.

REVERSIBLE GLYCOSYLATED POLYPEPTIDE IS ASSOCIATED TO GOLGI MEMBRANES AS A PROTEIN COMPLEX

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Reversible glycosylated polypeptide is a self-glycosylating plant protein, believed to be involved in polysaccharide synthesis, however its function remains unclear. RGP protein has been localized in the trans-Golgi vesicles by immunogold labeling in pea, however little is known about the nature of its association. Previously, we described that the potato RGP protein presented different native molecular sizes with singular glycosylation properties. It is likely that the high molecular forms may be originated by an inter-glycosylation action of RGP molecules protein similarly to the mechanism proposed for oligomeric forms of glycogenin, proposed initiator of mammalian glycogen biosynthesis. Here, we analyzed the size of the RGP forms, which are associated to Golgi vesicles. To analyze native RGP protein associated to Golgi membranes, the protein was extracted and sedimented in a 5-20% sucrose density gradient. Western blot analysis showed a distribution of RGP throughout the gradient. In addition, we analyzed the presence of RGP in protein complexes using Blue Native Gel Electrophoresis (BNGE). The results showed that RGP is part of different protein complexes associated to Golgi membranes. When these complexes were analyzed in a second dimension by SDS-PAGE followed by western blot analysis, the 38-kDa monomer of the RGP protein was found. Therefore, these results indicated that RGP associated to Golgi vesicles is part of protein complexes.

PL-C3.

MITOCHONDRIAL LOCALIZATION OF TWO FUNCTIONAL ARGINASES IN *ARABIDOPSIS*

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Arginase hydrolyzes arginine (Arg) to ornithine and urea. During seedling growth it remobilizes Arg N in the seed storage proteins. A tomato arginase has been shown to be responsive to wounding and to jasmonic acid. The first plant arginase gene was identified, in *Arabidopsis thaliana,* by functional complementation in yeast. A second arginase has since been identified in the *Arabidopsis* genome. The two arginases demonstrate different developmental and tissue specificity. We show that this second gene also encodes a functional arginase. T-DNA insertion mutants indicate that the two arginases are non-redundant during germination and early seedling growth. We also examined the effect of each mutation on mitochondrial basic amino acid carriers, predicted to be responsible for regulating arginase action by limiting access of Arg to arginase. Though plant arginase activity has been associated with the mitochondrion, and though both *Arabidopsis* and soybean arginases contain mitochondrial targeting sequences they have yet to be localized microscopically. Interestingly another group has postulated that one of the two *Arabidopsis* arginases resides in the chloroplast. We utilized transient expression of both arginases with a C-terminal *myc*-epitope in *Arabidopsis* cell culture to determine their subcellular, i.e. mitochondrial, locations. Ultimately, we seek to identify a link among arginase, Arg pools and nitric oxide synthase (AtNOS) which utilizes Arg as a substrate to generate NO.

PL-C4.

IDENTIFICATION OF AQUAPORINS IN FRAGARIA x ANANASSA AND ANALYSIS OF THEIR EXPRESSION PATTERN DURING FRUIT RIPENING

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In strawberry, fruit develops and ripens rapidly, forcing a brief commercial life. Although fruit softening is partially due to cell wall degradation, and a growing number of enzymes related to this process have been characterized, the contribution of fruit turgor and water movement pathways should not be neglected. We therefore focused our work in the identification of water channels in order to characterize their expression and putative contribution to strawberry fruit ripening. A cDNA library from ripe strawberry fruit (cv Chandler) allowed us isolating a 1.2 Kb fragment (FaMIP). The phylogenetic analysis of the full length deduced amino acid sequence confirms that FaMIP has high homology with plasma membrane aquaporins, more specifically PIP1 subgroup. Northern-blot analysis revealed that FaMIP expresses specifically in fruit tissue and that its expression increases through strawberry fruit ripening. Moreover, the expression is repressed by auxins. We conclude that water channels are present in strawberry fruit and modify their expression *pattern during fruit ripening.*

PL-C5.

SALICYLIC ACID IS INVOLVED IN ARABIDOPSIS DEFENSE AGAINST Cd-INDUCED OXIDATIVE STRESS

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Salicylic acid (SA) has been identified as an important signaling molecule involved in plant disease resistance and in hardening responses to abiotic stressors. The response of wild type Arabidopsis seedlings (Col) and of a SA-deficient transgenic line (NahG) to Cd exposure (500 µM) was studied. The effect of pretreating NahG plants with SA (100 µM) was also examined. A rapid accumulation of H_2O_2 was observed by *in situ* localization with DAB, however no visible symptoms of Cd toxicity could be detected until day 5. Catalase activity was significantly diminished 24 h after Cd treatment in Col plants and in the SA-pretreated NahG line, while in NahG plants, it was significantly increased. MDA content was significantly enhanced in Col plants 60 h after Cd treatment while NahG plants showed MDA levels similar to those of untreated Col plants. By day 5, chlorophyll content of Col and NahG plants amended with SA was significantly reduced. SA-unamended NahG plants retained their chlorophyll levels. Superoxide anion (O_2^-) rapidly accumulated in detached leaf segments exposed to 500 µM Cd, except in NahG line. The transcript level of AtSOD3 gene was negligible in NahG plants. However, when exposed to Cd, the expression of this gene was rapidly induced in all treatments. Our results indicate that constitutive SA tends to potentiate the Cd-induced oxidative damage by activating the expression of SOD and repressing catalase activity and/or expression. Exogenous SA added to SA-deficient Arabidopsis plants seems to have positive long-term effects by slowly reverting the deleterious effects that Cd exerts on the H_2O_2 detoxifying system.

PL-C6.

TRANSGENIC TOBACCO PLANTS EXPRESSING A BACTERIAL FLAVODOXIN EXHIBIT ENHANCED TOLERANCE TOWARDS INFECTION WITH THE PATHOGEN *XANTHOMONAS CAMPESTRIS*

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Oxidative stress resulting from environmental hardships and iron deficiency lead to decreased levels of the electron transport protein ferredoxin (Fd). Under these conditions, cyanobacteria and some phototrophic eukaryotes 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 display enhanced tolerance to oxidative stress conditions and iron deficiency. Upon pathogen infection, which usually leads to an oxidative stress scenario, these plants displayed enhanced tolerance in comparison to the wild-type siblings. We performed a metabolic profiling of the plants inoculated with *Xanthomonas campestris,* as well as a thorough study of the antioxidant components of the cells and transcription pattern of genes involved in the defense against pathogens. The results indicate that the increase tolerance may be due to the role of Fld as antioxidant thorugh replacement for oxidatively-damaged Fd.

PL-C7.

POLYSACCHARIDES FROM THE GREEN SEAWEEDS *CODIUM FRAGILE* AND *C. VERMILARA*. STRUCTURE, LOCALIZATION AND ANTIVIRAL ACTIVITY

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Three different polysaccharides were present in the water extracts from both seaweeds; a water soluble sulfated arabinogalactan, a starch-type polysaccharide and a β(1→4)-D-mannan. The major product for the first species is a highly branched proteoglycan composed by a central chain of 3-linked arabinopyranose residues with long side chains of β-D-galactopyranose, partially sulfated on C-6 and also partially pyruvilated.

Fine sections of the milled seaweeds and the residues of extraction were studied by LM using different dyes. Results of these observations correlated with the chemical analysis.

All the extracts showed an important antiviral activity against herpes simplex viruses HSV-1 and HSV-2 (IC_{50}< 1µg/ml, for some of them), however this activity is lost on partially degraded derivatives and they showed no important virucidal effect.

PT-C1.

USING PROTEOMICS TO IDENTIFY POTENTIAL VACCINES COMPONENTS AGAINST BORDETELLA PERTUSSIS

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B. pertussis (Bp), the etiological agent of whooping cough in humans, is currently re-emerging despite vaccination. The study of the infective phenotype of this pathogen showed that under iron deprivation it differentially expresses *in vivo* immunogenic proteins that are not included in any of the currently used vaccines. Additionally, an increased virulence was detected in this phenotype. The proteomic approach was used to analyze the pattern of differential protein expression during iron deprivation of *Bp*. Independent gel duplicates of both iron-restricted or iron-sufficient growth conditions were analyzed. In average gel 900 spots of each condition were detected. Only the spots with a relative intensity difference ≥ 300% were considered as differential. Using tryptic digestion and MALDI-TOF MS we identified four proteins with differential expression in absence of iron: a superoxide dismutase (SodA), an hemin transport protein (BhuR), an hypothetical protein, and a putative lipoprotein. The identified proteins might help to explain the increased virulence of this phenotype. Intracellular survival of the bacteria during oxidative burst in phagocytes may be at least partly favored by the increased expression of SodA under iron-restricted conditions whereas BhuR, being an outer membrane protein, might be involved in bacterial interaction with the host. Further studies are being conducted to gain a better insight into their role in bacterial pathogenesis and their potential as vaccine components.

PT-C2.

A PROTEOMIC APPROACH TO DEFINE THE CONOTOXIN BINDING SITES IN THE NICOTINIC ACETYLCHOLINE RECEPTOR

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The nicotinic acetylcholine receptor is a membrane pentameric protein ($\alpha_2\beta\gamma\delta$) with two acetylcholine binding sites with different ligand affinity. α-Conotoxins bind those sites allowing differentiation of receptors from different genera and even from different species of the same genus. We utilized ^{125}Iα-Conotoxin MI modified with a photoaffinity reagent, thus enabling the adduct covalent binding. The work is aimed as defining the high affinity binding site of the *Torpedo marmorata* receptor for α-Conotoxin MI. We previously showed that α and δ subunits are involved in such site and described an α-subunit fragment located in it (SAIB 2001; 2004). In this work the adduct-receptor complex was submitted to SDS-PAGE and the α and δ ^{125}I-subunits digested with V8 proteinase. Labeled fragments were digested with trypsin and the peptide mixture was submitted to MALDI-TOF-MS to find those molecular masses indicating receptor tryptic sequences modified with the adduct. The δ 118-125 peptide was identified. We modeled said subunit by using the acetylcholine binding protein structure as the template; fragment localization fully agrees with that previously described for other nicotinic receptor binding sites.

PT-C3.

IDENTIFICATION OF COMPONENTS OF THE POLYADENYLATION COMPLEX IN *TRYPANOSOMA CRUZI*: ANALYSIS OF THE INTERACTION INTERFACE BETWEEN TCCPSF30 AND TCFIP1 FACTORS AND ITS POTENTIAL USE AS DRUG TARGET

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In Trypanosomes, the regulation of gene expression operates primarily at the post-transcriptional level. Transcription is polycistronic and pre-mRNA maturation is carried out by two coupled reactions: Trans-splicing/ Polyadenylation. Data mining of *T.cruzi* genome using as query the proteins of the human and yeast Polyadenylation complexes lead us to the identification of 10 putative orthologues. Yeast two hybrid assays using the identified factors allowed us to build an interaction matrix array. From this analysis, we selected the interacting pair TcCPSF30/TcFIP1 as a putative drug target. We next performed a deletion mutational analysis to establish the minimum contact surface. To determine the essential amino acids implicated in that contact, we designed an Alanine Scan mutation approach that covered the entire interaction surface of each protein. Finally, these data was integrated to generate an *in silico* structural model of the TcCPSF30/TcFIP1 interaction interface. Simultaneously, we compared the results obtained for the *T.cruzi* interacting pair with its human counterpart. Our results showed significant differences between them that could be exploited as targets for rational drug design.

ST-C1.
COORDINATED ACTION OF PKA AND ERK IN CHOLESTEROL TRANSPORT ACTIVATION

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We have shown a strict dependence of ERK1/2 activation for PKA-activated cholesterol transport in MA-10 Leydig cells. The goal of the present study was to analyze the interaction of PKA, ERK and StAR (Steroidogenic Acute Regulatory) protein on mitochondria isolated from MA-10 cells. The interaction of ERK1 with PKA and StAR was demonstrated by co-precipitation and Western blot of PKA and StAR from isolated mitochondria derived from stimulated cells and ERK1-GST bound to agarose. Mitochondria derived from cells transfected with either sense or antisense StAR cDNA were incubated with active or mutated inactive (Lys71 → Ala) ERK and catalytic PKA subunit. Mitochondrial StAR content increased after sense cDNA transfection and production of progesterone increased in the presence of PKA and wild-type ERK from 32±3 to 70±7 ng/ml (+112%, $p<0.05$). The effect was abolished by the use of mutated ERK or antisense StAR cDNA. The results indicate that an interaction between PKA, ERK and StAR is required to activate cholesterol transport. The presence of a consensus site for ERK phosphorylation within StAR protein suggests that, apart from PKA, ERK activity would also be required for the formation of a molten-globule StAR conformation leading to cholesterol interaction with hydrophobic domains.

ST-C2.
INTRACELLULAR SIGNALING FOR ESTRADIOL INDUCED AXOGENESIS

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We have previously shown that 17-β-estradiol (E2) (and the plasma membrane-non permeating E2BSA) induces a sustained and strong phosphorylation of ERK (pERK) that is required for E2-induced axogenesis in cultured neurons from male fetuses. Moreover, the Ca^{2+} antagonist BAPTA and the PKC inhibitor Ro 32-0432 completely abolished the neuritogenic effect of E2. In order to investigate if PKC and intracellular Ca^{2+} were involved in MAPK-activation, after 2 DIV hypothalamic neurones were pre-treated for 1h with Ro 32-0432 or BAPTA-AM, pulsed for 15 min with E2 and harvested for Western blotting. Both treatments significantly reduced pERK, indicating the confluence of signals on the MAPK pathway. We asked whether the changes in pERK were significant enough to affect transcriptional activation. To examine this, we studied the level of CREB phosphorylation, a downstream transcription factor target of MAPK. E2 induced phosphorylation of CREB at Ser133 and the inhibition of MEK1/2 by UO126 completely blocked this effect. In summary, these results demonstrate that E2 induces axogenesis in male-hypothalamic neurons through activation of the MAPK pathway. Concurrently, calcium signalling through activation of PKC converges onto the same pathway. ERK1/2 is phosphorylated, triggering effects on gene transcription via CREB and regulation of the cytoskeletal machinery, both required to induce axon growth.

ST-C3.
DUAL ROLE FOR MAPK'S IN GLIAL CELLS SURVIVAL AND DEATH

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We have previously shown that ethanol induces apoptosis in glial cells, and GDNF trophic treatment diminishes it. We investigated here the role of MAPKs signaling pathways in ethanol-triggered cell death and GDNF delivery protection. B92 glial cells were cultured on D-MEM (FBS 10%). After 24 h medium was replaced by medium containing 86 or 172 mM EtOH, for 15' to 120', alone or supplemented with GDNF (30 ng/ml). Then, cultures were prepared for Western blot technique or, for identification of apoptotic cells by DAPI DNA staining. We observed that ethanol activated JNK and that its pathway inhibitor (SP600125) neutralized ethanol-induced cell death, suggesting a role for JNK in EtOH toxicity. However, treatment with GDNF, that neutralizes ethanol-induced cell death, also activates JNK, showing a dual role in survival and death signaling. Moreover, the inhibition of both PI3K/AKT and MEK/ERK pathways (by LY29002 and UO126 respectively) blocked the protective effect of GDNF, suggesting a role for both signaling cascades in the GDNF protection. Nevertheless, ethanol also activates ERK, which indicates its participation in cell death and survival events. Preliminary assays show that p38 activation is not involved in ethanol-induced cell death. These findings provide further insight into the mechanism involved in ethanol-induced apoptosis and trophic protection of glial cells, and suggest that either JNK or ERK MAPK kinases need another signal to define their function as survival or death inducing-factors.

ST-C4.
MODULATION OF ERK1/2 AND P38 MAPK SIGNALING PATHWAYS BY ATP IN OSTEOBLASTS

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This study investigated the modulation of ERK1/2 and p38 MAPK signaling pathways by ATP in a rat osteoblast-like osteosarcoma cell line (ROS17/2.8). We found that ERK1/2 was activated in a dose-dependent manner from 1 to 100 μM ATP, whereas p38 activation reached the maximum at 10 μM ATP. The time-response showed maximum levels of MAPK phosphorylation within 5 min of treatment with 10 μM ATP. This activation was almost completely blocked using neomycin (2.5 mM), an inhibitor of PI-PLC, Ro 318220 (1μM), a PKC inhibitor, and PP1 (50 μM), a potent and selective inhibitor of the Src-family of tyrosine kinases. In addition, ATP stimulated MKK3/6 and c-Src(tyr416) phosphorylation which were inhibited by Ro 318220, suggesting that PKC is an upstream mediator in the signaling cascade. Immunocytochemistry studies supported p38 MAPK activation by ATP and interestingly revealed a nuclear localization of this kinase and a translocation of c-Src into the nucleus. These results show that ATP stimulates the ERK1/2 and p38 MAPK pathways in osteoblasts involving as upstream mediators PI-PLC and PKC, and translocation to the nucleus of Src familiy kinases. In addition, this study demonstrates for the first time nuclear localization of p38.

ST-C5.
ROLE OF $P2Y_2$ RECEPTOR AND MECHANICAL STRESS-ACTIVATED Ca^{2+} INFLUX (SACI) IN ERK1/2 AND P38 MAPK STIMULATION BY ATP IN OSTEOBLASTS

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We studied the modulation of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and its relation to ERK1/2 and p38 MAPK activation by ATP in ROS 17/2.8 osteoblastic cells. ATP, UTP or ADP (10μM) similarly induced an increase in $[Ca^{2+}]_i$ showing that more than one P2Y purinoreceptor subtype is expressed in these cells. Moreover, ATP and UTP, but not ADP, activated ERK1/2 and p38 MAPK suggesting a role for $P2Y_2$ receptors in the ATP action on MAPK. This effect was abolished by cell treatment with 2.5-5 μM Gd^{3+} or a free- Ca^{2+} buffer (plus 0.5 mM EGTA) but not by the intracellular Ca^{2+} chelator BAPTA or voltage dependent Ca^{2+} channels inhibitors (5 μM nifedipine and 5 μM verapamil). Confocal microscopy analysis suggested that purinergic-dependent $[Ca^{2+}]_i$ rise was mainly nuclear and due to Ca^{2+} release from intracellular stores. Stimulation by mechanical stress activated a transient Ca^{2+} influx sensitive to Gd^{3+} (SACI) in cells pretreated with ATP or UTP but not ADP. Both ATP dependent -$[Ca^{2+}]_i$ mobilization and -SACI were suppressed by suramin (purinergic antagonist) and neomycin (a PI-PLC inhibitor). The results suggest that $P2Y_2$ receptor stimulation by ATP in osteoblasts sensitizes mechanical stress activated Ca^{2+} channels involving PI-PLC activation and leading to calcium influx and a fast phosphorylation of ERK 1/2 and p38 MAPK.

ST-C6.
IP_3 STIMULATES *IN VITRO* TRANSCRIPTIONAL ACTIVITY IN C2C12 AND HELA CELL PROTEIN EXTRACTS

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Skeletal muscle cells increase their nuclear Ca^{2+} concentration when depolarized. This Ca^{2+} appears to be involved in regulation of transcription (mediated by CREB phosphorylation); the mechanism how Ca^{2+} exerts this function it is still not well understood. Recently, the presence of IP_3 receptors in nuclear envelope of muscle cells has been described. Isolated-nuclei stimulated with IP_3 increase nucleoplasmic Ca^{2+} and P-CREB levels. In order to understand how Ca^{2+} activates transcription, we performed *in vitro* transcription assays with HeLa nuclear extracts (HNE) and whole cell extracts (WCE) from C2C12 cells obtained by mechanical disruption. Extracts were used to transcript a reporter plasmid containing 4 CRE-box in the promoter, in the presence of (α-P^{32})-UTP. IP_3-stimulated HNE and C2C12 WCE increase transcription 3 and 1.8 fold over control, respectively. Treating both extracts with EGTA abolished the IP_3 induced transcription rise, which was entirely recovered by adding an excess of Ca^{2+}.
These results suggest a Ca^{2+}-mediated effect of IP_3 on transcription, which may allow the study of the mechanisms involved in Ca^{2+} -mediated regulation of gene expression.
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ST-C7.
A FATAL AFFAIR: BDNF LEAVES TRKB FOR P75NTR AFTER SEIZURES

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It has been shown *in vitro* that Brain-Derived Neurotrophic Factor (BDNF) induce cell death through the neurotrophin receptor p75 (p75ntr), while its binding to the TrkB receptor induce their survival. In animals, Status Epilepticus (SE), a condition of prolonged seizures, causes neuronal apoptosis in the hippocampus and at the same time a notable increase in BDNF. Then, it is possible that, *in vivo*, the binding of BDNF and/or a deregulation in the balance of its receptor´s expression, lead to the cell death observed after SE. Thus in the present study we performed Western Blot analysis and co-immunoprecipitation assays in hippocampal samples from animals after SE to detect receptors modifications and ligand-receptor complexes. We found a significant decrease in TrkB expression and a subsequent increase in p75ntr. Then, both pro- and mature forms of BDNF were immunoprecipitated (IP) and analyzed for its binding to p75ntr and/or TrkB. An increase in p75ntr co-IP at 12 and 72 h after SE was observed. In a remarkable contrast, during excitotoxic death a noteworthy decrease was observed in TrkB of co-IP samples without any modifications of truncated TrkB forms. The SE-induced up-regulation in the binding of BDNF to p75ntr, with the accompanying down-regulation of its binding to TrkB, suggests that BDNF may contribute to cell death signaling activity *in vivo* due to modifications of its receptors.

ST-C8.
GENOME WIDE RNAi SCREEN FOR GENES INVOLVED IN THE TRANSCRIPTIONAL RESPONSE TO HYPOXIA

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Cellular responses to low oxygen tension (hypoxia) involve changes in gene expression that promote adaptation to hypoxic stress. This transcriptional response to hypoxia is a highly conserved mechanism mediated by a heterodimeric (α/α) transcription factor called HIF (hypoxia inducible factor). We have previously defined a hypoxia inducible transcriptional response in *Drosophila* that is homologous to mammalian HIF, being the *Drosophila* bHLH-PAS proteins, Similar (Sima) and Tango (Tgo) the functional homologues of HIF-α and α subunits, respectively. As reported in mammals HIF-α/Sima is regulated by oxygen at several different levels, that include protein stability and subcellular localization. To gain insights about the HIF/Sima transcriptional response to hypoxia we have established a *Drosophila* S2 stable cell line bearing a HIF Responsive Element (HRE)-Luciferase reporter that is induced in hypoxia in a Sima/Tgo-dependent manner. The HRE-reporter was then used in a genome wide dsRNAi-based screen to identify novel genes involved in the hypoxic response. The screen led to the identification of about 220 genes presumably required for the transcriptional response to hypoxia, among which we identified components of the insulin-signaling pathway. We demonstrate by pharmacological and genetic experiments that insulin can trigger the hypoxic response through the PI3K/AKT and TOR pathways.

ST-C9.

THE PMRA/PMRB AND RCSC/YOJN/RCSB SYSTEMS CONTROL EXPRESSION OF THE *SALMONELLA* O-ANTIGEN CHAIN LENGTH DETERMINANT

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The lipopolysaccharide (LPS) is the outermost component of the Gram-negative envelope. It consists of the hydrophobic lipid A, a short non-repeating core oligosaccharide and a distal polysaccharide known as O antigen. The *cld* gene product determines the modal distribution of chain length on the O-antigen. However, little is known about how *cld* expression is regulated. We now report that the PmrA/PmrB and RcsC/YojN/RcsB two-component systems independently promote *cld* transcription. We show that the response regulators PmrA and RcsB footprint different regions of the *cld* promoter but promote transcription using the same start site. Conditions that promote *cld* expression increase the amount of O-antigen L-type in the LPS, leading to heightened resistance to serum complement. The LPS profile of the *rcsB* mutant was also altered in the size of the O-antigen subunits attached to the lipid A-core. The lipid A structure of a *cld* null mutant differed from that of the wild-type strain, suggesting a novel role for the Cld protein in lipid A modification. The participation of the Cld protein in the modification of lipid A and in the regulation of O-antigen length may be the reason *cld* expression is controlled by multiple regulatory systems.

ST-C10.

INFILTRATION OF INFLAMMATORY CELLS PLAYS AN IMPORTANT ROLE IN MATRIX METALLOPROTEINASE EXPRESSION AND ACTIVATION IN THE HEART DURING SEPSIS

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Septicemia is an emerging pathological condition that involves among other effects, refractory hypotension and heart dysfunction. We have investigated the contribution of resident non-myocytic cells to heart alterations in wild type and NOS-2-KO mice under septic shock condition by LPS administration. These cells contribute to the rapid infiltration of additional inflammatory cells that enhance the onset of heart disease through the release of inflammatory mediators. Our data shows that early activation of resident monocytic cells plays a relevant role on the infiltration process, mainly of MHC II and CD11b positive cells determined by Western blot and immunohistochemistry. The infiltration was significantly impaired in NOS-2-KO mice or after pharmacological inhibition of NOS-2 or COX-2 since cardiomyocytes failed to express these enzymes. However, heart tissue and cardiomyocytes cells lines express and activate MMP-9 through mechanisms regulated, at least in part, by NO and PGs in an additive way, as was studied by real time PCR, Western blot, immunohistochemical and enzyme activity assays. These results provide a direct link between the inflammatory response in the heart and ECM remodelling by the MMPs released by the cardiomyocytes. These data suggest that the activation recruitment of inflammatory cells to heart is a major early event in the cardiac dysfunction promoted by septicemia and septic shock.

BT-P1.

E2 GLICOPROTEIN EXPRESSION IN ALFALFA TRANSGENIC PLANTS AND CHO-K1 CELLS TO PRODUCE SUBUNIT VACCINES AGAINST BVDV

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The Bovine Viral Diarrhea Virus (BVDV) is responsible for world wide economic loses mainly due to reproductive failure in the cattle population. The control of fetal BVDV infection through vaccination is very important to avoid the birth of persistently infected calves. Therefore, to overcome the drawbacks of the traditional vaccines we have decided to express the BVDV E2 glicoprotein in two eukaryotic systems. The expression of the protein in transgenic plants suggests that inexpensive vaccines could be produced. On the other hand using a mammalian cell system will provide us the chance to obtain the protein with the correct post translational modifications. We are reporting here the development of alfalfa transgenic plants expressing the structural protein E2 of BVDV. We have also transiently expressed the E2 and a truncated version of it in Chinese Hamster Ovary cells (CHO-K1). We have obtained the complete version of the protein from cells extract and the truncated one from the supernatant tested by Western blot. The results presented here aimed us to produce stable cell lines which will constitutively express the glicoprotein. We are currently evaluating the immunogenicity of the E2 expressed in both systems in guinea pigs. The results suggest it could be used in the formulation of subunit vaccines.

BT-P2.

PRODUCTION OF NATIVE AND MODIFIED BIOACTIVE RECOMBINANT hEGF PEPTIDES

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Epidermal Growth Factor (EGF) is a soluble peptidic hormone that binds to ERB tyrosine kinase receptors which regulate cell migration, proliferation and differentiation in various epithelial and nervous tissues. Alterations of this signaling pathway is found in many human tumors and it is associated to poor prognosis and resistance to chemo and radiotherapy.
We developed an EGF expression system in the yeast *Hansenula polymorpha* and a cell culture system for assaying the biological activity. A synthetic gene coding for hEGF was designed considering the host codon usage. It was cloned and expressed in parallel with the human cDNA sequence. The biological activity of the recombinant peptides was assessed by stimulation of proliferation of B16F1 melanoma cells. Also, variant EGF peptides were designed in order to modify the affinity and regulation of receptors; their expression and characterization is currently under way. This expression system could be improved to produce native EGF for epithelial healing pharmaceuticals. The strategy of designing tailor-made modified peptides can be used for development of antitumor agents targeted to the ERB family receptors. M.C. Master Degree scholarship was funded by Lab. Celsius SA.
Supported by CSIC; Universidad de la República, Uruguay.

BT-P3.

DEVELOPMENT OF A rhIFN-â1a PRODUCING MAMMALIAN CELL CLONE GROWING IN SUSPENSION MODE AND SERUM-FREE MEDIUM

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The recombinant human interferon beta 1a (rhIFN-â1a) is one of the most important cytokines that have been used as therapeutic agent for a variety of diseases, being the medicine of choice for multiple sclerosis. In the present work we show producing rhIFN-â1a mammalian cell clones achieved by CHO.K1 cells transfected with different vectors (pCIneo-Beta, pcDNA3.1-Beta and pZC-Beta3). Two stable cell clones (303 and 311) were selected by immunodot and western blot screening of culture supernatants. In order to optimize the bioprocess, the 303 clone was selected and cultured in a serum-free medium to obtain cells adapted to suspension growth. Densities of 1.5 - 2 10^6 cells/ml were achieved in spinner flasks at 37ºC with 5% CO_2 containing individual cells and producing rhIFN-â1a in a ratio of 1.5-2 IU/10^2 cell . day. The results indicate that the clone and conditions are appropriated for the scale up for industrial purposes.

BT-P4.

RECOMBINANT HUMAN GRANULOCYTE COLONY-STIMULATING FACTOR (rhG-CSF): PRODUCTION OF GLYCOSILATED FORMS IN CELL CULTURES

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The rhG-CSF is a monomeric glycoprotein widely used in patients with neutropenia. Our goal was to obtain this protein in mammalian cell cultures.
The rhG-CSF gene and cDNA were isolated and then used separately to construct two plasmids, pCI-neoG and pKG_4G, to be expressed in eukaryotic cells. Transfections of CHO.K1 and BHK-21 cells by lipofection were performed, and geneticin was used for the selective pressure. Cell lines were first analyzed by immunodot to test their productivity and then cloned by the limit dilution method. The resulting clones were studied by ELISA, Western Blot and *in vitro* bioassay. In order to develop the ELISA and the Western Blot, monoclonal antibodies and policlonal serum anti rhG-CSF were produced in our laboratory.
The detection limits were 0,5 ?g/ml for the ELISA and 0,2 UI/ml for the *in vitro* bioassay.
Several clones were obtained, expressing active rhG-CSF in a stable form. They showed different levels of productivity, ranging from 100 ng to 2 ?g of rhG-CSF per ml of supernatant. The clones containing the cDNA produced lower levels of the protein than those containing the gene.

BT-P5.

FORMULATION OF A CULTURE MEDIUM FOR MAMMALIAN CELLS USED IN RECOMBINANT PROTEIN PRODUCTION

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In this work the effect of a mixture of three hexoses (H1, H2 and H3) is evaluated, combined with a mixture of three energy-provider compounds (E1, E2 and E3), to determine their optimal proportion in a medium being developed for culturing recombinant mammalian cells. The effect of hexoses was analyzed using an augmented *simplex lattice* {3,2} design, while a constrained {3,2} simplex-centroid design was chosen for the mixture of E compounds. These experimental designs are combined in a crossed mixture, giving a total of 62 experiments.
The empirical data were used to train an artificial neural network (ANN) for each response. The ANNs were then used to predict the responses for 300 different combinations of the six compounds, and based on this information a partial desirability function (d_i) was generated for every response. The d_i were then combined in a global desirability function D, and the blend for which it is predicted to obtain the highest value of D was selected. This combination was a mixture of H3=20.33 mM, H2=2.33 mM, H1=2.33 mM, E1=1.000 mM, E2=0.775 mM and E3=3.325 mM. Remarkably, H3 is not frequently found in commercial media.

BT-P6.

ANALYSIS OF RECOMBINANT HUMAN ERYTHROPOIETIN N-GLYCOSYLATION PRODUCED UNDER DIFFERENT CULTURE SYSTEMS

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CHO cells producing recombinant human erythropoietin (rEPO) were cultivated during 3 months growing in suspension in a perfused stirred-tank bioreactor. Additionally, adherent CHO cells that produce EPO were cultivated using a multitray system during a similar period. rEPOs corresponding to different stages of both cultures were purified and *in vivo* biological potency was determined, resulting higher than 100,000 IU/ml for all samples. Comparative MALDI/MS analysis of endoproteolytically cleaved peptides demonstrated the N- and C-termini integrity of the rEPO preparations. MALDI/MS both in positive and negative ion mode and MALDI-PSD tandem MS/MS spectra of oligosaccharide mixtures showed that the glycans N-linked to rEPOs belong to the complex type and essentially consist of fucosylated biantennary, triantennary and tetrantennary structures carrying a different number of sialic acid residues. Our studies showed no major differences in the N-glycan structures of samples produced under different bioprocess conditions and not significant changes in the extent of protein glycosylation over the course of both cultures.

BT-P7.

PRODUCTION OF GLYCOSYLATED RECOMBINANT HUMAN INTERFERON BETA (rhIFN â) IN MAMMALIAN CELLS

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IFN â is the therapeutic agent used in sclerosis multiple disease. In the present work different strategies were applied to produce glycosylated IFN â. Hence, CHO, CHO dhfr$^-$, BHK and HEK cells were transfected with different expression vectors using LipofectAMINE. All recombinant cell clones produced glycosylated and non-glycosylated forms of rhIFN-â but in strongly different proportions. A unique CHO clone was chosen based on preferential production of the glycosylated isoform and the higher productivity. The clone was grown on porous and non porous microcarriers in stirred systems. Batch and perfusion cultures were carried out using spinner flasks and a bioreactor. When the perfusion system was employed, the cell densities were 10 times greater than the obtained with the batch system. The protein was partially purified over 50% by dye affinity and ionic exchange chromatography. On the second stage the glycosylated species could be separated from the non-glycosylated ones. The biological specific activity was maintained after the processes and complies with the pharmacopoeia standards.

BT-P8.

EXPRESSION OF RECOMBINANT THROMBOPOIETIN (hTPO) IN *Trypanosoma cruzi*

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The fast clearance of otherwise promising therapeutic proteins frequently constitutes a major hampering in the development of new clinical approaches. Starting from two *T. cruzi* repetitive antigens that provide proteins with the ability to persist in blood, a five amino acid motif known as HRM was developed (JBC 279: 3375, 2004). To test for it possible use in biotechnology, a genetic fusion of this motif with the hTPO was done. The construct contains 26 repeats of the HRM motif located at the C-terminus. Unfortunately we were unable to express this protein in bacteria. Since the HRM motif contains *O*-glycosylation consensus sequences, its expression as a bare protein in mammalian cells is precluded. Therefore we transfected *T. cruzi* epimastigotes. To this purpose a pTREX vector-based plasmid was designed containing the secretory signal of the TcSMUG epimastigote mucin and a 6xHis tag. G418-selected parasites were subjected to PCR on genomic DNA and RT-PCR searching for the insertion and transcription of the gene. In the first attempts of protein expression almost nil quantities were obtained. Therefore cystein-proteinases inhibitors able to permeate cells were assayed. We found that Z-Phe-Phe-diazomethylketone allowed the recovery of recombinant protein from the culture supernatants. This protein was not *O*-glycosylated as demonstrated by serological reactivity. Determination of the *in vivo* TPO activity will establish the utility of the HRM motif in pharmacology to extent the half live of proteins in blood. Eventually, *T. cruzi* might be used for the expression of proteins for veterinary use.

BT-P9.

HIGH LEVEL EXPRESSION OF HORSERADISH PEROXIDASE ISOZYME C IN INSECT LARVAE

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Peroxidase from *Amoracia rusticana* roots (HRP) is mainly used in medical diagnosis kits. All HRP for local industries must be imported. As HRP is highly glycosylated, its expression in the active form in prokaryotes is not possible. The baculovirus-insect cell system is an interesting alternative for its expression. So far, we have expressed 20 mg/l in *Spodoptera frugiperda* cell line cultures. The enzyme was genetically modified to increase its isoelectric point through a 6xArg fusion tail addition, thus allowing an easier purification by cation-exchange chromatography. Our goal in this work was the design of other alternative expression system in insect larvae (*Rachiplusia nu*). The recombinant baculovirus contained the HRP6xArg gen under the polyhedrin promoter control and the GP67 secretion signal of *Autographa californica*. After amplification, a viral clone was used to infect larvae by subcutaneous inoculation. At day-3 post-infection hemolimph was collected and extracts of total larvae were prepared. HRP concentration was 105 and 24 mg/l respectively. To keep samples free of melanization, 0.05 M sodium phosphate buffer, pH 6.0, 5 mM EDTA, 0.15 M KCl and glutation cristals was selected after assaying different buffers. Native PAGE and IEF with specific staining showed a band corresponding to HRP and pI 9.5 in both samples.

BT-P10.

THE LEADER INTRON PRESENT IN THE CYTOCHROME OXIDASE SUBUNIT 5C GENE STRONGLY ENHANCES TRANSLATIONAL ACTIVITY OF THE SUNFLOWER *HAHB-4* PROMOTER

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Arabidopsis thaliana cytochrome oxidase subunit 5c is encoded by three genes that present a leader intron in a conserved position upstream the first ATG. It has been reported that removal of this DNA segment produces a strong decrease in the expression of a reporter gene in transgenic plants. *Hahb*-4, a member of the HD-Zip I family of transcription factors, is regulated at the transcriptional level by drought and ABA. Constitutive expression of this protein confers to transgenic plants a strong tolerance to water stress, associated to a slight delay in development. *Arabidopsis thaliana* plants transformed with *Hahb-4* whole gene present water stress tolerance, but not as high as plants bearing the 35S CaMV promoter. In order to optimize drought tolerance and avoid negative phenotypic characteristics, we have introduced the *COX5*c leader intron into the *Hahb-4* promoter region and transformed plants with this construction. Histochemical analysis show additional tissue/organ expression and fluorometric measurements indicate an increase of 5-6 folds in GUS activity. The results indicate that this heterologous chimeric construction is well recognized in *Arabidopsis,* presenting a high reporter gene activity and constitutes a new biotechnological tool for transgenic expression of this, and other genes in plants.

BT-P11.

HYOSCYAMINE 6β-HYDROXYLASE EXPRESSION IN *SACCHAROMYCES CEREVISIAE*

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Saccharomyces cerevisiae is a host of choice for the expression of heterologous proteins due to its ability to produce recombinant proteins correctly folded and modified at the posttranslational level. In addition, this organism has been classified as "Generally Regarded As Safe" (GRAS). The reasons mentioned above made yeast a very attractive organism to work with on biotechnological processes. Hyoscyamine 6β-hydroxylase (H6H) catalyzes the conversion of hyoscyamine into scopolamine. The latest is widely used as a pharmaceutical. We isolated the h6h mRNA from different *Brugmansia candida* organs. In this work we report the cloning and expression of H6H in *S. cerevisiae* for future industrial applications. The gene was cloned into the yeast pYES2.1-TOPO TA expression vector. The constructions were confirmed by sequencing. Recombinant yeast clones were isolated by cultures in YNBD medium containing histidine, leucine and tryptophan without uracil. The expression of the h6h cloned was induced by changing the dextrose carbon source for galactose. The expression of the enzyme as a fusion protein allowed us to detect it by Western blot using antibodies against V5 epitope. The protein is expressed after 4 hours induction. The functionality of the cloned enzyme will be assayed by scopolamine HPLC detection.

BT-P12.

***Toxoplasma gondii* ANTIGEN PRODUCTION IN TOBACCO LEAVES**

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We adapted a previously described *Agrobacterium*-mediated transient expression system to test the expression level of two antigenic proteins of *T. gondii*: dense granule 4 (Gra4) and serin proteinase (TgPI). We used two different strategies for plant expression cassette construction to determine which of them represents the best choice to generate transgenic plants in the future. One construct was based in the PVX amplicon, where each gene was placed under the control of an additional coat protein subgenomic promoter. In the other construct each gene was fused to an apoplastic peptide signal under the *CaMV* 35S promoter. *T. gondii* antigen expression levels in tobacco leaves were quantified by Western blot using *E. coli*-expressed these antigens as control. Depending on the expression cassette used and the antigen expressed, the antigen yields in leaves infiltrated ranged from 0.2% to 0.01 % of total soluble protein. Gra4-expressing leaves showed the highest expression levels. The Gra4 and TgPI accumulation levels were higher with the amplicon PVX. Gra4 and TgPI will be included in future studies to evaluate the biological activity of these recombinant proteins.

BT-P13.

CLONING AND EXPRESSION OF A CYSTEIN PROTEASE FROM LATEX OF *Asclepias fruticosa* L. IN THE METHYLOTROPHIC YEAST *Pichia pastoris*

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Papain-like proteases are the most numerous family of the cysteine protease class. They have been produced by heterologous expression in a variety of systems, but the *Pichia* system appears to be the most efficient when high yields of active protease are required at low costs. The clearest advantage of this system is that it does not express and secretes endogenous cysteine proteases and there is a very low level of secretion of endogenous protein.

Cloning and expression of asclepain f, a plant cysteine peptidase isolated from latex of *A. fruticosa*, is now reported. A full-length 1152 bp cDNA was cloned by RACE PCR from latex RNA. The sequence showed many characteristics elements of the papain-like cysteine endopeptidases. It was ligated to pPICzá vector and expressed in the methylotrophic yeast *Pichia pastoris*. Endopeptidasic activity was detected using PFLNA as substrate in the extracellular medium. The Peptide Mass Fingerprint identified by MALDI-TOF MS corresponds to that of the enzyme.

[*]CONICET Fellow, [§]CONICET and [#]CIC Researcher Career. Supported by grants from CYTED, ANPCyT, CONICET, CIC and UNLP.

BT-P14.

CONSTRUCTION AND EVALUATION OF A NEW RECOMBINANT *ESCHERICHIA COLI* STRAIN FOR POLY(3-HYDROXYBUTYRATE) PRODUCTION FROM CHEAP SUBSTRATES

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Polyhydroxyalkanoates (PHAs) are thermoplastic biopolyesters, accumulated as energy reserve and electron sink by numerous microorganisms under unfavorable growth conditions. Poly(3-hydroxybutyrate) (PHB) is the best characterized PHA; and has drawn much attention as an environmentally harmless plastic because of its complete biodegradability. PHB biosynthetic genes from a natural isolate of *Azotobacter* sp. were cloned in our laboratory and expressed in *E. coli* under the control of the *lac* promoter (plasmid pJP24). The resulting strain was able to accumulate PHB using milk whey and corn steep liquor as main carbon and nitrogen sources, respectively. However, our previous findings showed that ampicillin resistance was not the best selection for PHB production at high-cell-density culture in bioreactors, since plasmid-borne resistance was lost during cultivation. To enhance plasmid stability, *aph* gene (coding aminoglycoside 3'-phosphotransferase) was amplified by PCR and cloned in the PHB-expression plasmid resulting in pJP24K, resistant to kanamycin. Biomass and PHB production during fed-batch culture were determined for the resulting recombinant strain. These parameters, together with plasmid stability were compared to those obtained for the ampicillin resistant strain, demonstrating that the new recombinant is more suitable for PHB production.

BT-P15.

BIOSYNTHESIS OF ERYTHROMYCIN IN *E. coli*: IMPROVEMENT OF THE MYCAROSYLATION STEP

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Biosynthesis of erythromycin C involves the synthesis of the polyketide 6-deoxyerythronolide B through the condensation of one proprionate and six methylmalonyl-CoA units. Further modifications of the macrolactone ring are sequentially introduced, which comprise a number of enzymatic activities encoded by 20 genes. These "tailoring" steps involve a hydroxylation at C-6, the sequential addition of the deoxysugars mycarose and desosamine, and a second hydroxylation at C-12. Recently, we developed an *E. coli* strain that functionally expressed two operons for the biosynthesis and transfer of mycarose and desosamine deoxysugars from *Micromonospora megalomicea*, together with the 6-dEB synthetase genes from *Saccharopolyspora erythraea*. This allowed us to obtain erythromycin C from batch cultures, together with other intermediate compounds. The characterization by NMR and LC/MS of one of these products indicated the absence of a methyl group in the mycarose moiety. In the present work, we were able to identify the biosynthetic step responsible for the accumulation of this intermediate product. Surprisingly, this was not due to a failure in the methyl transferase step but the previous enzyme in the pathway of dTDP-L-mycarose biosynthesis, the ketoreductase MegBII. The addition of *megDVII* gene from megalomicin cluster to the mycarose operon allowed improving the mycarosylation efficiency. Structural analysis suggests that MegBII and MegDVII interact to form a functional complex.

BT-P16.

LIPOSOMES AS DNA VEHICLES APPLIED TO A MODEL OF ANTITUMORAL THERAPY

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In this work we describe the development of a plasmid DNA vehicle based on cationic lipids. The liposome-DNA systems were assayed in vitro on murine melanoma B16F0 cell line to test their transfection efficiency. The liposome formulations tested were: DOPE:DOTAP (1:1), (DC8,9PC:DMPC)pol: Vectamidine (1:1), DOPE:DOTAP:Chol (0,5:1:05), DOPE:DOTAP:Chol (0,8:1:0,2) in the indicated molar ratios.

Melanoma was induced on C57BL6 male mice 6-8 weeks old by subcutaneous injection of B16F0 cells. Liposome formulations were tested for in vivo transfection efficiency in this model using pCH110 plasmid and â–galactosidase activity was measured in situ as reporter of transfection efficiency.

Citotoxicity was tested in vitro over B16F0 cells and BHK21 cells as a reference and measured using crystal violet dye.

In order to enhance the interaction between liposomes and target cells, folate-conjugated lipids have been developed and folate-targeted liposomes are being evaluated.

The results of transfection efficiency obtained so far, suggest that some of the liposome formulations assayed could be used as effective vehicles for the delivery of plasmid DNA to tumor cells. The low toxicity of liposome formulations indicates that they could be suitable for in vivo delivery.

BT-P17.
POLYMERIC CATIONIC LIPOSOMES AND THEIR POTENTIAL USE AS TRANSFECTION VECTORS

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To investigate the possible applications of polymeric liposomes in transfection techniques, DC8,9PC and DMPC were co-polymerized or mixed with diC_{14}amidine (vectamidine) at different ratios. Vectamidine is a liposome forming double chain cationic amphiphile. These formulations were assayed for their transfection efficiency on mammalian cell lines with pCH110 vector, determining B-galactosidase activity in situ. Also, biodistribution studies were performed on female Balb-c mice, 6 to 8 weeks old. Blood samples were extracted 1 hour after intraperitoneal inoculation. The liver, kidney, lungs, intestines and blood were extracted after 24 hours. The presence of the plasmidic probe (pGEMT-vp7) was tested by polymerase chain reaction (PCR) amplifying vp7 sequence with specific primers.
On the other hand DNA percentage association as well as citoxicity of the selected formulations was determined.
The results obtained show that these formulations could be suitable for in vitro and in vivo delivery of plasmid DNA with low toxicity.

BT-P18.
DESIGN OF ULTRADEFORMABLE LIPOSOMES AS NANOVEHICLES FOR PHTHALOCYANINES

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With the aim of developing a novel therapy against cutaneous leishmaniasis we combined photodynamic theraphy with a nano-controlled release system capable of experience locomotion towards leishmania-infected macrophages in the deeper layers of epidermis. We developed ultradeformable liposomes (UD) loaded with two zinc phthalocyanines (ZnPc) as photosensitizers, to be used as vehicles able to squeeze through the skin barriers such as nanopores and stratum corneum. The UD were prepared by resuspension of a film made by evaporation of Phospholipon-90 and Sodium Cholate (border activator), followed by sonication and extrusion. ZnPc were incorporated at different stages depending on their hydrophobic or hydrophilic nature. Ultradeformability was tested by submitting UD under pressure through a 50nm-pore filter, and citotoxicity was tested on Vero cells. We found that both ZnPcs were incorporated into UD without altering their ultradeformability, and that viability of cells was not significatively affected by UD containing ZnPc until up to a concentration of 1 ?M of the photosensitizer.

BT-P19.
PHARMACEUTICAL NANOTECHNOLOGY: ABZ INTO SOLID LIPID NANOPARTICLES

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Echinococcosis, cystic echinococcosis, alveolar echinococcosis and neurocysticercosis are treated with albendazole(ABZ). ABZ is water insoluble, with low systemic bioavailability and efficacy due to scarce adsorption after oral or intrarruminal administration to bovines, caprines and ovines. The bioavailability could increase if ABZ was released from a sc depot. For this reason, we have incorporated ABZ into solid lipid nanoparticles(SLN). As a controlled released system, SLNs are capable of capturing, retaining and controlling the releasing of lipophilic drugs. In this study, we designed a home made device to prepare SLN by dispersing warm o/w microemulsions in cold water. A multifactorial design was used to establish optium combination of lipid (stearic acid), drug, surfactant (phospholipon), and co-surfactant(taurocholate sodium) to achieve maximum percent drug entrapment. We caracterized ABZ-loaded SLN using DSC, Dynamic Light Scattering (DLS), TLC and UV spectroscopy(298 nm). We have studied their reproducibility and stability in different critical conditions. ABZ-loaded SLNs for sc administration were freeze-dried using fructose. The mean particle size was 243 nm. Most of SLNs showed a polymorphism state between α-β'. ABZ into SLN was more than 22%. The results demostrate, by optimizing critical process parameters sc injectable SLN- dispersions can be freeze- dried, preserving their small size. It is a simple, available and effective method to produce SLNs.

BT-P20.
DIVALENT METALS TRANSPORTING PEPTIDES: *IN VITRO* ACTION OF COPPER COMPLEXES ON THE HUMAN HAIR FOLLICLE STRUCTURE

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Certain types of divalent metal peptides complexes posses both tissue protection and repair properties. Human peptides complexed particularly with copper have protective and regenerative actions on several organ systems including skin and hair follicles.
The effect of bivalent metal transporting peptides, specifically complexed with copper – copper peptide- was analyzed in our laboratory. In that sense, the morphological and molecular structures of human hair follicles obtained from hair scalp biopsies were assessed.
The structural analysis of the hair follicle related to the presence of the cupric peptide was performed according to morphological, histological and molecular criteria with the aim of correlating the vital state of its component cells and the copper peptide complex exposition kinetic. Moreover, we assessed the peptide effect on follicle extracellular matrix structure and molecular components. In this view, the relationship of peptide exposure and apoptosis/ necrosis was assessed through several molecular markers analysis. We discuss perspectives and dermatological potential uses in hair transplant technique.

BT-P21.

THREE SOL-GEL STRATEGIES FOR THE IMMOBILIZATION OF BACTERIA AND THEIR CAPACITY OF SURVIVING AT DIFFERENT TEMPERATURES

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In order to show the influence of bacterial membrane and wall architecture in their susceptibility to the immobilization process, we selected three types of bacteria: E. coli and S. aureus as representative of Gram negative and Gram positive families respectively, and R. loti as a model of a microorganism enclosed within a natural capsule.

We also chose three different sol- gel precursors to evaluate which of them had less deleterious effects on immobilized bacteria and allowed us to preserved them for longer periods of time. These precursors were silicon dioxide/citric acid, sodium silicate/citric acid and tetraethoxysilane/hydrochloric acid/glycerol.

Thermal stability of immobilized bacteria preserved in hermetically closed tubes at room temperature, 4ºC, -20ºC and –70ºC will be presented. The number of viable cells will be studied for a period of 120 days.

By the moment we have found that the three microorganism survive the encapsulation process performed with the different matrices with high viability rates (data to be shown). After freezing them the number of living cells falls down, but just in one or two orders of magnitude and then remains constant.

BT-P22.

MESORHIZOBIUM LOTI IMMOBILIZATION USING SOL – GEL PROCESS

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Rhizobia are soil and rhizosphere bacteria of agronomic importance because they perform nitrogen-fixing symbioses with leguminous plants.

The aim of this work was to generate an alternative way of inoculating Rhizobia in the fields and to obtain a higher number of living cells for a longer period of time in comparison with commercial formulas.

Two different matrices, silicon dioxide/hydrochloric acid and sodium silicate/citric acid, were used in the immobilization of these bacteria. Immobilized Rhizobium was preserved at 4º C for a period of a month. We observed the best results for the second matrix (sodium silicate/citric acid) suggesting that the presence of the organic acid plays a role in the protection of the bacteria. The number of bacteria falls from 10^8 to 10^7 CFU/ml due to the immobilization process itself, but then remains constant for a month. In the first matrix (silicon dioxide/hydrochloric acid) the number of initial cells falls from 10^8 to 10^6 CFU/ml but then also remains constant in the time. Other studies were performed to confirm that the immobilized rhizobium were still able to nodulate plants, showing positive results.

BT-P23.

OBTEINING OF CHEMICALLY MODIFIED SOL-GEL MATRICES, APPLICABLE TO PSEUDOAFFINITY CROMATOGRAPHY FOR PROTEINS CONCENTRATION

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Affinity chromatography is a well know technique for purification processes. Conventional soft gel affinity media has unstable mechanical characters. Cibacrom Blue is a dye that has high affinity for several proteins and enzymes. For this reason it has been bound to a great variety of supports, such as Sephadex, Sepharose, poliacrilamide and others.

In this work we used the sol-gel technique to obtain a high mechanically stable, polymeric matrix of tetraethoxysilane and aminepropyltriethoxysilane (APTES). The dye was covalently bound to APTES free amine groups.

We studied the adsorption of albumin from an aqueous solution, simulating urine conditions. It was found that the system binds 7 mg of albumin per 1 g of matrix.

This technique could be useful in biotechnology and clinical analysis to obtain porous supports for pseudoaffinity chromatography columns, which will be able to concentrate albumin or other proteins from complex samples.

BT-P24.

IMMOBILIZATION AND STABILIZATION OF CYCLODEXTRIN GLYCOSYLTRANSFERASE

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Cyclodextrin glycosyltransferase (CGTase) catalyses the formation of cyclodextrins (CD) from starch. Several free CGTases have been used in industrial CD production. However, immobilized enzymes are an alternative to allow the recovery and reuse of the biocatalysts in large-scale applications. The aim of this work was to develop an effective method for immobilizing a CGTase from *B. circulans* DF 9R. Covalent immobilization of CGTase on glyoxyl agarose (GA) beads promoted a very high stabilization of the enzyme against any distorting agent (strong stirring, temperature, pH and organic solvents). Other immobilized preparations (CNBr, PEI or MANAE agarose and Eupergit C) exhibited lower activity after incubation under similar conditions. In spite, optimized CGTase-GA derivative expressed a high percentage of catalytic activity (70%). Stabilization of the enzyme immobilized on GA is higher when using the highest activation degree (75 µmol of glyoxyl per mL of support) as well as when performing long enzyme-support incubation times (4 h) at room temperature. Multipoint covalent attachment seems to be responsible for this very high stabilization. The CGTase-GA presented very good prospects as an industrial biocatalyst.

BT-P25.

A DNA APTAMER DEVELOPED BY SELEX AGAINST UBIQUITIN RECOGNIZES SPECIFIC PROTEIN POPULATIONS IN CELLULAR EXTRACTS

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Aptamers are short strands of DNA or RNA that have the ability to specific recognize ligands by forming binding pockets. The targets can be small organic compounds, nucleic acids, proteins and even supramolecular structures. They are selected from a vast population of random sequences by a process called SELEX, which consist of iterative rounds of competitive selection and amplification.
To obtain an aptamer specific for ubiquitin, we performed SELEX starting from a random population (~10^{14}) of 62 nt long ssDNA. The target for selection was ubiquitin bound to nitrocellulose, and the selected ssDNA molecules were amplified by PCR. After 12 rounds of selection-amplification, the enriched population was cloned and 25 of these clones were sequenced. One of them, Apt18, was sinthesized with a 5' modification (biotin), and used in western-blot-like experiments. After detection with streptavidin-AP, we obtained specific patterns of bands in total cellular extracts from Rat or *T. cruzi*. In *E. coli* extracts, where ubiquitin is not present, no protein was detected using this method. We are at present trying to determine if these bands correspond indeed to the ubiquitinated pool of cellular proteins.

BT-P26.

A NOVEL hGM-CSF-DERIVED TAG FOR IMMUNO-DETECTION OF RECOMBINANT PROTEINS

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Epitope tagging with peptides has become an important tool for detecting, localizing and purifying recombinant proteins. Moreover, the development of small tags acquires high relevance for fusion protein design where its functionality should not be affected by tag addition.
A continuous epitope A_1PAR_4, derived from human Granulocyte-Macrophage Colony-Stimulating Factor (hGM-CSF), was delineated by an overlapping peptide scan employing an anti-cytokine monoclonal antibody (mAb CC1H7).
The peptide APAR was fused to the N- and C- terminal region of the recombinant human interferon-á2b (rhIFN-á2b) using several linkers (APARGGG, GGGAPAR and APARSPS, being SPS the natural consecutive amino acid sequence of APAR in the GM-CSF molecule). Fusion proteins were expressed in eukaryotic (CHO.K1 cells) and prokaryotic (E. coli) systems. Only APARGGG and APARSPS-carrying proteins were recognized by mAb CC1H7. Both proteins were strongly recognized by western blot but only APARSPS-fused protein was specifically recognized by methods that evaluate native proteins. These features suggest the use of the epitope APARSPS as a tag for fusion protein design, conferring the property of being recognized by procedures that preserve the native conformation as well as by others that promote the partial or complete denaturalization.

BT-P27.

RATIONAL DESIGN OF AN IMMUNOCHEMICAL ASSAY FOR QUANTIFYING SEVERAL THERAPEUTIC FORMS OF RECOMBINANT HUMAN INTERFERON-?2

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Recombinant hIFN-?2a, hIFN-?2b and the corresponding long lasting forms of pegylated IFN-?2 have undergone extensive clinical investigations and have become useful drugs for viral and oncological indications. Therefore, methods for quantifying them are relevant in order to evaluate different stages of their production processes and their final formulation, where the molecule is not pure and conventional methods cannot be applied.
Three anti-IFN-??2b monoclonal antibodies (mAbs CA5E6, CB15D7 and CA1A3), mapping three distinct epitopes, were selected for immunochemical procedures. A sandwich mAb-polyclonal antibody ELISA and a competitive ELISA with each mAb were carried out. mAb CB15D7 was suitable to quantify rhIFN-??2b and 12-kDa monomethoxy polyethylene glycol (PEG)-conjugated rhIFN-??2b in both ELISA versions. Nevertheless, the 40-kDa PEG-rhIFN-??2a derivative was only accurately quantified by the competitive assay using mAb CB15D7 or CA5E6. The sandwich ELISA comprises some restraints for the native cytokine recognition that were clearly evidenced with the highest pegylated form of the IFN. The competitive assay is appropriated for all therapeutic forms of rhIFN-?2 independently of the solution to be tested.

BT-P28.

IDENTIFICATION OF PEPTIDE LIGANDS FOR A MAb anti rh-INTERFERON-á2b PURIFICATION USING ONE-BEAD-ONE-PEPTIDE LIBRARIES

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Nowadays affinity chromatography with protein A as the ligand is preferred for industrial purification of MAbs. However, protein A is prone to degradation and thermolabile. Short peptides, instead, are suitable ligands due to their higher stability.
In this work, a simple and non-expensive method for affinity ligand selection over a combinatorial peptide library using mass spectrometry sequencing (MS/MS) is described. A one-octapeptide-one-bead library was synthesized on the PEG-based solid support Aminomethyl ChemMatrix™ with HMBA as the linker. The HMBA resistance to TFA allowed side chain deprotection without releasing the peptide from the resin. The library was synthesized by the Divide-Couple-Recombine (DCR) method using Fmoc chemistry. An immunoaffinity screening against an MAb anti-rh-IFN-á2b over the library containing the octapeptides XXXXGGGG was performed. Beads showing a positive reaction were mechanically isolated and subjected to ammonia/THF cleavage. Free peptides were sequenced by MS/MS. Five peptides were identified as possible ligands for the MAb. They will be synthesized, attached to Sepharose and their ability to purify the MAb will be assessed.

BT-P29.

CYTOTOXIC ACTIVITY OF LEPTOCARPIN ON UNRELATED HUMAN CANCER CELL LINES

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Leptocarpin, a sesquiterpene lactone isolated from Chilean plant *Leptocarpha rivularis*, has been tested for its cytotoxic effect on five human cancer cell lines: breast adenocarcinoma (MCF-7), pancreatic adenocarcinoma (NP-9 and NP-18), histiocytic lymphoma (U937), and myeloblastic leukemia (HL-60). In cells grown in appropriate media leptocarpin elicited cytotoxicity as assessed by Trypan blue dye exclusion, cell number determination in a Cell Coulter, and MTT reduction. On microscopic examinations, leptocarpin treated cells exhibited typical morphological features of apoptosis, such as cell shrinkage and formation of apoptotic bodies. Cytoplasmic and nuclear fluorescent staining revealed distinctive chromatin condensation and nuclear fragmentation. Likewise, DNA laddering also was observed. The apoptotic percentage paralleled with cytotoxic parameters. In conclusion, leptocarpin demonstrated to be active on human tumor cells of different origin by means of apoptosis induction, this cytotoxic activity was very higher than the observed in normal blood cells, suggesting a potential and wide antitumor role.

BT-P30.

ANTIANDROGEN OLIGONUCLEOTIDES: A NEW PHARMACOLOGICAL CLASS ACTIVE ON CELL LINES AND PRIMARY CULTURE OF HUMAN HAIR FOLLICLE

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Androgens mediate many metabolic responses and are implicated in oncological and dermatological disorders acting through the androgens receptor (AR). Antisense oligonucleotides (ODN), aptamers ODN, small interfering RNA (siRNA) and peptides constitute a new pharmacological class, the "tides". Accordingly, we aimed to inhibit the AR expression in cell models, for the development of new pharmaceuticals. We disclosed acceptor sites in the human AR mRNA by a "mRNA walking" approach. Designed ODN were able to inhibit, in primary culture of human hair follicle cells, at mM concentrations, the AR expression from 80% to 35-40%, depending on AR mRNA accessibility. Synthetic siRNA, designed to target the disclosed acceptor sites inhibited AR mRNA expression in cell cultures at nM concentrations between 100% and 30–40%. Functional assays on cell lines showed that synthetic siRNA efficacy correlates with: a) access to AR mRNA b) the efficacy of ODN even thought the inhibition mechanisms of antisense ODN and siRNA are completely different. Thus in vitro accessibility mapping allowed us to design potent antiandrogen ODN suitable in the treatment of androgen related disorders.

BT-P31.

ANTIMUTAGENIC ACTIVITY OF A PROTEIN ISOLATED FROM *Cyphomandra betacea* MATURE FRUIT

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Cyphomandra betacea (Cav.) Sendt is a subtropical fruit native from Andean region of Perú, Argentine and Chile. It has been used like food in salad and jellie preparations. Nevertheless, their functional properties have not been exploited yet. In a previous work, we have isolated and purified an invertase inhibitory protein (IIP) from mature fruit. This protein exerts *in vitro* inhibitory action on hydrolases isolated from phytopathogenic bacteria and fungi. It also can inhibit the growth of them. Recently, we demonstrated their antioxidant capacity, reactive oxygen species scavenging activity ($O_2^{\bullet -}$ and HO•) and DPPH-radical scavenging activity. In this work, we evaluated the toxic, mutagenic and antimutagenic effects of IIP by using a short-term test. The assay using *Salmonella typhimurium* TA 98 and TA 100 was performed in presence of two mutagens, isoquinoline (IQ) and 4-nitro o-phenylenediamine (NPD), with and without metabolic activation. IIP showed no toxicity to assayed strains at concentrations that have antimicrobial and antioxidant activities (5-250 µg/ml). The IIP showed MR (mutagenicity relation) values below 2, which indicate that the protein does not exert effect mutagenic. IIP was able to inhibit the NPD-mutagenicity. According with our results, we could suggest the potential application of this protein in the agriculture control of phytopathogenic organism development in post-harvested fruit and vegetable.

BT-P32.

ANTIGENOTOXIC EFFECT OF *Zuccagnia punctata* Cav. AND ARGENTINE PROPOLIS EXTRACTS

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Zuccagnia punctata Cav (Fabaceae), a South American plant species, is the botanical origin of propolis from Amaicha del Valle, Tucumán, Argentina. In previous works it has been reported that ethanolic extracts of both the plant and the propolis showed antimicrobial, free-radical scavenging, antioxidant and antimutagenic (Ames test) activities. For this, both natural products were used in blue berries conservation. Recently, an antibacterial compound (i.e. 2',4' dihydroxychalcone) was isolated from them.

The aim of the present study was to investigate the antigenotoxic effects of both extracts using the alkaline Comet assay on metabolically competent human-derived hepatoma (Hep G2) cells. We showed that propolis and *Z. punctata* extracts were able to diminish the DNA damage induced by benzo(a)pyrene (B(a)P) and 4-nitroquinoline-*N*-oxyde (4-NQO) in Hep G2 cells. This effect could be explained by their 2',4' dihydroxychalcone contents. Otherwise, the tested natural products did not show neither cytotoxic nor genotoxic effects on Hep G2 cells. Our results justify its use in food and pharmaceutical industries.

BT-P33.

A MONOCLONAL ANTIBODY THAT NEUTRALIZES AN HETEROGENEOUS COLLECTION OF hIFN-ALPHA

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Increased expression of human interferon alpha (hIFN-?) correlates with acute viral infections, inflammatory disorders and several autoimmune illnesses where the cytokine may be a component of either initiating or maintaining the disease. Nowadays, the therapeutic use of monoclonal antibodies (mAbs) has gained acceptance, representing IFN-??an attractive target for antibody-based therapy.
A panel of 11 murine mAbs was prepared using recombinant hIFN-?2b??Taking into account the affinity constant, the inhibition of IFN-? biological activity and the recognition of different molecular areas of the cytokine, four mAbs were selected to produce scFv fragments. One of these fragments (CA5E6) was able to neutralize a wide spectrum of IFN-?s including the recombinant human cytokines IFN-?2a and IFN-?2b and an heterogeneous collection of IFN-?s produced by activated leukocytes and Namalwa cells (lymphoblastoid IFN). On the other hand, binding experiments showed a very low decrease in scFv affinity in comparison with the parent murine antibody. Consequently, having established the ability of scFv CA5E6 to neutralize a broad diversity of IFN-?s, it is a potential candidate to be used for the treatment of diseases such as systemic lupus erythematosus, multiple sclerosis and rheumatoid arthritis.

BT-P34.

IMMUNOGENICITY AND PASSIVE PROTECTION INDUCED BY IMMUNIZATION OF MICE WITH BACULOVIRUS-EXPRESSED VP6 PROTEIN FROM BOVINE ROTAVIRUS

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Group A bovine rotavirus (BRV) is the principal aetiological agent of severe neonatal gastro-enteritis in mammalian species worldwide. It is responsible for extensive economical losses especially to the cattle industry. BRV VP6 constitutes the middle capsid layer of the virus and it is the major and more immunogenic viral protein.
In this work we report the expression of BRV (C486) VP6, in SF9 cell cultures in suspension as well as in insect larvae by a recombinant baculovirus technology. Female BALB/c mice were immunized with one, two and three doses of the antigen (n=10/group). Immunized mice developed high titers of anti-VP6 serum and milk IgG and IgA as measured by ELISA. Mice born to immunized dams were protected against challenge (protection level of 63%). The results presented here indicate that it was possible to express large quantities of recombinant VP6 and that the expressed antigen was able to induced a strong immune response and protect the offspring against viral challenge as it was evaluated using the suckling mice model.

BT-P35.

IMMUNOCAPTURE DEVICE FOR PATHOGEN MICROORGANISMS AISLATION USING SILICON OXIDE MATRICES

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The sol-gel chemistry is utilized frequently in glass derivatization in order to attach reactives groups carried in a silicon oxide matrix to the surface. Tetraethoxysilane and 3-Aminopropyl-triethoxysilane were used to generate a silicon polymer with amine groups capable to form a Schiff base with glutaraldehyde, leaving the other end of the glutaraldehyde molecule free to react with an amine group of an antibody. In this work, in order to demonstrate biological activity of the immobilized antibody, polyclonal antiserum directed against *Escherichia coli* O157 was used to prepare immunocapture devices using glass slides. Different mixtures of *E. coli* O157 and generic *E. coli*, with and without an enrichment step, were used in the evaluation of the specificity and sensibility of the device. At the moment specificity was demonstrated with a minimum background of unspecific bound. With the enrichment step 10 specific cells were detected among 100 total cells. The system appears as a promising technology for pathogen detection.

BT-P36.

CYSTEIN PEPTIDASES AND INHIBITORS PRESENT IN THE LATEX OF *Carica quercifolia* (ST.HIL.) HIERON. (CARICACEAE)

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The latex of *Carica quercifolia* fruits was obtained doing superficial incisions in the fruit and receiving it in citric-citrate buffer 0.1 M pH 5.6 containing EDTA 5mM. The preparation was centrifuged at 11500g during 20 min. Gums and other insoluble materials were discarded, and a limpid supernatant was obtained (crude extract). This extract 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 pH profile showed that the activity is manifested in a wide pH range (more than 80% of maximum activity among pH 6,9 and 9,1). The enzymatic activity was inhibited by E-64 and iodoacetic acid, but it was not affected for 1-10 phenantroline, pepstatine neither PMFS. Therefore, the new endopeptidases should be included in the cysteine group, as all the other proteases isolated from plants belonging to the *Caricaceae* family. Chromatographic purification of crude extract was achieved by FPLC using Sephacryl S-100 and then CM-Sepharose FF (pH 7,5). Three fractions with proteolyitic activity were isolated and a fourth with inhibitory activity of papain.
[1]CONICET fellow, [2]CONICET and [3]CIC Researcher Career. Supported by grants from ANPCyT, CONICET, CIC, UNLP and CYTED.

BT-P37.
CLONING AND SEQUENCING OF CYSTEIN PROTEASES FROM LATEX OF *Philibertia gilliesii*

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We have previously determined the presence of several cysteine proteases in the latex of *P. gilliesii* by biochemical studies. They differ in their pI as well as their molecular weight. The most alkaline of these endopeptidases (philibertaine g I) was purified, and its N-terminal sequence was obtained. The aim of this work was to clone and sequence the cDNA of cysteine proteases expressed in the latex of *P. gilliesii*. We designed degenerate primers based on the N-terminal sequence of philibertaine g I, extracted total RNA from latex, obtained cDNA (about 0.8 kb) by RT-PCR, and cloned these products into pGEM-T Easy Vector (Promega). At least two different cysteine proteases were identified within the sequenced clones. Deduced amino acid sequences from both clones showed similarities of 64 and 56% with papaya proteinase omega (CAA49504). In addition, highly conserved domains characteristic of these endopeptidases can be identified in both groups of sequences. However, none of the obtained sequences matched the N-terminal sequence of philibertaine g I. Therefore, latex of *P. gilliesii* can be a promising source of novel proteases with potential technological applications.

BT-P38.
USE OF PLANT PROTEASES IN CHEESE MAKING

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The coagulating power of partially purified extracts of plant endopeptidases (balansain, hieronymain, philibertain g and asclepain f) isolated in the LIPROVE has been determined on pasteurized bovine skim milk. The power of these vegetal curds at 30°C were compared with commercial chymosin. Milk coagulation behaviour was different for the assayed proteases: balansain and hieronymain have a similar behaviour (0.41 and 0.29 clotting units, respectively), whereas asclepain f exhibits the lower coagulation activity. In absence of cysteine, philibertain g did not exhibit clotting activity, but the addition of low cysteine levels (10 mM) promoted the higher coagulation activity. The rate and extension of hydrolysis products in the early process of cheese making was followed by SDS-Tricine gels electrophoresis and analysis of the corresponding densitograms. Hieronymain was use like vegetal curd to manufacture model miniature cheeses Holland type from pasteurized cow milk. Finally, preliminary analyses of this material are being carried out, evaluating the variation of several parameters (fat, humidity, content, salts, etc.) that are used for the formulation of a final product of good quality.

[1]UNLP Fellowship, [2]Member of CIC Researcher Career. Supported by grants from ANPCyT, CONICET, CIC, UNLP and CYTED.

BT-P39.
HERBICIDE DISSIPATION AND ITS EFFECTS ON PLANTS IN MICROCOSM ASSAYS

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In the last decades, the high yield expected from crop cultures and the wide implementation of "no till-system" farming techniques, lead to an extensive use of agrochemicals, like herbicides. This fact makes it very important to understand the fate of the herbicides and its metabolites in the ecosystems because their accumulation can represent a serious risk for the environment and human health. To study these complex interactions, microcosms experiments were set under laboratory conditions. Agricultural soils with and without history of treatment with herbicides were used; they were amended with different concentrations of the herbicide 2,4-dichlorophenoxybutyric acid (2,4-DB). Appropriate samples were taken in order to assess the herbicide and its metabolites dissipation and the effects of those chemicals on soil microbial communities and plants. Analysis by HPLC revealed that the soils with history of herbicide use had a faster dissipation of 2,4-DB (initial amount 5 mg/Kg) and no metabolites accumulation, when compared to soils not previously exposed to herbicides. No differences in the degradation rates were found between planted and unplanted microcosms; nevertheless, chlorophyll A/B ratio and total thiols content showed changes in leaves and roots tissues respectively, when the herbicide was present.

BT-P40.
TRANSGENIC EXPRESSION OF ANTIBACTERIAL PROTEINS AP24, LISOZYME AND DERMASEPTINE IN *Solanum tuberosum* (var. Spunta) PLANTS CONFERS BROAD SPECTRUM RESISTANCE TO PATHOGENS

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Engineered resistance to micro-organisms represents a promising approach for generating broad-spectrum pathogen-resistant potato plants. *Solanum tuberosum* (var. spunta) plants were transformed with genetic constructions constitutively expressing the chicken lysozyme, the tobacco AP24 osmotine or the *Phyllomedusa* dermaseptine coding sequences, respectively. Transgenic potato lines transformed with either the lysozyme and AP24, the dermaseptine or the three antimicrobial genes were assayed under controlled conditions for resistance to the phytopathogenic bacteria *Erwinia carotovora* spp. *atroseptica* and to the fungal pathogen *Fusarium eumartii* Carp. Transgenic plants and tubers were inoculated with *Erwinia* and *Fusarium* fresh cultures. Disease development was measured using arbitrary semi-quantitative scales. Transgenic lines respectively transformed with the dermaseptine, lysozyme, AP24 plus lysozyme or dermaseptine plus lysozyme and AP24 sequences were screened. Transgenic plants expressing combinations of two or three antimicrobial compounds exhibited increased resistance to both pathogens as compared to those expressing only one of them. Higher degrees of resistance were roughly correlated with higher expression of foreign genes.

BT-P41.

CLONING AND EXPRESSION OF CRY GENES FROM A *Bacillus thuringiensis* STRAIN WITH ENTOMOPATHOGENIC ACTIVITY AGAINST *Haematobia irritants*

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We previously described the developing of a semisynthetic medium that allowed the developing of adult *H. irritants* in the laboratory. It was also described the finding of a *B. thuringiensis* strain (BtHf) with entomopathogenic activity against the larvae stage of the insect. Toxicity analysis indicated that the activity was present in the bacteria paraesporal crystal, which was composed of at least one protein of 130-140kD. Here we described the cloning, identification and heterologous expression in *E. coli*, of two Cry genes present in the BtHf strain. By using a set of primers complementary to genes encoding high MW Cry proteins, we were able to PCR amplify two genes from BtHf DNA. The nucleotide sequence of these genes reveled their identity as related to Cry 1 type. One of these genes was recently described and its insect specificity is at present unknown. Using the pET expression system, several attempts to express deleted versions of these genes in *E. coli* were unsuccessful however; the complete versions of both genes were successfully overexpressed. This will allow us to carry out experiments in order to verify that the toxic activity resides in the paraesporal crystal proteins of BtHf as well as to analyze the toxic potency of each protein.

BT-P42.

ESTIMATING BACTERIAL DIVERSITY IN ACTIVATED SLUDGE FROM THE FULL-CYCLE rRNA APPROACH

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A novel strategy based on the full-cycle rRNA approach is proposed to describe the richness of bacterial populations present in activated sludge, circumventing the limitations of PCR-based analysis. DNA was isolated from sludge taken from the aeration basin of a full-scale industrial treatment plant receiving pretreated wastewater of an oil refinery. A total of 139 16S rRNA gene clones were sequenced for phylogenetic analysis and assigned to 46 OTUs. We have designed 20 rRNA-targeted oligonucleotide probes for quantitative FISH analysis, specific for OTUs represented by at least two clones in the library. 63 $\pm$ 3% of the DAPI-stained cells were accounted for by hibridization with probes targeting OTUs represented by more than four clones in the library, which belonged to the classes *Alpha* and *Beta* of the *Proteobacteria*, and to different members of the phylum *Acidobacteria*. Other bacteria in the sludge, determined using phylum-level probes were *Actinobacteria*, *Verrucomicrobia, Bacteroidetes and Firmicutes*, each one represented with a single clone in the library, and *Deltaproteobacteria* and *Planctomycetes*, for which representative clones were not found in the 16S rRNA gene clone library. Using FISH data instead of library abundance to fit distribution models shows promise for estimating total richness, because they display unbiased abundance of bacterial populations in a sample.

BT-P43.

DNA CONTENT OF *Anticarsia gemmatalis multinucleopolyhedrovirus* POLYHEDRAL INCLUSION BODIES PRODUCED IN INSECT CELL CULTURES

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Baculovirus polyhedral inclusion bodies (PIBs) are formed by virions occluded in a cristalline matrix of polyhedrin, a highly expressed viral protein. The DNA content of PIBs is proportional to the quantity of occluded virions, and then it could be considered as a parameter of PIB quality. A rapid flourimetric technique was optimized to quantify the DNA content, in order to evaluate the quality of *Anticarsia gemmatalis* multinucleoplyhedrovirus (AgMNPV) PIBs produced *in vitro* in cultures of the UFLAg-286 cell line in different serum-free and serum-supplemented culture media. The technique has a detection limit of 0.023 ìg/ml of DNA, and its response is linear until 0,74 ìg/ml of DNA. UFLAg-286 cell cultures infected with AgMNPV in UNL-8 serum-free medium produced $3x10^8$ PIBs/ml, with a mean DNA content of 0.84 ìg/ $1x10^8$ PIBs (final yield of occluded DNA: 2,67 ìg/ml culture medium). Serum addition to UNL-8 medium did not increase PIBs yield nor DNA content. Under similar infection conditions, UFLAg-286 cultures in the commercial serum-free medium Sf900II gave a final yield of 1,35 ìg of occluded DNA/ml culture medium. These data will be useful to perform a stoichiometric balance of AgMNPV production process in insect cell cultures.

BT-P44.

ISOLATION OF A NAPHTHALENE-DEGRADER *Pseudomonas sp*. AND PRELIMINARY EVALUATION OF ITS USE FOR BIOAUGMENTATION

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The survival of a naphtalene-degrader bacterial strain isolated with classical enrichment methods within autochthonous and allochthonous was compared. A bacterial strain, NR2, which could use naphthalene as the sole source of carbon and energy was isolated from sludge taken from the aeration basin of a system treating wastewater from an oil refinery. NR2 was subsequently identified as *Pseudomonas putida*. Four aerobic microcosms containing naphthalene as the only source of carbon were prepared. Two of them included sludge obtained from the oil refinery from where NR2 was isolated, and the other two contained sludge belonging to a pharmaceutical treatment plant, which hadn't been previously exposed to hydrocarbons. One of each of the two groups of microcosms were bioaugmented with 3.4×10^6 CFU/ml of the strain NR2. The active bacterial populations were monitored over a period of 26 days by RT-PCR/DGGE and it was observed that NR2 didn't integrate in neither of the communities. However, the sludge from the oil refinery exhibited a significant change in the active microbial community structure, compared to control sludge. In addition, the naphthalene-degrading ability was maintained. These results underline the importance of considering several ecological aspects when planning the use of isolated strains for using in processes of bioaugmentation.

BT-P45.
PHENANTHRENE BIOREMEDIATION IN SOILS USING AN ANTARCTIC BACTERIAL CONSORTIUM

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A bioremediation assay was carried out with soil from Jubany Station. Soil from 75 cm depth having a low level of PAHs contamination (1200 ppb) was removed, added with 1.5 gr of phenanthrene per kg and placed in 1 l flasks. The assay comprised a community control (CC), a biostimulated system (FMB700), a bioaugmented system (M10) and a biostimulated - bioaugmented system (M10-FMB700). In the biostimulated systems, a 9:1 mix (FMB700) of an organic source of P and N (fish meal) and a surfactant (Brij700) was added (3% w/w). Bioaugmentation was carried out using a PAHs-degrading Antarctic bacterial consortium (M10). Total heterotrophic aerobic bacteria were determined on CPS agar plates and hydrocarbon-degrading bacteria on agarized saline basal medium with diesel fuel and phenanthrene as sole carbon and energy source. Soil samples were extracted with dichloromethane and extracts cleaned up. Phenanthrene concentration was determined by GC-MS. Results were analyzed by One-way ANOVA**.** After 56 days, phenanthrene concentration decreased by 46% in the M10-FMB700 system, showing significant differences compared with M10 system (28.4% of removal), FMB700 system (21.9% of removal) and the CC system (18.9% of removal). Bioaugmentation combined with biostimulation was the most effective strategy for phenanthrene bioremediation under Antarctic conditions.

BT-P46.
CHANGES IN SOIL BACTERIAL COMMUNITIES DURING A HYDROCARBON-BIOREMEDIATION MESOCOSMS ASSAY IN ANTARCTICA

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An *in situ* bioremediation assay was performed in mesocosms prepared in 1m^2 plots with diesel-oil polluted soil from Jubany station, Antarctica. Significant hydrocarbon degradation was observed in all treated plots. Bacterial communities in control (CC) and in plots subjected to different treatments [biostimulation (AB) and biostimulation + consortia bioaugmentation (M10 and J13)] were characterised by T-RFLP analysis of PCR amplified 16S rDNA, at three different times, followed by cluster analysis of the electrophoretic patterns. Bacterial communities in all plots were similar at the beginning of the experiment, but there were rapid changes (within three weeks) in the structure of communities in the treated plots, which were highly similar (82%) irrespective of the treatment applied. Consortia M10 and J13 were not detected as established components of the community in bioaugmented plots. The relative abundance of the predominant bacterial population in CC decreased by more than 40% in treated plots, while at least other five bacterial populations increased in those. Therefore, the treatments caused an increase in the equitability (J') of the communities, although no major variations in Shannon diversity (H') was observed. Our results show that hydrocarbon bioremediation in treated mesocosms was successful and it resulted in changes in bacterial communities.

BT-P47.
LIQUID ENVIRONMENT MEDIUM FOR DETERMINATION OF PLASTIC BIODEGRADABILITY

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The increasing problems caused by waste management of packaging plastics have stimulated interest in biodegradable materials. There fore, methods are required to accurately assess degradation in the environment. In this study a culture medium for testing the biodegradability of plastic in liquid environment is analyzed. A biodegradable plastic material prepared from proteins and starch in the Biochemical Engineering Laboratory, G:I:B, FI-UBA, was used as a test material. Liquid environment were performed in two liters flasks, connected to an alkaline solution trap. A culture medium, favorable for grow of *Streptomyces* species and filamentous fungus, was chosen. Microorganisms inoculum was prepared from the supernatant of fertile superficial soil mixed with the culture medium. Incubations were performed with shaking. The degree of degradation was determined by carbon dioxide production trapped in the alkaline solution. After sixty days of incubation the samples were degraded until 36% of the total organic carbon (TOC) content was oxidized to carbon dioxide. We conclude that the used of this culture medium improved the efficiency of liquid environment method in approximately 28%, in comparison with other minimal medium, previously used in this laboratory, which reached no more than 29% of TOC degradation, in the same period.

BT-P48.
SIMAZINE BIOREMEDIATION USING NATIVE SOIL BACTERIA

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Simazine is a *s*-triazine herbicide widely used in agriculture. Its extensive use and toxicity cause environmental and health concern. Soil microbiota is involved in s-triazines attenuation. Bioaugmentation with an s-triazine degrading bacteria was studied in microcosms using pristine and annually simazine-treated soils. An orchard soil was used for pristine simulation. Long-term simazine exposed soil was collected from an avocado plantation. *Pseudomonas* sp. strain P41, was added to soil as alginate-immobilized cells. Total heterotrophic bacteria (THB) were grown in nutrient agar plates. Simazine catabolic activities (SCA) were estimated by most probably number. Soil simazine was determined by HPLC. In pristine soils, addition of bacteria increase SCA and THB. Simazine attenuation was observed only in inoculated soil (simazine half-life time 15 days). Annually simazine-applied soils have an active native simazine degrading microbiota. However, bioaugmentation with strain P41 significantly increases SCA in soil and reduces simazine half-life to 3 days. As *Pseudomonas* sp. strain P41 is able to increase the simazine attenuation in soils, it will be used for field bioremediation.

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BT-P49.
DEGRADATION OF PHENOL IN ACTIVATED SLUDGE
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We have analyzed the enrichment of microorganisms involved in the biodegradation of phenol in activated sludge. Four sequential batch laboratory scale reactors were supplied with saline medium, yeast extract and peptone. Two of the reactors received additionally 80 mg of phenol per day. Time-dependent changes in the structure of microbial communities were analyzed by RT-PCR coupled to denaturing gradient gel electrophoresis (DGGE). Whereas the rate of phenol degradation evaluated in batch assays was 10 folds greater in treated reactors sludges compared to control reactors, banding patterns of treated and control reactors (of both rDNA and rRNA) were not significantly different. A quantitative real time PCR detection system was developed for the different subclasses of the major subunit of phenol hydroxylase enzyme (LmPH) genes, previously described as high, medium and low media saturation constant (K_s). The number of total copies of LmPH gene (per mg of MLSS) was $(1.3\pm0.1) \times 10^9$ and $(3.2\pm0.4) \times 10^9$ for the treated reactors, and $(7.3\pm1.6) \times 10^7$ and $(1.6\pm0.1) \times 10^8$ for the control reactors. Assuming a single copy of LmPH gene/cell, these values represent 8.1% of total bacteria in the treated reactors and only 1.0% in the controls ($p<<0.001$). The real time PCR assay revealed the enrichment in phenol degraders through the increase in the number of genes of low K_s and high K_s in phenol-amended reactors, allowing the detection of functional differences between communities, which were not evidenced by DGGE.

BT-P50.
LAB-SCALE TREATMENT OF FOODSTUFF WITH THE CILIATE *Tetrahymena termophila* USING A SPECIALLY DESIGNED BIOREACTOR
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The eukaryotic cell *Tetrahymena termophila* has a number of unique features. So far no industrial process in which this non pathogenic organism could be used for the treatment of food has been developed. The advantages of this microorganism include the conversion of cholesterol into provitamin D, the formation of conjugated linoleic acid (CLA) and large quantites of coenzyme Q. Moreover *Tetrahymena* sequesters sterols in the forms of steryl steres that are acumulated in intracelular lipid droplets. Due to these qualities, *Tetrahymena termophila* can be used to improve the characteristics of special foodstuff, like milk. The development of a special bioreactor that allows the microorganism to grow and the treatment of the foodstuff on a continuous form is therefore a challenging undertaking. The stages of this process include (A) The biomass production in a specially formulated economical medium (B) The harvesting of the cells (C) Their addition to the foodstuff (D) An adequate treatment for separation of the cells from the foodstuff and finally, (E) The recovery of the improved foodstuff and the colection of the cell biomass. Therefore with the application of the bioreactor in this process an improved foodstuff can be obtained. The cell biomass recovered can be exploited to extract usefull compounds including the already mentioned. These substances may find important applications in food and drug biotechnology.
Acknowledgments to the support of Banco Río and Medipharma.

BT-P51.
IMPROVEMENT OF FOODSTUFF TREATMENT USING A *Tetrahymena sp.* MUTANT DEFICIENT IN HYDROLASES SECRETION
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The ciliate *Tetrahymena sp.* is a free-living eukaryotic organism that has ideal properties for the treatment of milk and egg. These include the ability to bioconvert cholesterol into provitamin D by virtue of the unique occurrence of delta-7 cholesterol desaturase. In addition it acylates sterols, accumulating them in the form of steryl esthers inside intracellular lipids droplets. Because of these properties, it can be employed to eliminate cholesterol from milk, egg yolk and finely ground meat particles. An undesirable trait of *Tetrahymena* is that it secretes large amounts of hydrolases to the medium, lake lipases, phospholipases and proteases. These enzymes may confer unpleasant sensory properties on the treated foodstuffs. We therefore explored the use of mutant cell lines specifically blocked in hydrolase release. The most efficient mutant, termed MS-1 has be obtained by N-nitrosoguanidine mutagenesis of the wild type cell line. It is a stable homozygous micronuclear mutant. We found that this mutant has reduced extracellular lipase and phospholipase release. Previously it had been shown to be deficient in extracellular protease activity. This cell allows extended periods of incubation without coagulation of the milk and therefore a more intense cholesterol bioconversion. Thus it appears as an advantageous alternative to the wild type CU-399 which was used in our prior studies.
Acknowledgments to the support of Banco Río and Medipharma.

BT-P52.
EXPLORING DIFFERENTIAL SCANNING CALORIMETRY AS A TOOL FOR EVALUATING FREEZING AND THAWING SENSITIVITY IN Saccharomyces cerevisiae
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Differential Scanning Calorimetry (DSC) was used to evaluate the freezing and thawing (F/T) resistance of two commercial *S. cerevisiae* strains having high (O) and low (NO) tolerance to osmotic stress. Cells in stationary phase of growth were exposed to two cycles of freezing at -20ºC for 20 and 4 h, respectively, and thawing at 25ºC, after being exposed to various physical and chemical mild stresses. Cells were used for DSC runs performed at 10°C/min from -100 to 110°C. The most adequate transitions to analyze in relation to cell viability after F/T treatments were those attributed to protein denaturation. Thermograms showed two protein populations corresponding to low and high denaturation temperatures: 43-55ºC (P1) and 65-80ºC (P2) respectively. Apparent enthalpy (J/g protein) change showed that strain O had a relatively higher resistance to F/T treatment than strain NO (ΔH = 30 J/g protein). On the other hand some pre-treatments (*v.g.*, temperature, ethanol and H_2O_2) increased both protein denaturation temperature and thermal resistance after F/T. The analysis of data clearly showed that the DSC can be a very useful tool to analyze the impact of different stress factors on F/T tolerance.

BT-P53.
PRELIMINARY CHARACTERIZATION OF RAM SPERM-SEMINAL PLASMA INTERACTIONS

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Cryopreservation reduces the fertilizing ability of ram sperm, due to membrane alterations similar to capacitation leading to non functional sperm. The addition of seminal plasma (SP) reverts this effect, by stabilization of the plasma membrane. Our work is focused to improve the fertilizing ability of cryopreserved ram sperm, based on the knowledge of the interactions between SP and sperm surface. In order to study the proteins of ram SP, we compared different ram strains (Frison, Pampinta and Corriedale) and conservation temperatures (-20º and -196ºC) by SDS-PAGE. In all the cases similar profiles were obtained. SP from different seasons were evaluated, showing variations in the protein composition. To evaluate the ability of SP proteins to bind onto the sperm membrane, co-incubation of sperm and SP from different conservation temperatures and seasons was performed and the sperm and SP proteins were analyzed by SDS-PAGE. The same proteins were associated to the sperm membrane at both conservation temperatures. Also, SP proteins associated to sperm membrane reproduce the variation observed in the protein profile of SP from each season. In conclusion, many SP proteins bind to the sperm surface. This interaction is not altered by the conservation temperature. Further studies would be done to investigate if the seasonal variations in the SP proteins that interacts with sperm may have physiological effects in the stabilization of the sperm membrane.

BT-P54.
LINDANE REMOVAL FROM CONTAMINATED SOIL BY *STREPTOMYCES* M7

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Lindane, that has been extensively used worldwide for the control of agricultural and medical pests, is a common pollutant found ubiquitous in the biosphere. The use of indigenous actinomycetes for bioremediation of lindane contaminated soils is an attractive approach, since these microorganisms are already adapted to the habitat. The aim of this work was to investigate the ability of *Streptomyces* M7 to remove lindane from contaminated soils.
Sterile soils contaminated with lindane 100 µg Kg^{-1} were inoculated with *Streptomyces* M7 0.5 g/Kg and incubated by 28 days. Bacterial growth was determined by UFC/g and residual lindane by GC; previously extractions with acetone and hexane were made.
Two weeks after inoculation of *Streptomyces* M7 into lindane-amended autoclaved soil, the strain removed 25% of the pesticide. The microbial population increased from $6.7.10^5$ (0 day) to $3.7.10^9$ (14 days). Similar results were observed in the bacterial growth in control non contaminated soils.

BT-P55.
BIOSORPTION OF CADMIUM BY *STREPTOMYCES* SP. F4

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Cadmium is a highly toxic heavy metal that has not known biological use, is very mobile on soil, and can be transfered and acumulated in the roots, leaves and stems of plants.
Diferents microorganisms like bacteria and fungi including yeasts, have a potential ability of metal biosorption, but there is very few information over actinomycetes for this purpose.
A cadmium resistant actinomycete was previously isolated from a former uranium mine, in Thuringia, Germany. This strain was identified as *Streptomyces* sp. F4.
A phylogenetic tree constructed with the 16S rDNA of *Streptomyces* sp. F4 (GenBank DQ141201) and 10 other *Streptomyces* sequences detected close similarity to *S. tendae*.
The *Streptomyces* sp. F4 was grown on media with Cd 8 ppm and without this metal and after 4 days of growing, an inhibition of 80% was observed compared to the control. On a biosorption assay on medium with 8 ppm Cd, the Cd biosorbed reaches the maximum after 6 days of culture, with 3,76 mg of Cd and 47% of biosorption. The specific biosorption was 60.6 mg Cd/g cell. This strain could be usefull for Cd bioremediation purposes.

BT-P56.
A COPPER ACCUMULATING ACTINOMYCETE STRAIN: MORPHOLOGICAL, BIOCHEMICAL AND MOLECULAR CHARACTERIZATION

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A copper resistant actinomycete strain AB0, previously isolated from polluted sediments, has the ability of reducing copper present in a synthetic medium in a 70% after seven days of growth. Advances in understanding the way in which this microorganism accumulates the metal as well as its characterization are very important to develop novel copper bioremediation processes. The aim of this work is to demonstrate the copper accumulation capacity of this strain using a transmission electron microscopy histochemical technique as well as performing a fully completed characterization of this strain. Using the Timm´s reagent, Cu^+ appeared as reaction deposits of reduced silver within the cell, mostly in the tips where typical actinomycete apical growth is produced but some metal deposits were also found associated with the exopolimer. Morphological and biochemical assays were performed using microtiter techniques thats includes lisozime and NaCl resistance, melanin production, pH tolerance, carbohydrate assimilation. According to the obtained results and 16s rDNA sequencing, the isolated strain was characterized as *Amycolatopsis sp.* AB0.

BT-P57.

CHROMATE REDUCTASE ACTIVITY OF *Streptomyces* MC1

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In natural water and subsurface soils chromium occurs in two major oxidation states III and VI. Cr (VI) is approximately 1,000-fold more cytotoxic and mutagenic than Cr (III). As the application of Cr is extensive in several industries, chromium-associated pollution is of increasing problem. Biological transformation of Cr (VI) to Cr (III) by enzymatic reduction is a means of decontamination. Actinomycetes are the dominant population in soil. Their metabolic diversity and the particular growth characteristics indicate them as agents for bioremediation.
The aim of this work was to study the chromate reductase activity of *Streptomyces* MC1, a chromium resistant strain.
Streptomyces MC1 was grew in minimal medium with Cr (VI) 1 mM, and without this metal, using glucose or glycerol as carbon source. After seven days, cells were harvested, crushed and resuspended. After centrifugation, enzyme activity was determinated using sodium phosphate buffer, NADH and Cr (VI) as substrate. The activity was 49 and 22 nmol/min/mg protein when the strain was grew with glucose or glycerol without the metal, however this activity was reduced at 12 or 11 nmol/min/mg protein after seven days of growth. Maybe Cr(III) makes a retro-inhibition effect. *Streptomyces* MC1 could be used for Cr (VI) reduction in biotechnological processes.

BT-P58.

DEVELOPMENT OF A PLANAR GOLD MICROELECTRODE ARRAY ARRANGEMENT COMPATIBLE WITH ATTACHMENT AND CULTURE OF NEURONAL CELLS: CLINICAL AND CELL-BASED SENSOR POTENTIAL

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The construction of a planar array of microelectrodes (MEA) following an easy and inexpensive method previously described was optimized, in order to allow higher microelectrode density; in the array obtained, each gold electrode can be measured independently. The polymeric material than surrounds each electrode was assayed about its ability to allow neuronal growth. The design of our MEA allows easily replacement and its use as detector in a FIA system. The FIA system is composed by a laminar flow cell, peristaltic pump and a standard manifold, a pseudo-reference (Ag/AgCl) electrode as reference and counterelectode was used. The dimensions of each microelectrode (20-40 µm) are compatible with the neuronal cellular soma, and could allow as following the spontaneous electrical extracellular activity of the cells growing over the electrodes. Moreover, MEAs can be used to examine the activities of whole cells and tissues rather than single receptors (as in patch clamp experiments), and this approach can be very efficient as sensitive biochemical detectors.

BT-P59.

PRELIMINARY STUDIES OF NAPHTHALENE-DEGRADING BACTERIA ISOLATED FROM MUELLE STORNI SEDIMENTS

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In order to isolate naphthalene-degrading bacteria from sediments of a highly contaminated site with polyaromatic hydrocarbons, three successive enrichment cultures were made in a synthetic marine media with 0.5 % naphthalene as a sole carbon and energy source. Growth was performed with agitation in darkness at 25ºC and 15ºC. After incubation, several colonies were isolated on agar plates at the different enrichment conditions. DGGE procedure was applied in order to compare the resulting biodiversity of culturable bacteria and ARDRA method to select potentially different species of bacteria for further identification.
In a culture-independent approach, total DNA was purified from the same sediments, and alpha subunits of initial PAH-dioxygenase genes were amplified using a degenerate primer set, cloned and sequenced. All analyzed clones in the library were closely related to *phnAc* genes from the phenanthrene-degraders *Burkholderia* sp. Ch1-1, Ch3-5, Cs1-4 and Eh1-1 (AY367784-7; 99.5 to 99.8% similarity at the nucleotide level) and *Alcaligenes faecalis* AFK2 (AB024945; 95.7-95.9% similarity). The presence of these catabolic genes was also search on the isolated bacteria.

BT-P60.

LARGE-SCALE PRODUCTION OF *Serratia entomophila*

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The bacterium *Serratia entomophila* has been developed as a biological control agent for grass grub, *Costelytra zealandica*
S. entomophila is usually produced in large-scale reactors at concentration of 4–6 x 10^{10} cfu/ml. Although this concentration is commercially profitable, an increase in cell density is desirable to improve volumetric productivity. Therefore, the influence of different carbon sources and dissolved oxygen concentration (DOc) on cell growth was investigated to optimise industrial production in experiments performed both shake-flasks and bioreactors.
Molasses, fructose, glucose, and a combination of glucose and fructose were tested against sucrose, the standard carbon source used in *S. entomophila* production. The cell density achieved with these different carbon sources did not improve that reached using sucrose. In contrast, the level of DOc in the culture medium had an important influence on cell density. DOc set-point levels of 5, 20 or 50% were used during the fermentations. Clear differences in cell number as well as cell growth kinetics were achieved between 5 % (2-3 x 10^{10} cfu/ml) and 20 or 50% DOc (6-8 x 10^{10} cfu/ml), revealing the important role that dissolved oxygen level plays in this fermentation system.
Therefore, the challenge to further improve *S. entomophila* cell density will need to take into account the selection of an appropriate reactor design and reactor configuration, the use of advanced, robust, fast and reliable methods for fermentation control, and the use of fed-batch as fermentation strategy.

CB-P1.

EFFECTS OF SESQUITERPENE LACTONES ON LIFE CYCLE OF *LEISHMANIA MEXICANA*

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Leishmania mexicana is the etiological agent of cutaneous leishmaniasis that affects to millions of people in the world. Here, we evaluated the effect of natural compounds on parasites cultured in liquid medium. Three sesquiterpene lactones; helenalin (HLN), mexicanin (MXN) and dehydroleucodine (DhL) obtained from native plants were tested on viability, proliferation, morphology and invasive capability of *L.mexicana*. The lactones inhibited proliferation of the parasites at very low concentrations (0.25-2.5ug/ml), being HLN and MXN more effective than DhL and than Ketokonazole (used as control). Moreover, cytotoxicity of these compounds was lesser in mammalian cells. We also observed that HLN and MXN induce apoptosis from concentrations of 1 µg/ml as judged by DNA fractionation. Infective capability of the parasites on Vero cells was also evaluated, and observed that MXN and HLN (DhL at lesser extent) reduce significantly the number of infected cells. Ultrastructurally, HLN and MXN induce intense vacuolization in the cytoplasm. We concluded that the lactones studied are active against *L. mexicana* at doses no lethal for the host cells. These compounds could be used as agents against leishmania in future, although further studies about the mechanism of action should be addressed.

CB-P2.

***TRYPANOSOMA CRUZI*: INVOLVEMENT OF PKC ISOFORMS IN METACYCLOGENESIS**

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Trypanosoma cruzi, the agent of Chagas´disease, possesses a complex life cycle, that develops in an hematophagus insect vector, *Triatoma infestans* and in a mammalian host. In the vector´s intestinal tract, epimastigote differentiation to the infective metacyclic forms depends on a network of multiple signaling molecules. This process can be explained partially by the stimulatory effects of the free-fatty-acid fraction present in the vector's intestinal extract, mainly oleic acid (OA), that stimulated *de novo* diacylglycerol formation and protein kinase C (PKC) activity in the parasite. PMA a well known PKC activator also induced metacyclogenesis (Wainszelbaum *et al*., Biochem J., 2003). We also determined in the epimastigote stage the presence of β, γ, δ, ε and ζ PKC isoforms. To elucidate which of these PKC isoforms are involved in *T. cruzi* metacyclogenesis induced by OA, epimastigotes were stimulated with 300 µM OA or 32nM PMA (control) for 15 min at 28ºC. Samples of parasite suspentions were taken every 15 min to 60 min and subjected to subcellular fractioning. The membrane and cytosol fractions obtained were analyzed by Immunoblot using specific PKC isoform antibodies. Results showed that in the first 15 min PKC δ translocated to membranes, demonstrating its participation on this process. This activation was extinguished after this time (transcient signal). The PKC δ translocation was also observed with the PMA stimulus. Accordingly various lines of evidence indicate that PKC δ plays a crytical role in cellular processes such as differentiation.

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CB-P3.

PURIFICATION AND CHARACTERISATION OF MYO-INOSITOL HEXAKISPHOSPHATE DEPOSITS FROM LARVAL *Echinococcus granulosus*

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The larval stage of the cestode parasite *Echinococcus granulosus* makes vesicular deposits of a calcium salt of *myo*-inositol hexakisphosphate (InsP_6) and exocytoses them onto its host-exposed layer of extracellular matrix. Over one-third of the dry mass of this otherwise mucin-rich extracellular structure (termed laminated layer) is composed of calcium InsP_6. We now show that prolonged mild alkaline digestion of the laminated layer mucins allows to purify the extracellular calcium InsP_6 deposits as a solid residue, which was determined to be composed of a salt with the same stoichiometry as the calcium salt of InsP_6 obtainable *in vitro*, i.e. $Ca_5H_2L{\cdot}16H_2O$ (L representing fully deprotonated InsP_6). In addition, the solid was observed to contain minor amounts of Mg. The purified deposits appeared similar to microcrystalline solids under the electron microscope. The major inositol phosphate in deposits other than InsP_6 was found to be *myo*-inositol (1,2,4,5,6) and/or (2,3,4,5,6) pentakisphosphate. This was also found to be the major InsP_5 both in the intact laminated layer and in the parasite cells giving rise to it. The compound was also the InsP_5 isomer labelled when larval parasites were cultured with tritiated inositol.

CB-P4.

ASSOCIATION OF HOST-DERIVED S100 PROTEINS AND ANNEXINS WITH THE LAMINATED LAYER OF THE *Echinococcus granulosus* LARVA

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The larva of *E granulosus*, the hydatid cyst, is protected by an acellular laminated layer (LL) composed by a meshwork of mucin-based fibrils and deposits of the calcium salt of *myo*-inositol hexakisphosphate (InsP_6). The LL binds host-derived proteins *in vivo*. We hypothesize that the adsorption of an "appropriate" set of host proteins must be a key element of the parasite's mechanisms for controlling host inflammation. We are thus identifying proteins bound by the LL under conditions of resolved (human, mice) and chronic inflammation (cattle), by mass spectrometry and Western blotting. Annexins (Ax) and S100 proteins are calcium-binding proteins that include members secreted by non-conventional mechanisms and have extracellular roles in inflammation. The anti-inflammatory protein AxA1 was found to be much more abundant on LL from human than from bovine infections; these latter have abundant AxA2. S100A12, a pro-inflammatory mediator, was found in the LL from bovine but not in LL from human hosts. Using the purified synthetic salt, we found that AxA1 but not AxA2 is capable of binding to calcium InsP_6 deposits. S100A8 and S100A9, of postulated pro- and anti-inflammatory activities, were found in LL from human and mice infections, their presence in bovine LL is still to be determined. Future studies will address the cellular sources of these proteins at the host-parasite interface, as well as the effects of their multivalent (LL-associated) presentation on cells of the innate immune system.

CB-P5.
INTERACTION OF *MYCOBACTERIUM MARINUM* AND THE AUTOPHAGIC PATHWAY
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Mycobacterium marinum (MM) is a natural pathogen of fish that occasionaly affects humans causing the "fish tank granuloma". These granulomas are very similar to the granulomas caused by *M. tuberculosis* in the lungs of humans. Its pathological similarity to *M. tuberculosis* infection and the more rapid growth and increased safety, makes MM a useful model to study pathogenic mycobacteria. Raw 267.4 cells infected with MM were analyzed by confocal microscopy. We have observed an increase in the processing of the protein LC3 upon infection with MM, which is indicative of autophagy. Our results indicate a high degree of colocalization between LC3 and MM phagosomes, implying an interaction between the autophagic pathway and MM. The analysis of bacteria survival was performed by determining the CFU (Colony Forming Units). Similar to *M. tuberculosis* we observed that when autophagy was induced, by incubation with starvation medium or by rapamycin treatment, there was a decrease on the survival of MM. Using electron microscopy, we observed a few phagosomes containing the bacteria, and an important number of "multilamelar bodies". As previously described, we have also observed that after 15 hours infection, MM escapes from the phagosomes and develops actin tails in the cytoplasm. In summary, our results show for the first time that MM interacts with the autophagic pathway and that its survival is hampered by autophagy induction.

CB-P6.
***Helicobacter pylori* TRIGGERS THE AUTOPHAGIC PATHWAY IN GASTRIC EPITHELIAL CELLS**
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H. pylori (HP) is a microaerophilic gram-negative bacterium that is able to invade epithelial cells in the gastric mucosa and survive inside large vacuolar compartments. Several pathogens have developed strategies to disrupt and utilize the autophagic pathway for their own benefit to generate their replicative compartments. Therefore, we assessed the possible engagement of autophagy during the invasion process of gastric epithelial cells by HP.
With this aim, gastric epithelial cells (AGS) and *atg5*-knockout mouse embryonic fibroblasts (MEFs), a cell line which is defective in autophagy, were infected with wild type and mutant strains of HP. Autophagy was monitored by expression of LC3, an autophagosome specific marker. The infected cells were analyzed by confocal microscopy. HP infection of AGS cells caused the recruitment of GFP-LC3 to a sub-population of the intracellular compartments where HP resides, and this effect was dependent on the post-infection time. When we infected *atg5*-/- MEFs, HP wasn't able to recruit LC3 to the vacuolar membrane at any time point. The engagement of autophagy was dependent on the presence of the bacterial toxin VacA, since autophagy was not detected when VacA isogenic mutant bacteria were utilized.
Our results demonstrate a potential role for VacA-mediated autophagy in the biogenesis of this unique bacterial niche.

CB-P7.
RAB11-FAMILY OF INTERACTING PROTEIN 3 - FIP3-IS RECRUITED TO PHAGOSOMES VIA ITS C-TERMINUS DOMAIN
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FIP3 also known as arfophilin1 belongs to a novel family of Rab11-interacting proteins (FIPs), which all share a highly conserved motif at the C-terminus of the proteins, the Rab11-binding domain. It has been recently shown that FIP3-containing vesicles are recruited to the cleavage furrow and are required for a late stage of cytokinesis. To determine whether FIP3 participates in the phagocytic pathway, we investigate its intracellular localization in macrophages. By immunofluorescence, we found that the endogenous protein is predominantly membrane-bound and FIP3-positive vesicles are concentrated close to the perinuclear region of macrophages. Overexpression of FIP3 fused to the green fluorescent protein displays a similar pattern. We have also observed that Rab11-FIP3 colocalizes with Rab11 at the recycling endosomal compartment. Furthermore, FIP3 is highly enriched at the early phagosomal membranes. We show that the targeting of FIP3 to phagosomes is Rab11 dependent, since a truncated C-terminal region of FIP3 ($FIP3_{244-756}$) remains membrane bound and associated to phagosomes. We have previously demonstrated that Rab11 is required for an efficient particle uptake and our present data suggest that FIP3 may act in concert with Rab11 to regulate membrane availability for particle uptake. Rab11-and FIP3-containing vesicles could be required at cellular processes where it will be necessary the delivery of large amounts of membrane such as cytokinesis and phagocytosis.

CB-P8.
RAB COUPLING PROTEIN BINDS TO PHAGOSOMES BY DIRECT INTERACTION WITH RAB11
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*R*ab *C*oupling *P*rotein (RCP) is a member of the newly identified class I Rab11 *F*amily of *I*nteracting *P*roteins (FIPs). RCP binds Rab11 tightly via a Rab Binding Domain located at its C-terminus. The N-end region of RCP has a C2 phospholipid-binding domain. Using confocal microscopy and biochemical assays, we have previously shown that RCP regulates phagocytosis and recycling from the phagosomal compartment in macrophages. To further characterize the binding of RCP to phagosomal membranes, we use truncated forms of this protein fused to the green fluorescent protein. The C-terminus of RCP (CT-RCP) displays a membrane-bound pattern and alters endosomal compartment. We observed CT-RCP associated as discrete patches to phagosomes. On the contrary, N-terminus of RCP (NT-RCP) has a diffuse distribution resembling a soluble protein. Neither association to early and recycling endosomes nor to early phagosomes could be observed. Stimulation of phosphatidic acid synthesis (a lipid to which RCP preferentially binds) results in the translocation of NT-RCP from the cytosol to the plasma membrane. However, we still could not observe any association of NT-RCP to early phagosomes after PMA treatment. Therefore, these results suggest that RCP is associated to phagosomes via a direct interaction with Rab11 and that the C2 domain is required to target vesicles departing from the phagosomal compartment to the plasma membrane. These events could be important for the understanding of molecular mechanisms involved in antigen presentation.

CB-P9.
RAB1 GTPase INTERACTS WITH COPII AND MODULATES ITS DYNAMIC

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Rab1 is an essential regulator of ER to Golgi transport and participates in traffic events at the donor and acceptor membranes through its interaction with effectors. A dominant negative Rab1 mutant (Rab1N121I) inhibits sorting of cargo proteins into ER exit sites (ERES) suggesting that Rab1 modulates cargo sorting. COPII protein complex (Sar1GTPase, Sec23/24 and Sec13/31) is required for sorting and concentration of cargo into ERES. To test if COPII components act as Rab1 effectors we performed GST-pull down assays using Rab1GTP and rat liver cytosol. Analysis of the bounded proteins indicates that Rab1, in its active form (Rab1Q67L), interacts with Sec23. In agreement, double immunofluorescence assay showed that Rab1Q67L colocalized with COPII structures. Furthermore, FRAP experiments performed in HeLa cells co-expressing GFP-Sec13 and Rab1Q67L indicate that Rab1 activity affects Sec13 membrane association-dissociation kinetics at the ERES.
These data show that Rab1 interacts with a COPII component, modulates COPII membrane association dynamic and strongly suggest that Rab1 activity modulates COPII sorting function at ERES interfaces.

CB-P10.
RAB1 DIRECTLY INTERACTS WITH GBF1 AND MODULATES ARF1 DYNAMICS

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Assembly of cytosolic coat proteins I (COPI) complex at the ER-Golgi interface is directed by the small GTPase ADP ribosylation factor 1 (Arf1) and its guanine nucleotide exchange factor, GBF1. Our previous results indicated that overexpression of the GTP-restricted Rab1 mutant (Rab1Q67L) increases association of GBF1 and COPI in ER exit sites interface. Moreover, an interaction between Rab1 and GBF1 was reported. Here, we further characterized this interaction. The ability of recombinant Rab1 to bind either the N-terminal or the C-terminal domains of GBF1 was tested using GST-pull down assays. We show that GTP form of Rab1 directly binds the N-terminal domain of GBF1. To evaluate how Rab1-GBF1 interaction influences Arf1, we measured Arf1 dynamics in presence of active Rab1. We performed FRAP assays in cells co-expressing Arf1-GFP and Rab1Q67L. Arf1 membrane association-dissociation kinetics at the Golgi complex was delayed. Moreover, Rab1Q67L inhibits Arf1 membrane dissociation induced by Brefeldin A suggesting that Rab1 modulates Arf1 membrane association. Taken together our data support a model where Rab1 directly interacts with the N-terminal domain of GBF1 to modulate Arf1 dynamics and activity.

CB-P11.
MICROARRAY ANALYSIS ON CELLS EXPRESSING RAB1GTPase MUTANTS

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Rab GTPases cycle between GDP and GTP bound forms. Rab1 localizes in the ER-Golgi intermediate compartment and is essential for ER to Golgi transport. Expression of the Rab1 mutant with impaired GTPase activity (Rab1Q67L) induces enlargement of the Golgi compartment. To explore how cells respond to Rab1-regulated Golgi enlargement we performed a global gene expression microarray analysis on cells expressing Rab1 constructs. RNA isolation, quantification, assessment of RNA integrity, labeling and hybridization procedures compatible with the Affymetrix GeneChip® platform were performed on samples from control cells (expressing GFP empty vector, or GFP-Rab7Q67L) and from cells expressing GFP-Rab1Q67L and GFP-Rab1wt. Thus, we identified genes with significant changes ($P<0.05$) in expression levels, encoding for proteins that might be involved in Rab1-regulated pathways (i.e. specific transport proteins; transcription factors, phosphatases-kinases and others). Our study shows specific Rab1-dependent changes of gene transcription. Additional analysis will provide information of the possible mechanisms that allow cells adaptation to an increase in Rab1 activity and will permit to establish a correlation with physiological situations where Rab1 is highly expressed.

CB-P12.
RIM PARTICIPATE IN THE ACROSOMAL EXOCYTOSIS OF HUMAN SPERM

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RIM is a Rab3A effector that binds the active form of this small GTPase and participates in exocytosis in several neuroendocrine cells. The protein has an N-terminus Zn finger domain, a central PDZ domain and two C-terminus C2 calcium and phospholipids binding domains. Activation of Rab3A initiates acrosomal exocytosis; hence, we are interested in the function of Rab3A effectors in the process. RIM52, an N-terminal fragment of human RIM1α (residues 1-399) that contains the Rab3A and Munc13 binding domains was produced in bacteria. Rabphilin3A, another Rab3A effector was also expressed in bacteria and purified. Human sperm cells permeabilized with streptolysin O were incubated with increasing concentrations of RIM52 and rabphilin. Only RIM52 was able to block calcium-triggered exocytosis. We speculate that the truncated recombinant protein competes with the endogenous factor and blocks membrane fusion. To investigate whether endogenous RIM is required for secretion, a polyclonal antibody against RIM52 was generated in mice. This antibody was added to permeabilized sperm and exocytosis was initiated adding calcium. The antibody completely abrogated acrosomal secretion. These results indicate that RIM is implicated in acrosomal exocytosis, likely as a Rab3A effector.

CB-P13.
MEMBRANE BINDING PROPERTIES OF K-RAS
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Ras proteins (H, N and K) localize at the inner leaflet of plasma membrane, where they activate diverse signal transduction pathways. K-Ras is farnesylated and contains a polybasic domain (KKKKKK) at the C-terminal domain. These elements are necessary for proper subcellular localization of this protein. To evaluate the significance of membrane properties on plasma membrane association of K-Ras, constructs were engineered to express the full-length and C-terminal domain of K-Ras fused to fluorescent proteins. By biochemical approaches, we evaluated *in vitro* the effect of ionic strength, polyelectrolyte and Ca^{2+} concentration on membrane binding properties of K-Ras. Results from these experiments suggest that, electrostatic interactions contribute to plasma membrane association of this protein. To investigate the role of calcium on the subcellular distribution of K-Ras, we analyzed in live cells the effect of the calcium ionophore A23187 on the plasma membrane localization of K-Ras. The ionophore induced a significant and fast redistribution of both full-length and C-terminal domain of K-Ras fused to fluorescent proteins from plasma membrane to cytoplasm. This redistribution was inhibited when cells were incubated with Ca^{2+} chelators (BAPTA-AM, EGTA). Together, these results suggest that the dynamic nature of the interactions between K-Ras and membranes, and its modulation by intracellular Ca^{2+} might be relevant for subcellular localization and function of this protein.
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CB-P14.
SUBCELLULAR LOCALIZATION AND TRANSPORT OF P8
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P8 is an 8kDa protein induced in human pancreatitis. It is expressed in response to stress, could be involved in tumour development and act as a growth factor. It is phosphorylated *in vitro* by PKA and acetylated by p300. Immunocytochemistry showed that p8 localizes to the nucleus in subconfluent cell cultures and throughout the whole cell under superconfluence. p8 was found to have a functional nuclear localisation signal, necessary and sufficient to localise the green flourescent protein (GFP) to the nucleus. The histone deacetylases inhibitor trichostatin A provokes delocalisation, suggesting a role for acetylation. Through the Cytotrap two-hybrid system we could identify several partners of p8, among which we chose to study the small GTPase Ran as well as its binding protein RanBP1 since they are involved in nuclear transport, spindle assembly and post-mitotic nuclear envelope reassembly. Ran shifts between GDP and GTP-bound states thanks to accessory proteins, such as RanBP1. They where expressed in *E. coli* as GST fusion proteins. Pull down technique confirmed that both Ran (GDP or GTP-bound) and RanBP1 directly interact with p8. In RanQ69L, a mutant unable to hydrolyse GTP, the interaction is lost, suggesting a crucial site for complex formation.

CB-P15.
EXPRESSION OF PROTEINS THAT REGULATE INTRACELLULAR TRAFFIC IS CONTROLLED BY ANDROGENS IN RAT EPIDIDYMIS
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Mammalian epididymis provides the proper environment for sperm maturation thanks to the secretory activity of its epithelium. As occurs in other organs of male reproductive tract, epididymis is highly influenced by hormones, since androgen deprivation induces morphological and biochemical changes. The presence of high activities of acid hydrolases in epididymal lumen is still poorly understood, and these enzymes could play a role in sperm maturation. In other cell types, transport of these enzymes to lysosomes is regulated by specific receptors (MPRs) that recognize mannose-6-phosphate on the enzyme molecule. Two types of receptor have described so far, the cation-dependent (CD.MPR) and the cation-independent (CD-MPR), and little is known about the co-existence of both proteins in most of cell types. We wondered if MPRs participate in transport and secretion of acid hydrolases in rat epididymis and if they are influenced by hormonal changes. Regarding these questions, we measured expression of MPRs in epididymis of rats castrated or treated with the antiandrogenic drug Flutamide (Ft). By Western blot studies we observed that both CD- and CI-MPR expression is increased either in castrated or in Ft treated rats. However, no variations in mRNA levels were observed by RT-PCR, indicating that the effect could be post-transcriptional. We also observed an increased synthesis and secretion of cathepsin D, mostly as precursor of 54 kDa. We concluded that castration or treatment with Ft induces variations in transport and secretion of certain enzymes by changes in expression of MPRs.

CB-P16.
SUBGOLGI LOCALIZATION OF GAL-T1/SIAL-T1/SIAL-T2 MULTIENZYME COMPLEX DEPENDS ON EXPRESSION LEVELS OF SIAL-T2
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Results from this laboratory have shown that ganglioside glycosyltransferases form multienzyme complexes in the Golgi apparatus. One of these complexes is formed by GalT1 /SialT1/ SialT2, and another by GalNAcT/GalT2. Previously we communicated that in CHO-K1 cells that do not express SialT2, GalT1 and SialT1 behaved to Brefeldin-A treatment as residents of proximal Golgi compartments. However, when these cells were stably transfected with full length SialT2, GalT1 and SialT1 changed to a behavior compatible with residence in more distal Golgi compartments.We now add that these effects are reverted by knocking down the expression of SialT2 with a specific siRNA. Western blot experiments showed a 30% decrease of SialT2 expression in siRNA treated cultures. Examination of BFA treated cells under the fluorescence microscope showed that in cells receiving the siRNA, SialT2 expression was under the limit of detection and GalT1-CFP and SialT1-YFP showed increased redistribution into ER membranes in comparison with cells that were siRNA-tracer negative. Metabolic labelling from ^{14}C-galactose showed a pattern of radioactive glycolipids in siRNA treated CHO_{ST2} cells that fitted quite well the expected theoretical for a mix of 30% of CHOwt and 70% CHO_{ST2} cells. Results indicate that subGolgi localization of glycosyltransferase complexes may change according to relative levels of expression of participating enzymes and suggest a capacity of the organelle to adapt the topology of the machinery for synthesis of glycolipids in response to different cell functional states.

CB-P17.

INTERNALIZATION OF V1a VASOPRESSIN RECEPTOR THROUGH LIPID RAFTS AND CLATHRIN PATHWAYS IN MDCK II CELLS

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V1a vasopressin receptor undergoes agonist-induced internalization like others GPCRs, as an important level of regulation. In this study, flotation gradient centrifugation in absence or presence of non-ionic detergents showed the localization of V1a-R in lipid rafts of stable transfected MDCK II cells, without an apparent change after vasopressin stimulation. Confocal microscopy studies showed a high degree of co-localization of V1a-R with the specific marker of lipid rafts, cholera toxin B subunit (CTB) but absence of co-localization with caveolin-1, indicating the presence of V1a-R in lipid rafts different from caveolae. Confocal microscopy and internalization assays using [^{3}H]AVP showed that treatment with cholesterol binding agents (MβCD, Nystatin and Filipin) clearly inhibited receptor endocytocis, demonstrating a crucial participation of lipid rafts in V1a-R endocytosis. Inhibition of clathrin pathway with K^+ depletion, hypertonic sucrose, acid medium and clathrin negative dominant mutant (EH29), also showed inhibition of V1a-R internalization but in a different extents. We discuss possible models of V1a-R internalization in MDCK II cells and the importance of each pathway in the whole complex process.

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CB-P18.

RAFT-PHOSPHATIDYLINOSITOL-4,5-BISPHOSPHATE (PIP2) IS ESSENTIAL FOR TUBULAR ORGANIZATION OF RENAL PAPILLA

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Tubular organization of renal papilla depends on cell-cell and cell matrix attachment, which are principally mediated by adherens junctions (AJ) and focal adhesions (FA), respectively. Vinculin (V) is present in both type of adhesions, and talin (T) only in FA. To investigate whether PIP2 constitutes a molecular platform for FA and AJ assembly, we treated collecting duct cells with the raft-disruption agent cyclodextrin (CD), and cell adhesion structures were observed by confocal microscopy. CD caused important changes in cell morphology with loss of cell-cell adhesions and cell retraction, dissipation of V, T and PIP2 from FA accompanied by a distribution in certain zones of the plasma membrane suggesting a delocalization of these proteins from raft, where FA were located before treatment. We also treated cultured cells with the specific PIP2 sequestering agent neomycin, and with the PIP2 synthesis inhibitor LiCl. Neomycin induced an almost complete loss of V from FA and AJ, and T from FA, and intensity of fluorescence of PIP2 staining appeared to be increased. LiCl induced a dissipation of V from FA and AJ, whereas T - immunostaining FA remained intact, and PIP2 immunoreactivity was substantially reduced. Westernblot analysis correlated with the above observations These results demonstrate that the existence of PIP2-enriched raft is a requirement for maintenance of FA and AJ which ensures tubular organization of renal tissue.

CB-P19.

TNF-α PRODUCTION BY HUMAN LYMPHOCYTES IS REDUCED BY *LACTOBACILLUS REUTERI* CRL 1098: EFFECT OF LOW CELLULAR MEMBRANE CHOLESTEROL

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Cholesterol-rich microdomains, named lipid rafts, present in the membrane of eukaryotic cells, play a critical role in several biological processes such us production of cytokines. We investigated the effect of probiotic *Lactobacillus reuteri* CRL 1098 on the proinflamatory cytokine TNF-α production by human lymphocytes with low membrane cholesterol content. Human lymphocytes were isolated with a density gradient centrifugation and treated with 10 mM methyl-beta-cyclodextrin (MßCD) for 12 min at 37°C to reduce cholesterol levels. Under these experimental conditions cell viability was higher than 90%, and cholesterol was reduced by 55%. TNF-α was detected by chemisluminescent assay. The lymphocytes treated with and without MßCD were then incubated with *L. reuteri* at different MOI and times of incubation. The results show that TNF-α production of 149pg/ml/ 1.00x 10^6 by normal lymphocytes, was reduced by 23% when the cells were incubated with *L. reuteri* at 20 MOI for 4 h. When low-cholesterol lymphocytes were incubated with *L. reuteri* under the same conditions, a substantial reduction of 51% of TNF-α amount was observed. These results show for the first time that a probiotic bacteria reduced TNF-α production by human lymphocytes and suggest that lipid rafts could be involved in this cellular response.

CB-P20.

MICROFILAMENTS ARE REQUIRED FOR THE BIOGENESIS OF THE COXIELLA-REPLICATIVE COMPARTMENT

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Q fiber is a disease caused by the intracellular pathogen Coxiella burnetii. This bacterium generates a large replicative compartment in the host cell, known as parasitophorous vacuole (PV). We have investigated the role of the actin cytoeskeleton in the biogenesis of the PV by using different agents. CHO cells were infected with C. burnetii Phase II for 16 hours and actin was visualized by fluorescence microscopy using phalloidin, at different times post infection. At early times after infection some small Coxiella-containing vacuoles were decorated by actin. However by 24-48 hs post infection there was a marked increase in the size of the PV and the colocalization with actin increased to 68-95%. Treatment of infected cells with the actin polymerization inhibitor latrunculin completely blocked PV formation. Interestingly, this inhibitory effect was reversed by washing out the drug and the large PV reformed after few hours. Similarly, PV generation was hampered by the miosin inhibitor butanodionemonoxime (BDM) although in an irreversible way. These results indicate that actin and miosin motors play a critical role in the biogenesis of the C. burnetii replicative niche.

CB-P21.
ASSEMBLY OF STRESS GRANULES REQUIRES MICROFILAMENTS AND MICROTUBULES
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Stress granules (SGs) are phase-dense organelles that appear transiently in the perinuclear region of eukaryotic cells upon induction of enviromental stress (e.g. heat, oxidative agents, UV, etc). SG assembly is triggered by an abortive translational initiation that leads to the accumulation of stalled preinitiation complexes along with RNA-binding proteins (TIA-1/R, HuR, Staufen, etc). SGs are proposed to serve as a triage site that controls the fate of untranslated mRNAs. Our goal is to evaluate the participation of the cytoskeleton and molecular motors in the collapse into SGs of the translational machinery, normally dispersed throughout the cytoplasm. We tested the effect of different microtubule and microfilament-disrupting drugs on SG formation upon exposure of cultured cells to oxidative stress-inducing agents. We found that cytoskeleton-disrupting treatments cause dramatic changes on SG assembly. Disruption of the microtubule network prior to stress induction provokes the formation of SGs of normal size that fail to localize perinuclearly. Disruption of the actin network induces the accumulation of a larger number of smaller SGs that remain dispersed throughout the cytoplasm. Our results suggest a role for the cytoskeleton for the anchorage and/or for the transport of stress-granule components to the perinuclear region.

CB-P22.
MODIFICATION OF THE C-TERMINUS OF α-TUBULIN BY SITE-DIRECTED MUTAGENESIS: VISUAL ANALYSIS OF THE CYTOSKELETON OF TRANSFECTED CELLS
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The cytoskeleton of eukaryotic cells comprises an interconnected network of microtubules, microfilaments, and intermediate filaments. Microtubules are dynamic structures that play a major role in a wide range of processes, including cell morphogenesis, cell division, intracellular transport and signaling. Microtubules are formed by heterodimers of αß-tubulin. The α-tubulin gene is highly conserved in eukaryotes. Its C-terminal amino acid is tyrosine which can be removed by tubulin carboxypeptidase and reincorporated by tubulin tyrosine ligase. This post-translational reaction is called the tyrosination/detyrosination cycle, and its physiological role is still unknown. Site-directed mutagenesis was performed on commercial vector pEGFPtub (Clontech), changing the C-terminal tyrosine codifying codon by another one that codifies for arginine, glycine or aspartic acid. These mutants are not substrate for tubulin carboxypeptidase. Obtained vectors were transfected into NIH 3T3 cells to analyze, by immunofluorescence, the expression of each mutant tubulin, its capacity to assemble into microtubules, and the localization of other cytoskeleton proteins.

CB-P23.
INCORPORATION OF TYROSINE ANALOGUES INTO THE C-TERMINUS OF ?-TUBULIN
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α-Tubulin is biosynthesized with a tyrosine at its C-terminus which can be removed by tyrosine carboxypeptidase and re-incorporated by tubulin tyrosine ligase. We studied the capability of the ligase to incorporate tyrosine analogues into tubulin. Azatyrosine induces the reversion of cancerous phenotype and can be incorporated into the tubulin's C-terminus as well as into proteins via *de novo* synthesis. Which of these two mechanisms is responsible of this effect remains unclear. The introduction of a nitro group into the position 3 of the phenolic ring of tyrosine avoids its incorporation into proteins via *de novo* synthesis, but not into tubulin's C-terminus. Therefore, 3-Nitroazatyrosine was synthesized, purified by TLC and characterized by UV-Visible, IR, mass and RMN-^{1}H spectroscopy. It was found that 3-nitroazatyrosine cannot be incorporated into proteins via *de novo* synthesis nor into tubulin's C-terminus. No effect was found in cellular proliferation. We also studied if the ligase was able to incorporate other tyrosine analogues. Melphalan could not be incorporated; Thienylalanine and p-aminophenylalanine were incorporated with low affinity, and m-fluoro-tyrosine was incorporated very efficiently *in vitro*. m-F-tyrosine stopped proliferation of C6 cells and changed their morphology. An antibody against the C-terminal m-F-Tyr residue was developed.

CB-P24.
COMBINED TREATMENT OF *IN VIVO* PANCREATIC CANCER WITH OLIGOELEMENTS Se, Zn, Mn PLUS LACHESIS MUTA AND GEMCITABINE
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We have previously reported the use of oligoelements Se, Zn, Mn plus Lachesis muta (O-LM) as antitumor drug in pancreatic cancer, both *in vivo* and *in vitro*. Besides, gemcitabine (G) have been adopted in recent years in pancreatic carcinoma. In the present work we have investigated the *in vivo* use of G, O-LM and G+O-LM, in pancretic carcinoma xenografts, which were developed in nude mice, by sc inoculation of Panc-1 cells derived from a human ductal pancretic adenocarcinoma. When tumor mean diameter reached 0.6 cm, mice were treated with G, O-LM or both. Relative tumor size at 30 days in O-LM and control (C) groups was significantly greater than in G and combined groups. Haematoxylin-eosin (H.E.) stained sections were examined for histopathological changes. The combined treatment showed a significantly minor number of mitosis per field (40x) G, O-LM or C. Sections were also examined for apoptosis, proliferating cell nuclear antigen (PCNA) and vascular endothelial cell factor (VEGF) expressing cell. Apoptosis in G and combined groups was greater than O-LM and C groups and the opposite for PCNA. VEGF was expressed almost uniformly in all groups. In conclusion, G and O-LM *in vivo* combined treatment seemed to be effective in pancretic carcinoma, presumibly due to an increased apoptosis and a decreased proliferation.

CB-P25.

ANTIPROLIFERATIVE ACTIVITY OF HOP EXTRACTS IN TUMOR CELLS

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Previous reports demonstrated multiple biological effects of humulones or α-acids from hop. The aim of this study was to evaluate the antiproliferative and antioxidant activity of hop extracts in human tumor cell lines (MCF-7, T47D, PAJU). Hop extracts used herein are pure, standardized solutions of iso-α-acids: hexahop gold (HH), tetrahop gold (TH) and isohop (IH) (Haas Hop Products, Inc.). Cell cultures were treated with the different extracts (0-100 μg/ml) for 24 h and dose-response curves were obtained. Proliferative activity was evaluated by MTT assay, apoptosis by staining with Hoescht33258 and antioxidant capacity by determining the oxidation of 2',7' dichlorofluorescin (DCFH). Results demonstrated a dose-dependent inhibition of cell growth in all the cell lines treated with HH ($p<0.001$) and TH ($p<0.001$). This inhibition was significant as from 25 μg/ml in MCF-7 and PAJU cells and as from 100 μg/ml in T47D. Conversely, no significant growth inhibition was induced by IH in these cell lines. Cells treated with HH, TH or IH did not show significant induction of apoptosis. Regarding DCFH oxidation a dose-dependent decrease was observed in all the cell lines treated with TH and HH. In conclusion, TH and HH induced cell growth inhibition, unrelated to induction of apoptosis and possibly associated to the antioxidant capacity of these extracts.

CB-P26.

MOLECULAR MECHANISM OF HUMAN LEUKEMIA JURKAT T-CELL APOPTOSIS INDUCED BY PLANT TRYPSIN INHIBITORS

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Plants constitute an important source of compounds which can induce apoptosis in a variety of cells. Previously, we reported the isolation of a trypsin inhibitor from *Peltophorum dubium* seeds (PDTI). This inhibitor, as well as soybean trypsin inhibitor (SBTI), has lectin-like properties and triggers rat lymphoma cell apoptosis. In the present study, we demonstrate that PDTI and SBTI induce human leukemia Jurkat cell death. Induction of apoptosis was confirmed by flow citometry after propidium iodide labeling, showing a significant increase of the sub G_0/G_1 fraction. To understand the mechanism of apoptosis, we evaluated caspases involvement and showed caspases-3 and -8 activation by PDTI or SBTI treatment. Consistent with these results, pan caspase inhibitor and caspase-8 inhibitor protected Jurkat cells from apoptosis. However, there was no caspase-9 activation, confirmed by the failure of caspase-9 inhibitor to prevent cell death. We detected a moderate decrease of mitochondrial membrane potential but no significant release of cytochrome c from mitochondria. These results suggest that the intrinsic mitochondrial pathway is not predominant in the apoptotic process. On the other hand, we observed the recruitment of Fas-associated death domain (FADD) to the cell membrane indicating the involvement of this adaptor protein in PDTI- and SBTI-induced apoptosis in Jurkat cells.

CB-P27.

ACTION OF TAMOXIFEN ON THE UTERUS OF RATS BEARING EXPERIMENTAL MAMMARY TUMORS

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Extended tamoxifen (Tam) administration in breast cancer therapy leads to an increased risk for endometrial neoplasia. The objective of this work was to analyze the effect of Tam on the uterus of rats bearing N-nitrose-N-methylurea induced mammary tumors: A) Tam (1 mg/kg.day, sc) was administered for 120 days beginning when rats were 40 days old. B) Tam was administered for 30 days. Control groups not receiving Tam treatment or NMU injection were included. Results disclosed in the investigated uteri were: in A) highly significant decrease of uterine weight and absence of steroid receptors expression. Histological studies showed very thin, nearly atrophic endometrial mucosae and also atrophic smooth muscle wall. In B) significant diminution of estrogen receptor expression, increase of progesterone receptor and cystic glandular endometrial hyperplasia. Data were correlated with the expression of: PCNA, c-fos, bax, bcl-2 and insulin growth factor type-I. In A and B, rat estral cycle was arrested at diestrum (*o.m.* analysis of vaginal smears). Nuclear anisocaryosis and hyperchromasia was observed in the endometrium of NMU-injected rats regardless of Tam treatment. Results show the wide range of biological interrelations established at the end of treatments.

CB-P28.

THE INSULIN GROWTH FACTOR SYSTEM ON MAMMARY TUMORIGENESIS

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Insulin growth factors (IGFs) are important mediators in the growth, development, differentiation and survival of normal and transformed mammary tissue. The aim of this study was to investigate the expression and localization of the insulin growth factor-type 1 receptor (IGF-IR) and insulin receptor (IR) during the mammary carcinogenesis induced in rats by N-Nitroso-N-methylurea (NMU). NMU was injected to 50, 80 and 110 days old rats. Samples of mammary tissue were collected at days 55, 85 and 115, immunohistochemical analysis and Western blot were performed. The antibodies used were a polyclonal rabbit Insulin-Rα and IGF-IRα. Expression of both receptors was increased in epithelial and mioepithelial cells of mammary gland ducts throughout the observation period. At day 115, a significantly higher expression of both receptors was found when mammary tissue of NMU injected rats was compared to tissue of normal rats. Our results suggest that both IGF-IR and IR, components of the IGF system, have an important role in the promotion/progression phase in the experimental model tested. At present the expression of both receptors is being determined on mammary glands of NMU injected rats under tamoxifen treatment.

CB-P29.
ANALYSIS OF THE REGULATORY MECHANISMS THAT CONTROL DLG TUMOUR SUPPRESSOR EXPRESSION
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High risk HPVs play a causal role in the development of cervical cancer. h-Dlg (Dlg) oncosuppressor is associated with cell polarity and tissue architecture, and HPV E6 oncoproteins target Dlg for ubiquitin-mediated proteolysis. We showed that the expression of Dlg is altered during the progression of high-risk HPV-related cervical lesions and that Dlg levels are highly reduced in invasive carcinoma. E6 phosphorylation by protein kinase A (PKA) reduces E6 capacity for binding and degrading Dlg. We analyzed PKA activity by immunohistochemistry and demonstrated variations in PKA activity during the development of cervical tumours that could be altering E6 phosphorylation status and hence, Dlg levels. However, the mechanisms controlling Dlg expression at the transcriptional level are still unknown. In order to understand the basic Dlg transcriptional regulation we proposed the cloning of Dlg promoter region and its functional analysis. By bioinformatic analysis, a potential Dlg promoter region was identified on the 5´flanking region of Dlg ORF, contained into a P1-derived artificial chromosome. Fragments of this region were amplified, cloned into a reporter vector and the promoter activity was determined in cultured cells by luciferase assays. Using bioinformatic tools, binding sites for different transcription factors were found in this region. The involvement of two tumour-related factors in Dlg regulation is currently being analysed.

CB-P30.
MELANOMA GROWTH AND PROGRESSION IS ALTERED BY THE OVEREXPRESSION OF VERSICAN V3, AN EXTRACELLULAR MATRIX COMPONENT
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Versican is a large chondroitin sulfate proteoglycan produced by several tumor cell types, including malignant melanoma. Retroviral overexpression of the V3 isoform of versican in human melanoma cell lines markedly reduces cell growth. The V3-overexpressing melanoma cells retain their diminished growth potential *in vivo*, since primary tumors arising from these cell lines growth more slowly than their vector-only counterparts. This effect was accompanied by increases in cell adhesion on hyaluronan and an enhanced ability to migrate on hyaluronan-coated transwell chambers. This enhanced migration is blocked when cells are preincubated with soluble hyaluronan, or anti-CD44 antibodies, suggesting that V3 acts by altering the hyaluronan-CD44 interaction. Conversely, migration on plastic and collagen type I is reduced in V3-overexpressing cells. Cell communication through gap-junctions is also increased in the transduced cells. Primary V3 tumors show a lower mitotic index and Ki67 staining as well as an increase in the apoptotic ability visualized by a TUNEL assay. The expression of other tumor components as versican, CD44, CD31, or integrins αv and $\beta 1$ is not modified, whereas an increase in the hyaluronan content of V3-tumors can be observed. Our results show that V3 overproduction modulates the *in vitro* behavior of human melanoma cell lines and reduces their tumorigenicity *in vivo*.

CB-P31.
SMALL EXTRACELLULAR CHONDROITIN- DERMATAN SULFATE PROTEOGLYCANS EXPRESSION IN ENDOTHELIAL CELL LINES
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Small chondroitin-dermatan sulfate proteoglycans (SPRG) biglycan and decorin are characteristically present in vascular endothelial cell (EC) basement membranes and may affect several stages in the cell adhesion process. We have examined the expression of SPRGs in different vascular ECs lines. EC lines H5V, RECA and 1G11 were grown to confluence. Cell number and viability determinations were performed. Partial purification of SRPGs was carried out by ion exchange chromatography by applying equal aliquots of the ECs culture supernatant to a DEAE-Sephadex column. After a linear gradient, the eluted fractions were assays by the metachromasy with 1,9-dimethyl methylene blue. Recovered materials, digested and undigested with 0.02 unit of chondroitin ABC lyase, were applied to 8% SDS-polyacrilamide gel for analytical identification. Chromatography of H5V and 1G11 supernatants showed two peak corresponding to 0.35 M and 1 M NaCl. Densitometric profiles showed that relative amounts in the second peak was two times greater in 1G11 supernatant than H5V. No peaks were detected for RECA culture. A sharp band visible at 45 kDa was detected in both culture supernatants. This band became three times more intense after enzymatic degradation, and therefore may be considered a core protein of chondroitin-dermatan sulfate PRGs. Consequently, this methodology is useful to highlight different profiles of SPRGs in ECs and the role in migration.

CB-P32.
EXPRESSION OF *GD3 Synthase* AND *paraxis* TRANSCRIPTION FACTOR IN MESODERMAL LAYER FORMATION DURING *Xenopus laevis* EMBRYOGENESIS
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Glycosphingolipids (GSL) are believed to be integral for the dynamics of many cell membrane events, including cellular interactions, signaling and trafficking. In previous studies we investigated the ganglioside profile, cellular expression, and biosynthesis during the early development of the *Xenopus laevis* amphibian embryo. In this work we report the molecular cloning of α2,8-sialyltrasnsferase (GD3 synthase, GD3s), a key enzyme that catalizes the first step of b-series gangliosides synthesis. We have isolated a 2.88 Kb length transcript for GD3s. Expression of this enzyme was analyzed during early development of *Xenopus* embryos by RT-PCR and in situ hybridization. We detected transcripts of GD3s from stage 10 onward. The spatio-temporal analysis of expression patterns of enzyme and specific markers by in situ hybridization reveals that it is developmentally regulated. GD3 is mainly expressed in dorsal blastopore lip at gastrula stages and in the migrating mesodermal cells. In later stages of development it is expressed in the presomitic mesoderm together with the *paraxis* transcription factor. Experiments using a resin microsphere embedded with PPMP (phenyl palmitoyl morpholino propanol), a specific inhibitor of glycosphingolipid byosinthesis, was implanted in the right side of stage 10 embryos. The inhibitor produced embryos with abnormal expression of mesodermal markers, such as *goosecoid, chordin*. Knock-down experiments using expression of a dominant negative form *paraxis* revealed severe defects in somite formation. Rescue experiments confirmed the specificity of this experiment. We are currently investigating a possible role of *paraxis* transcription factor in mesodermal formation. Together, our results strongly argue for a role of gangliosides in the formation presomitic area and raised the questions if *paraxis* is implicated.

CB-P33.
THE TRANSLATION REPRESSOR SMAUG FORMS STRESS GRANULE-LIKE FOCI
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Cytoplasmic events depending on RNA binding proteins contribute to the fine-tuning of gene expression. Sterile Alpha Motif (SAM)-containing RNA binding proteins constitute a novel family of post-transcriptional regulators that recognize a specific RNA sequence motif known as Smaug Recognition Element (SRE). The Drosophila member of this family, dSmaug, triggers the translational repression and deadenylation of maternal mRNAs by independent mechanisms, and the yeast homologue Vts1 stimulates degradation of SRE-containing messengers. Two homologous genes are present in the mammalian genome. We found that hSmaug 1 represses the translation of reporter transcripts carrying SRE motifs. When expressed in fibroblasts, hSmaug 1 forms cytoplasmic granules that contain polyadenylated mRNA and stress-granule markers. The murine protein mSmaug 1 is expressed in the central nervous system and is abundant in post-synaptic densities, a subcellular region where translation is tightly regulated by synaptic stimulation. Biochemical analysis indicated that mSmaug 1 is present in synaptoneurosomal 20S particles. These results suggest a role for mammalian Smaug 1 in RNA granule formation and translation regulation in neurons.
Supported by NIH and Wadsworth Foundation (USA), and ANPCyT (Argentina).

CB-P34.
TSH-INDUCED THYROPEROXIDASE (TPO) GENE EXPRESSION IS INHIBITED BY NITRIC OXIDE AT TRANSCRIPTIONAL LEVEL BY INVOLVING TTF-2 THYROID TRANSCRIPTION FACTOR IN FRTL-5 CELLS
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Thyroid-specific gene expression is mainly regulated by thyrotropin (TSH). The cell signaling molecule nitric oxide (NO) is involved in diverse biological processes. Our previous reports indicated that the NO donor, sodium nitroprusside (SNP), inhibited TSH-induced iodide uptake and TPO and thyroglobulin mRNA expression in the rat thyroid cell line FRTL-5. This study aimed to analyse the mechanism involved in NO-induced reduction of TPO gene expression in TSH (0.5mIU/mL) stimulated FRTL-5 cells. In transcription assays SNP (200-500μM,12h) decreased transcriptional activity of the minimal TPO promoter linked to luciferasa reporter gene (p420TPOLuc). It is known that TTF-2 is crucial for TPO gene regulation. The functional activity of 12ZLuc, a construct with 12 tandem repeats of TTF-2 binding site, was decreased by SNP (12h). Preliminary data indicated a reduction of TTF-2 mRNA level (Northern Blot / RT-PCR) in SNP treated cells (100-500μM,24h). In conclusion, this study revealed for the first time the ability of NO to reduce TPO gene expression at transcriptional level possibly by involving TTF-2. Since TPO is essential for thyroid hormone biosynthesis, these findings could be of pathophysiological interest.

CB-P35.
CHARACTERIZATION OF THE VERSICAN PROMOTER IN HUMAN MELANOMA CELL LINES
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Versican is an extracellular matrix proteoglycan expressed in many tumors. In human melanoma, we have previously described that a correlation exists between versican expression and the progression of the tumor: there is no expression in benign nevi, the expression in dysplastic nevi is proportional to the degree of atypia and it is highly expressed in malignant and metastatic melanoma. *In vitro*, there is an inverse correlation between versican expression and cell differentiation degree, i.e., differentiated melanoma cell lines do not express versican, whereas undifferentiated cells do express this proteoglycan. We have cloned the versican promoter and analyzed its activity in human melanoma cell lines producing or not this proteoglycan. We have analyzed the versican promoter *in silico* to determine the transcription factors able to bind to specific sites in the promoter. Deletion constructs have been designed to analyze the role of these transcription factors in promoter activity. Electrophoretic mobility shift assays and supershifts have shown that TCF4, Sp1 and AP2 are involved in the regulation of the versican promoter activity in human melanoma cell lines.

CB-P36.
A PUTATIVE ROLE FOR THE DOUBLE-STRANDED RNA-BINDING PROTEIN *STAUFEN 1* IN STRESS GRANULES´ PHYSIOLOGY
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Eukaryotic cells respond to stress by dramatically altering mRNA metabolism in phenomena such as localization, translational state and rate of decay. The most studied event involved in these changes is the assembly of stress granules (SG). We have shown previously that under oxidative stress, mammalian Staufen 1 undergoes aggregation in SG (Thomas *et al*., MBC 2005). The colocalization of Staufen 1 with SG markers was also demonstrated in many different stress conditions and cell types, and thus the possibility for an active role in the stress response was suggested. In this work we show that cell lines overexpressing Staufen 1 fail to assemble SG, in response to different stressors. However, phosphorilation of eIF2-α -a required event in the stress response cascade, and in SG formation- is normal in Staufen 1 overexpressing cells, suggesting a downstream action of Staufen. We used several constructs bearing deletions, isolated double-stranded RNA-binding domains (dsRBDs) and oligomeric domains, to identify the protein region(s) responsible for SG inhibition. This study presents the first physiological evidence of a role for Staufen 1 in the regulation of SG assembly.
Supported by NIH and Wadsworth Foundation (USA), and ANPCyT (Argentina).

CB-P37.
RNA GRANULES, STRESS GRANULES AND PROCESSING BODIES

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Cytosolic mRNA granules are the functional units for mRNA transport, silencing, activation and degradation. Staufen, FMRP, SMN and other RNA-binding proteins are conserved markers of distinct RNA granules. When exposed to stress conditions, eukaryotic cells built up larger RNA-protein aggregates, known as stress granules (SGs). SGs are transient structures that contain housekeeping mRNAs which are not translated during the stress response. We found that SMN, FMRP and Staufen RNA granules are plastic, respond to the cellular translational state and can be converted into SGs upon different stressors. SGs formation involves the remodeling of normal RNA granules as SGs include Poly(A)-binding protein (PABP), the RNA-binding proteins HuR and TIAR/TIA-1, and small but not large ribosomal subunits. We also analyzed the relationship between SGs and Processing Bodies (PBs), cytosolic structures involved in mRNA decay. We found that the PBs-components exoribonuclease Xrn1 and GW182 are recruited into SGs whereas the decapping enzyme Dcp1 is not. Upf1, a component of the SMD and NMD pathways, is also present in SGs. Our results suggest an equilibrium between RNA granules, polysomes, SGs and PBs that is modulated to help post-transcriptional regulation of the genome.
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CB-P38.
CO-EXPRESSION AND LOCALIZATION OF 6-PHOSPHOFRUCTO -2- KINASE / FRUCTOSE - 2,6 - BISPHOSPHATASE AND FBPase IN VARIOUS CELL LINES

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The bi-functional enzyme, 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK/ FBPase2) catalyzes the synthesis and degradation of fructose-2,6-bisphosphate. This metabolite is the principal inhibitor of the gluconeogenic enzyme fructose-1,6-bisphosphatase (FBPase). We have shown that the FBPase subcellular localization is modulated by metabolic conditions. But, there is no information about the modulation of PFK-FBPase2 localization by the metabolic state. Thus, we proposed to study the expression and subcellular localization of PFK-FBPase2 in diverse cell lines under different metabolic conditions. We determined by PCR and Western blot that PFK-FBPase2 is expressed in FTO2B, HEPG2, EBNA, HELA and LLCPK1. Additionally, we cloned this enzyme and fused it to green fluorescent protein to determine it subcellular distribution. The results show that PFK-FBPase2 and FBPase both localized in cytoplasm and nucleus depending on the cellular metabolic conditions. Further, it was possible to establish that in the absence of glucose both enzymes co-localize in the cytoplasm of the liver and kidney cell lines.
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CB-P39.
METABOLIC AND HORMONAL CONDITIONS MODULATE THE SUBCELLULAR LOCALIZATION OF HEPATIC FBPase IN LIVER OF NORMAL AND DIABETIC RATS

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Recently, we demonstrated in isolated rat hepatocytes that fructose-1,6-bisphosphatase (FBPase) translocates from the cytoplasm to the nucleus in response to glucose and insulin. However, it is unknown if this dynamic subcellular location occurs in hepatocytes located in different zones of the liver. Thus, by immunohistochemistry we studied the influence of the metabolic state of the rat over FBPase subcellular distribution in liver. Our results show that FBPase was localized in periportal hepatocytes of fasted and re-fed rats. After re-feeding of starved rats, FBPase translocates from a mainly cytosolic localization to the nucleus. Moreover, the enzyme also concentrates in cell periphery compartments. As in isolated hepatocytes, these results suggest that hyperglycemia and hyperinsulinemia are the stimuli inducing FBPase translocation to the nucleus in fed rats. However, analyzing the FBPase subcellular distribution in liver of diabetic rats, with hyperglycemia and hypoinsulinemia, we found that FBPase has an exclusive cytosolic localization. These data allow us to postulate that insulin is the main physiological factor involved in the signaling of FBPase nuclear import *in vivo*. The present study reveals a further sophistication of the regulatory mechanisms that control the gluconeogenic pathway.
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CB-P40.
GLUCOCORTICOIDS AND TOAD TESTIS

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Several aspects are involved in the sensitivity of testicular cells to glucocorticoid (GC) action. Plasma GC levels and characteristics of binding globulins (CBG) determine the amount of GC that enters the tissue. But, the number and affinity of GC receptors (GR) and 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) are also important. To study that interaction in the testes of *Bufo arenarum*, 11β-HSD activity, plasma corticosterone (B) concentration, CBG properties, GR characteristics and the role of 5α-reductase (5αRed) were studied during the breeding. 11βHSD and 5αRed were assayed using ^{3}H-B in microsomes. Plasma B and testosterone (T) were determined by RIA. GR was assayed in cytosol with ^{3}H-Dexametasone (Dex) while CBG binding properties were studied using B and T. The effect of GC on T production was assayed by culturing testes with different concentrations of DEX and 5α-dehydroB (5α-DHB). Our results indicated that the activity of 11β-HSD converts B into 11-dehydroB (11-DHB) that binds to GR with much less affinity than B. However, 5α-Red transforms B into 5α-DHB that binds GR with similar affinity than B. CBG binds B and T thus level of both steroids affects the availability of each other. Since during the breeding plasma B concentration increases and T level decreases, the magnitude of the increase in free B would be lesser than expected. Dex treatment decreases testosterone by inhibiting the activity of $CypP450_{c17}$. However, 5α-DHB showed to be more potent than Dex suggesting that 5α-Red could be an activating mechanism. In conclusion, the effect of GC on testicular function is the result of several interacting factors.

CB-P41.
MELATONIN INHIBITS THE NUCLEAR TRANSLOCATION OF THE GLUCOCORTICOID RECEPTOR (GR): AN ANSWER TO ITS ANTI-APOPTOTIC EFFECT ON MOUSE THYMOCYTES

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In previous works, our group have demonstrated that the antagonistic effect of melatonin on the glucocorticoid-mediated apoptosis on mouse thymocytes involves the prevention of cytochrome C release and a decrease *in bax* expression. Despite that several reports have shown the antagonism melatonin/ glucocorticoid, little is known about the molecular mechanisms involved on this process. In the present work we analysed the ability of melatonin to modulate GR activation. We found, by performing competence assays that melatonin does not affect the binding of dexamethasone to GR. However, according to immunofluorescence confocal microscopy and subcellular fractionation followed by western-blot assays, melatonin prevents the dexamethasone-mediated GR nuclear translocation. Moreover, we also observed, by co-immunoprecipitation experiments, that the methoxyindole completely blocks the GR/Hsp90 disssociation. Theses effects seem to be tissue-specific as it does not occur on another cell types like HC11 and Cos-7 cell lines. Taken together the results suggest that melatonin is working as a modifier that influences the transformation of GR upon hormone induction, in a tissue specific manner.

CB-P42.
CONFORMATIONAL VARIATIONS FOR HUMAN ESTROGEN RECEPTOR ALPHA SYTHESIZED IN DIFFERENT CELLULAR EXTRACTS

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Conformation of estrogen receptor alpha (ERα) has been shown to modulate its own biological activity. Also, some of its functional properties are tissue-specific. To explore weather the cellular context of synthesis could favor alternative conformations of ERα we focused on the translation process in two systems, rabbit reticulocyte lysate (RRL) or wheat germ extract (WGE), and compared the accessibility of chymotrypsin to the newly synthesized product. The receptor synthesized in WGE was proteolyzed much more slowly compared to that synthesized in RRL. We demonstrate that this is not due to a protease inhibiting activity in the WGE. To analyze further conformation differences, functional properties of ERα were studied. The incubation of ERα synthesized in RRL with the hormone analog DES protected a degradation intermediary from further cleavage, whereas no significant changes were observed with WGE. Determination of affinity constant for estradiol binding showed significant differences for the ERα synthesized in each system. Our data suggest that the translation process in different cellular contexts could affect the folding pathway of the receptor leading to conformational variants with different functional properties.
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CB-P43.
LOCALIZATION OF ALDOLASE B IN LIVER AND PANCREAS OF DIABETIC AND NORMAL RATS

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Endogenous glucose production takes place mainly in liver and kidney tissues from non-glucidic intermediates such as glycerol, amino acids and lactate. Fructose-1,6-bisphosphate aldolase (aldolase) catalyzes the conversion between fructose-1,6-bisphosphate and glyceraldehide-3-phosphate/ dihydroxyacetone phosphate. Previously, we have showed the participation of muscle and hepatic isoenzymes of aldolase in glycolysis and gluconeogenesis, respectively. Aldolase B localizes specifically in rat kidney proximal tubules, while aldolase A localizes in rat kidney distal tubules. Aldolase B shows a compartmentalized distribution in rat liver: periportal hepatocytes show higher expression levels, with diminishing values toward perivenous hepatocytes. Here, we show that the aldolase B subcellular localization changes depending on the rat metabolic and hormonal conditions. In re-fed rat liver, aldolase B showed a nuclear localization, while in fasted rats was mainly cytosolic. In streptozotocin-induced diabetic rat liver, this isoenzyme was found preferentially in the cytosol. Finally, aldolase B was detected essentially in islets in rat pancreas, and its expression was diminished in diabetic rat pancreas. In conclusion, our findings demonstrate that aldolase B localization in rat is modulated by the nutritional and hormonal state.
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CB-P44.
BMP /SMAD SIGNALS IN THE INTESTINAL TRACT DURING DIABETES

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Bone morphogenetic proteins (BMPs) are pleiotropic secreted proteins, structurally related to transforming growth factor beta and activins. BMPs act early in the gut morphogenesis, also regulate specification and differentiation in the developing enteric nervous system (ENS) and have recently been considered to play a pivotal role in limiting the number of enteric neurons and promote the development of a particular subset. Gastrointestinal disorders are common complications in diabetic state. Under our experimental conditions we observed an increase in apoptosis and a decrease in proliferation within the muscular layer particularly at myenteric ganglia level. This results are in correlation with a significant decrease in the expression of Vasoactive intestinal polypeptide mRNA, an inhibitory transmitter secreted by a subpopulation neurons. These facts may contribute in part to the abnormal gut motility observed in diabetes. In this study, we have investigated the possible involvement of BMPs in the gut of diabetic rodents. We show that BMP4 expression is active in the sumbmucosae and in the ENS of normal gastrointestinal tract but is more widespread in diabetic gut. Furthermore, the mRNA profiles for BMP ligands, receptors and cytoplasmic mediators were significantly altered during diabetic animals. We observed an induction of BMPRII, BMPRI and Smad3 and also a downregulation of BMP 2. Collectively, our data demonstrate for the first time active BMP/Smad signaling in diabetic gut and thus raise the possibility that BMPs could play a determining role in intestinal pathophysiology during diabetes. We are currently investigating what BMP ligand is involved in the activation of this pathway.

CB-P45.

INFLUENCE OF DIETARY METHIONINE ON THE UBIQUITINATION OF MOUSE LIVER CYTOSOLIC PROTEINS

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Protein content decreases 50% in the liver of mice fed with a protein free diet. The ubiquitin-dependent system is responsible for the degradation of short-lived and abnormal proteins in eukaryotic cells. This work was designed to examine the effect of diets on the ubiquitination of cytosolic proteins *in vivo* and in isolated hepatocytes. Balb-c female mice were fed during 5 days with normal (23% of casein), protein free, and protein free containing Met, respectively. The isolated hepatocytes were incubated for 4h with a complete medium, minimal medium, and minimal medium supplemented with Met. The ubiquitin conjugates were analysed by Western blot with polyclonal antibodies. The results indicated that: a) the total protein content of undernourished hepatocytes decreases as observed *in vivo*; b) the main size range of ubiquitinated proteins *in vivo* and *in vitro* was 89000-18000 and 73000-34000, respectively; c) the level of some ubiquitinated proteins was modified by diets (while some increased others decreased); e) supplementation with Met caused a pattern of ubiquitinated proteins similar to that of controls. Then dietary Met can increase in both systems the stability of some proteins.

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CB-P46.

ANTI-APOPTOTIC EFFECTS OF CALBINDIN D_{28K} ON GERMINAL CELLS OF SEMINIFEROUS TUBULES FROM HYBRID MOUSE

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Hybrid male mice (2n=32), derived from crosses between CD1 female mouse (2n=40) and Milan II male mouse (2n=24), have variations of the standard diploid number of chromosomes due to Robertsonian fusions. This affects the germinal cell survival, and these mice show sub-fertility. The purpose of this work was to evaluate the calbindin D_{28k} (CB) capacity to protect hybrid mouse germinal cells from apoptosis. Testis from 3 and 5 months old (m.o.) CD1, Milan II and hybrids male mice were dissected and fixed. CB expression was determined on transverse sections of the seminiferous tubules, employing immunoperoxidase technique with policlonal antibody against CB. Apoptosis was studied via DNA fragmentation by the TUNEL assay. The meiotic germinal cells from the stage XII presented positive TUNEL staining in the hybrid mice, specially at 5 m.o. In CD1 and Milan II mice, a few cells revealed apoptosis signal. CB expression appeared in a high number of germinal cells in the stage XII from 3 m.o. hybrid mice, but it was absent in cells of the same stage from CD1 and Milan II mice. At 5 m.o., CB expression was much higher in the hybrid mice. Only scarce cells showed double staining of CB and TUNEL at both times. The results suggest that CB over-expresses in the meiotic germinal cell from hybrid mice, probably to buffer intracellular calcium increase and to protect cells from apoptosis.

CB-P47.

MOLECULAR CHARACTERIZATION OF A *BUFO ARENARUM* OVIDUCTAL PROTEASE

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In amphibians, the envelope surrounding the egg exhibits differences according to the functional state of the female gamete. The eggs obtained from the coelomic cavity are not fertilized, but they are after their passage through the Pars Recta (PR) portion of oviduct. This acquisition of egg fertilizability is due to an oviduct-induced alteration of egg envelope. The biochemical and ultrastructural vitelline envelope modifications are caused by the action of a trypsin-like protease named oviductin.

The aim of this work was to clone the oviductin complete cDNA and to analyze their functional domains. Total RNA was isolated from oviductal PR of hormonally stimulated animal. Sets of primers were designed based on homology sequences. We first amplified, cloned and sequenced an internal 530 pb partial cDNA. The 5' cDNA end was amplified using a new group of specific designed primers. To complete the mRNA sequence, a 3' rapid amplification of cDNA ends (3' RACE) was performed. The overlapping sequence showed a 3,203 bp-long oviductin cDNA with one open reading frame coding for a 980 aminoacids protein. The molecular structure comprise two protease domains (α and β) and three CUB domains. The α domain has three important aminoacids for catalytic activity (His, Asn, Ser), while in the β domain a His residue was replaced by Asp. Thus, this domain is not likely to be proteolytically active. These results would indicate that the *Bufo arenarum* oviductin α domain produce the partial hydrolysis of the envelope glycoproteins. At this moment, the exact function of the CUB domains and β domain is still unknown.

CB-P48.

OXIDATIVE STRESS IN INTESTINAL MITOCHONDRIA UPON MENADIONE TREATMENT

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A single large dose of menadione (MEN) depletes cytosolic glutathione (GSH), resulting in inhibition of intestinal Ca^{2+} absorption. The aim of this work was to elucidate whether the redox molecular systems from intestinal mitochondria are also altered by a high MEN dose. Four week old chicks were divided in two groups 1) control, 2) treated i.p. with 2.5 μmol of MEN/kg of b.w. 30 or 60 min before sacrifice. In mitochondria, GSH content was determined by enzymatic method and the activities of antioxidant enzymes and oxidoreductases were measured by spectrophotometric procedures. DNA fragmentation was studied by the TUNEL assay in intestinal slices. Data revealed that 30 min after MEN injection, GSH content was decreased, malate dehydrogenase (MDH) activity decreased and superoxide dismutase (SOD) and GSH peroxidase (GPX) activities increased. DNA fragmentation was also increased by MEN treatment. GSH content returned to control values 1 hour after dosing, but not MDH and α-ketoglutarate deshydrogenase (α-KGDH) activities. SOD and GPX remained increased 1 hour after dosing, while catalase (CAT) activity was not modified by the treatment. Conclusion: a large dose of MEN produces oxidative stress in intestinal mitochondria by GSH depletion, which inhibits Krebs cycle oxidoreductase activities. The antioxidant enzymes increase their activities as a compensatory mechanism. Nuclear DNA fragmentation increased by MEN indicates that enterocytes are no able to overcome the oxidative stress.

CB-P49.
CENTRAL ROLE OF VITAMIN C IN ANTIOXIDANT DEFENSE IN HUMAN ENDOTHELIAL CELLS

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Although the role of glutathione and vitamin C in antioxidant defense has been addressed in numerous studies, we know very little on how they interact with each other. This is important because all cells are capable of *the novo* synthesis of glutathione while vitamin C is obtained from extracellular sources. We analyzed the role of the pair vitamin C-glutathione in the antioxidant defense mechanisms of human endothelial cells, using primary cultures of human umbilical endothelial cells (HUVEC) and human tonsil endothelial cells (HTEC). Our data indicate a two-way regulation of the antioxidant capacity of the endothelial cells. Vitamin C affected the steady-state glutathione content and played a central role in the fate of glutathione and the capacity of the endothelial cells to survive under oxidative stress. On the other hand, although glutathione affected the capacity of the endothelial cells to accumulate vitamin C, it appears to play a secondary role in the defense against acute oxidative stress. We conclude that vitamin C, and not glutathione, is central to the capacity of endothelial cells to survive in the presence of moderate level of oxidants such as those expected *in vivo*.

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CB-P50.
ASTROCYTES FATE: OXIDATIVE STRESS VERSUS HO1/ MAPK

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Mn is an essential trace element, required for many ubiquitous enzymatic reactions. Mn toxicity is known to occur in certain occupational settings through inhalation of Mn-containing dust. Accumulation of Mn in brain can cause a neurodegenerative disorder with similar features to those described as Parkinson-like symptoms. Previously, we demonstrated that $MnCl_2$ treated-astrocytes suffered apoptotic death involving caspases 3/6 activation. The aim of the present work was to investigate MAPK mediated modulation of HO1 cytoprotection in Mn^{2+}-induced apoptosis in rat astrocytes. Cellular viability decreased (36%, $p<0.001$, MTT assay) by treatment with $MnCl_2$ 1mM during 24hs. We detected ROS generation employing DCFH-DA and fluorescence microscopy. However, pre-incubation with different antioxidants such as MLT (0,1mM), GSH (0,5mM) and NAC (10
mM) were unable to prevent cellular viability decrease. Furthermore, HO1 overexpression (70%, $p<0.01$) was detected by western blot which correlated with a decreased in ERK½ expression (64%, $p<0.05$; 75%, $p<0.01$). We also found that p38 is involved in HO1 modulation. Our results suggest that HO1 and MAPK signalling pathways play an important role in Mn^{2+}-induced apoptosis in astrocytes.

CB-P51.
PROCYANIDINS PROTEC CACO-2 CELLS FROM BILE SALTS- AND OXIDANT-INDUCED DAMAGE

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We investigated the capacity of an hexameric procyanidin (Hex) isolated from cocoa to protect Caco-2 cells, as well established model of intestinal epithelium, from: a- bile salt-induced cytotoxicity and b- from increased cell oxidants and loss of barrier integrity induced by a lipophilic free radical generator (2,2'-azobis (2,4-dimethylvaleronitrile) (AMVN). Hex interacted with the cell membrane as evidenced by Hex-mediated increase of the transepithelial electrical resistance. Hex acted inhibiting bile-induced cytotoxicity. The release of LDH and GOT to the media was inhibited by Hex in a dose (2.5-20 μM)-dependent manner. Hex (1.25-10 μM) inhibited AMVN-induced increase in cell oxidants. AMVN promoted a loss of integrity of polarized Caco-2 monolayers (measured as the apical to basolateral transport of fluorescein sulfonic acid) that was completely prevented by Hex. Results show that Hex can interact with cell membranes and protect Caco-2 cells from aggressors such us bile salts and oxidants. Therefore, high molecular weight procyanidins, that are poorly absorbed in the intestinal lumen, could exert cytoprotective, antiflammatory and anticarcinogenic actions at the oral cavity and gastrointestinal tract, providing beneficial health effects in pathologies such us inflammatory diseases, alterations in the intestinal barrier permeability and cancer.

CB-P52.
P19INK4D OVEREXPRESSION ENHANCE DNA REPAIR EFFICIENCY AND INCREASE RESISTANCE TO UV DAMAGE IN MA-10 LEYDIG CELLS

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We have previously reported that p19INK4d, a member of INK4 cell cycle inhibitors, is also involved in DNA repair, as it´s induced by UV light and its overexpression remarkably improves repair of a foreign damaged DNA in MA-10 Leydig cells. Going further, this work aims to study on one hand if p19 modifies the ability of endogenous DNA repair in MA-10 Leydig stable cell lines that overexpress or silence p19 expression, and on the other hand, to evaluate in this cell lines the ability to recover from UV damage. MA-10 Leydig stable cell lines were established containing p19 sense or antisense gene under a metallothionein promoter inducible by Zn^{2+}. The clones obtained were assayed by Northern blot for p19 expression and tested for DNA repair ability by UDS and comet assay. We found out that overexpression of p19 strongly enhances DNA repair efficiency and besides enlarge the number of cells going through DNA repair process. Adding to that, MTT, clonogenic and chromosomic aberrations assays showed that p19 also greatly raises the faculty of the cells to get over UV damage and continue growing. To conclude, not only does p19 increase DNA repair efficiency but enables the cells to resist better to DNA damage as well.

CB-P53.
IRON-DEPENDENT METABOLISM IN MOLLUSCS FROM THE BEAGLE CHANNEL
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Iron role in oxidative metabolism in two subantarctic limpets *Nacella magellanica* and *Nacella deaurata* was studied. In spite of living in the same area, the difference in shore level location affects animal exposure to aerial and environmental conditions. Total iron (Fe) content in digestive glands was 1.99 ± 0.03, and 0.99 ± 0.07 nmol/mg FW for *N. deaurata* and *N. magellanica*, respectively. Ferritin was extracted from digestive glands. Samples were purified in a Sephacryl-S-300 column and ferritin was identified by SDS-PAGE. Ferritin content was 5.15 and 4.73 µg/g FW for *N. deaurata* and *N. magellanica*, respectively. Ascorbyl radical content (A·) was evaluated as a reaction product of available catalytic iron in the hydrophilic medium. A· content was 1.0 ± 0.4 and 1.1 ± 0.6 pmol/mg FW for *N. deaurata* and *N. magellanica*, respectively. Lipid radical content was assessed as the result of oxidation catalyzed by Fe in the lipophilic phase. Lipid radical content was 192 ± 31 and 122 ± 29 pmol/mg FW for *N. deaurata* and *N. magellanica*, respectively. Since total Fe content was significantly increased in *N. deaurata* as compared to *N. magellanica*, this results showing that iron content in ferritin is not different between the two limpets suggest that the excess iron is mainly stored in the cellular lipid phase and is responsible for lipid damage.

CB-P54.
LOCALIZATION OF PTP1B IN NEURONS AND ASSOCIATION WITH N-CADHERIN
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PTP1B is an endoplasmic reticulum (ER) phosphatase that associates with the intracellular domain of N-Cadherin. In neurons, N-cadherin is transported in dense-core vesicles to the axonal terminals. In this work we describe the localization of PTP1B in neurons, both *in vitro* and *in situ*. Furthermore, we examine whether the association of PTP1B with N-cadherin occurs during its transit along the neuronal processes or after expression at the cell surface. To analyze the distribution of PTP1B in neurons *in situ* we electroporated whole mounted chick retinas with GFP-PTP1B. Confocal analysis of retinal ganglion cells shows that GFP-PTP1B localizes in cell bodies, dendrites and axons. In cultured neurons endogenous PTP1B shows a punctuate distribution among all neuronal domains, including growth cones and filopodia. At neuronal processes we see poor co-localization of PTP1B with cadherin, as revealed by a pan-cadherin antibody. Similarly, we do not see co-localization with synatophysin, a marker of a different type of transport vesicles. Inmunoclusterization of cell surface N-cadherin induces a selective co-clustering of PTP1B and beta catenin. Our results support the notion that PTP1B associates with cell surface N-cadherin.
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CB-P55.
ROLE OF PROTEIN TYROSINE PHOSPHATASE PTP1B IN N-CADHERIN BIOSYNTHESIS
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N-cadherin function at the cell surface depends upon binding of β-catenin to its cytosolic domain, event that requires PTP1B association with cadherin. Beta-catenin binds to the precursor of N-cadherin early during biosynthesis, traveling as a complex to the cell surface. Since PTP1B is associated to the cytosolic face of the endoplasmic reticulum (ER) we asked: 1) whether PTP1B is associated with the N-cadherin precursor; and 2) whether β-catenin binding to the N-cadherin precursor is regulated by PTP1B. To identify and isolate the N-cadherin precursor we inserted the HA epitope in the propeptide sequence of the N-cadherin-GFP construct (HA-N-cad-GFP). HA-N-cad-GFP expression reveals that HA immunoreactivity concentrates at the ER and Golgi. As expected, only GFP fluorescence was detected at the intercellular junctions where the processed N-cadherin resides. HA immunoreactivity co-localizes with β-catenin but not with PTP1B at the intra-cellular compartments. N-cadherin, β-catenin, and PTP1B co-localizes at cell junctions. PTP1B could not be detected in immunoprecipitates of the N-cadherin precursor. In contrast, in cells with different PTP1B backgrounds β-catenin associates with the N-cadherin precursor in a similar proportion. Our results suggest that PTP1B targets N-cadherin complexes at the cell surface.
Supported by ANPCyT.

CB-P56.
MODULATION OF PROTEIN TYROSINE PHOSPHATASE 1B BY ERYTHROPOIETIN IN UT-7 CELL LINE
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The central role played by tyrosine phosphorylation of the erythropoietin receptor (EpoR) in cell activation by erythropoietin (Epo) has focused attention on protein tyrosine phosphatases (PTPs) as candidates implicated in the pathogenesis of resistance to therapy with human recombinant Epo. The prototype member of the PTP family is PTP1B, a widely expressed non-receptor PTP. It was suggested that PTP1B plays a central role in downregulation of EpoR and insulin receptor signaling. We have explored the Epo effect on PTP1B expression in the Epo-dependent UT-7 human cell line. For different periods Epo was added to Epo-deprived cells. After cell lysis, total proteins or immunoprecipitates with anti-PTP1B, were subjected to Western Blot using anti-PTP1B, anti-PTyr or anti-EpoR. Induction of PTP1B expression was already observed at 3 h of Epo stimulation while PTP1B Tyr phosphorylation was detectable after 5 min Epo stimulation and continued within 6 h. The interaction between PTP1B and EpoR was studied by reciprocal immunoprecipitation. In conclusion, we have found an Epo-induced expression of PTP1B, associated with increased PTP1B Tyr phosphorylation, suggesting that besides modulating Epo/EpoR signaling, PTP1B suffers a feedback regulation by Epo.

CB-P57.

CNBP IS PHOSPHORYLATED BY Ser/Thr KINASES DURING ZEBRAFISH EMBRYONIC DEVELOPMENT

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Cellular nucleic acid binding protein (CNBP) is a small single-stranded nucleic acid binding protein with nucleic acid chaperone activity. It is made up of seven tandem CCHC-type zinc knuckle domains and an RGG box between the first and second zinc knuckles. CNBP primary structure shows a nuclear localisation signal PKKEREQ and a PEST proteolytic site that yields two peptides of approximately 4000 and 15000 Da. Moreover, *in silico* analysis revealed the existence of several putative phosphorylation sites in Ser, Thr, and Tyr residues. We have previously shown that zebrafish CNBP is differentially phosphorylated both *in vitro* and *in vivo* during embryogensis. In this report, we show that a natural proteolytic CNBP product is differentially phosphorylated during early development, showing a phosphorylation pattern coincident with the one observed with the whole protein. We determined that CNBP is phosphorylated by Ser/Thr kinases present in zebrafish embryonic extracts. Using bidimentional electroforetic analysis, we observed that CNBP is phosphorylated in more than one amino acid residue, regardless the developmental stages used as source of kinase activity. To address which kinase is required to CNBP phosphorylation, we performed phosphorylation assays in presence of specific inhibitors of different kinases involved in defferentiation and cell proliferation.

CB-P58.

***IN VIVO* PHOSPHORYLATION OF ROTAVIRUS` NSP5 PROTEIN BY CASEIN KINASE 1α CK1α**

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NSP5 is a nonstructural rotavirus protein which is very important for the viroplasm formation in infected cells. The protein can be found in different stages of phosphorylation in the cell. Previous "*in vitro*" experiments showed the involvement of protein kinase CK1α in the phophorylation of serine 67 of NSP5. This modification allows subsequent phosphorylations of NSP5 possibly also catalyzed by CK1α or by another similar kinase. Co-transfecting different constructs into MA-104 cells, we have investigated this phenomenon "*in vivo*". Transfection of specific siRNA for CK1α (siCK1α) caused depletion of this isoform and resulted in a strong reduction of NSP5 hyperphosphorylation. In addition, co-transfection of $NSP5^{S67D}$, a mutant of NSP5 that mimic the phosphorylation, demonstrated that CK1α is not necessary for the hyperphosphorylation of the protein. Using immunofluorescence against NSP2, a different Rotavirus protein, it was observed that the morphology of the viroplasms was imapaired in cells depleted of CK1α through siCK1α transfection. This result suggests the involvement of CK1α phosphorylation of NSP5 in viroplasms formation.

CB-P59.

STUDY OF LOW DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN-1 (LRP-1) EXPRESSION AND α_2-MACROGLOBULIN (α_2-M) EFFECT ON MURINE MÜLLER CELLS

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Müller cells (MC) constitute the main glial cells of the retina, which are involved in retinal neovascularization. Althrough MC facilitates the neovascularization in hypoxic conditions, the molecular mechanisms of this event has not been established. Previously we demonstrated that the α_2-M/LRP-1 system is expressed in rat retinas with ischemia-induced neovascularization. In addition, we also showed that α_2-M activates (ERK)-MAPK pathway and cellular proliferation in cells expressing LRP-1. Herein we investigated the LRP-1 expression and analyzed the α_2-M effect on intracellular signaling pathways in primary cultures of MC, which were isolated from C57BL6 mice. MC were characterized by immunofluorescence detecting specific protein (CRALBP). LRP-1 expression was detected by Western blotting and immunocytochemistry. (ERK)-MAPK and PKB pathways were analyzed by Western blotting in MC cultured in presence of α_2-M. We showed that nearly 95-100% of cells expressed CRALPB and LRP-1. In addition, α_2-M generated ERK1/2 phosphorylation. Nevertheless, α_2-M no promoted PKB (Akt phosphorylation) activation. In conclusion, we demonstrated that LRP-1 is expressed in murine MC, which can mediate intracellular signaling pathway activation by α_2-M. Thus, we proposed that α_2-M/LRP-1 system is implicated in the retinal neovascularization.

CB-P60.

c-FOS INTERACTS AND ACTIVATES SPECIFIC PHOSPHOLIPID SYNTHESIS ENZYMES

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The oncoprotein c-Fos, in addition to its transcription factor activity as a member of the AP-1 family of inducible transcription factors, has the capacity to associate to the ER and activate the synthesis of phospholipids in events associated to nerve cell growth, differentiation and synaptic transmission. Of the 380 amino acids that form c-Fos, the basic domain (aa 139 to 159) is required to activate specific phospholipid synthesis enzymes, some of which are integral ER membrane proteins and others that are translocated to attain their activated state. Herein, in NIH 3T3 cells, we co-express c-Fos and different myc-tagged phospholipid synthesis enzymes. Co-immunoprecipitation experiments showed interaction between c-Fos and the enzymes CTP: Phosphocholine cytidylyltransferase α (CCT α) and PtdIns4 Kinase IIa (PI4KIIα) whereas no co-IP was observed with Phosphatidyl inositol Synthase (PtdIns S) or choline/ethanolamine phosphotransferase 1 (CPT1) which, in addition, were not found activated in a c-Fos dependent manner either in assays performed *in vivo*, *in vitro* or by the addition of recombinant c-Fos to the assays. To confirm these interactions, we carried out confocal FRET measurements (fluorescent resonance energy transfer). Positive FRET values were found between c-Fos and PI4KIIα and between c-Fos and CCTα whereas FRET was not observed between c-Fos and either CPT1 or Ptd InS. The results presented herein disclose a physical interaction between c-Fos and the enzymes of the pathway of phospholipid synthesis that this protein activates and points to a new regulatory mechanism for the genesis of these lipids in events associated to cell growth and differentiation.

CB-P61.
DESCRIBING THE ROLE OF c-FOS IN NEUROFIBROMATOSIS TYPE I
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c-Fos is rapidly induced in response to a plethora of stimuli. It heterodimerizes mainly with the protein c-Jun, thus constituting the AP-1 family of transcription factors, that regulate the expression of genes involved in processes such as mitosis and differentiation. We have demonstrated that c-Fos, in addition to its transcription factor activity, has the capacity to associate to the endoplasmic reticulum (ER) and activate the synthesis of phospholipids for the genesis of membrane required for cell growth. This process occurs in tumor nervous system cells.
Herein, we determined if this phenomena exists in a cancer animal model of physiological relevance; for this purpose, c-Fos expression and cellular localization was examined in brain and peripheral tumors (neurofibromas) occurring spontaneously in B6 mice heterocigotes for the *p53* and *Nf1* genes (NPcis mice), an animal model for the human Neurofibromatosis Type I (Nf1) disease. We also examined stable cell lines generated from both types of neoplasias High levels of c-Fos expression were observed co-localizing with ER markers both in brain tumors and in neurofibromas. In neurofibromas c-Fos is mainly found in Schwann cells, which is thought to be the cell type responsible for driving the disease.
In the stable cell lines, high levels of c-Fos expression were found in all the lines considered in each tissue, whereas Fra-1 levels, another member of the AP-1 family, were under the limit of detection.
These results support the importance of c-Fos in supporting normal and exacerbated growth of cells derived from the nervous system.

CB-P62.
LOCALIZATION AND EXPRESSION DIFFERENCES OF IP3 RECEPTORS IN DUCHENNE MUSCULAR DYSTROPHY
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In cultured skeletal muscle cells after depolarization, a slow calcium rise, unrelated to muscle contraction, but related to gene expression takes place. This calcium rise is mediated by IP_3 receptors (IP_3Rs). Duchenne muscular dystrophy is characterized by muscle cell calcium increases. We evaluated the distribution and expression of the different IP3 receptors isoforms in dystrophyc (RCDMD) and normal (RCMH) human cells lines by Western Blot. In RCDMD cell line we found an important increase of type-2 IP_3R levels (5-fold), and also a decrease in type-3 IP_3R receptor (2-fold) compared to normal cells. Notably, type-2 IP_3Rs are located in the nuclear envelope in RCDMD, while in RCMH it was only in the sarcoplasmic reticulum. Type 1 IP_3R was located in the sarcoplasmic reticulum in RCMH, and in both reticulum and nucleus in RCDMD. Otherwise, type-3 IP_3R was founded in both reticulum and nucleus in RCMH cells and only in the reticulum of RCDMD cells. We also determined the phosphorylation levels of ERK1/2 (down river of calcium increase) after depolarization in both cell lines. The dystrophic human cell line was unable to raise a response similar to normal cells. These differences in expression and localization of IP_3Rs probably could help to explain the calcium increases seen in Duchenne muscular dystrophy.
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CB-P63.
REGULATION OF VITAMIN C TRANSPORT IN RAT HEPATOCYTES AND HEPATOMA CELLS
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We studied the transport of reduced and oxidized vitamin C and the expression of the respective transporters in freshly isolated rat hepatocytes and in rat hepatoma cells H4IIE, and analyzed the effect of the pharmacological depletion of glutathione. Transport studies, immunolocalization analysis and RT-PCR assays revealed that rat hepatocytes express the ascorbic acid (AA) transporter SVCT1 and the dehydroascorbic acid (DHA) transporter GLUT2, while the hepatoma cells express the AA transporters SVCT1 and SVCT2 and the DHA transporters GLUT1 and GLUT2. The absence of glutathione failed to affect the expression of the DHA transporters and the capacity of the cells to transport DHA, but greatly decreased the expression of the AA transporters SVCT1 and SVCT2 and obliterated the capacity of the cells to transport AA. Thus, in normal and transformed cells, the glutathione content controls specifically the expression and function of the reduced vitamin C transporters without affecting the transporters of oxidized vitamin C.
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CB- P64.
BACTERIAL LIPOPOLYSACCHARIDE INCREASES THYROTROPIN-INDUCED IODIDE UPTAKE AND SODIUM-IODIDE SYMPORTER (NIS) GENE EXPRESSION IN FRTL-5 THYROID CELLS
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Lipopolysaccharide (LPS) is an endotoxin from Gram-negative bacteria that exerts a wide variety of biological responses and is present in blood during certain infectious processes. Thyroid function and specific gene expression are regulated by thyrotropin (TSH). The first step in thyroid hormonogenesis is the NIS-mediated iodide uptake. NIS has been proposed as a possible autoantigen in autoimmune thyroid disease. The aim of this work was to analyse the effect of LPS on iodide uptake and NIS gene expression in TSH treated FRTL-5 cells. We observed that iodide uptake was stimulated by LPS (maximum 0.1µg/ml, 48h). LPS increased NIS protein level (immunofluorescence /confocal microscopy) (maximum 0.1µg/ml, 48h). The NIS mRNA level was increased by LPS (RT-PCR). In transient transfections assays, LPS increased NIS promoter (-2841 to +13 bp) activity in a concentration and time-dependent manner (18-24h). The expression of the LPS receptor, TLR4 was evidenced (RT-PCR). In conclusion, LPS was able to stimulate NIS gene expression at transcriptional level. The increment of the NIS level could explain the increase of iodide uptake produced by LPS. A novel property of thyroid cell to express TLR4 was revealed. These findings support a potential role of LPS in thyroid pathophysiology.

CB-P65.

GLYCOSYLATION DEFICIENCY MODIFIES LEUCINE TRANSPORT IN *SACCHAROMYCES CEREVISIAE*

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In the yeast *Saccharomyces cerevisiae*, amino acid transport is mediated by permeases of a broad and restricted specificity.
In a previous communication with the purpose of studying the effect of glycosylation reactions on the activity of L-leucine permeases, we described the isolation of sodium vanadate resistant mutants (Vanadate^R) from MMY2 strain. In the presence of this ion, it is possible to isolate putative glycosylation mutants.
In the isolated colonies (Vanadate^R) we evaluated: a) the growth in media with L-leucine as the only nitrogen source b) resistance to the L-leucine toxic analogue, trifluoroleucine (TFL) and c) L^{14}C-amino acid uptake.
We carried out Western blot assays with an antibody raised against β subunit (*WBP1*) of yeast oligosaccharyltransferase (*OST1*), a well acknowledged reporter of glycosylation levels in the endoplasmic reticulum. Three out of ninety-five isolated mutants showed not only an increase in electrophoretic mobility of glycoforms associated with this subunit but L-leucine transport deficiency as well.
Our results indicate that alteration of glycosylation pathway in MMY2 derived strains leads to a change in the capacity of the cell to import amino acids and in the kinetical parameters of the different amino acid permeases involved in the uptake process.

CB-P66.

MYELOSUPPORTIVE ROLE OF GANGLIOSIDES

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The myelopoiesis dependent upon myelosupportive stroma required production of growth factors and heparan-sulfate proteoglycans, and generation of negatively-charged sialydase-sensitive intercellular environment between the stroma and the myeloid progenitors. We studied production, distribution and role of gangliosides in the AFT-024 murine liver-derived stroma, and the FDC-P1 cell line as myeloid progenitors. GM3 was the major ganglioside produced by stroma, but not by myeloid cells, and it was required for the optimal stroma myelosupportive function. It was released into the supernatant and selectively incorporated into the myeloid progenitor cells, where it is colocalized with the GM-CSF receptor a chain. It was also further metabolized by myeloid cells into gangliosides of the *a* and *b* series, similar to the endogenous GM3. In these cells, GM1 was the major ganglioside, but although it also segregated at the interface with the stroma, it did not colocalize with the growth factor receptor. We conclude that gangliosides, which are transferred from stroma to target cells, generating on the latter ones specific membrane domains with molecular complexes that include growth factor receptors.
CAPES-SPU; PEI of CONICET, CNPq, FAPERJ; FAPERGS and PROPESQ-UFRGS.

CB-P67.

GANGLIOSIDES REGULATE THE CAPACITY OF STROMAL CELLS TO SUPPORT MYELOPOIESIS

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Stroma-mediated myelopoiesis depends upon growth-factors and the appropriate intercellular microenvironment, whose polarity is relevant for granulocyte-macrophage colony stimulating factor (GM-CSF) mediated myeloid cell proliferation. We have addressed the question of whether gangliosides participate in formation of the microenvironment required to sustain myelopoiesis. We analysed the ganglioside synthesis, expression and shedding by two primary liver stromal cell cultures isolated from the wild type and the interferon gamma receptor knockout mice with different myelopoiesis support capacity. FDCP-1 myeloid growth factor-dependent cell line survival and proliferation were used as the reporter system. Our results demonstrated that although the two stromal cells synthesise the same gangliosides, their relative content and distribution were distinct. The FDCP-1 cell proliferation correlated with, and was dependent upon, the presence of GM3. FDCP-1 cells do not accumulate GM3, but they are able to take up the stroma-produced GM3. The role of the stroma in sustaining myelopoiesis is thus double: it provides simultaneously the growth factor and the ganglioside required for the optimal stimulation of the myeloid cell proliferation.
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CB-P68.

HIGH SPECIFICITY ANTIBODIES LOCALIZE HIGH-RISK HPV16 AND HPV18 E6 ONCOPROTEINS IN CULTURE CELL LINES AND CERVICAL BIOPSIES

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High-risk human papillomaviruses (HPVs) are major etiological agents of cervical cancer. The E6 oncoproteins of high-risk HPVs 16 and 18 are involved in the tumorigenesis of human cervical cells by targeting numerous cellular proteins. We show that recombinant high risk E6 oncoproteins form large soluble oligomers, which were used to obtain anti-E6 polyclonal antibodies and used them for precise localization of the E6 oncoproteins within cell lines and cervical adenocarcinoma samples from patients. Endogenous E6 proteins were observed distributed in both nucleus and cytoplasm of HeLa and CaSki cells, established cell lines transformed with HPV18 and HPV16, respectively, by immunofluorescence and confocal microscopy. Immunohistochemistry of cervical biopsies shows that E6 is associated with nuclear and plasmatic membrane areas. Finally, electron microscopy combined with immuno-gold techniques confirm that this oncoprotein is mostly localized at the periphery of condensed chromatin and randomly distributed in cytoplasm. The preferential nuclear distribution of this viral oncoprotein in HPV-infected cells correlates with this activities at the transcriptional level.

CB-P69.

INHIBITORY EFFECT OF QUERCETIN ON METALLOPROTEINASES EXPRESSION AND LPS-INDUCED NITRIC OXIDE PRODUCTION

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Quercetin (QT) is a flavonoid with anti-inflammatory activity, although the molecular mechanisms are unclear yet. During inflammation Metalloproteinases (MMPs) and Nitric Oxide (NO) play a key role; however, in severe inflammatory stages, they produce deleterious consequences. In this work, the main objective was to investigate the anti-inflammatory effect of Qt, evaluating the inhibitory action on MMPs (MMP-2 and MMP-9) and NO production. The inhibitory Qt effect on MMPs activity was analyzed by zymography in conditional culture medium of CHOK-1 cells incubated in the presence of QT (25 - 100 µM). In addition, levels of MMP-2 and MMP-9 mRNAs were determined by reverse transcriptase (RT)-PCR. Finally, LPS-induced NO production was measured by the Griess reaction in the conditional culture medium of J774 macrophage cells incubated in the presence of Qt (25 - 100 µM). The results showed that Qt inhibited the activity and mRNA expression of both MMPs. Inhibitory effects were also observed in LPS-induced NO production. These results taken together indicate that Qt exerts its anti-inflammatory effect by inhibiting the main components of inflammation stages, such as MMPs and NO, which could suggest that this flavonoid affects the signalling pathways involved in the regulation of these inflammatory mediators.

CB-P70.

ACTION OF TAMOXIFEN ON THE UTERUS OF RATS BEARING EXPERIMENTAL MAMMARY TUMORS

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Extended tamoxifen (Tam) administration in breast cancer therapy leads to an increased risk for endometrial neoplasia. The objective of this work was to analyze the effect of Tam on the uterus of rats bearing N-nitrose-N-methylurea induced mammary tumors: A) Tam (1 mg/kg.day, sc) was administered for 120 days beginning when rats were 40 days old. B) Tam was administered for 30 days. Control groups not receiving Tam treatment or NMU injection were included. Results disclosed in the investigated uteri were: in A) highly significant decrease of uterine weight and absence of steroid receptors expression. Histological studies showed very thin, nearly atrophic endometrial mucosae and also atrophic smooth muscle wall. In B) significant diminution of estrogen receptor expression, increase of progesterone receptor and cystic glandular endometrial hyperplasia. Data were correlated with the expression of: PCNA, c-fos, bax, bcl-2 and insulin growth factor type-I. In A and B, rat estral cycle was arrested at diestrum (*o.m.* analysis of vaginal smears). Nuclear anisocaryosis and hyperchromasia was observed in the endometrium of NMU-injected rats regardless of Tam treatment. Results show the wide range of biological interrelations established at the end of treatments.

CB-P71.

THE INSULIN GROWTH FACTOR SYSTEM ON MAMMARY TUMORIGENESIS

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Insulin growth factors (IGFs) are important mediators in the growth, development, differentiation and survival of normal and transformed gland mammary tissue. The aim of this study was to investigate the expression and localization of the insulin growth factor-type 1 receptor (IGF-IR) and insulin receptor (IR) during the mammary carcinogenesis induced in rats by N-Nitroso-N-methylurea (NMU). NMU was injected to 50, 80 and 110 days old rats. Samples of mammary tissue were collected at days 55, 85 and 115, and binding assay (conventional technique), immunohisto- chemical analysis (monoclonal antibody) and Western blot (monoclonal antibody) were performed. On day 55, neither IGF-IR nor IR were expressed. On day 85, low expression of both receptors were detected in NMU-rats. On day 115, a significantly higher expression of both receptors vs. normal glands were revealed. Our results suggests that both IGF-IR and IR, components of the IGF system, have an important role in the promotion/progression phase in the experimental model tested.

CB-P72.

OVEREXPRESSION AND KNOCK-DOWN OF THE LARGE GTP EXCHANGE FACTORS GBF1 AND BIG1 DIFFERENTIALLY ALTER THE GOLGI COMPLEX

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The recruitment of several types of coat proteins involved in protein secretion is dependent on the activation of ADP-ribosylation factors (ARFs) by guanine nucleotide exchange factors (GEFs). Two such GEFs, GBF1 and BIG1 localize to mutually exclusive regions of the Golgi complex and exhibit different substrate specificity and Brefeldin A (BFA) sensitivity *in vitro*. We have examined the relative impact of over-expressing GBF1 and BIG1as well as siRNA-mediated knock-down assays on various biological assays. We prove that GBF1 is cytotoxic at high levels but confers resistance to BFA when moderately over-expressed. Over-expression of GBF1 also led to a marked decrease of BIG1 levels. In contrast, over-expression of BIG1 was not toxic and did not produce BFA resistance, but did interfere with the resistance induced by GBF1. The BFA resistance produced by GBF1 was limited to the core Golgi stack and did not extend to the *trans* elements of the Golgi complex. Lowering the expression of these GEFs led to disruption of the Golgi structure and selective dissociation of various Golgi markers. These results provide evidence that GBF1 and BIG1 perform different but interdependent functions on the Golgi complex.
Additionally, we are currently testing multiple site-specific GBF1 mutants and their effect on protein secretion in a human T lymphocyte system.

CB-P73.
SIGNS OF DEATH IN SPERMATOCYTES OF THE RAT TESTIS: GLUCOSE AS A POSSIBLE MODULATOR
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The spermatogenesis is the process by which diploid spermatogonia become haploid spermazotoa. During this process of differentiation, apoptosis supports the homeostasis of each cellular type. The objective of this study was to determine the effect of glucose in germ cell survival *in vitro*. Methods: Primary culture of isolated spermatocytes from 25 days old rats were cultured between 0-30 h in KH-medium with or without 10 mM glucose. Cell viability was evaluated by LDH activity and trypan blue exclusion. Fas and p53 levels were evaluated by western blot. Co-localization of Fas and TUNEL was made by immunofluorescence. Results: First, we studied the identity of apoptotic cells in the testis. We found that Fas positive cells co- localized with TUNEL in 25 days old rat germ cells which, according with their localization in the seminiferous tubules, were spermatocytes. Cultured spermatocytes in the presence of glucose showed an increased level of Fas and their transcriptional activator p53. After 24 hrs of culture, 60% of viable spermatocytes were found in glucose-containing medium, but 80% in medium with lactate. Immunofluorescence showed increase of the level of Fas in cells incubated with glucose. Conclusion: Our results indicate that the germ cells undergoing apoptosis are spermatocytes, and that glucose could be a modulator of germ cell survival.
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CB-P74.
HEPARANASE EXPRESSION IN BREAST CANCER
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Heparanase is an endo-beta-glucuronidase that degrades heparan sulfate proteoglycan and seems to be involved in cancer development and metastasis. We had the objective to evaluate heparanase mRNA expression in mononuclear fraction of peripheral blood of 30 breast cancer patients compared with 20 healthy women by semiquantitative RT-PCR. Association between variables as heparanase expression, type of treatment, presence or absence of metastasis was assessed using univariate analysis by Students t-Test for parametric variables. Data management and analysis were done using the SPSS software, version 10 (SPSS Inc., IL, USA). Patients expressed significantly high levels of heparanase, while healthy women no heparanase expression was observed. We obtained a correlation ($p = 0.04$), between low heparanase expression and patients submitted to tamoxifen treatment (78.82 ± 54.20) compared with chemo- or radiotherapy treatment (136.28 ±57.32). It was also found a significant heparanase expression decreased after surgery ($p = 0.002$). In addition, an increased of heparanase expression was also obtained before metastasis (81.01 ± 17.01) compared after metastasis (142.90 ±59.71) ($p = 0.027$). Seventy percent of lymphocytes of breast cancer patients were labeled with heparanase polyclonal antibody while only 10% of healthy women lymphocytes were labeled, but this expression could be increased by the presence of the plasma patients.

ES-P1.
CHARACTERIZATION AND PARTIAL CLONING OF ARGINASE FROM *Pleurotus ostreatus*
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Arginase (EC 3.5.3.1) catalyzes the hydrolysis of arginine to ornitine and urea. The enzyme is widely distributed in living organisms and plays several important biological functions, including urea genesis and regulation of cellular arginine levels. At present, the best characterized are the mammalian and some bacterial and yeast arginases. However, there is no information about the enzyme from the fungi species *Pleurotus*, which we are characterizing in view of their potential use for waste degradation and as a nutritional source of proteins. In this study, the arginase activity was found to depend on the stage of development of *P. ostreatus*; significant arginase activity was detected in carpophores, but not in mycelia. The enzyme was purified and kinetically characterized. The specific activity was 1,8 µmoles of urea/mg protein/min and the *Km* for arginine was 4,7 mM. The enzyme was activated by low concentrations of Mn^{2+} y Ni^{2+} and completely inactivated for Zn^{2+}. The inhibitory actions of products and substrate analogs was also examined. By sequence analysis and RT-PCR experiments using total RNA of *P. ostreatus* carpophores, we have amplified a fragment of 650 base pairs, which most probably correspond to the arginase gene.
Supported by Grant LASPAU (Lewis Tyler Trusted Found).

ES-P2.
THE MEMBRANE PEPTIDASE PHEX INTERACTS WITH CELL SURFACE GLYCOSAMINOGLYCANS
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PHEX gene (phosphate-regulating gene with homologies to endopeptidase on the X chromosome), encodes a M13 family zinc metallo-endopeptidase identified as a mutated gene in patients with X-linked hypophosphatemic (XLH), an inherited disorder characterized by defective calcification of cartilage and bone. The aim of this work was to verify the interaction of glycosaminoglycans (GAGs) with membrane peptidase PHEX. All the GAGs tested inhibited PHEX activity and among them heparin showed the highest inhibitory activity ($K_I = 2.5 \pm 0.2$ nM) depicted by a competitive tight-bound model. The Alexa Fluor 488-PHEX conjugated interacted with heparin-Sepharose resin, being eluted at 0.8 M of ionic strength. The PHEX-GAGs interaction did not change significantly the enzyme secondary structure. However, protein fluorescence assays indicated that heparin can stabilize the tertiary structure at high temperatures. Confocal microscopy and flow citometry analysis of CHO cells showed that Alexa Fluor-PHEX conjugated binds at CHO-K1 cell surface, but did not bind at heparin sulfate defective cells CHO-745. In addition, the endogenous PHEX and heparan sulfate are colocalized at CHO-K1 cell surface, and in CHO-745 only cytoplasmatic staining was detected. These findings suggest that proteoglycans present at cell surface, can anchor and modulate the membrane PHEX protein. *Support: FAPESP, CNPq and FADA.*

ES-P3.
BIOCHEMICAL AND INMUNOHISTOCHEMICAL STUDIES OF RODENT BRAIN AGMATINASE

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Agmatinase catalyzes the hydrolysis of agmatine to putrescine and urea. Among other important actions, agmatine (decarboxylated derivative of arginine) is considered a neurotransmitter / neuromodulator in the brain. In spite of the detection of significant amounts of agmatinase mRNA in human brain, only extremely low agmatinase activities are commonly detected in mammalian brain. Here, we report the histochemical detection of agmatinase protein in neurons of arquate nucleus, ciliated ependymal cells, hypothalamic tanycytes, ependymal cells of the ventricules and the pericarium of trigeminal ganglion neurons. Ependymal agmatinase would regulate the agmatine concentrations in the cerebral spinal fluid. In attempting to isolate and characterize the enzyme, an agmatinase inhibitory activity was detected in a partially purified rat brain homogenate. The inhibition, detected by a decreased activity of an externally added *E. coli* agmatinase, was also produced by recombinant human agmatinase. Both inhibitory species were recognized by an anti-*E. coli* agmatinase antibody. We suggest that the inhibition, which results from reversible interactions between agmatinase molecules, would explain the low levels of agmatinase activity commonly detected under *in vitro* conditions.
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ES-P4.
KINETIC AND ENZIMATIC STUDIES OF A PEROXIREDOXIN

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Peroxiredoxins (Prx) are thiol-dependent antioxidant enzymes that reduce peroxides at expenses of thioredoxin (Trx) or other thiols. The reaction mechanism implies the oxidation of a critical cys by the peroxide to yield sulfenic acid, and subsequent reaction of this intermediate with another thiol. Prx are classified into two classes (1-Cys or 2-Cys) according to the number of cys involved in the catalytic mechanism. The 2-Cys Prx have been extensively studied primarily in the context of cellular physiology, but little is known about its enzymology. They are assumed to be poor peroxidatic enzymes with ping-pong kinetics and the K_M reported for the mammalian isoforms are in the order of 5-20 µM for the peroxide substrate. Most of the kinetics have been performed using a coupled assay with Trx, Trx reductase (TR) and NADPH. Kinetic parameters of the human 2-Cys Prx from erythrocytes were determined using the coupled assay with human and *E.coli* Trx/TR system, as well as the loss of Trx fluorescence, and were confirmed by simulations using Gepasi software. In addition, the bimolecular rate constants for the reaction between reduced Prx and different peroxides were determined by stopped-flow spectrophotometry and competition kinetics. Our results show that the K_M for H_2O_2 is unusually low (< 0.5 µM), yielding a high catalytic efficiency (10^6 $M^{-1}s^{-1}$) albeit a low kcat (~1 s^{-1}) which challenge the previous concept of peroxiredoxins as poor peroxidases compare with the well known mammalian catalase and glutathione peroxidase with catalytic efficiencies of 10^7 $M^{-1}s^{-1}$.

ES-P5.
CONDUCTION OF LIGTH IN A MINIMUN MODEL FOR A ROD IN PBS FROM *GRACILARIA CHILENSIS*

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Phycobilisomes are multiproteic complexes present in red algae and cyanobacteria; they perform an accessory function responsible of the light harvesting and conduction of light to Photosystems. Phycobilisomes from *G. chilensis* are formed by phycobiliproteins organized in a core formed by Allophycocyanin (APC), and radially disposed rods formed by Phycocyanina (PC) and Phycoerythrin (PE). This special arrangement of proteins constitutes the most efficient conductor of light (~95%). This work describes the building of a structural model of a minimun rod formed by two hexamers of PE and two hexamers of PC and proposes preferential pathways for light conduction able to explain the high efficiency of the system. The methodology to obtain the model involved the three-dimensional structures of both phycobiliproteins and the use of ZDOCK to obtain a rigid docking model of the complexes PC-PC, PC-PE, PE-PE. The refinement was performed by Molecular Dynamics after a carefull evaluation of the force fields available in GROMACS. Preferential pathways for light conduction were proposed based on the values of the Transfer constants obtained for each pair of chromophores in the rod. The values obtained for the rate of conduction agree with the experimental data reported in the literature.
Proyect DIUC Nº 205.037.002-1.0.

ES-P6.
COMPARATIVE STUDIES OF RECOMBINANT HUMAN MEMBRANE AND CYTOSOLIC AMINOPEPTIDASE P.

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Aminopeptidase P (APP) is a ubiquitously expressed metallopeptidase found in several organisms including bacteria, yeast and vertebrates. The enzyme hydrolyzes exclusively the Xaa-Pro bond of oligopeptides. Human APP exists as a membrane-bound (mAPP) and a cytosolic homologue (cAPP). The enzyme is involved in the processing of bradykinin (BK) being the main metabolic pathway of BK 1-8 and the only degrading enzyme in the presence of ACE inhibitors. We performed a comparative study of catalytic and physicochemical properties of human recombinant mAPP and cAPP using as substrates internally quenched fluorescent BK derivatives containing *o*-aminobenzoic acid (Abz) as fluorescent group and 2,4-dinitrophenyl (Dnp) or (2,4-dinitrofenil)-etilenodiamino (EDDnp) as quencher. The results indicated that cAPP hydrolyzed all the peptides with catalytic efficiencies 10 to 20 times higher than mAPP. NH_2-K(Dnp)-PPGK-Abz-NH_2, hydrolyzed by both forms with the highest catalytic efficiency, was used in the studies of the influence of pH, salt, thermal decomposition and stability of the enzymes. The effects of Cl- and pH were very similar for both forms. The cAPP completely lost activity after lyoplylisation while mAPP did not change the catalytic properties after this procedure. Further studies are now in process to better characterize the differences in the thermal stability of the enzymes.
Support: CNPq, FAPESP and FADA.

ES-P7.
WRAPPING THE PRION PROTEIN: STRUCTURAL BASIS FOR NUCLEIC ACID BINDING TO PrP

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The infectious agent of transmissible spongiform encephalopathies is believed to comprise, at least in part, the prion protein (PrP). Other molecules can modulate the conversion of the normal PrP^{C} into the pathological conformer (PrP^{Sc}) but the identity and mechanisms of action of the key physiological factors remain unclear. PrP can specifically recognize nucleic acids with high affinity, resulting in a proteinase K-resistant and β-sheet-rich protein. Here, we report small-angle X-ray scattering, nuclear magnetic resonance spectroscopy and binding assay measurements of the soluble 18-base pair DNA:PrP 1:1 complex. We demonstrate that, although interaction is mediated mainly through the PrP globular domain, the unstructured region is also recruited to the complex. This visualization of the complex provides insight into how nucleic acid binds to PrP and how it chaperones conformational conversion.
Supported by: CNPq, FAPERJ, CAPES, PRONEX, PADCT.

ES-P8.
MUTATIONAL ANALYSIS OF SUBSTRATE RECOGNITION BY HUMAN ARGINASE TYPE I

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Argininase and agmatinase catalyzes the production of urea from arginine and agmatine, respectively. They requires Mn^{2+} for catalytic activity and conserve all residues involved in metal coordination and catalysis, but their substrates are not intechangeable. Our sugestion has been that the substrate specificity rely maily in a loop located at the entrance of the active site (residues Ala-125 to Pro-144, in arginase I). We have, therefore, generated several mutations in this area. The N130D variant of arginase I exhibited about 20% of the wild-type activity and about 5 fold increased K_m for arginine. In contrast with wild-type species, the N130D mutant exhibited a significant catalytic activity on agmatine and its arginase activity was significantly more sensitive to inhibition by agmatine and putrescine. The observed kinetic changes were not accompanied by gross structural alterations, as indicated by unaltered fluorescence spectra and sensitivity of arginase I to thermal inactivation. Similar functional alterations were produced by multiple mutations and deletions around Asn-130. Results obtained favours a critical role for Asn-130 in substrate recognition by arginase.
Grant FONDECYT 1030038 and CONYCYT Doctoral Scholarship to M.S. Orellana.

ES-P9.
PURIFICATION AND CHARACTERIZATION OF AN α-AMYLASE PRODUCED FROM *Paecilomyces variotii* USING ELECTROELUTION

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Amylases constitute an important class of enzymes with many biotechnological applications such as starch hydrolysis. As previously reported, *Paecilomyces variotii* produces a glucoamylase and an α-amylase in submerged cultures supplemented with oat flour. The two enzymes were separated by chromatography. α-amylase purification was carried out by sequential elution in DEAE-cellulose, Sephadex G-100 gel filtration and electroelution, resulting in an enzyme purified 16.1-fold, with 8.9% recovery. The main hydrolysis products using starch as substrate, revealed by TLC, were maltose and maltotriose, confirming the character of the enzyme in study as α-amylase. Optimal pH and temperature were 4.0 and 60ºC, respectively. Thermostability analysis showed the enzyme was fully stable up to 60ºC. The α-amylase was stable at pHs 2.5-8.0. Isoelectric point and carbohydrate content were 4.5 and 23%, respectively. The α-amylase hydrolyzed preferentially starch, amylose and amylopectin (low Km and high Vmax values) and it was activated by Ca^{2+} (65%) and Co^{2+} (60%).
Financial support: CNPq, CAPES and FAPESP.

ES-P10.
XYLANASE REGULATION IN *Aspergillus phoenicis*

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Xylanase is an important enzymatic system with several industrial applications, such as supplementation of feed animal, biobleaching of cellulose pulp, and others. We previously investigated the effect of growth temperature of the *A. phoenicis* on thermostability of endoxylanase. Here, we are showing that xylanase activity was induced when *A. phoenicis* was pre-grown in glucose, at 42ºC, for 72h, without agitation, followed by induction with 0.1% xylan, xylose or β-methylxyloside (6h, 42ºC, under agitation). Addition of 0.1% glucose resulted in catabolic repression, but the effect was reverted by 100 µM cAMP or dibutiril-cAMP. A fragment of the gene encoding xylanase was amplified by PCR and used as probe in the Northern blot assay. The fungus was grown under conditions that induced or repressed xylanase activity, and total RNA was extracted. Gene expression was coincident with the physiological observations, i.e., gene transcription was induced in the presence of xylan, xylose or β-methylxyloside, and repressed in the presence of glucose. In addition, the repression by glucose was reverted by cAMP or dibutiril-cAMP.
Financial support: CNPq, CAPES and FAPESP.

ES-P11.
EXTRACELLULAR ADENINE NUCLEOTIDES HYDROLYSIS IN GRX CELLS

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Extracellular nucleotides regulate diverse biological functions in the liver and their levels are controlled by the activity of a cascade of cell surface-bound enzymes such as ectonucleoside 5'-triphosphate diphosphohydrolases (E-NTPDase) and ecto-5'nucleotidase. Hepatic stellate cells (HSCs) present myofibroblast or lipocyte phenotypes and participate in homeostasis of liver extracellular matrix and control retinol metabolism. We studied the extracellular nucleotides hydrolysis in the two phenotypes of a cell line (GRX) representative of the HSCs. Ectonucleotidases activities were measured by the release of inorganic phosphate. The expression of E-NTPDases and ecto-5'nucleotidase was determined using RT-PCR. The results showed that the ATP/ADP hydrolysis ratio was 2:1 for myofibroblasts and 5:1 for lipocytes. Lipocytes showed increased AMP hydrolysis compared to myofibroblasts. Myofibroblasts and lipocytes synthetize mRNA for the NTPDases 3, 5 and 8 and ecto-5'-nucleotidase. Only myofibroblasts express NTPDase 6. Our data suggest that the hydrolysis of ATP and ADP is mainly due to the NTPDases 3 and 8 because they are expressed on the cell surface and hydrolyses ATP better that ADP. The increase in AMP hydrolysis in lipocytes could represent an important hepatoprotective effect by increasing the levels of adenosine.
Supported by CNPq, FAPERGS, PROPESQ-UFRGS.

ES-P12.
GINGIVAL CREVICULAR FLUID COLLAGENASE-3 IN PROGRESSION OF PERIODONTAL DISEASE

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Collagenase-3 (MMP-13) is one of the interstitial matrix metalloproteinases, expressed in sulcular epithelium in periodontitis-affected subjects, being associated with tissue degradation and bone resorption. We studied both MMP-13 levels and activity in gingival crevicular fluid (GCF), because it could be involved in bone lost during periodontitis progression.
GCF from active and inactive sites from periodontitis-patients, and normal sites from healthy subjects were obtained. MMP-13 expression was followed by immunological methods, and the determination of human active MMP-13 was done using a quenched fluorogenic substrate. Also immunohistochemical analysis of gingival tissue was done.
In GCF we visualized the presence of MMP-13 only patient sites, but not in healthy sites. A larger proportion of active MMP-13 form was found in patients compare with controls. This enzyme was visualized in diseased gingival tissue.
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ES-P13.
EXPRESSION AND PURIFICATION OF WILD TYPE/ MUTANT HYBRID TETRAMERS OF FRUCTOSE-1,6-BISPHOSPHATASE

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The gluconeogenic enzyme fructose-1,6-bisphosphatase (FBPase), is a square planar tetramer of identical subunits, which does not contains tryptophan residues. This enzyme, catalize the hidrolysis of fructose-1,6-bisphosphate to fructose-6-fosfate and inorganic phosphate. This activity is synergistically inhibited by AMP and fructose-2,6-bisphosphate. Also high concentrations of substrate can elicit inhibition of the enzyme. But the mechanistic basis of these inhibitions is not completely understood. To study this we expressed the wild type and a mutant enzyme containing a tryptophan residual in position 219 which sense the binding of the ligands. The tetramers hybrids were obtained by mixing equivalent quantities of these enzymes for 12 hours at 4ºC. This procedure allow the subunit exchange producing five hybrid forms 4:0, 3:1, 2:2, 1:3, 0:4 which were FPLC separated by an annionic exchange chromatografic and visualized by non denaturing PAGE. We founded that: the wild enzyme, the mutant Phe219Trp, the hybrid 3:1; 2:2 and 1:3 present similar kinetic characteristic. Interestingly, the hybrid concentration formation is affected by substrate concentrations but by inhibitor and salt concentration. The use of these fluorescents hybrids will allow detecting the binding constant and better understanding of signal propagation in FBPase Fondecyt 1051122, MECESUP AUS0006.

ES-P14.
TUBULIN MUST BE ACETYLATED AT LYS 40 OF THE α-CHAIN TO INHIBIT THE Na^+,K^+-ATPase ACTIVITY

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A subpopulation of membrane tubulin is associated with Na^+,K^+-ATPase in neural and non-neural cells. This association both confers hydrophobic properties to tubulin and inhibits the Na^+,K^+-ATPase activity. Treatment of cells with 1mM L-glutamate provokes dissociation of the complex, leading to increment of active enzyme. The acetylated form of tubulin is present in the tubulin fraction associated with Na^+,K^+-ATPase, however we do not know whether this type of tubulin is indispensable for interaction with the enzyme. To investigate this question, we used 6-11B-1 antibody specific to acetyl group bound to Lys at position 40 of the α-chain of tubulin, and CAD cells, which lacks acetylated tubulin. In these cells, L-glutamate was unable to stimulate the Na^+,K^+-ATPase. Using immunoprecipitation procedures, we showed that the Na^+,K^+-ATPase/tubulin complex was absent in CAD cells. Treatment of cells with deacetylases inhibitors (TSA and tubacin), led to appearance of a significant amount of acetylated tubulin. Under these conditions, the Na^+,K^+-ATPase/tubulin complex was found in membranes, and Na^+,K^+-ATPase activity was inhibited. In addition, L-glutamate was now able to dissociate the complex and to increase enzyme activity. These results indicates that tubulin must be acetylated at Lys 40 to interact with the plasma membrane Na^+,K^+-ATPase.

ES-P15.
ANALYSIS OF C-TERMINAL DELETED VERSIONS OF *Escherichia coli* MutS
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Mismatch Repair System (MMRS) contributes to genetic stability by correcting point mutations or small insertion/deletion loops and preventing recombination between partially homologous DNA sequences. In *Escherichia coli*, the assembly of the MMRS is initiated by MutS, an 853 amino acids protein that recognizes and binds to mispaired nucleotides. *In vitro*, MutS exists as dimers and tetramers in equilibrium. Deletion of the 53 C-terminal amino acids shifts the equilibrium to the dimer form and avoids the formation of inactive high order oligomers. Recently it has been found that this C-terminal region is important for MutS function *in vivo* suggesting that tetramerization would be critical for the protein activity. In order to determine the role of the C-terminal region on the protein oligomerization and function, several C-terminal deletion mutants of *E. coli* MutS were constructed. We characterized their oligomerization capacity by light scattering and density gradient sedimentation, and determined their biochemical activity. It was found that the deletion of just 7 amino acids (Δ848 mutant) resulted in the loss of tetramerization and high order aggregate formation. However, Δ848 retained its ATPase activity and DNA binding capability. All C-terminal deletion versions were able to partially complement an *E. coli* mutS deficient strain suggesting that they are very likely competent for mismatch repair.

ES-P16.
CHARACTERIZATION OF A *Bothrops alternatus* METALLOPROTEINASE
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Snake venoms metalloproteinases (SVMPs) play an important role in hemorrhage and disturbances of the hemostasis. *Bothrops alternatus* is the species responsible for most of snakebites in North-eastern Argentina. In this work a metalloproteinase from *Bothrops alternatus* venom was isolated and its functional and structural aspects studied to attempt a description of molecular features involved in its hemorrhagic activity.
The enzyme (50 kDa) was digested "in gel" by trypsin and the peptide mixture submitted to MS (LCQ-DUO ESI-Ion Trap). Sequest analysis allowed identification of three fragments belonging to the carboxyl cysteine-rich domain of some members of this protein family apart from one fragment of the proteinase domain. The tryptic digest was also submitted to RP-HPLC. Edman degradation of several fractions followed by sequence alignment was carried out. Studies indicated that the protein has a high degree of homology with jararhagin from *Bothrops jararaca*.
Results, taken as a whole, lead to the conclusion that the enzyme belongs to the P-III type of SVMPs.

ES-P17.
COMPARISON OF TWO CYSTEINE ENDOPEPTIDASES FROM *Bromelia hieronymi* Mez (*Bromeliaceae*)
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Hieronymain I and II are cysteine endopeptidases isolated from fruits of *Bromelia hieronymi* Mez and show different biochemical and physicochemical properties. The enzymes were purified by acetone fractionation followed by ionic exchange chromatography (FPLC). The isolated proteases presented optimum alcaline pH ranges (8.5-9.5 and 7.3-9.0, respectively) and the enzymatic activity was completely inhibited by E-64 and iodoacetic acid, confirming that belong to the catalytic group of cysteine endopeptidases. Molecular masses of hieronymain I and II (MALDI-TOF) were 24,066 and 23,411 Da, and isoelectric points were >9.3 and 7.6-8.3, respectively. Each enzyme showed specific kinetic parameters, determined on Z-Phe-Arg-pNA and N-α-CBZ-L-amino acid *p*-nitrophenyl esters. The N-terminal sequences of the proteases (ALPESIDWRAKGAVTEVKRQDG and AVPQSIDWRVYGA, determined by Edman's automated degradation), differ slightly and show a great deal of sequence similarity to other cysteine endopeptidases from Bromeliaceae.
[1]UNLP Fellowship, [2]CIC and [3]CONICET Researcher Career. Supported by grants from ANPCyT, CONICET, CIC, UNLP and CYTED. (Project IV.22). Mass spectra and N-terminal sequences were carried out in IBB, UAB, Spain, by Sebastián Trejo and Francesc Canals.

ES-P18.
PURIFICATION AND CHARACTERIZATION OF A NEW LECTIN FROM THE EDIBLE MUSHROOM *BOLETUS EDULIS* WITH ANTIPROLIFERATIVE EFFECT ON TUMOR CELL LINES
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A new lectin was isolated from the fruiting bodies of the edible mushroom *Boletus edulis* by affinity chromatography on a chitin column. *Boletus edulis* lectin (BEL) exhibited a molecular mass of approximately 15 kDa in SDS-PAGE under reducing conditions. BEL cDNA was cloned and the deduced primary sequence, corresponding to 142 amino acids and a calculated molecular mass of 15597 Da, showed a high similarity with the members of the fungal saline-soluble lectin family. The binding properties of the lectin were studied by competitive enzyme-lectin assays (CELA). The results show, as it was previously described for the *Agaricus bisporus* lectin (ABL), that BEL would have two sugar binding sites with different specificity, one for Thomsen Friedenreich antigen (TF antigen, Galβ1-3GalNAcα-O-Ser/Thr) related residues and the other for N-acetyl glucosamine. The lectin also exhibited a potent dose-dependent antiproliferative activity toward some human tumor cell lines with no apparent cytotoxicity.

ES-P19.
ACETYLATED TUBULIN INHIBITS THE ACTIVITY OF PMCA THAT IS RECOVERED BY ADDITION OF ETHANOL
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It was previously demonstrated that acetylated tubulin associates with Na^+,K^+-ATPasa inhibiting its enzymatic activity, and that dissociation of the complex restores its activity. In the present work we demonstrate similar behaviour of plasma membrane Ca^{+2}-ATPasa (PMCA). Studies were carried out by using synaptosomes from rat brain and COS cells in culture. Results indicate that: 1) the higher the amount of tubulin bound to membrane, the lower the enzyme activity; 2) the activation of enzyme by 1% ethanol produces diminution in the amount of tubulin bound to membrane; 3) addition of tubulin to synaptosome preparations inhibits the activity of PMCA that is then recovered by addition of ethanol. These changes of enzyme activity are accompanied with, respectively, higher and lower amount of tubulin bound to membrane; 4) in COS cells, acetylated tubulin/PMCA complex is dissociated as ethanol concentration increases in the culture medium; 5) Treatment with tubulin-Sepharose beads of a detergent-solubilized brain membrane preparation produced precipitation of PMCA. These results show that acetylated tubulin physically interacts with PMCA inhibiting its activity, and that the acetylated tubulin/PMCA complex can be dissociated by ethanol with recovery of PMCA activity.

ES-P20.
INVOLVEMENT OF ACETYLATED TUBULIN IN THE ACTIVATION OF PLASMA MEMBRANE H^+-ATPase OF *Saccharomyces cerevisiae* BY GLUCOSE
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Plasma membrane H^+-ATPase of *Saccharomyces cerevisiae* is activated by D-glucose. We found that in the absence of glucose, this enzyme forms a complex with acetylated tubulin. When cells were treated with glucose, the H^+-ATPase/tubulin complex was dissociated increasing the enzyme activity with concomitant decrease of acetylated tubulin in plasma membrane. The effect of glucose is inhibited by 2-deoxy-D-glucose, a competitive inhibitor of glucose uptake. Whereas total tubulin is distributed uniformly throughout the cell, acetylated tubulin is concentrated near the plasma membrane. Results from immunoprecipitation experiments indicated physical interaction between acetylated tubulin and H^+-ATPase which was disrupted by treatment with 1 mM glucose. Double immunofluorescence observed by confocal microscopy indicated that H^+-ATPase and acetylated tubulin partially co-localize at the periphery of glucose-starved cells, with predominance at the outer and inner side of the membrane, respectively. Co-localization was not observed when cells were pretreated with 1 mM glucose. Biochemical experiments using isolated membranes from yeast and purified tubulin from rat brain demonstrated inhibition of H^+-ATPase activity by acetylated tubulin, and concomitant increase of H^+-ATPase/tubulin complex.

ES-P21.
CLONING, EXPRESSION AND CHARACTERIZATION OF THE FERREDOXIN-NADP$^+$ REDUCTASE FROM *LEPTOSPIRA INTERROGANS*
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Ferredoxin-NADP(H) reductases (FNR) are ubiquitous flavoenzymes that deliver NADPH or low potential one-electron donors (ferredoxin, flavodoxin) to redox-based 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 photosynthetic process, 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. Recently, we have determined that FNR from *Leptospira interrogans* (LepFNR), a parasitic bacterium of animals and humans, belong to an already defined monophyletic group composed entirely of parasitic species and it is included among the plastidic FNR class, probably as the result of a lateral gene transfer event. Therefore, we decided to study the function and structure of this enzyme. We cloned the LepFNR gene from the bacterium genome, and developed an efficient expression and purification system of the protein. We found that LepFNR displays similar spectral properties, functional characteristics and kinetic parameters than the pea FNR. We have obtained protein crystals to elucidate its structure by means of x-rays crystallography and to compare it with the structures of other FNRs.

ES-P22.
MODULATION OF THE ENZYME CATALYTIC EFFICIENCY BY AMINO ACID VOLUME
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Ferredoxin-NADP(H) reductases (FNR) are ubiquitous flavoenzymes that participate in a broad range of redox metabolic pathways. Several structural features of these enzymes remain yet to be explained. FNRs consist of two domains; one involved in the binding of the prosthetic group FAD and the other in the binding of NADP. The residue Y308 in pea FNR is stacked near parallel to the *re*-face of the flavin and is highly conserved among members of the family. Computing the relative free energy for the lumiflavin-phenol pair with the relative positions found for Y308 in pea FNR we have concluded that this amino acid is constrained against the isoalloxazine. This effect is probably performed by amino acids C266 and L268, which are facing the other side of this tyrosine, forcing it to adopt a more planar orientation with respect to the flavin. Simple and double FNR mutants of amino acids C266 and L268 were obtained and characterized. We observed that reducing amino acid volume decreases the catalytic efficiency without altering the protein structure, probably due to an increase of the Y308 - isoalloxazine interaction. Our results allows to suggest that these amino acids have been evolutively selected by volume and that they participate in the fine tuning of the enzyme efficiency, modulating the interaction of the Y308 with the isoalloxazine.

ES-P23.
MODULATION OF THE PEA FERREDOXIN-NADP$^+$ REDUCTASE ACTIVITY BY A METAL SITE
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Ferredoxin-NADP(H) reductases (FNR) are ubiquitous flavoenzymes that participate in a wide range of redox metabolic pathways in a variety of organisms. The plant-type reductase displays strong preference for NADP(H) and is a very poor NAD(H) oxidoreductase. In contrast, a number of redox compounds, can replace its natural substrates (ferredoxin or flavodoxin) as electron acceptors *in vitro*. FNRs consist of two domains, one involved in the binding of the prosthetic group FAD and the other responsible for binding of $NADP^+$. Two tyrosine residues lie close to each side of the isoalloxazine. The highly conserved Y308 in pea FNR is stacked near parallel to the re-face of the flavin and should be displaced by the nicotinamide ring of NADP(H) for productive binding to the enzyme. Searching for evidences of the mentioned displacement we constructed a mutant FNR in which the Y308 is followed by a tail of nine amino acids, including four histidine residues. In the presence of a metal ion this added structure could fold itself and, as consequence, would partially impair the tyrosine residue movement. Kinetics studies of this mutant FNR showed a decline in enzyme catalytic efficiency either in the presence of Zn^{2+} or Co^{2+}. However, the enzyme affinity for NADP(H) was not changed under this conditions. Our results are the first experimental evidence that Y308 mobility is essential for obtaining a high enzyme catalytic efficiency.

ES-P24.
STRUCTURAL GROUNDS FOR THE SUBSTRATE SPECIFICITY OF THE FERREDOXIN-NADP(H) REDUCTASE
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Ferredoxin-NADP(H) reductases (FNR) are ubiquitous flavoenzymes that catalyse the reversible electron transfer between NADP(H) and obligatory one-electron carriers as ferredoxin. A highly conserved C-terminal tyrosine (Y308 in pea) is stacked near to the flavin in plant type FNRs. This amino acid performs a fundamental role in nucleotide discrimination and in increasing catalytic efficiency. Y308 is apparently displaced when the nicotinamide ring of NADP(H) is bound. We have investigated the structural grounds of the FNR-NADP(H) interaction and the involvement of Y308 on it. Differential spectroscopy analysis of the mutant FNR Y308S and analogues of $NADP^+$ shows an autonomous binding of 2'phospho-AMP or nicotinamide moieties with similar spectroscopy changes. In addition, nicotinamide mononucleotide reduced (NMNH) oxidase activity is detected in Y308S but not in wild-type FNR indicating that Y308 preventing of NMNH to act as substrate. Moreover, the artificial substrate ferricyanide inhibits FNR increasing NADPH and NADH Michaelis constants and, the specificity for NADPH relative to NADH. Our results indicate that binding of any of the two moieties of the substrate NADP(H) affects the prosthetic environment, reflecting a conformational change on the enzyme which may tailor the enzyme for catalysis.

ES-P25.
UDPG-DBM GLUCOSYLTRANSFERASE ACTIVITY AND A 38 KDA PROTEIN RELEASED FROM PURIFIED *ESCHERICHIA COLI* PROTEOGLYCOGEN BY AMYLOLYSIS
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Glycogenin (GN), the protein moiety of mammalian and yeast proteoglycogen, initiates the polymerization of glucose by autoglucosylation from UDPG, a reaction that requires Mn^{++}. The polymerization is continued by glycogen synthase (GS), which as GN utilize UDPG but no ADPG. Contrary to the mammalian and yeast enzymes the bacterial GS is specific for ADPG and it was claimed to be also responsible for initiation of the polymerization. We had described a 31 kDa protein linked to proteoglycogen in *Escherichia coli*, but no further characterization was done. Now we show that the rM of the glycogen-bound protein is 38-kDa. The protein is present in a proportion lower than the GN of liver proteoglycogen. Due to this, the search for a glycogenin-like activity was done without separation of amylase from the amylolyzed mixture, a procedure which might result in lost of the released protein. Thus, only the transglucosylation of DBM, whose reaction product is not substrate for amylase, was measured. When treatment with caotropic agent was omitted, the purified *E. coli* glycogen preparation contained GS activity which glucosylated DBM from ADPG but no from UDPG. The treatment with 3.6 M KI eliminated the GS activity. Free of GS, the amylolyzed glycogen glucosylated DBM from labeled sugar nucleotide. The reaction was Mn^{++}-dependent and utilized UDPG but no ADPG. Our results are consistent with the presence of glycogenin-like protein bound glycogen in bacteria.

ES-P26.
SURFACE ACTIVITY AND INTERACTION WITH PHOSPHOLIPIDS OF NON GLUCOSYLATED AND GLUCOSYLATED GLYCOGENIN
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We described the amphiphilic character of glycogenin (Gn), which forms by itself Gibbs and Langmuir monolayers at the air-buffer interface and associates to phospholipids (FEBS Lett., 509, 323-26, 2001). Now we compare the surface activities and phospholipid interactions of non-glucosylated apoglycogenin (a-GN) and slightly-glucosylated glycogenin (sl-GN). The sl-GN contained up to 8 (sl-8-GN) or 13 (sl-13-GN) linked glucoses. Injected into the subfase, a-GN and sl-13-GN induced a rapid increase of the surface pressure, reaching 14 mN/m in 6 min. They spread as stable monolayers from aqueous solutions; the surface pressure- and surface potential-molecular area isotherms of compression and expansion were remarkably different, the sl-13-GN occupying a higher area than a-GN at a given surface potential. When sl-8-GN, labeled by autoglucosylation from UDP-[14C]glucose, was mixed and over night incubated with pre-formed large unilamelar vesicles of palmitoyl-oleoyl-glycerophosphocholine, and vesicles separated from the labeled GN by exclusion on a Sephacryl column, about 9-24 % of the labeled protein coeluted with the vesicles, indicating interaction of the sl-8-GN with phospholipid. These results further sustain our hypothesis that GN, before or after autoglucosylation, can associate to ER membranes, were the biosynthesis of proteoglycogen might initiate, followed by dissociation when reaching a higher glucosylation degree.

ES-P27.
FINDING KEY RESIDUES FOR SITE-DIRECTED MUTAGENESIS OF HEMOLYTIC PHOSPHOLIPASE C FROM *Pseudomonas aeruginosa* THROUGH BIOINFORMATIC ANALYSIS

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P. aeruginosa hemolytic phospholipase C (PlcH) is a pathogenic factor that together with PChP supplies Pi and choline. To determine the residues involved in catalysis, bioinfomatic analyses were performed. Sequence profile using BLASTp and CDD revealed that PlcH architecture consists in a highly conserved phosphoesterase domain at N-terminus between residues 51 and 454. This domain is shared with eukariotic acid phosphatases and Gram-negative bacterial Plc. None of these family members have a resolved structure. Neither sequence nor structure homology with bacterial Gram-positive Plc was observed. LOOPP fold recognition method allowed to identify structural homologues into alkaline phosphatase-like superfamily. The best ranked proteins were *E. coli* and human alkaline phosphatases. Considering that these enzymes share ligands like Pi and Zn^{2+}, two models of phosphoesterase domain were built to compare the key residues described in these phosphatases. These bioinformatic studies will be useful to perform site-directed mutagenesis to know the role of the conserved aminoacyl residues in the phosphoesterase domain of PlcH.

ES-P28.
ROLE OF HISTIDINE RESIDUES IN THE CYCLODEXTRIN GLYCOSYLTRANSFERASE FROM *Bacillus circulans* DF 9R

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Cyclodextrin glycosyltransferase (CGTase; EC 2.4.1.19) catalyses the conversion of starch and α (164) related glucans into cyclodextrins through intramolecular transglycosylation. The enzyme is also capable of catalysing starch hydrolysis and transglycosylation intermolecular reactions. Three histidine (His) residues are conserved in all CGTases described. Three-dimensional structure of CGTases from other sources has shown that such residues are located at or near the active site. Besides, it has been proposed that they are involved in the substrate binding site. On the other hand, protein reaction with diethyl pyrocarbonate (DEP) under mild conditions, leads to N-ethoxyformyl-derivatives. To search the role of His residues in functional aspects of the *Bacillus circulans* DF 9R CGTase, the effect of protein DEP-modification on hydrolytic and ß-cyclizing activities were measured. Results indicated that, with a DEP/His ratio of two, ethoxyformylation follows a pseudo first order kinetics and only one His residue is modified. This fact, leading to a drop of 75% in both activities, may be due to an alteration of the enzyme-substrate affinity.

ES-P29.
CYTOCHROME P450 REDUCTASES IN *Trypanosoma cruzi*. CLONING AND CHARACTERIZATION

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Recombinants genomic sequences of *Trypanosoma cruzi*, orf 639 and orf 819 encoding for homologues of FAD and FMN containing NADPH dependent Cyt P-450 reductases have been cloned and expressed in BL 21(DE3) pLys E cells by using pRSET-A-Tc-639 and pRSET-A-Tc-819 recombinant vectors. Their structural similarities to Cyt p-450 reductases include a NADPH, FAD, FMN, and BH4 domains. single gene copies for both sequences have been demonstrated by southern blot analysis. the transcription of both genes was demonstrated by RT-PCR. Northern blot analysis showed a single transcript of 1.8 kb of ORF 639. Predicted protein molecular masses of 67 kDa and 81 kDa were corroborated by western blot using respective polyclonal antibodies raised against the purified expressed proteins as well as against the His-tag. Both enzymes reduce Cyt c using only NADPH as cofactor. Km for NADPH was 18 µM and 17 µM for Tcr-639 and Tcr-819 respectively. the ability for both enzymes to reduce alternatives substrates and participate in Cyt p-450 dependent dealkylation process was demonstrated in a heterologous system. Tc-639 and Tc-819 reductase activities were inhibited by the flavoprotein specific inhibitor diphenyleneiodonioum (DPI).

ES-P30.
RESIDUES INVOLVED IN THE CATALYTIC MECHANISM AND SUBSTRATE SPECIFICITY OF THE b SUBUNIT IN ACIL-CoA CARBOXYLASES OF *S. coelicolor*

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Acetyl-CoA carboxylase (ACC) and propionyl-CoA carboxylase (PCC) of *Streptomyces coelicolor* are multisubunit complexes. Each complex consist of three different subunits α, β y ε. Both complexes share the same biotinylated subunit α, AccA2. The β and the ε subunits are specific from each of the complexes (AccB-AccE and PccB-PccE to ACC y PCC respectively). ACC and PCC catalyze the carboxylation of acetyl- and propionyl-CoA to generate malonyl- and methylmalonyl-CoA, respectively. The core catalytic β subunits, PccB and AccB, catalyze the transcarboxylation between carboxi-biotin and acyl-CoAs. The crystal structure of Apo and substrate-bound PccB showed a hydrophobic and highly conserved biotin-binding pocket. Biotin and propionyl-CoA bind perpendicular to each other in the active site, where two oxyanion holes were identified. N1 of biotin is proposed to be the active site base. Based on the amino acid sequence alignment and structure comparison between AccB and PccB we identify some key residues. The molecular basis of substrate specificity and the essentiality of oxyanions hole was investigated by mutagenesis. Understanding the substrate specificity of ACC and PCC will help to develop novel structure-based inhibitors that are potential therapeutics against infectious disease. It would also facilitate bioengineering to provide novel extender units for polyketide biosynthesis.

ES-P31

CHARACTERIZATION OF AN ESSENTIAL ACYL-COA CARBOXYLASE FROM *Mycobacterium tuberculosis*

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Pathogenic mycobacteria contain a variety of unique fatty acids which have methyl branches at even-numbered position at the carboxyl end, and a long n-aliphatic chain. One such group of acids are the mycocerosic acids and their biosynthesis is essential for growth and pathogenesis. Therefore, the biosynthetic pathway of the unique precursor of such lipids, methylmalonyl-CoA, represents an attractive target for developing new antituberculous drugs. Heterologous protein expression and purification of the individual subunits allowed the successful reconstitution of an essential acyl-CoA carboxylase from *M. tuberculosis*. The enzyme complex was reconstituted from the a biotinylated subunit AccA3, the carboxyltransferase b subunit AccD5 and the? e subunit AccE5 (Rv3281). The kinetic properties of this enzyme showed a clear substrate preference for propionyl-CoA compared with acetyl-CoA (specificity constant five fold higher), indicating that the main physiological role of this enzyme complex is to generate methylmalonyl-CoA for the biosynthesis of branched-chain fatty acids. the crystal structure of AccD5 was determined at 2.9 A. Extensive in silico screening resulted in the identification of two inhibitors whose K_i are 1-10 μM. Our results pave the first step towards understanding the biological roles of the key ACCases that commits acyl-CoAs to the biosynthesis of cell wall fatty acids, as well as providing a new structure-based drug design target for tuberculosis therapeutic development.

ES-P32.

ISOLATION AND PARTIAL CHARACTERIZATION OF A NEW LIPO-GLYCO-CAROTENOPROTEIN FROM *Pomacea scalaris* (GASTROPODA, AMPULLARIIDAE)

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Carotenoid-binding proteins are commonly found in invertebrates playing roles such as photoprotection, singlet oxygen scavenging or camouflage. Its carotenoids form non-covalent complexes with proteins giving tissues a variety of colors. In mollusks they have been described in only a few species. Within the Ampullariidae, our group has characterized the main perivitellin of *Pomacea canaliculata*, ovorubin, which is a multifunctional glyco-lipo-carotenoprotein complexed with astaxanthin. In the present work we isolated and partially characterized scalarin, a perivitellin of *P. scalaris*, a close relative to *P. canaliculata*. Scalarin was isolated from egg homogenates by ultracentrifugation, and purified by size exclusion chromatography. Protein moiety was characterized by PAGGE, determining its degree of glycosilation and MW. Lipids were analyzed by TLC-FID. Carotenoid extracts were analyzed by HPTLC and spectrophotometry. Scalarin was found to be the major egg carotenoprotein with characteristics of a VHDL (δ=1.26 g/ml) representing 51% by wt. of the total protein of the egg perivitellus. The particle is glycosilated, has a MW of 380 kDa, and it is composed of three subunits of ca. 35, 28, and 24 kDa. Lipids represent a small percentage. Carotenoid analysis revealed the presence of free and sterified astaxanthin, in a pattern similar to that of ovorubin, though the absorption spectrum was blue-shifted. The complex is thermostable up to 100°C.

ES-P33.

CRYSTALLIZATION AND FIRST STRUCTURAL FEATURES OF GUMK, A BETA-GLUCURONOSYLTRANSFERASE INVOLVED IN XANTHAN BIOSYNTHESIS

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Glycosyltransferases (GTs) 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-ß-1,4-glucose-P-P-polyisoprenyl, a intermediate step in the synthesis of the pentasaccharidic subunit of xanthan, an exopolysaccharide produced by *Xanthomonas campestris*. We established crystallization conditions for this membrane-associated protein, obtaining crystals that diffracted to 1.9 Å with synchrotron radiation. Crystals belonged to P6122 group with cell dimensions a= 123.95 Å, b= 123.95 Å, c= 174.50 Å, α= 90°, ß=90°, γ=120°. The observed structure for this enzyme shows a Rossmann-type fold, consisting primarily of α/ß/α sandwiches. The overall fold consists of two separate Rossmann domains connected by a linker region. This structural arrangement corresponds to that seen for the GT-B superfamily. Moreover, this is in agreement with previous results from our laboratory, showing that GumK reaction could proceed even in the absence of divalent cations. Structural alignments shows a very good structural conservation among members of this family, despite poor sequence identity.

ES-P34

BACTERIAL UDP-GLUCOSE PYROPHOSPHORYLASE: MOLECULAR CLONING AND STUDY OF THE RECOMBINANT ENZYME FROM *Streptococcus mutans*

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Streptococcus mutans, a gram-positive bacteria, is a main ethiologycal agent of human dental caries. For this, the interaction between bacteria and biofilms formation are critical issues. Concurrently, synthesis of oligo- and polysaccharides in this organism is central for pathogenicity. Two main enzymes utilize Glc-1P in bacteria: ADPGlcPPase (EC 2.7.7.27), for the synthesis of reserve polysaccharides; and UDPGlcPPase (EC 2.7.7.9), for carbohydrates interconvertion and structural polymers buid-up. These enzymes have been poorly studied in *S. mutans*. We present the molecular cloning of the *galU* gene, coding for UDPGlcPPase from genomic DNA of *S. mutans* ATCC 25175. The identity of the cloned gene was confirmed by complete sequencing. The *galU* was cloned into the pRSET B vector for expression. The purified recombinant exhibited typical hyperbolic saturation kinetics for substrates. Values of K_m for UDPGlc (0.83 mM), PPi (0.40 mM) and for Glc-1P (0.30 mM) were determined. Comparative studies with UDPGlcPPase from other bacteria, as well as with truncated (C-term) mutant enzymes suggest that the N-term and the central domain are responsible for catalysis and substrate binding.

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ES-P35.
ADP-GLUCOSE PYROPHOSPHORYLASE ALLOSTERIC REGULATION. A STUDY ON THE MECHANISM OF ACTIVATION

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ADPGlcPPase (EC 2.7.7.27) is allosterically regulated by metabolites related with the main carbon pathway of the source organism. Residues relevant for activator binding were identified, but the activation mechanism is poorly understood. A single mutation in the sequence of the *Escherichia coli* enzyme, W113A, abolishes activation by fructose-1,6-bisphosphate (FBP). A comparison between the wild type and the mutant enzymes showed that: 1) Both enzymes are tetramers and exhibit identical migration in native PAGE. 2) Both enzymes bind FBP with similar affinity by capillary electrophoresis analysis. 3) Inhibition by chloride ions was notably enhanced by FBP in the wild type but not in the mutant enzyme. Results suggest that W113 is not directly interacting with FBP. However, the W residue would play a relevant role in activation after that a conformational change (induced by FBP) occurs in the loop where it is located. It is hypothesized that the W residue is involved in the translation of the conformational change induced by ligands between subunits.
Granted by: UNL, CAI+D2002; ANPCyT, PICTO'03 01-13241, PICT´03 01-14733, PAV´03 137.

ES-P36.
ANCHOR RESIDUES IN 14-3-3 AND PHOSPHOPROTEIN COMPLEXES DETERMINED BY MOLECULAR DYNAMICS

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Proteins named 14-3-3 can bind more than 200 different proteins, mostly at a phosphorylated state. These partner proteins are involved in different cellular processes, as cell signaling, transcription factors, cellular morphology and metabolism; which suggests pleiotropic functionality for 14-3-3 proteins. Recent efforts to establish a rational classification of 14-3-3 binding partners showed neither structural, nor functional relatedness. We propose that a disorder-to-order transition occurs in the binding of 14-3-3 proteins with their partners. This induced fit process contributes to form the final high-affinity complex. To avoid a kinetically costly search for correct structure, it is necessary that specific amino acid side chain (structurally constrained) helps to stabilize a native-like bound intermediate. We identify the anchor residues in a protein-protein complex of 14-3-3 by using molecular dynamics protocols in explicit solvent box and verify that, even in the absence of their interacting partners, the anchor side chain is found in conformations similar to those observed in the bound complex. Kinetic and thermodynamics implications for the binding between 14-3-3 and its partners are discussed.

ES-P37.
DOMAINS INVOLVED IN ALLOSTERIC REGULATION OF ADP-GLUCOSE PYROPHOSPHORYLASE

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Previous studies in ADPGlcPPase from bacteria evidenced a region critical for activation in the N-term domain. Sequence alignment of ADPGlcPPases from different organisms showed that conserved residues located in loops positioned between regulator- and substrate-binding sites play a role in allosteric activation. Site-directed mutants in these domains of the *Escherichia coli* and *Agrobacterium tumefaciens* enzymes were insensitive to allosteric effectors, fructose-1,6-bisP and fructose-6P, respectively. Interestingly, the *A. tumefaciens* mutants were still activated by pyruvate. To evaluate if this mechanism depends on the type of regulatory metabolite, two equivalent residues in the enzyme from *Anabaena* sp. PCC 7120 (activated by 3P-glycerate) were mutated. Mutants Q58A and W96A of the cyanobacteria enzyme were characterized. Results revealed that, while substrate kinetics were similar to those of the wild type, the mutant enzymes were unable to be activated by 3P-glycerate. Apparently, the activation of cyanobacterial ADPGlcPPase by this metabolite is similar to that exerted by hexose-P in other bacterial enzymes but different to the activation by pyruvate.
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ES-P38.
EFFECT OF ANTI-IDIOTYPE ANTIBODIES AGAINST THOMSEN-FRIEDENREICH DISACCHARIDE ON PROLIFERATION OF EPITHELIAL TUMOR CELLS

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Oligosaccharide chains of mucin-type O-glycans are frequently over-expressed and aberrantly glycosylated in epithelial tumor cells. Core 1 (Galbeta1-3GalNAcalpha-O-) of mucin-type O-glycans, called Thomsen-Friedenreich disaccharide (TFD), is an example of that. *Agaricus bisporus* lectin (ABL) recognizes mainly TFD, showing inhibitory tumor cell proliferation property. The purpose of present project is to obtain antibodies with similar biological characteristic to ABL. Anti-idiotype antibodies was developed using ABL as first template, in rabbit. IgG anti-ABL antibodies purified by affinity chromatography reveal inhibitory ability on interaction between ABL and TFD exposing ligands. This rabbit anti-ABL IgG was used as immunogen in mice and humoral response was analysed. Raised anti-idiotype antibodies (IgG and IgM) recognize glycoproteins secreted by T47D human tumor cell line by ELISA and are inhibited by related TFD molecules. Also, they recognize T47D, MCF7 and HT29 human epithelial tumor cell lines by immunofluorescence and CellELISA. Anti-idiotype antibodies, here developed, show inhibitory activity on proliferation of HT29 and MCF7 tumor cells, with a minor efficiency that ABL. However the inhibitory effect of these anti-idiotype antibodies on tumor cell proliferation is clearly evidenced.

ES-P39.
IMMUNOGENICITY OF BENZYL ALPHA THOMSEN-FRIEDENREICH DISACCHARIDE USING A LINKER ON ITS CARRIER LINKAGE

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Mucin-type O-glycans are upregulated and aberrantly glycosylated in many carcinomas. O-glycan Core 1 (Galβ1-3GalNAcα-O-), called Thomsen-Friedenreich disaccharide (TFD), is an example of a cryptic structure that is over-expressed in cancer cells by changing its glycosyltransferase profile. This molecule is an attractive model to study carbohydrate immunogenicity and a potential candidate for active specific immunotherapy of patients with cancer. The aim of present work is study the influence of a linker in sugar conjugation of BzlαTFD to carrier protein, in the attempt to address the carbohydrate immunogenicity. As linker arm was used Lys_2 and Lys_5, which are covalent linkage to succinylated KLH. The synthetic glycoconjugate was used as immunogen in Balb-C mice. Antibody titers were measured by using ELISA against several antigens. They recognize BzlαTFD and related TFD structures by ELISA. Direct immunofluorescense and CellELISA evidenced that yielded IgG and IgM antibodies bind epithelial tumor cell lines (T47D, HT29 and MCF7), which are partially mediated by related TFD molecules. These antibodies have inhibitory capacity on proliferation of epithelial tumor cells. This work reveals beneficial properties on the use of Lys_n as linker arm of BzlαTFD, in the attempt to direct the immune response to related TFD molecules expressed on epithelial tumor cells.

ES-P40.
PURIFICATION AND CARBOHYDRATE-BINDING CHARACTERIZATION OF *ARUNDO DONAX* LECTIN

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Glycans are key structures involved in biological processes such as cell attachment, migration and invasion. Information coded on cell-surface glycans is frequently deciphered by proteins, as lectins, that recognize specific carbohydrate topology. Here, we describe the purification and carbohydrate specificity of *Arundo donax* lectin (ADL). ADL was purified by affinity chromatography using rabbit erythrocytic stroma incorporated into a polyacrylamide gel. Elution of lectin was carried out with NH_4OH. The purity of ADL was controlled by polyacrylamide electrophoresis (SDS-PAGE), yielding a single proteic band of approximately 35 kDa. ADL shows hemagglutinating activity with blood red cells from rabbit, pork and rat. GlcNAc and related GlcNAc molecules are the more important inhibitors of hemagglutinating activity of ADL. N-acetyl and equatorial C4-hydroxyl residues from GlcNAc are important loci in the carbohydrate-binding recognition of ADL. Adjacent hydrophobic group to GlcNAc, such as alpha benzyl, also shows a partial contribution in the interaction. ADL shows ability in binding to HT29 and T47D human epithelial tumor cells by direct lectin fluorescence. ADL could be a useful tool in detection of Glc NAc glycoconjugates, with potential application in oncology area.

ES-P41.
SIALYLATION BY RECOMBINANT TRANS-SIALIDASE OF THE SYNTHESIZED OLIGOSACCHARIDES, *O*-LINKED IN *Trypanosoma cruzi* GLYCOPROTEINS

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The mucin-like glycoproteins of *T. cruzi* have novel *O*-linked oligosaccharides which are acceptors of sialic acid in the *trans*-sialidase (TcTS) reaction. This process is involved in infection and pathogenesis. The *O*-chains may be derived from the two cores, Gal*p*(β164)GlcNAc or Gal*f*(β164)GlcNAc by further branching with various units of Gal*f* and/or Gal*p*. The presence of galactofuranose is related to the lineage and was found in the G, DM28C and Tulahuen strains of *T. cruzi*.
We have chemically synthesized the oligosaccharides containing 3-5 sugar units. The acceptor properties were studied in order to correlate their structure with the ability to act as substrates. Recombinant TcTS (A. C. C. Frasch (UNSAM)), and sialyllactose as donor were used. The reactions were analyzed by HPAEC-PAD. The Km values were calculated for the free sugars and the benzyl glycosides. The highest affinity was shown by the trisaccharide.
The pentasaccharide, the major *O*-linked sugar in the mucins, presents two terminal β-D-Gal*p* for possible sialylation. A preparative TS reaction was performed with the benzyl glycoside of the pentasaccharide. One of the two external Gal*p* units was selectively sialylated, as shown by NMR spectroscopy.

ES-P42.
DETECTION OF HELIX-HAIRPIN-HELIX DNA GLYCOSYLASE PROTEINS IN PLANTS

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The Helix-hairpin-Helix (HhH) DNA glycosylase is a taxonomic widespread superfamily of DNA binding proteins involved in repairing a spectra of oxidated and alkylated bases from DNA. This structural motif appeared before the origin of the three domains of life explaining the high sequence diversity found in this superfamily. This high sequence divergence makes difficult the recovery of homologous sequences. For that reason in this work we used structural representative members of this superfamily taken from CAMPASS structural database. With one of each protein we did sequential searches using PSI-BLAST until convergence. From these sets of sequences we retrieved 71 plant representatives. In order to obtain a reliable alignment, we detected the HhH domain using CCD and with this domain we obtained an alignment. With this alignment we obtained a maximum likelihood phylogeny which mainly represents the functional divergence of the families. We found that plants contains representatives of AlkA -Alkyladenine glycosylase-, Endonuclease III (Nth), MutY, Oxoguanine glycosylase and 3-Methyl Adenine DNA Glycosylase I (TAG). With this phyogeny we studied the distribution of HhH DNA glycosylases in plants and also their possible fuctional adaptations.

ES-P43.
STARCH-SYNTHASE III FAMILY ENCODES A TANDEM OF THREE STARCH-BINDING DOMAINS
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The starch-synthase III (SSIII) is one of the enzymes that participate in the synthesis of starch in plants. SSIII from *Arabidopsis thaliana* contains a 557-amino acid N-terminal SSIII-specific domain with three internal repeats. Based on similarity searches, fold class assignment methods and homology modeling, we found that each of the repeats encodes for a starch-binding domain (SBD). Although the SSIII from *A. thaliana* and its plants and algae homologous show no detectable sequence similarity with characterized SBD, the sequential and spatial conservation of residues known to be involved in binding of starch gives a further support for this assignment. As the majority of SBD are found in degrading starch and glycogen microbial enzymes, this is the first report of an SBD both in plants and in a synthesizing enzyme. This results offer important information not only for the evolutionary and functional-structural aspects of the SBD domain, but also for physiological issues in plant starch metabolism.

ES-P44.
THE CHAPERONE HOLDASE ACTIVITY OF HUMAN PAPILLOMAVIRUS E7 ONCOPROTEIN
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E7 oncoprotein is the major transforming activity in human papillomavirus and shares sequence and functional properties with adenovirus E1A and SV40 T antigen, in particular by targeting the pRb tumor suppressor. HPV 16 E7 forms spherical oligomers that display chaperone activity in thermal denaturation and chemical refolding assays of two model polypeptide substrates: citrate synthase and luciferase, and it does so at sub-stoichiometric concentrations. We show that the E7 chaperone stably binds the polypeptides en route to denaturation or renaturation and holds them in a near-native state, but does not bind the fully native proteins. However, the E7 oligomers bind native pRb without the requirement of it being partially unfolded. A fragment containing the N-terminal domain of E7 can interfere with pRb binding but not with the chaperone activity. Thus, the E7 oligomer displays a non-specific chaperone activity that could explain its wide target specificity. The ability to bind up to ~72 molecules of pRb appears as essential either for sequestering pRb from Rb-E2F complexes or for targeting it for proteasome degradation.

ES-P45.
INCREASED STABILITY AND DNA BINDING OF MONOMERIC VARIANTS OF THE DIMERIC DNA BINDING DOMAIN OF THE HUMAN PAPILLOMAVIRUS E2C TRANSCRIPTIONAL REGULATOR
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Human papillomavirus infects millions of people worldwide and is a causal agent of cervical cancer in women. The expression of all viral genes is controlled by the E2 transcriptional regulator, which also participates in DNA replication, and the recognition of DNA is carried out by the C-terminal dimerization domain (E2C). In late stages of the infection, E2 represses the expression of the two main oncoproteins E6 and E7, and disruption of the E2 ORF upon insertion in the host genome was proposed as an irreversible step towards cell transformation. In order to test the hypothesis of improving stability and DNA binding affinity of E2C we constructed monomeric variants by inserting a neutral linker between two consecutive E2C genes, generating a monomeric species. The two best expressing variants, with 6 and 12 residue linkers (E2Csc-6 and E2Csc-12), were purified and characterized. Both show overall identical conformation to the natural dimer as judged by CD and fluorescence spectroscopy. Urea denaturation experiments show cooperative transitions with their stabilities largely improved by 4.7 kcal.mol^{-1} for the E2Csc-12, and 3.0 kcal.mol^{-1} for the E2Csc-6 residue linker. At 150 mM phosphate, E2Csc-12 displays a *KD* of 0.5 ± 0.1 nM compared to 2.52 ± 0.3 nM for E2C. Binding of E2Csc-6 shows a deviated salt dependence indicative of differential release of ions upon binding and suggesting a strained conformation compared to the 12 residue linker and E2C. Finally, calorimetric experiments show very similar ΔH values around 22 kcal.mol^{-1}, further supporting an identical interface to the reference dimer, with similar and low entropic contribution, indicative of a mainly enthalpically driven process.

ES-P46.
UNFOLDING OF DNA BINDING HELIX OF HPV E2 DNA BINDING DOMAIN LEADS TO AMYLOID FORMATION VIA A β-SHEET OLIGOMERIC INTERMEDIATE
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The DNA binding domain of papillomavirus E2 proteins is at the centre of the regulation of gene transcription and replication of the virus. It is a dimeric ß-barrel domain which combine an eight-stranded dimeric ß-barrel core interface with two symmetrical DNA binding α-helices and other two helices, all packed against the central barrel. Treatment with low amounts of trifluoroethanol at pH 5.6 and room temperature leads to the slow loss of α-helix content to yield a mostly ß-sheet species. This species subsequently undergo an even slower transition into amyloid aggregates, as determined by light scattering, Congo Red and Thioflavin T binding, reporters of amyloid-like structures. Electron microscopy shows the presence of short amyloid fibres with a curly aspect. The strong concentration dependence of formation of the ß-sheet intermediate indicates an oligomerization process. The ß-oligomeric intermediate losses its near-UV circular dichroism signal, indicating the exposure of the native buried tryptophan residues to the solvent. The formation of the oligomeric intermediate is completely prevented by addition of stoichiometrical amounts of a DNA duplex containing the specific E2 binding site strongly suggesting that stabilization of the DNA binding α-helix prevents the formation of the intermediate. This, together with the clear loss of α-helix, indicates an a-helix to ß-sheet transition for the formation of the intermediate oligomer.

ES-P47.

MOLECULAR BASIS FOR PHOSPHORYLATION DEPENDENT PEST MEDIATED PROTEIN TURNOVER

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Rapid protein turnover regulates the intracellular levels of proteins involved in key cellular processes. In many cases, proteasomal-mediated degradation of these proteins is modulated by phosphorylation of short PEST sequences. The E2 protein from bovine papillomavirus participates in essential steps in the life cycle of this transforming virus, such as gene transcription, DNA replication and episomal genome maintenance. Phosphorylation of a PEST sequence located in the flexible hinge region of E2 accelerates its degradation, determining the level of viral genome copy number. To investigate the effects of phosphorylation on the conformation and stability of this region, we determined the structure of a p29 amino acid peptide fragment containing the PEST sequence (E2-PEST) by NMR methods. The E2-PEST peptide has pH dependent polyproline II and α-helix structures, connected by a turn around the actual PEST sequence. The overall structure is disrupted by phosphorylation, in particular that at serine 301, and substitution of different residues within this region can have either stabilizing or destabilizing effects. There is an excellent correlation between the structural stability of different peptides *in vitro* and the resulting half-life of full-length E2 proteins containing the same mutations *in vivo*. The structure around PEST region is not unfolded and appears to have evolved to display a marginal stability, finely tunable by phosphorylation or mutations. We propose that conformational stability, rather than recognition of a phosphate modification, modulates the processing of PEST sequences by the proteasome machinery or other proteases.

ES-P48.

STRUCTURAL BASIS FOR SPECIFIC DNA RECOGNITION BY AN ANTIBODY

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We have obtained high affinity monoclonal antibodies against the 18mer duplex DNA site of the human papillomavirus E2 protein, and characterized their unusual thermodynamic properties. We found that the antibody ED10 can recognize one of the strands of the duplex DNA with subnanomolar affinity, but not the other. Shortening the oligonucleotide down to 4 bases while leaving the 5' intact, display a binding affinity as high as the 18mer single stranded DNA. We crystallized the free Fab and bound to a 6mer single stranded oligonucleotide, which diffracted to 2.7 and 1.9 Å, respectively. Only the first two bases from the 5' end (dTdC) display electronic densities at the antibody binding site, the rest are flexible. We observe at least 4 hydrogen bonds and the bases make substantial hydrophobic interactions with aromatic residues. There is a substantial conformational change to allow DNA binding. The antibody can thus recognize two bases at the 5'end, even in the 18mer duplex, but recognition of duplexes without dTdC at the 5' end strongly suggest two recognition modes, a preferential high affinity single-stranded mode we show here and a double stranded mode for which we have no structural evidence. The flexibility of the antibody or the existence of conformers in solution can explain this mode.

ES-P49.

STRUCTURAL BASIS FOR THE ENHANCED TRANSCRIPTIONAL CONTROL BY THE HUMAN PAPILLOMAVIRUS STRAIN-16 E2 PROTEIN

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High risk HPV16 is responsible for the largest percent of the cervical cancers linked to human papillomavirus infection and the E2 protein is a key factor for transcriptional regulation of all viral genes. We present the first structure for the DNA binding domain of HPV16 E2 bound to DNA, and in particular, a natural cognate sequence. The structure was obtained by refining the structure of the free protein using NOE and RDC restrains from the complex. The NMR structure reveals that the overall conformation remains virtually unchanged and chemical shift analysis of the protein bound to a shorter duplex uncovered a contact out of the minimal E2 DNA binding sequence, made by lysine 349, located in ß2 loop, packed against the DNA binding helix. This contact was confirmed by titration calorimetry and mutagenesis, with a contribution of 1.0 kcal mol of this interaction to the overall binding energy. HPV16 E2 is known to have the strongest DNA binding affinity, related to a strict positive and negative control, where the latter translates into the repression of the expression of the E6 and E7 oncogenes, responsible for carcinogenesis. The novel features not previously observed in any related structure provide structural and thermodynamic basis for the tight transcriptional control of this high risk strain.

ES-P50.

ENZYMATIC HYDROLYSIS OF MILK PROTEINS BROUGHT ABOUT BY AN ASPARTIC PEPTIDASE FROM *SILYBUM MARIANUM* FLOWERS

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The flowers of different species of cardoon (*Asteraceae*) have been characterized once they are a rich source of aspartic peptidases with milk clotting activity, such as *Cynara cardunculus* L. flowers which are used in traditional cheesemaking in the Iberian Penynsula. This study was aimed at characterizing the enzymatic action of the aspartic peptidase presents in flowers of *Silybum marianum* (L.) Gaertn. (*Asteraceae*) in terms of the degradation of caseins. The proteolytic activities toward Na-caseinates from caprine and ovine milks were studied in a comparative fashion using urea-PAGE, tricine-SDS-PAGE, densitogram analysis, electroblotting and sequencing. Caprine α_{s1}- and β- caseins were degraded up to 68%, and 40% respectively during 24 hours of incubation. Only one important and well defined band of 14,4 kDa, a fragment of β-casein, was observed after 12 hours of hydrolysis. On the other hand, after 24 hours of incubation, the ovine α_s- and β-caseins were degraded up to 76%, and 19 % respectively. In what concerns to specificity of the proteolytic activity towards ovine caseinate the major cleavage site was Leu99-Arg100 in α_{s1}-casein.

ES-P51.
PROPERTIES OF AN ASPARTIC PROTEINASE ISOLATED FROM *SILYBUM MARIANUM* FLOWERS
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Plant aspartic proteinases (Aps) have been purified and characterized from a variety of tissues from several species, even though its functions are still unclear. The aim of this work was to study the specificity toward insulin of a peptidase from flowers of *Silybum marianum* (L.) Gaertn. (*Asteraceae*).
The proteolytic activity toward the oxidised insulin ß-chain was studied at differents conditions of time, pH and in the presence or absence of peptidase group specific inhibitors.
The enzyme was inhibited only by pepstatin, an aspartyl endopeptidase specific inhibitor. The hidrolysis of the insulin ß-chain was highly specific at pH 3,0 and less specific at pH 6,0. N-terminal amino acid sequence determination of peptides released at pH 3,0 indicated that the first peptide bound hidrolysed was Tyr16-Leu17, and after an extensive incubation at the same pH the Phe25-Tyr26 bound was also cleaved.
Thus AP from flowers of *S. marianum* shows, like most APs, specificity for peptide bonds located between or next to amino acid residues with large hydrophobic side chains. However, its selectivity on oxidised insulin ß-chain is much higher than reported for any other plant APs tested with this substrate.

ES-P52.
STUDY OF THE INTERACTION BETWEEN THE R AND C SUBUNITS OF THE PROTEIN KINASE A IN THE FUNGUS *MUCOR CIRCINELLOIDES*
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Protein kinase A is a tetramer composed of a dimer of regulatory subunits (R) and two monomeric catalytic subunits (C). In the holoenzyme C interacts with the pseudosubstrate site in R. This pseudosubstrate site and additional contact sites in R render an affinity in the nanomolar range. The affinities between R and C subunits of PKA are quite different depending on the specie, finding the highest values in *M.rouxii* and *M.circinelloides*.
Our hypothesis is that in the amino-termini region of R subunits exist elements that interact with C subunits that are responsible for the different affinity. The wild type R subunit and two mutant, R1 laking the N-terminal and R2 lacking a region of acidic residues were cloned and expressed in a *S.cerevisiae* strain tpk2$^-$ tpk3$^-$ bcy1$^-$ (133). Interaction between wtR or mutants R, and homologous or heterologous C subunit was evaluated by *in vivo* and *in vitro* assays. *In vivo* assays were made in yeast analyzing differences in phenotypes and growth between the different transformants. *In vitro* assays were made assaying the inhibition of C homologous or heterologous by each R subunit and dissociation of holoenzymes reconstituted. The IC50 for wtR was lower than those for mutant Rs. Dissociation of reconstituted holoenzyme showed that the concentration of cAMP required to dissociate the wtR-C holoenzyme was higher than the concentration requeried to dissociate the mutant R-C holoenzyme These results indicates that the acidic residues region may be important in the high affinity interaction R-C found in *M.rouxii* and *M.circinelloides* PKA.

ES-P53.
ACTIVATION OF THE PLASMA MEMBRANE Ca^{2+} PUMP AS REVEALED BY FRET BETWEEN BFP AND GFP FUSED TO THE N AND C TERMINUS
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The autoinhibition of the plasma membrane Ca^{2+} pump (PMCA) is believed to involve the interaction of the C-terminal domain with the catalytic region which contains the domains A and N (Bredeston and Adamo. 2004. *J. Biol. Chem.* 279:41619-41625). Calmodulin binds to the inhibitory sequence and promotes an activated state characterized by high affinity for Ca^{2+} and high transport activity. The BFP was inserted after Thr2 of the human PMCA isoform 4xb while the GFP was located at the COOH-terminal. Excitation of the BFP-PMCA-GFP at 387 nm resulted in a differential GFP emission by energy transfer (FRET) at 509 nm. The calculated average distance between chromophores (r) in the BFP-PMCA-GFP was 45 Å. The activation of the PMCA by the addition of 10 µM Ca^{2+} and 200 nM calmodulin increased the value of r to 50 Å. A similar value of r was obtained when the PMCA was activated by phosphoinositides in the presence of 0.5 mM EGTA. Under these conditions the addition of Ca^{2+} calmodulin caused no further change. According to these results, the NH_2 terminus and the COOH terminus of the PMCA would re-orient or slightly separate during activation.
Supported by grants of CONICET and UBA

ES-P54.
STUDY OF THE PARTICIPATION OF THE HINGE REGION OF THE REGULATORY SUBUNIT IN THE INTERACTION WITH THE CATALYTIC SUBUBNIT OF FUNGAL CAMP-DEPENDENT PROTEIN KINASES (PKA) THROUGH ENZYMATIC KINETICS
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There are great differences among species in the affinity between regulatory (R) and catalytic (C) subunits of PKA. These differences can not be explained by the present structural data. There is a striking sequence diversity in the hinge region. The aim of this work is to test if the hinge region interacts with C and if it can account for the differences observed among species in the R-C affinity. To approach this issue we have first compared the ability of several peptides to inhibit the phosphorylating activity of *Mucor rouxii*, *Saccharomyces cerevisiae* and bovine heart C. The peptide sequences correspond to the autophosphorylation site plus a variable region toward the N-termini of R. We did not observe the competitive inhibition expected, but a dual effect of peptides: they inhibited the phosphorylation as well as they activated it. The whole R had also this dual effect on kemptide phosphorylation.The results are in accordance with idea of an activated holoenzyme. In addition, we compared the kinetic parameters of the peptides phosphorylable version. The results show for the three enzymes that the longer the peptides, the higher were their V_{max} and the lower their K_m. We conclude that the R region studied participates in the interaction and does not account for the differences observed in the R-C affinity among species.

ES-P55.

TERATOGENICITY TESTING OF HEXACHLORO-BENZENE (HCB). ITS INFLUENCE ON CHICK EMBRYO HEAM METABOLISM

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42 fertile eggs were incubated and after 12 days were injected in the yolk with 4-8-20-30 and 50 mg HCB/egg using dimethilsulfoxide (DMSO) as vehicle. To dose-dependence studies, incubation "in ovo" continued for 4 hs: 16 and 32% of mortality was registered only for 30 and 50 mg HCB/egg. Developmental anomalies were sporadic being the liver the organ mainly affected. To time-dependence studies (4, 12, 36 and 60 hs) 20 mg HCB/egg was chosen. It was observed a time-dependence relationship out lighted by the increased fluorescence in liver, revealed under UV lamp. Four heam biosynthetic pathway enzymes activities were determined "*in vivo*" on liver and yolk sac membrane (YSM). DMSO increased enzymes activities significantly, and in the same magnitude, due to the requirements of Cyt P-450 synthesis to help drug detoxification. Deltaminolevulinic acid dehydratase activity was not significantly modified along the period assayed. Inhibition of Uroporphyrinogen Decarboxilase and Coproporhyrinogen Oxidase, and the slight activation of Porphobilinogen Deaminase, the rate limiting enzyme of this metabolic pathway, explain the fluorescence observed in liver due to porphyrins accumulation. Liver enzymes were more affected than YSM ones. This study shows that HCB affects heam biosynthesis in chick embryos and it is more affected in liver than in YSM. We can also conclude that the xenobiotic is neither particularly letal nor teratogenic.

ES-P56.

RELATIONSHIP BETWEEN HEME OXYGENASE-IRON RELEASE AND GLUTATHIONE SYNTHESIS

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Glutathione (GSH) is the most abundant nonprotein thiol in mammalian cells, and it is responsible for maintaining an intracelular reducing environment. GSH depletion induces heme oxygenase (HO-1) activity, which catalyzes heme degradation to carbon monoxide, free iron and biliverdin. It had been demonstrated that iron causes a biphasic change on GSH content. The object of this study was to evaluate whether exists a relationship between HO-1 induction, free iron and restoration of GSH level in rat liver. Albino Wistar rats (160-180 g) were injected ip. with diethyl maleate (DEM) (a GSH depletor) (4 mmol/kg). After DEM treatment, a 58% decrease in GSH was observed (3h), increasing thereafter up to control levels (9 h) and reaching a higher level (27% over to control animals) at 12 h. HO-1 activity showed a maximum at 9 h (200% over the controls) and remained increased until 18 h after DEM treatment. The administration of a HO inhibitor, ZnPPIX (100 µmol/kg), simultaneously with DEM decreased HO activity at 12 h, and consequently GSH levels remained diminished. The iron chelator, 1-10-phenanthroline (20 mg/kg), abolished GSH restoration when DEM was administered. To sum up, our results support the hypothesis that HO-iron release is necessary to recover GSH content.

ES-P57.

BACTERIAL GLYCOGEN SYNTHASE: AUTOGLYCOSYLATION PRODUCTS CHARACTERIZATION

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Bacterial and plant glycogen synthase (GS), the enzymes responsible for the synthesis of glycogen and starch, the most widespread energy storage compounds belong to GT5 Glycosiltransferases family.
Agrobacterium tumefaciens GS catalyses two reactions: initiation (unprimed reaction) and elongation (primed reaction), however the intimate mechanism by which glycogen/starch is synthesized remains unsolved. Kinetics studies revealed a biphasic reaction during unprimed reactions. The identification by HPAE-PAD of the products formed during the initial phase revealed the accumulation of two intermediaries, maltotriose and maltotetraose that decayed during the time course of the reaction.
Combining both, kinetic studies and product characterization let us to suggest that maltotriose and maltotetraose are the primers of the high molecular weight oligosaccharides formed during the elongation reaction.

ES-P58.

REMOTE MUTATIONS ENHANCE METALLO-b-LACTAMASE ACTIVITY

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Metallo-β-lactamases (MBLs) represent the latest generation of β-lactamases. The structural diversity and broad substrate profile of MBLs allows them to confer resistance to most β-lactam antibiotics. In order to explore the evolutionary potential of these rather incipient enzymes, we have subjected the *Bacillus cereus* MBL (BcII) to a directed evolution scheme, that resulted in an increased hydrolytic efficiency towards cephalexin. A systematic study of the hydrolytic profile, substrate binding and active site features of the evolved lactamase reveals that directed evolution has shaped the active site by means of remote mutations to better hydrolyze cephalosporins with small, uncharged C-3 substituents. One of these mutations is found in related enzymes from pathogenic bacteria, and is responsible for the increase in the enzyme's hydrolytic profile in the clinical setting. It can be concluded that: (1) MBLs are able to expand their substrate spectrum without sacrificing their extant hydrolytic capabilities, (2) directed evolution is able to mimic mutations that have occurred in Nature, (3) the metal-ligand strength is tuned by second shell mutations, thereby influencing the catalytic efficiency, (4) changes in the position of the second Zn(II) ion in MBLs affect the substrate positioning in the active site. Overall, these results show that evolution of enzymatic catalysis can take place by mutations that exert a supramolecular control of reactivity.

ES-P59.
AN INSIGHT INTO THE SECOND ZN SITE OF THE DINUCLEAR BCII METALLO β-LACTAMASE
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Metallo-β-lactamases (MBLs) are zinc-dependent enzymes able to hydrolyze a broad range of β-lactam antibiotics. Their active sites are able to bind up to two Zn(II) ions. The MBL B. cereus BcII enzyme contains three his residues and one OH^- as Zn1 ligands, while the Zn2 ligands are His, Asp and Cys residues. Up to date, the role of each site in the enzyme has not been elucidated.

In an attempt to study the function of Zn2 site, we constructed a mutant in which all the Zn1 ligands were replaced by ser residues. This mutant enzyme (Zn2-BcII) was able to bind only 1 Zn(II)/ mole. The activity against several b-lactams compounds was severely compromised. Electronic spectra of the Co(II)-substituted species suggest that the metal ion is tetracoordinated with one Cys ligand. Paramagnetic NMR spectra of the Co(II)-substituted enzyme allow us to confirm that this mutant binds only one metal ion in the Zn2 site. Circular dichroism results suggest a structural role for the Zn(II) ion. Overall, our results imply that the Zn1 site is essential for the enzyme activity, unlike the CphA and ImiS MBLs which are fully active only with one Zn(II) in the Zn2 site.

ES-P60.
ISOLATION, PURIFICATION AND PHYSICOCHEMICAL CHARACTERIZATION OF A NOVEL TRYPSIN INHIBITOR ISOLATED FROM *CALLIANDRA SELLOI* SEEDS
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Plants synthesize a variety of molecules including proteinase inhibitors which play an important role as defense proteins against plant predators. In this work, a novel trypsin inhibitor (CSTI) was isolated and characterized from *Calliandra selloi* seeds. Seeds were ground and the saline extract was submitted to affinity chromatography on a trypsin agarose column followed by ion exchange chromatography on a DEAE cellulose column. SDS-PAGE showed a band of *Mr* 20000 and mass spectrometry indicated the presence of two proteins of *Mr* 20100 and 20299, probably due to glycosylation. Tryptic inhibitory activity was measured using BAEE (N-Benzoil-L-arginine ethyl ester) as a substrate, and chymotryptic inhibitory activity using BTEE (N-Benzoil-L-tyrosine ethyl ester). CSTI was able to inhibit the activity of both trypsin (Ki: 2.61 10^{-7} M) and chymotrypsin. CSTI amino terminal sequence was determined by automatic Edman degradation (NQQEVLLDTDGDILRNGGXYYI…) showing homology to plant Kunitz inhibitors. Furthermore, circular dichroism on the far UV of CSTI was similar to soybeans Kunitz trypsin inhibitor, showing that it belongs to the β-II protein family.

ES-P61.
CRYSTAL STRUCTURE OF THE E2C SINGLE CHAIN DNA-BINDING DOMAIN FROM HUMAN PAPILLOMAVIRUS
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The papillomavirus E2 proteins regulate the transcription of all papillomarirus genes and are necessary for viral DNA replication. The E2 DNA binding domain (E2C) displays an unusual dimeric ß-barrel topology only shared by the Epstein-Barr origin binding protein EBNA1. Monomeric variants of the HPV16 E2C were constructed in the lab by inserting a neutral linker (6, 9 and 12 residues) between two consecutive E2C genes, to address folding, thermodynamics and DNA binding issues. The E2Csc-12 was purified crystallized by the vapor diffusion method using hanging drops at room temperature. The drops were formed by mixing an equal volume of a 6 mg/ml protein solution with the reservoir solution that contained 80 mM sodium citrate (pH 5.21), 1.53 M $(NH_4)_2SO_4$ and 150 mM Na^+ and K^+ tartrate. Hexagonal crystals grew in few weeks and diffraction data were recorded on a Mar CCD detector from a crystal maintained at -170ºC. All images were indexed; the reflections were integrated, scaled and postrefined with MOSFLM and CCP4 package. The data diffracted down to 1.8 Å. The structure was determined by molecular replacement using a model of dimeric E2C HPV16 and the program AMORE and finally refined using CNS program. The structure revealed two additional residues in the ß3 sheet, A327 and A328 and the presence of a sulphate anion near two lysine residues, K352 and K359 of a symmetry molecule.

ES-P62.
KINETIC CHARACTERIZATION OF CYSTEINE ENDOPEPTIDASES FROM SOME MILKWEED LATEX
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Milkweed latex endopeptidases have been demonstrated to show a strong biochemical similitude to papain. To confirm their similar hydrolytic properties, different synthetic substrates were assayed for some *Asclepiadaceae* species latex endopeptidases. The synthetic substrates used were: Z-aa *p*-nitrophenyl esters derivatives, Bz-Phe-Arg *p*-nitroanilide, Bz-Arg-Arg *p*-nitroanilide and PFLNA. Preferencies and kinetic parameters (Km, Km/kcat and Vm) were calculated and compared. Z-Gln *p*-nitrophenyl ester was the most preferred substrate for asclepains (Km 0.0503 mM and 0.1634 mM, resepectively) and araujiains from *Araujia hortorum* (Km 0.024 mM, 0.238 mM and 0.099 mM, respectively). Likewise, araujiain a II from *A. angustifolia* also showed the highest preference for the same derivative, whereas araujiain a I, a III and funastrain c II (Km 0.024 mM) from *Funastrum clausum* for the Z-Ala *p*-nitrophenyl ester derivative. The allosteric behaviour of araujians from *A. angustifolia* prevented the calculation of their kinetic parameters. For PFLNA as substrate, the Km values were: asclepain c I 0.8183 mM, araujiain a II 0.18 mM, araujiain a III 5.14 mM and funastrain c II 0.1011 mM. The other endopeptidases showed no affinity for this substrate. As a conclusion, the endopeptidases studied showed a remarkable preference for two Z-aa *p*-nitrophenyl ester derivatives: Ala and Gln. The Km values obtained for the PFLNA derivative were in the same order than the one for papain; all these data support the idea that these endopeptidases belong to the papain-like family.

ES-P63.

COMPATIBLE SOLUTE (TREHALOSE)-MEDIATED STABILIZATION OF PROTEINS. STUDIES ON THE PLASMA MEMBRANE H^+-ATPase OF *Kluyveromyces lactis*

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Many industrial applications require the use of proteins and enzymes under conditions that may result in inactivation, making it desirable to develop methods that stabilize the structure and function of proteins. In nature, when cells from different organisms are subjected to adverse conditions, they present the stress response, i.e. the expression of heat shock proteins and the production and accumulation of compatible solutes. As soon as the stress condition passes, compatible solutes are rapidly degraded. We have observed that compatible solutes stabilize the function and structure of the isolated plasma membrane H^+- ATPase from *K lactis* against high temperature or dessication. Among the different compatible solutes tested, trehalose exhibited the highest protective activity. In addition, we have observed that upon returning to ideal conditions, compatible solutes inhibit the activity of the H^+-ATPase, explaining why these solutes have to be eliminated as soon as possible. Profiting from the compatible solute-mediated stabilization of H^+-ATPase, the isolation method was modified. The resulting, highly pure, enzyme was used for kinetic studies. High variations in fluorescence intensity were observed upon substrate binding. The H^+-ATPase exhibited cooperative behavior and migrated in non-denaturing gels as an oligomer.

ES-P64.

DESIGN AND EXECUTION OF AMINO ACID MOLECULAR MODELS TO BUILD 3-D PROTEIN STRUCTURES: UNDER GRADUATED STUDENTS' LEARNING EVALUATION

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The understanding of the protein structure has fundamental importance so that the students understand its function; however they found difficult to visualize the three-dimensional structure of these molecules. The graphic computation has facilitated the understanding, but there is not the freedom for handling and interaction. To date, models that represent the three-dimensional structure of the biomolecules, built from representative units, have been supplied for students and teachers enabling a better visualization of the molecular phenomena and its importance in the structure/function relationship. In this sense, the present proposal will be defining models that simulate the units of the 20 amino acids with the aim of producing them in industrial scale. The models will be composed of plastic pieces that can demonstrate the interaction among the functional groups (polarity, electro negativity, mass) that will be represented by colors, shapes, appropriate sizes, facilitating the assembly and interpretation of the 3-D structure. For the drawing the softwares of CAD (Computer Aided Design) Rhinoceros® and Autodesk Inventor® have been used. They allow the visualization of the 3-D amino acid structures, minimizing mistakes and optimizing the prototype construction. Were designed Cα content connection points to amine, hydrogen and side chains; hydrogen atoms and connections pieces that represent hydrogen and covalent bonds will be represented by different standard pieces. The pieces for amino and carboxyl groups have different colors and shapes, besides the connection points that will allow the bond in the Ca representing the peptide bond. From computational models, prototypes will be made so that they will turn out into physical models, and then some tests will be applied to check pieces fitting and assembly to build protein secondary structures. The models will be presented to professors, researches, and graduated students in the X PABMB meeting for a first evaluation. In this term it will be realized adaptations and/or corrections in the proposed and the CAD design will be used to build molds in steel that will allow the industrial production by thermoplastic injection process. The last term will consist of an effective evaluation as a facilitative didactic tool of the teaching/learning process in the Structural Molecular Biology area.

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ES-P65.

STRUCTURAL BASIS FOR 8-OXO GUANOSINE INSERTION AND EXTENSION BY T7 DNA POLYMERASE

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8-Oxo-guanosine (8oG) is a highly mutagenic DNA lesion. The mutagenic capabilities of 8oG reside in its capabilities to base pair with dCTP and dATP. We used the technique of X-ray crystallography to solve structures of complexes containing the 8oG lesion, forming base pairs with dCTP and dATP. We observed that an 8oG$dATP mismatch is not recognized as a mutagenic event, because the 8-oxo group mimics the position of the O_2 group of a Thy$dATP pair. We determined the crystal structure of an insertion 8oG$dCTP complex and observed that Lys536 forms a hydrogen bond with the 8oG lesion in its *anti* conformation, favoring dCTP incorporation. A structural model suggested that Lys536 impinges dATP misincorporation by preventing the 8oG (*syn*) conformation, as crystallization attempts to trap an insertion 8oG$dATP mismatch were futile. We engineered a Lys536Ala mutant whit altered kinetic parameters, which preferentially misincorporates dATP. We solved the crystal structure of this mutant enzyme with an 8oG$dATP mismatch, and observed that dATP incorporation is impaired by steric-electrostatic forces.

GE-P1.

THE TRANSCRIPTION FACTOR KLF6 BINDS *IN VIVO* TO THE ADENOVIRUS E2A PROMOTER AND PHYSICALLY INTERACTS WITH ATF7

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Krüppel-like factor 6 (KLF6) is a ubiquitously expressed member of the Krüppel/Sp1-like family of transcription factors sharing three zinc fingers as a signature DNA binding motif with specificity for GC-boxes promoter elements. Current data support a KLF6 function in the regulation of cell proliferation though the target genes for *in vivo* binding of KLF6 have not been still identified. Putative consensus KLF6 target sites were identified within the E2A transcription unit of *Adenovirus* neighbouring to the ATF/CRE binding motif as determined by *in vitro* PCR-assisted binding selection assays. In this work we demonstrate that KLF6 binds to the E2A promoter *in vivo* by ChIP and Real-time PCR approaches in lung epithelial cells upon *Adenovirus* infection. Interestingly, expression of endogenous KLF6 protein was modified during the infection course reaching a maximum level after 6 h. and then decreased correlating with enhanced expression of the E1A viral oncoprotein. On the other hand we determined that KLF6 interacts physically with ATF7 whose role in the E2A transcriptional activation has been demonstrated. Thus we provide evidences that the physiological function of KLF6 and ATF7 transcription factors is subverted during *Adenovirus* infection to assure transcription of viral genes and thereby disturbing their control of normal cell proliferation.

GE-P2.
ZFHEP-1 AND -2 BINDING IS REGULATED BY PKC-INDUCED PHOSPHORYLATION

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The activity of a transcription factor (TF) can be controlled, for instance by post-translational modification such as phosphorylation. Zfhep (Zinc Finger Homeodomain Enhancer-binding Protein) is a TF involved in lymphopoiesis, neurogenesis and myogenesis and it is expressed as two isoforms, Zfhep -1 and -2 which lacks the N-terminal DNA-binding domain of the larger Zfhep-1. Zfhep exists as two phosphorylated forms. Our goal was to examine the effect of phosphorylation on Zfhep binding to its promoters. Zfhep expressing cell lines were treated with 10 ng/ml phorbol esters (PMA) and 500 ng/ml ionomycin (IO) for 30 min. Nuclear extracts (NE) from untreated cells were incubated with calf intestinal phosphatase (CIP) or with CIP + phosphate. Band shift assays were performed with Jurkat/CHO-K1/COS-7 NE (CIP, CIP+phosphate or PMA/IO treated) and Zfhep-2 programmed rabbit reticulocyte lysates in the presence of [32P]-labeled oligonucleotides harboring Zfhep binding sites from Zfhep, α4integrin, CD4 and p73 promoters. Probes and NE were incubated for 1 h at 20ºC. CIP- treated samples increased their binding capacity to all the probes assayed. Zfhep -1 and Zfhep -2 showed similar results. Retardation complexes were competed by either anti-Zfhep antibodies or an excess of cold probe. PMA/IO- treated cells shown no band of retardation. The results show that phosphorylation induced by PKC changes the affinity of Zfhep for its physiologically important target genes.

GE-P3.
TRANSCRIPTIONAL EFFECT OF ZFHEP IS REVERTED BY TPA TREATMENT

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The activity of a transcription factor (TF) can be controlled by posttranslational modification such as phosphorylation. Zfhep (Zinc Finger Homeodomain Enhancer-binding Protein) is a repressor of several genes and it is involved in lymphopoiesis, neurogenesis and myogenesis. Zfhep exists as two phosphorylated forms (P-Zfhep). EMSA done in our lab showed that the hypophosphorylated Zfhep binds to target genes stronger than the hyperphosphorylated Zfhep. Our goal was to test whether *in vivo* transcriptional effect of Zfhep is also altered by phosphorylation. Cell lines expressing one or both P-Zfhep were transfected with Zfhep expression vector and CD4, p73 y Zfhep/luciferase promoters by O/N Ca-P precipitation. Then, cells were incubated with 10 ng/ml phorbol esters (PMA) for 8 h. As expected luciferase activities (normalized by βgal) were diminished by Zfhep. TPA treatment reverted Zfhep repression of its target genes. The results show that phosphorylation is a possible mechanism of regulation of the transcriptional activity of this TF. Zfhep has been shown as a Smad interacting factor for eliciting TGF-β dependent gene activation in myogenesis. Zfhep would act as a cell-specific factor, and its phosphorylation status would provide a way to modulate the activity of TGF-β signaling pathway. Although PKC pathway seems to be involved in Zfhep phosphorylation, the present results do not rule out the involvement of TGF-β signal (also able to be regulated by PKC) in the process.

GE-P4.
A MICROARRAY APPROACH TO THYMUS GLYCOBIOLOGY

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Glycosylation contributes with the necessary complexity to fine tuning several essential processes. Defined changes in the glycoconjugates allow to identify and to follow the steps of the lymphocyte maturation, although their actual biologic relevance remains largely unknown. To elucidate some of the molecules involved in the glycobiology of the thymus, a microarray approach was performed. We used two different Affymetrix glycochips available from the Consortium of Functional Glycomics. Assays were performed with RNA of thymocytes from C57BL/6J mice and from thymic epithelial cells (TEC) cultures. From a total of 752 glycobiology-related genes, 160 were detected with thymocyte samples. When TEC RNA was analyzed, 298 genes were detected from about 1560 genes. Transcripted mRNA mainly correspond to glycan degradation, glycantransferases, nucleotide sugar transport and synthesis, lectins, cytokines, adhesion molecules, interleukins and their receptors, growth factors and their receptors, chemokines, proteoglycans and Notch pathway molecules. Expression of several of these genes was confirmed by real-time PCR. These data will allow further insight into the thymocyte-TEC interaction.

GE-P5.
KEY REGULATORS OF P19INK4D IN RESPONSE TO DNA DAMAGE AND CELLULAR PROLIFERATION

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p19INK4 is a member of the INK4 family of proteins that regulate G1/S cell cycle transition by inhibiting the pRb kinase activity of CDK 4/6. We demonstrated that p19 a) is periodically expressed in a E2F-dependent manner b) its promoter presents binding sites for NFκB, c) is involved in DNA repair, d) is induced by UV irradiation. Little is known about the turn over of the signal of E2F and our results suggest that p19 could be involved in the negative feedback of E2F. The factors involved in p19 induction by UV remain elusive. The aims of the present work consist in 1) elucidate if p19 is involved in the negative feedback of E2F, 2) verify if NFκB and E2F participate in the induction of p19 in UV irradiated cells. To approach objective 1 we performed EMSA assays to assess the relative affinity of E2F for consensus sequences and p19 promoter sequences. E2F protein displayed a higher affinity for the consensus sequence than for p19 promoter elements. We compared the kinetics of cyclin E induction and p19, and observed a delay in p19 expression in comparison to cyclin E. To approach objective 2 we overexpressed NFκB in fibroblast and observed an induction of p19. p19 induction by UV irradiation was not modified by treatment with NFκB inhibitors but significantly decreased by E2F-decoy transfection. These results demonstrate that 1) p19 could be involved in a mechanism of negative feedback of E2F. 2) the UV induction of p19 is mediated by E2F and not by NFκB.

GE-P6.

CHARACTERIZATION OF THE MCE3 OPERON PROMOTER REGION OF *Mycobacterium tuberculosis*

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*mce*3 is one of the four mce (mamallian cell entry) operons of *Mycobacterium tuberculosis* that encode for exported proteins of unknown function but with a clear role in virulence (Gioffré et al 2005; Shimono et al 2003). The entry and survival of the pathogen in the host cell are crucial steps in pathogenesis, and *mce* conferred the ability on nonpathogenic *Escherichia coli* to enter nonphagocytic cells and survive (Arruda *et al.*, 1993). In addition to cell entry, *mce* operons perhaps have other functions. The expression profile of the *mce* operons is temporal and growth phase specific (Kumar *et al.*, 2003). In a previous study we described the role of Rv1963 (Mce3R), a transcriptional regulator member of the TetR family, which negative regulates the expression of mce3 operon (Santangelo *et al.,* 2002). Here we mapped the DNA binding site of Mce3R in the *mce*3 promoter region by footprinting assay and characterized the consensus region where it binds by gel shift assay and directed mutagenesis. With the aim of identify other genes that are transcriptional regulated by Mce3R we perform *in silico* analysis and RNA microarray assay. These genes, members of the same regulon as *mce*3 operon, were confirmed by RT-PCR. The identification of such genes could give sight on the function of *mce* operons.

GE-P7.

MCE2R (RV0586), A GNTR-LIKE TRANSCRIPTIONAL REGULATOR FROM *MYCOBACTERIUM TUBERCULOSIS*, REPRESS THE EXPRESSION OF THE VIRULENCE FACTORS ENCODED IN *MCE2* OPERON

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An invasin-like gene from *Mycobacterium tuberculosis* was first described that conferred the ability on non-pathogenic *Escherichia coli* to invade and survive within macrophages and human HeLa cells, and it was named the mammalian cell entry (*mce*) gene. Subsequently, the analysis of the complete sequence of the *M. tuberculosis* H37Rv genome revealed the presence of three additional paralogous mce genes, all encoding in an operon structures consisting of eight genes.

The biological function of Mce proteins is not known, but increasing evidences have demonstrated that they are clearly related to the virulence of *Mycobacterium tuberculosis* complex species. Regulatory mechanism which control *mce3* expression has been identified by our group. We have found that a TetR family transcriptional regulator down regulates *mce3* operon during the *in vitro* growing of *M. tuberculosis*. Other putative gene encoding transcriptional factor, Rv0586, is located immediately upstream of *mce2* operon. Here we report the initial characterization of Mce2R (Rv0586), a transcriptional regulator belonging from the GntR family. By using a promoter probe approach we found that Mce2R repress de expression of *mce2* operon. The specific binding of recombinant Mce2R to the promoter region of *mce2* operon was demonstrated by gel shift assay.

GE-P8.

GATA FACTORS CONTROL CARBON-RESPONSIVE UGA4 GENE EXPRESSION IN *SACCHAROMYCES CEREVISIAE*

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In *Saccharomyces cerevisiae*, delta-aminolevulinic acid (ALA) and gamma-aminobutyric acid (GABA) are imported into the cells through the Uga4 permease. Expression of the *UGA4* gene depends on GABA induction and nitrogen catabolite repression (NCR). In this work we studied the regulation of this gene by carbon source. Expression of *UGA4* was determined using the reporter gene LacZ under the control of *UGA4* promoter. Beta-galactosidase activity in wild type and mutant strains grown in the presence of different carbon sources under repressive and derepressive nitrogen conditions was measured. The main conclusions obtained from our results were: the effect of carbon source under nitrogen repression is not observed; induction of *UGA4* depends on carbon source since it is only detected on glucose; carbon regulation of *UGA4* is not mediated by the main positive GATA factor, Gln3, whereas the other positive GATA factor, Gat1, is involved in this regulation; subcellular localization of these positive GATA factors varies depending on carbon and nitrogen source used; Ure2, a cytoplasmic negative regulator of Gln3 and Gat1, is not mediating carbon regulation of *UGA4* through Gat1; the expression of *UGA43* and *GZF3*, the negative GATA factors involved in *UGA4* transcription, is also regulated by carbon source.

GE-P9.

STUDY OF THE EXPRESSION OF ALPHA PROTOCADHERINS AT THE SINGLE CELL LEVEL IN THE MOUSE BRAIN

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The mammalian protocadherin (Pcdh) proteins have been proposed to participate in specific synaptic connections based on structural similarities to cell adhesion molecules and their localization to synaptic junctions. They are encoded by three closely linked gene clusters (α, β, and γ). Multiple α and γ Pcdh mRNAs are generated by alternative pre-mRNA splicing between different "variable" (V) exons and three "constant" (Con) exons within each cluster. Each Pcdh V exon is preceded by a promoter and promoter choice determines which V exon is included in a Pcdh mRNA through its splicing to the constant exons. In order to understand the regulation of the expression of Pcdhs, we studied the expression of Pcdh αin single cells. We obtained Spret/EiJxC57BL6/J F1 mice to be able to identify paternal alleles. Antibody labeled-neurons from the brain corteces of postnatal day 2 mice were isolated using a cell sorter. RT-PCR analysis on these cells revealed that between one and two different V isoforms are expressed per chromosome in a stochastic monoallelic fashion while a specific subset of V exons is expressed biallelically. A model for the regulation of the expression of Pcdhs α will be discussed.

GE-P10.
PROBING THE COMPONENTS OF THE SR NETWORK IN TRYPANOSOMES
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The regulation of the gene expression in trypanosomatid protozoa is exerted mainly at the post-transcriptional level, including RNA processing, RNA stability and mRNA turnover. It is well established in higher eukaryotes that multiple serine/arginine-rich (SR) proteins and SR-specific kinases, which constitute the SR network, are critical for pre-mRNA processing. We have identified and characterized components of this network in *Trypanosoma cruzi* and *Trypanosoma brucei*: there is a single SR protein (TcSR and TbSR), as well as a single SR-specific kinase (TcSRPK and TbSRPK). These proteins were functionally characterized using *in vitro* phosphorylation assays, *in vivo* alternative splicing reactions in HeLa cells and complementation of *Schizosaccharomyces pombe* mutant strains. Our results showed that the two trypanosome proteins form an SR network and suggest an involvement in the trans- and/or cis- splicing processes. To further address the function of the SR network in trypanosomes, we used RNA interference to downregulate the proteins in *T. brucei*. So far, our studies revealed that TbSR is encoded by an essential gene. Preliminary results suggest an involvement of this SR protein in pre-mRNA processing. Experiments are in progress to pinpoint the role of this protein in trans- and/or cis-splicing.

GE-P11.
MOLECULAR STUDIES FOR *Triatoma infestans* THORACIC MUSCLES GLYCEROL-3-PHOSPHATE DEHYDROGENASE
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Triatoma infestans (*T.infestans*), Chagas disease vector, adquirers wings and the ability to fly at adult instar. In our laboratory it was demonstrated that glycerol-3-phosphate dehydrogenase (GPDH), an enzyme involved in the glycerophosphate shuttle, increases its activity 30 fold in adults thoracic muscles. Adults muscles should have higher glycolitic and respiratory capacity to support flight. Electrophoretic studies from thoracic muscles extracts showed two GPDH isoforms. Nymphs predominant isozyme has less movility. This work aim of beginning GPDH molecular studies about parts of coding regions from GPDH gen and its cDNA. Three insects species were selected for comparative studies: *T. infestans*, *Apis mellifera* (*A. mellifera*) and *Drosophila melanogaster* (*D. melanogaster*). On the basis of protein and nucleotide acid secuences alignament of GPDH from good flyers insects were designed specific primers for *A. mellifera* and *D. melanogaster* and the degenerate corresponding forms to be employed in *T. infestans* reactions. Primers were used to amplific DNA and cDNA secuences. As a result, 200 pb fragments were obtained at three insects species. The fragment size was the expected and corresponds to a portion of GPDH DNA and cDNA secuences. Secuencing results alow homology analysis.

GE-P12.
GENE EXPRESSION PROFILING ON ANTHRACYCLINE-TREATED erbB4 KO MOUSE
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Neuregulin (NRG) signaling through receptor tyrosine kinase erbB2 and erbB4 heterodimers is critical for the maintenance of adult heart function. Combined therapy of blocking antibodies of NRG signaling and anthracycline derivatives (AD), inhibitors of mammary tumor growth, could lead to a severe cardiomyopathy. We investigated the molecular modifications underlying the synergistic cardiotoxicity of AD under partial blockage of NRG pathway by injecting doxorubicin (D) in the ventricular muscle specific erbB4 knockout mouse (KO). We performed gene expression analyses by DNA microarrays and RT-PCR. Differentially expressed genes in D-KO were functionally grouped and compared to KO, Wt and D-injected Wt. There is a remarkable modification in the expression level of growth factors involved in cardiac growth and of signals implicated in inflammatory process. Although D triggered programmed cell death, the relative number of apoptotic cells was similar in WT and CKO mice monitored by TUNEL and Bcl-2/Bax ratio. In D-KO, there is a preferential loss in subcellular targeting of proteins normally localized to Z-line and intercalated disc as monitored by immunohistochemistry and Western blots. The functional identity and subcellular localization of some differentially expressed genes may assist understanding the potentiated toxicity of AD and blocked NRG.

GE-P13.
A POLAR MECHANISM COORDINATES DIFFERENT REGIONS OF ALTERNATIVE SPLICING WITHIN A SINGLE GENE
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Alternative splicing plays a key role in generating protein diversity. About 60% of human genes are alternatively spliced and ~30% of them have more than one alternative region. To evaluate if there is a link between the way in which two alternative events are processed in the same transcript, we transfected mammalian cells with minigenes carrying two alternative regions separated by three constitutive exons and analyzed the rate of exon inclusion into mature mRNA by RT-PCR. We found that, mutations which either inhibit or stimulate inclusion of the upstream alternative exon deeply affect inclusion of the downstream one. However, similar mutations at the downstream alternative exon have little effect on the upstream one. This "polar" effect is promoter specific and is enhanced by inhibition of transcriptional elongation. Consistently, cultured murine embryonic fibroblasts from mutant mice with either constitutive or null inclusion of a fibronectin alternative exon revealed coordination with a second alternative splicing region located far downstream . Using allele-specific RT-PCR, we demonstrate that this coordination occurs in cis. Bioinformatics supports the generality of these findings, identifying several genes with nonrandom distribution of mRNA isoforms at two alternative regions.

GE-P14.
AN INDUCIBLE SYSTEM TO ASSESS THE ROLES OF POLYMERASE II CTD IN ALTERNATIVE SPLICING
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Previous studies have demonstrated that RNA polymerase II (pol II) carboxy terminal domain (CTD) participates in co-transcriptional mRNA processing. In this work we have used an alfa-amanitin resistant pol II system and fibronectin minigenes to study the CTD role in the regulation of alternative splicing. Given the complexity and potential indirect effects on *in vivo* strategies, we developed an inducible reporter minigene so as to tightly restrict its transcription by wt and CTD deleted (delta-CTD) polymerases in a time controled manner. Since delta-CTD pol II does not support strong activator mediated transcription, we have used the glutamine rich Sp1 activation domain to trigger the inducible promoter. Our data with minigenes bearing constitutive promoters show that deletion of CTD inhibits exon skipping of fibronectin EDI exon, and this effect is more pronounced in inducible minigenes when delta-CTD pol II transcription is induced after complete inhibition of the endogenous pol II.
Despite no direct physical interaction has been described between pol II CTD and SR proteins, we found that the CTD is required for SRp20 inhibitory action on EDI inclusion, whereas SF2/ASF stimulating effect is independent of CTD. Together these results suggest that the CTD may act by recruiting an inhibitory splicing factor to the EDI alternative exon leading to exon skipping.

GE-P15.
CO-ACTIVATOR 150 (CA150) AFFECTS ALTERNATIVE SPLICING IN NON-STIMULATED AND UV IRRADIATED CELLS
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The transcriptional cofactor CA150 is a unique multi-domain protein. It contains 3 WW domains, 6 FF domains and a poly-proline region. CA150 interacts with several proteins involved in mRNA processing. Through its WW domains #1 and #2 it interacts with the splicing factor SF1. Through its FF domains interacts with the phosphorylated CTD of RNA pol II and with proteins involved in the response to UV light. On the other hand it was shown that CA150 is present in the spliceosome. We investigated if CA150 is involved in the coupling between transcription and alternative splicing in both, basal conditions and UV treated cells. We co-transfected Hep3B cells with different CA150 expression plasmids (wild type and mutated versions) and reporter minigenes containing the alternatively spliced EDI exon of the human fibronectin gene. We found that CA150 WT and a double mutant version afecting both WW1 and WW2 domains, which is unable to interact with SF1, stimulate inclusion of the EDI exon whereas the CA150 mutant in the WW3 domain has no effect on splicing. On the other hand, in UV treated cells, the inclusion of EDI exon was stimulated and this inclusion was inhibited by overexpression of CA150. These results suggest that CA150 is involved in the coupling between transcription and alternative splicing in both basal and stimulated conditions.

GE-P16.
DEPOLARIZATION-INDUCED REGULATION OF NCAM ALTERNATIVE SPLICING
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The nervous system shows many examples of alternative spliced-mRNAs that originate proteins with different regulation or function. It is tempting to look at possible roles of molecular mechanisms involved in neuronal plasticity, such as histone acetylation, in alternative splicing regulation. The NCAM gene is a suitable model to study this in the context of neuron differentiation and plasticity. The exon 18 (E18) of NCAM is alternatively spliced leading to two of the main isoforms of this protein: NCAM 140 and NCAM 180. The proportion of these two isoforms changes in the synapsis following Long Term Potentiation (LTP), suggesting a role in learning and memory. Previously, we have determined that histone acetylation leads to lower levels of E18 inclusion. We now show that depolarization by means of high extracellular potassium concentration, capable of modulating histone acetylation, induces E18 exclusion. Calcium balance might be involved in this mechanism, as an inhibitor of Ca^{2+} release from endoplasmic reticulum (2-Aminoethoxydiphenyl borate) has the opposite effect on E18 splicing. Futhermore, the balance between these two effects seems to be influenced by cellular differentiation. These results show a possible way through which balance between NCAM 140 and 180 could be regulated at the level of mRNA splicing.

GE-P17.
OPTIMIZED SEMIQUANTITATIVE RT-PCR TO STUDY EBAF TEMPORAL EXPRESSION PATTERN DURING THE EARLY PREGNANCY IN THE RAT OVIDUCT
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In previous work we have cloned and identified the *ebaf* (endometrial bleeding-associated factor) gene complete CDS (Accession No. AY758558) that is specifically expressed in the rat oviduct. To asses *ebaf* mRNA levels in early stages of pregnant rats, we optimized a semiquantitative RT-PCR standard procedure. The protocol was modified taking into account some aspects of the Real Time PCR technique, trying to: a) minimize the multiple variables that affect a single PCR reaction b) maximize gene expression differences, working at the exponential phase of the reaction. First we determined the amplification linear ranges for both *ebaf* and *β-actin*, as housekeeping gene, assaying different cycle numbers to obtain the complete profile of the PCR reaction (10 to 40 cycles). Each sample was then amplified in 6 individual PCR reactions for *ebaf* (21 to 26 cycles), and for the *β-actin* gene (18 to 23 cycles). By this way we ensure a linear amplification efficiency and avoid the reaction variability. Three different samples for each pregnancy day were assayed (day 1 to 7) and gene expression was expressed as *ebaf*/*β-actin* ratio. The experiment shows that *ebaf* expression was higher at day 4 of pregnancy compared to day 1 and 7 (five folds), suggesting a relevant function in the oviduct during the early stages of pregnancy.

GE-P18.
EXPRESSION PROFILE OF INDIVIDUAL PREGNANCY-SPECIFIC GLYCOPROTEIN GENES DURING SYNCYTIALIZATION OF HUMAN TROPHOBLAST CELLS
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Pregnancy-specific glycoproteins (PSG) are encoded by 11 highly similar genes tightly linked within a 700-kb region in Ch 19q13.2. PSGs are essential for gestation in mammalian species and their expression is markedly induced during trophoblast cells differentiation. However, the transcriptional induction and mRNA level of each PSG members are unknown. This report examines PSGs expression profile during *in vitro* syncytialization, as well as the contribution of the proximal promoter sequence in their transcription. Placental cytotrophoblast and choriocarcinoma Jeg3 cell line were induced to differentiate into syncytium. RNA was isolated from both cell types at different times and analyzed by semi-quantitative RT-PCR using PSG 1, 3, 5 and 7 gene specific primers. A differential expression pattern for each gene was found in both models. Luciferase reporter vectors bearing 600 pb of the promoter region of each gene, transiently transfected in Jeg3 cells, displayed promoter activities which were in agreement with endogenous transcripts levels. Multiple sequence alignments of the 600 pb and up to 3000 pb 5´ upstream the ATG codon revealed pairwise alignment identities of 92-94%. No significant correlation was found between previously characterized sequence motifs and transcription of individual PSG genes. The present study indicates a different expression profile of PSGs, suggesting a complex transcriptional regulation and a specific importance of each member in trophoblast differentiation.
Supported by CONICET, FONCyT and SECyT-UNC.

GE-P19.
INFLUENCE OF *bcl-x* ALTERNATIVE PROMOTER USAGE ON ITS SPLICING PATTERN
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Differential splicing from the *bcl-X* gene generates several isoforms with opposite effects on the apoptotic response (i.e. bcl-X_L and Bcl-X_S). We have demonstrated that the the 5' upstream region of the mouse *bcl-X* gene contains five different promoters and presented evidences suggesting that promoter selection influences the outcome of the splice process. To test this hypothesis we constructed minigenes containing each promoter region upstream a common sequence with both 5´ alternative splice sites and an unique 3´splice site. We confirmed the minigenes activities by transient transfection in three different cell types (HC11, Hela and HEK). In all cases, we observed a more abundant expression of *bcl-X_L*, however the ratio *bcl-X_L/bcl-X_S* was higher in cells transfected with the minigene that is under the control of promoter 3 (three times higher than minigenes from promoter 1), suggesting that promoter sequence could influence splice site selection. Co-transfections of constitutive STAT5A and B transcription factors (known as repressors of promoter 1 activity) together with the minigene containing the promoter 1 raised 4±1 times the ratio of *bcl-X_L/bcl-X_S* suggesting a splicing effect of these transcriptional factors. Taken together these results suggest a coupling between the regulation of transcription and splicing on *bcl-X* gene.

GE-P20.
SETTING UP OF A MICROARRAY PLATFORM
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In the last years, microarray technology has become a powerful tool for the study of gene expression. To date, there are numerous alternative protocols for every step, all of which may have an enormous influence in the final results. Our objective was to evaluate which of various alternatives for each of these steps produces the best quality results. Here we describe which are the most important variables that affect: Microarray printing, target sequences labeling, ARN amplification and hybridization and scanning. We have also developed and tested a new normalization method called Norm3D. Various experimental designs were analyzed using different statistics methods, like t-student, SAM, ANOVA and Limma. The presence of molecular signatures was also evaluated using various clustering algorithms. We found that the highest quality spots were obtained using DMSO 50% as printing buffer on an aminosilane-coated slide. We also found that the dynamic range of the scanner is maximized by successive scans at different sensitivity levels. The most efficient normalization was obtained using the Norm3D method, developed at our lab. Finally, we found that a dye swap design is critical for obtaining unbiased results.

GE-P21.
AN EARLY MOLECULAR SIGNATURE IN THE BRAIN INDUCED BY PERIPHERAL TUMORS
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The search for novel diagnostic tools in cancer based on gene expression studies often involves the comparison of malignant tumors and adjacent normal areas. Mutations arising in cancer cells during tumor development can generate alarm signals before the clinical detection of the disease. Since homeostatic processes are under strict control by the CNS, we hypothesized that the CNS might sense tumor-triggered alarm signals during its initial development. We report here an analysis of gene expression in different brain regions of mice injected s.c. with either mammary, lung or colon cancer cells. Mice were sacrificed at 18 h, 72 h or 8 days after the initiation of the experiment. The transcriptional profile of the brain response to the presence of a peripheral tumor was analyzed using in house-printed 10 K oligonucleotide-based microarrays. Of the 10 K spots surveyed, we observed that 11.4% of genes were differentially expressed in mice bearing tumors (fold > 1.15 and p-value < 0.05). Analysis of differentially expressed genes whose functional categories are known, indicates a significant enrichment in genes related to synaptic activity and sickness behaviour. A subset of these genes was validated by Real Time PCR. Unsupervised cluster analysis showed that the differentially expressed genes discriminated tumor-bearing mice from controls with high sensitivity and specificity. Moreover, we have also identified sets of brain-expressed genes suitable to discriminate between the three different cancer models.

GE-P22.
TRANSCRIPTIONAL ACTIVATION OF THE RAT FADS 1 GENE BY INSULIN
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The Fads 1 gene encodes the rat Delta-5 Fatty Acid Desaturase enzyme, one of the key steps in the biosynthesis of polyunsaturated fatty acids. In this study, we amplified approximately 1000 bps upstream from the Fads 1 gene by PCR, using as template a BAC clone obtained from a public rat genome library (CHORI). The resulting fragment was subcloned in the pGL3-basic vector (Promega). The final construct, together with the appropriate controls, were co-transfected in HTC rat hepatoma cells. Then, the luciferase activity produced by the cells carrying the putative promoter region, with or without insulin, was measured and compared with positive and negative controls. First, we found that the subcloned fragment had promoter activity. When adding insulin, we observed about 30% of transcritional activation, presumably due to the SREBP1-c transcription factor, which is consistent with the induction found in the enzymatic activity. Further experiments will be done to confirm the identity of the transcription factor(s) implicated, and to determine the localization of its/their binding site(s) inside the Fads 1 promoter.

GE-P23.
CELLULAR TOXICITY OF DUX4 DEPENDS ON NUCLEAR LOCALIZATION
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Fascioscapulohumeral muscular dystrophy (FSHD) is the third most common inherited myopathy after Duchenne muscular dystrophy and myotonic dystrophy. A 3.3-kb tandem repeat on chromosome 4q35 (D4Z4) is contracted in patients with FSHD. D4Z4 contains an ORF encoding a putative double-homeobox protein called DUX4. We prepared anti-DUX4 antibodies that specifically recognize an endogenously expressed protein in the nuclei of cultured human muscle-derived cells. Transient expression of DUX4 in cultured cells leads to cell death and no stable transfectants constitutively expressing DUX4 could be isolated. The functionality of two putative nuclear localization signals (NLS-1 and NLS-2) present in DUX4 was studied using PCR-mediated mutagenesis. Amino acid residues at NLS-1 and NLS-2 were independently replaced by threonines and the subcellular distribution of the various DUX4 mutants was analyzed in transfected cells. DUX4 mutants that do not localize to the nuclei highlight residues that mediate subcellular trafficking of DUX4. The typical DUX4-mediated cell death phenotype observed in transfection experiments was abolished in some DUX4 mutants. We propose that NLS-1 and NLS-2 are functional NLSs of DUX4 and that nuclear entrance is required for DUX4-mediated cell death.
Supported by FONCyT (Argentina), FSH Society (USA) and AFM (France).

GE-P24.
A GLOBAL NITROGEN REGULATOR INTERACTS WITH THE PUTATIVE PROMOTER SEQUENCES OF SUCROSE METABOLISM GENES OF *NOSTOC* SP. PCC 7120
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NtcA is a global nitrogen regulator that operates only in cyanobacteria. In *Nostoc* sp. PCC 7120, NtcA is required for heterocyst development and diazotrophic growth. It was shown that sucrose (Suc) has a crucial role in N2 fixation in that strain. Suc is synthesized in a two-step pathway involving sucrose-phosphate synthase and sucrose-phosphate phosphatase, and cleaved by sucrose synthase, enzymes encoded by *sps*A and *sps*B, spp, and *sus*A, respectively. We detected high levels of *sus*A and low levels of *sps*B transcripts in a *ntcA*$^-$ strain compared with the wild-type strain. We performed band shift experiments to investigate the interaction between NtcA and the promoter regions of Suc metabolism genes. Firstly, *ntc*A gene of *Nostoc* sp. PCC 7120 was cloned and expressed as a fusion protein. Band shifts assays were carried out with partially purified proteins NtcA-GST, GST, and also with a thrombin treated NtcA-GST. We used different sizes of promoter fragments to make EMSA. We detected interaction between NtcA and the promoter sequences of *sps*A, *sps*B (in two different places) and *sus*A *in vitro*. The NtcA putative binding sites in Suc gene promoters were found in the middle, upstream or downstream from the transcription start site of the promoters of *sps*A, *sps*B and *sus*A, respectively. These results support the hypothesis that Suc metabolism may be coordinated with nitrogen.

GE-P25.
GLYCOGEN BIOSYNTHESIS AND SUCROSE METABOLISM RELATIONSHIP IN *SYNECHOCYSTIS* SP. PCC 6803
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Cyanobacteria are prokaryotic microorganisms, which perform plant-like oxygenic photosynthesis. The unicellular moderately halotolerant cyanobacterium *Synechocystis* sp. PCC 6803 has been an important model organism to investigate the interrelationship between glycogen synthesis and sucrose metabolism. In this cyanobacteria, it has been reported that three enzymes are involved in glycogen synthesis: ADP-Glc pyrophosphorylase, glycogen synthase (GS) and the branching enzyme (Cid *et al*., 2002). We have identified (in *Synechocystis* genome database) and cloned two open reading frames (ORFs *sll*1393 and *sll*0945) homologous to GSs genes. Expression analyses of *glg* genes, and *sps*A (coding for sucrose-phosphate synthase, SPS), in the strain *Synechocystis* sp. PCC 6803 and in the insertional mutants lacking SPS (*Δsps*A) or GlgA (*Δglg*A) activities indicated that both GS contribute to glycogen biosynthesis. Northern blot analysis demonstrated that the transcript levels of the genes encoding enzymes of glycogen and sucrose metabolisms were significantly higher in the exponential growth phase. Differential expression was observed in different growth phases (exponential versus stationary) and in response to a salt shock. The glycogen determination indicated that the accumulation of glycogen is not essential for cellular growth. Additionally, our results suggest that sucrose may not be essential for glycogen accumulation under salt stress.
Supported by CONICET, ANPCyT, UNMdP and FIBA.

GE-P26.
THE CANONICAL INSULIN RESPONSE ELEMENT IS NECESSARY AND SUFFICIENT TO CONFER INSULIN RESPONSIVENESS TO ALAS PROMOTER
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Hepatic expression of several gene products is rapidly and completely inhibited by insulin. This inhibition is mediated through a DNA element present in the promoters, which we call the Insulin Response Element (IRE). We identified an heptanucleotide sequence (GGTTTTG) located at -386 to -380 bp of the human 5-aminolevulinate synthase (ALAS) gene that is highly homologous to the IRE present in the phosphoenol pyruvate carboxykinase and insulin receptor substrate 2 genes (TGTTTTG). We used reporter gene experiments to show that this sequence is necessary and sufficient for insulin-mediated repression of ALAS promoter. Transient tranfection assays in the presence of cell permeant inhibitors indicate that the hormonal regulation exherted through this IRE requires phosphatidylinositol 3-kinase and mitogen-activated protein kinase functionality. Competitive displacement using morpholino-type oligonucleotides, electrophoretic mobility shift assays and southwestern blotting using probes harboring two copies of the IRE motif, show that hepatic nuclear proteins bind specifically to this element. Northern blot analysis revealed that glycogen synthase kinase 3 activity is not involved in ALAS repression. Our results suggest that an IRE-binding factor, regulated by PI3K and MAPK effectors, mediates insulin response upon ALAS promoter.

GE-P27.
CHARACTERIZATION OF ACYL CARRIER PROTEIN EXPRESSION DURING SPORULATION IN *Bacillus subtilis*
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The acyl carrier protein (ACP) plays a key role in fatty acids biosynthesis, linking all the intermediates and presenting them to different enzymes of this metabolic pathway.
We have shown that sporulation, a differentiation process in *Bacillus subtilis*, requires *de novo* fatty acids biosynthesis, and by fluorescence microscopy we found that ACP is synthesized only in the mother cell compartment. In order to understand this observation, we constructed a transcriptional fusion of the *acp* promoter to GFP and we found that its compartmentalization is due to a preferential transcription of the gene in the mother cell. The same expression pattern was observed in mutants of *sigE* (mother cell specific) and *sigF* (preespore specific) transcriptions factor, indicating that other mechanism regulate *acp* expression.
By gel shift assays we determined the existence of a sporulation specific protein able to bind to the *acp* promoter, and that we purified and identified.
These results suggest that there is a regulatory mechanism that govern ACP expression during sporulation in *B. subtilis*.

GE-P28.
EXPRESSION OF TAGGED PROTEINS IN *T. cruzi*
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Trypanosomatids cell cycle control is a complex mechanism whose bases are not completely understood. To study the components of the cell cycle machinery, we investigate the expression of tagged proteins, as an approach to analyze cellular localization, protein-protein interactions, etc. Cell cycle is affected by over expression of its regulatory elements like cyclins or protein kinases. It is known that this over expression produce i.e. alterations of growth, phenotype and viability of the cells. To avoid these problems we are testing the expression of tagged proteins cloned in a low level expression vector pTEX with the TzCyc6 endogenous UTRs sequences. The trans-splicing process is regulated by UTRs. We were able to amplified by PCR the 5´ and 3´ UTRs of the mitotic cyclin TzCyc6, basing us on the genomic sequences (TIGR.org). These sequences were cloned in the orientation 5´UTR - GFP - 3´UTR in pET22(b)+ vector flanking the GFP gen. The full- length construction was amplified by PCR using primers containing restriction sites. To avoid interferences of pTEX regulatory sequences with the TzCyc6 regulatory ones, the construction was cloned in sense and antisense orientation in the pTEX vector. Parasites will be transfected and synchronize with hidroxyurea. It will allow to analyze the GFP expression levels by northern blot along cell cycle and to compare it with endogenous expression levels of TzCyc6 gen. This will provide information about the role of TzCyc6 in the cell cycle.
Supported by FONCyT, CONICET and UBA.

GE-P29.
EXPRESSION OF TRANSFORMING GROWTH FACTOR BETA (TGF-β) MEMBERS IN THE RAT OVIDUCT
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In mammalians, early embryonic development begins in the oviduct with active mitotic cycles. The embryonic genome activation and the reprogramming of the embryo gene expression take place after a few cleavage divisions before implantation in the uterus. During this preimplantation period, the oviduct creates a microenvironment that serves to facilitate the optimal early embryo development, in part by the secretion of embriotrofic factors.
We previously demonstrated the differential expression of the rat ebaf (endometrial bleeding-associated factor) gene, a member of the TGF-β family with regulatory properties on other TGF-βs, in the rat oviduct during early pregnancy. Few members of the TGF-β superfamily have been reported as being expressed in some mammalian oviducts. In order to discovery the role of ebaf in the oviduct, we studied the mRNA expression of seven TGF-β members in the rat oviduct by RT-PCR. To determine TGF-β1, TGF-β2, TGF-β3, BMP7, GDF9 and EBAF mRNAs expression, total RNA from rat oviducts were isolated and subjected to RT-PCR analysis. Results indicate that all of the studied genes are expressed in the rat oviduct. TGF-β signaling controls a diverse set of cellular processes; the oviductal expression of different TGF-βs suggests a delicate complex role for these factors in the mammalian oviduct during the reproductive process.

GE-P30.
IDENTIFICATION OF HYPOXIA RESPONSE ELEMENTS CONTROLLING THE EXPRESSION OF THE DROSOPHILA PROLYL-4-HYDROXYLASE GENE 'FATIGA', THAT OPERATES AS AN OXYGEN SENSOR

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HIF-1 is a heterodimeric α/β transcription factor essential for the adaptation of cells to reduced oxygen conditions. this heterodimer is present only in hypoxia to activate transcription of target genes by binding to hypoxia response elements (hres) in their regulatory regions. under normoxia hif-1α is degraded in the 26s proteasome, and the process depends on the hydroxylation of 2 key proline residues in HIF-1α polypeptide. hydroxylation is catalyzed by specific prolyl-4-hydroxylases (phds) that utilize o_2 as a co-substrate thus, functioning as oxygen sensors. we had previously identified sima and fatiga (fga) as the *drosophila* homologues of hif-1α and the phd, respectively. we found that *fga* gene generates 3 different transcripts by a combination of alternative splicing and alternative initiation of transcription (*fga a, b y c*). *fga b* and *c* but not *fga a* are induced in hypoxia or upon over-expression of sima, originating a negative feed back loop. we have used luciferase reporter constructs in *drosophila* s2 cells to identify the hres responsible for the hypoxic induction of fga *b* and *c*. we found a regulatory region containing 6 putative hres that conferred sima dependent inducibility and site directed mutagenesis of these motifs led to the identification of one particular hre responsible for sima /hypoxia-dependent inducibility.

GE-P31.
IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES IN THE BASIDIOMYCETE *Ceriporiopsis subvermispora* IN RESPONSE TO DIFFERENT MANGANESE CONCENTRATIONS BY cDNA-AFLP

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The aim of this work is to identify differentially expressed genes in an organism whose genome has not been sequenced. We investigated the global gene expression patterns regulated by manganese, in order to identify genes involved in its metabolism.
The model organism used is *Ceriporiopsis subvermispora*, a highly selective ligninolytic fungus, which possesses an enzymatic machinery composed by manganese-dependent peroxidases and laccase. When analizing cDNA-AFLP patterns obtained from the fungus grown in different concentrations of manganese, we observed changes in approximately 40 genes, suggesting the complex metabolism of manganese.
In this panel, we report the identification of 20 differentially expressed genes by the cDNA-AFLP technique. These genes were amplified, cloned and sequenced. Most of these were related to Fe-S proteins, ribosomal biogenesis and lipid metabolism. Some of these genes were analyzed by Northern blot, to confirm expression levels found in each condition.

GE-P32.
DEPOLARIZATION INDUCES STRUCTURAL MUSCLE GENES EXPRESSION IN SKELETAL MUSCLE CELLS

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Adaptive response of skeletal muscle to exercise or to electrical stimuli involves changes in specific gene expression. To identify genes that could be regulated by depolarizing stimuli, total RNA from C_2C_{12} myotubes, obtained 4 h after high K^+ stimulation, was analyzed using oligonucleotide microarrays. Within 35 genes differentially expressed, we identified two that encode structural muscle proteins, actin alpha skeletal muscle (Acta1) and troponin I (Tnni). Maximal mRNA increased levels at 4h (1.5 and 2-fold, respectively) was confirmed by RT-PCR. Depolarization of skeletal myotubes evokes slow calcium transients, mediated by IP_3 receptor (IP_3 R) activation mainly associated to cell nuclei. We focused our interest on the participation of the slow calcium transients in induction of both genes. Depolarization performed in the presence of either 2-APB, an inhibitor of IP_3 mediated Ca^{+2} release, or U73122, a phospholipase C inhibitor, did not prevent depolarization induced Acta1 mRNA increase, but decreased Tnni mRNA levels. These results suggest the involvement of slow Ca^{+2} transients in the regulation of Tnni transcriptional activity, but make unlikely the participation of IP3 generated calcium signals in Acta1 gene expression.
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GE-P33.
VEGF BUT NOT TGFβ1 EXPRESSION IS INCREASED BY NGF IN NEONATAL RAT OVARIES

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Infertility disorders such as ovarian hyperstimulation syndrome, pregnancy loss and ovarian tumours, are commonly associated to defects in angiogenesis. The nerve growth factor (NGF) promotes angiogenesis in several tissues. Delayed wound healing has also been associated with an impaired production of this neurotrophin. Although NGF is involved in a variety of ovarian functions, the effects of NGF on ovarian angiogenesis are unknown. Our aim was to elucidate if NGF has a role in the expression of the angiogenic factors VEGF and TGFβ1 in the rat ovary.
Neonatal rat ovaries were cultured with NGF 100 ng/ml for various times and processed for RT-PCR and immuno-histochemistry. NGF induced an early increase of mRNA levels for two isoforms of VEGF. VEGF protein was also increased in NGF-stimulated tissues whereas TGFβ1 mRNA content or protein had no significant change at any of the assayed times of stimulus.
The above results indicate that NGF increases the expression of VEGF in the ovary, which may be important in the maintenance of the follicular and luteal vasculature. This data also suggests that a disruption on NGF regulation could be a component in ovarian disorders related with impaired angiogenesis.
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GE-P34.

EFFECT OF OXIDANTS ON THE EXPRESSION OF LIGNINOLITICS GENES IN *Ceriporiopsis subvermispora*

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The white rot basidiomycete *Ceriporiopsis subvermispora* shows a high selectivity and ability to degrade lignin. Its ligninolytic system is composed of manganese peroxidase (MnP) and laccase (phenol oxidase). In addition, the fungus displays an extracellular hydrogen peroxide production system. Thus, ligninolysis is a highly oxidative process. Although the regulation mechanisms in basidiomycetes are basically unknown, it has been shown in some of these fungi that the oxidative stress generated by certain aromatic compounds such as H_2O_2 and O_2, lead to an increase of transcripts encoding MnP and laccase, as their corresponding enzymatic activities. In H_2O_2 supplemented cultures of *C. subvermispora* we were not be able to detect changes in the expression of MnP or laccase genes. However, in cultures treated with quinones, which are able to generate reactive oxygen species (ROS), expression of laccase increases dramatically, as measured by Northern hybridization and extracellular enzymatic activity. Treatments with quinones also has an important although transient effect on the amount of total glutathione in the fungus.

GE-P35.

MODULATION OF PPAR GENE EXPRESSION BY RESVERATROL IN A MODEL OF HEPATIC STELLATE CELL LINE

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Hepatic stellate cell (HSC) plays an important role in many types of acute liver injury and has been implicated in liver fibrosis. The HSCs maintain the homeostasis of the liver extracellular matrix, and under pathologic conditions they undergo a process of activation, developing a myofibroblast-like phenotype from the lipocyte quiescent original phenotype, with increased proliferation and collagen synthesis. Peroxisome Proliferator Activated Receptors (PPARs) regulates the homeostasis of secondary genes involved directly with the maintenance of the quiescent or the activated phenotype. Here we evaluated the capacity of resveratrol, a phytoalexin antioxidant, to modulate the expression of PPARs and related genes in a model of HSCs phenotype conversion. GRX was used as a representative HSC cell line, that can be induced to expresses the lipocyte phenotype by retinol or indomethacin treatment. These treatment differentially increased the expression of the PPARs and catalase and PEX16, genes well known to be regulated by PPARs. Co-treatment with resveratrol inhibited indomethacin and retinol induction of all PPAR isoforms. Catalase and PEX16 gene expression were also inhibited by resveratrol.
These results show a role for resveratrol in the modulation of HSC phenotype transition by modulating PPARα, -ß and -γ expression.

GE-P36.

EPIGENETIC ROLE IN SEASONAL ADAPTATION AND DEVELOPMENT IN FISHES

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Histones are a major component of chromatin, the protein-DNA complex essential for packing, function, and regulation of eukaryotic genome. A fraction of histones are nonallelic variants that have specific expression, localization, and specie-specific distribution patterns. The histone variant macroH2A (mH2A) has a remarkable function in gene silencing. Reprogramming of gene expression allows the adaptation of carp (*C. carpio*) to seasonal changes of its habitat. In the winter-adapted fish, the environmental conditions produce the shutdown of many genes, in contrast to the active expression of the same genes in the summer-acclimatized carp. Here we report the prominent expression of carp mH2A concurrently with hypermethylation of the rDNA gene promoter during winter, suggesting that mH2A should be involved in seasonal adaptation mechanisms at molecular level. Also, we address studies of mH2A in zebrafish. By immunodetection and whole mount *in situ* hybridization, we observed a broad pattern of expression, slightly stronger at embryo's head. Currently, we are performing experiments to knock down the expression of endogenous mH2A in zebrafish to evaluate the role of this histone variant during development. Our preliminary results suggest that macroH2A plays a fundamental role in control of gene expression in fishes.
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GE-P37.

THE TRANSCRIPTIONAL COREPRESSOR CoREST INTERACTS WITH THE MOLECULAR CHAPERONE HSP70 AND REPRESSES THE TRANCRIPTIONAL ACTIVITY OF HSF1

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The transcriptional corepressor CoREST is a component of a HDACs containing complex including the histone lysine specific demetilase, LSD1. By performing a yeast two hybrid screening assay with CoREST, we identified the molecular chaperone Hsp70. The association between both proteins was demonstrated by GST-pull down and co-immunoprecipitation assays. We delimited the interaction region of CoREST to its first SANT domain. Given that Hsp70 acts as a transcriptional repressor of HSF1 during the heat shock response, we analysed the effect of CoREST on the activity of a chloramphenicol acetyltransferase reporter gene commanded by the Hsp70 promoter. The results indicate that under a heat shock situation CoREST represses the induction of the reporter gene in a dose dependent manner even in the presence of HSF1. In conclusion, we demonstrated that CoREST represses the transcriptional activity of HSF1 during the heat shock response and we suggest that this repression is mediated by the interaction between CoREST and Hsp70.

GE-P38.
INTERFERENCE BY DOUBLE STRANDED RNA IN PLATYHELMINTHES: A NOVEL TOOL FOR THE STUDY OF PARASITIC INVASION

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The identification of the molecular mediators in the parasite-host interface is essential for the designing of diagnostic and therapeutic tools useful in the control of parasitic diseases. In the trematode *Fasciola hepatica* several secreted cysteine proteases (cathepsins) have been identified and proposed as key players in several processes such as invasion, immune evasion and feeding.
We decided to use RNA interference (RNAi), a new and potent tool of reverse genetics, in order to test this hypothesis. Our first aim is to provide evidence of the interference at the molecular level, by the analysis of the cathepsins mRNA levels by RT-PCR, and protein levels by Western blot and enzymatic activity assays. The second goal is the generation of quantitative phenotypes in interfered worms in order to evaluate the role of these proteases in the invasion process.We have standardized the synthesis of RNAi from cathepsins, generating a cathepsin L dsRNA. In preliminary assays with newly excysted juveniles (NEJ) of *F.hepatica* we have demonstrated a specific reduction in treated worms of the mRNA level by RT-PCR. We are currently optimizing the detection of the interference at the protein level.
Supported by CSIC-Iniciación, Fondo Clemente Estable-CONICYT.

GE-P39.
CYSTEINE PROTEINASES OF THE NEWLY EXCYSTED JUVENILE (NEJ) OF *FASCIOLA HEPATICA*

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The identification of potential targets for vaccination of the trematode *Fasciola hepatica* has become a major research focus for control of this parasitic disease of livestock. The secreted cysteine proteinases of the invasive stage, the newly excysted juvenile (NEJ) flukes are important candidates, as they are considered to play a central role in host invasion and immune evasion. The goal of our research is to characterize the expression profile of cathepsin B (CB) and cathepsin L (CL) proteinases of NEJ. RNA was extracted from NEJ obtained *in vitro* from metacercariae produced in our laboratory. Two plasmid minilibraries of cDNA fragments coding for CB and CL proteinases respectively were constructed. Primers were designed based on conserved regions of *Fasciola hepatica* and *Fasciola gigantica* CB and CL genes found in public databases in order to amplify most members of the multigenic family. 48 clones from each library were sequenced at random and good quality sequences were organized in contigs. Of the three CB and two CL contigs identified, two CB and one CL cDNAs are novel for *F. hepatica*, and none of them correspond to products detected in the adult stage. Protein profile was analized from mass spectrometry of protein fractions obtained from NEJ somatic extracts and excretion/ secretion products separated by SDS-PAGE. CB and CL family members corresponding to the cDNAs were identified among the secretory products. This approach has allowed us to correlate the cDNA and protein expression profiles and identify the secreted cathepsins involved in the early host-parasite interactions.
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GE-P40.
EFFECTS OF A DECREASE ON THE PROTEASOME ACTIVITY IN THE EXPRESSION OF MBP RNAm

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We have demonstrated that the addition of Lactacystin (L), an specific inhibitor of the 26S proteasome, to oligodendrocytes (OLG) cultures induce their exit from the cell cycle and differentiation. Recent studies demonstrated that a decrease in proteasome activity enhances the MBP promoter in N20.1 OLGc line, due to a stabilization of Sp1 and p27. Results in Swiss male mice intracraneally injected (ICI) in corpus callosum with L at day 5-7 post-birth and evaluated 7 days after, showed an increase in myelin deposition, total proteins, myelin galactolipids and in the different isoforms of MBP related to control. We have decided to evaluate consequences in MBP RNAm expression by the decrease in the proteasome activity "*in vivo*" during normal myelination. Mice were sacrificed at day 7, 9, 11, 13 post-birth and their brains dissected to make a total RNA isolation, PCR, semi quantitative PCR and Northern Blot. Measured levels of the MBP RNAm show a good correlation with animal age and normal myelination parameters. Supporting this, we found that Sp1 RNAm levels are diminished in L, possibly explained by the stabilization of Sp1 protein degradation by proteasome inhibition. We expect to demonstrate a correlation between the accelerated myelination previously demonstrated in animals ICI with L and the MBP RNAm levels compared to control.

GE-P41.
HYPOSMOLARITY INCREASES THE EXPRESSION OF P75NTR THROUGH CHANGES IN ACTIVITY OF SP1 TRANSCRIPTION FACTOR

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The p75NTR is involved in neuronal functions that range from induction of apoptosis and growth inhibition to the promotion of survival. p75NTR expression is induced in central nervous system by a wide range of pathological conditions (stroke, seizures, traumatic brain injury), but the cellular mechanisms regulating this response are unknown. Hyposmolarity increases p75NTR levels in several cell lines, raising the possibility that post-traumatic edema may contribute to injury-induced p75NTR expression. We studied the hyposmolarity-induced p75NTR expression to identify the elements controlling p75NTR expression. Our results showed that hyposmolarity robustly induces p75NTR expression in cell lines and in primary mouse cortical neurons. The hyposmolarity-exposed neurons were susceptible to the killing induced by Pro-NGF (a known p75NTR ligand) demonstrating the biological activity of the newly synthesized p75NTR. We performed reporter assays that demonstrated that a highly conserved Sp1-rich area in the proximal p75NTR promoter and a potential silencer element were involved in the increased transcriptional activity. Chromatin IP assays demonstrated that Sp1 was bound to the proximal p75NTR promoter after the hyposmolar exposure. Transfection of DN-Sp1 or siRNA for Sp1 dramatically reduced the hyposmolarity-induced p75NTR response. These results indicate that Sp1 transcription factor is an essential component for the induction of p75NTR expression after hyposmolarity and a novel silencer element may collaborate to regulate p75NTR transcription.
Supported by the CHRI and Killam Foundation.

GE-P42.
PVF1/PVR AND JNK IN DEVELOPMENT OF GENITAL DISCS IN DROSOPHILA
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We are interested in three particular cellular behaviours that ocurre during development of genital discs. The cells that constitute the discs are epithelial and performe morphogenetic movements to shape the structures so to move they must change their adhesion properties, apoptosis is necessary in the scultpture of these structures, and finally the discs indistinctly the sex are originated by the same three primordiums, depending the sex there is a primordium that growth less, as a result a diferent control of growth is established. Our results suggest that PVF/PVR, VEGF/PDGF/VEGFR/PDGFR homolog of mammals, activating JNK are regulating the procesees mention above. Ectopic expression of the ligand PVF1 is follow by ectopic expression of *puckered (puc)*the only MAPK phosphatase that regulate JNK in *Drosophila*, as JNK is the transcriptional activator of *puc*, this indicate PVF1/PVR activate JNK. The apoptosis is triggered by JNK as upregulating its activity rescue the phenotype of lowering apopotosis in the discs. The target expression of a constitutive active form of the receptor PVR, produces overgrowth and apoptosis; the overgrowth is rescued by lowering JNK. As PVF/PVR products are found all over the discs, its activity is restricted, indicating its activation respond to particular genetic context. The balance in the PVF/PVR as well JNK activities are important as we determine that more activity have the same phenotypic effects of lowering them.

GE-P43.
STRUCTURAL CHARACTERIZATION OF DENGUE VIRUS 2 FUSION PEPTIDE BOUND TO DETERGENT MICELLES
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Dengue fever (DF) is one of the most widespread tropical diseases in the world. One hundred million people living mainly in big cities and urban zones are infected with the DF virus every year, while about 2.5 billion people are at risk of infection. Penetration of the DF virus into the target cell involves endocytosis induced by interaction of the glycosylated E protein, which is the major surface protein of the viral particle, with cellular receptors yet unknown. Subsequently, a fusion peptide (part of E protein) inserts and fuses into the endosome membrane at low pH. This work aims at determining solution structure of dengue virus type 2 fusion peptide by NMR as well as to study structural changes occurred during membrane fusion. Using fluorescence spectroscopy, we observed that the decrease of pH can promote structural changes of fusion peptide. Furthermore, at pH 5.5, which is the same pH of the endossomal compartment, these structural changes were more pronounced. CD spectra of fusion peptide free in solution at pH 5.5 and at pH 7.3 revealed that this peptide has remained unstructured. Alternatively, in presence of SDS and DPC micelles, the dengue fusion peptide becomes more of alpha-helical either at pH 5.5 or pH 7.2. Currently, we are determining its 3D structure in detergent micelles.

GN-P1.
MAPPING OF SUBTILISIN LIKE SERINE PROTEINASES IN TWO *S. tuberosum x S. berthaultii* DIPLOID POPULATIONS
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P. infestans (P.i.) is the most important disease on potato. Subtilisin like serin proteinases (subtilisins) have been recently related to plant defense against P.i. and other pathogens, by means of elicitor recognition and programmed cell death activation. However, their function in plant-pathogen interactions is still poorly understood. Is our interest to map subtilisins on potato, determine their expression pattern and analyze their role on plant defense through silencing strategies. Based on known subtilisin sequences from different species, a blast search was performed on potato databases. Forty-five subtilisins were retrieved and selected, and intron-flanking primers were designed. Amplifications were first performed on 3 genotypes parental to two diploid interespecific populations (*S. tuberosum x S.berthaultii*) to detect polymorphisms. Sixteen pair of primers generated polymorphic bands on SSCP or denaturing polyacrilamde gels. Location of this first set of subtilisins will be presented. This task is a part of a bigger project that aims to build a molecular-linkage map based on functional gene markers to use candidate-gene approach to identify genes responsible for QTLs, and to provide information for structural and functional comparison between plant species.

GN-P2
IN SILICO RECONSTRUCTION OF THE AMINO ACID METABOLIM PATHWAYS OF *Trypanosoma cruzi*
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In this work we employ a computational approach to reconstruct the amino acid metabolic pathways of *Trypanosoma cruzi*, aiming to link genomic with functional information through an in silico analysis of the current knowledge on cellular processes and gene annotations. Amino acid metabolism is very important for the life cycle of *T. cruzi*; its study has the potential to disclose new therapeutic targets and foster the development of new drugs. For that, we downloaded a list of EC numbers and the protein sequences of all enzymes involved in this metabolism from KEGG. Clustering of the proteins belonging to each EC was performed using a similarity-based approach; statistical profiles for each cluster were constructed using Hmmer. Reconstruction of the metabolic pathways involved with the amino acid metabolism of *T. cruzi* was performed by the blastp program, using as query the dataset of predicted proteins of *T. cruzi* against the profiles of each individual cluster. We also analyze the degree of analogy in this particular metabolism, due to its importance from an evolutionary point of view and the great interest to find new pharmacological targets.
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LP-P1.

OXIDATIVE-NITRATIVE STRESS BIOMARKERS AND HORMONAL PROFILE IN VARICOCELECTOMIZED PATIENTS

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We studied oxidative biomarkers and sperm characteristics in 36 patients with varicocele and 33 healthy men. Samples were taken before and after varicocelectomy. Computer-assistant semen analysis (CASA), oxidative-nitrative stress biomarkers, and hormonal determinations (RIE) were performed in all subjects. Production of nitrates + nitrites and TBARS were elevated in seminal plasma from patients before surgery but were normalized after 1-month varicocelectomy whereas decreased levels of retinol, ascorbate, α-tocopherol, FRAP assay, and GSH / GSSG ratios were also measured. They were normalized after 3 months of varicocelectomy. Increased levels of Zn and Se in seminal plasma, protein carbonyls, and fragmented DNA in espermatozoa remained elevated up to 1 month after surgery. Complete recovery of the major sperm parameters was achieved only after a 3-month period post-varicocelectomy. Plasma testosterone was diminished in patients but was normalized soon after surgery. Estradiol content showed no significant differences between groups, while both LH and FSH levels, exhibited a biphasic behavior. This finding suggests a complex imbalance in the pituitary-gonadal endocrine system induced by surgical treatment of varicocele condition. Our results support a rationale for controlled clinical trials in antioxidant-supplemented infertile men with varicocele. Those changes could be related to the oxidative stress implicated in this experimental model.

LP-P2.

X-RAYS INDUCED CHANGES IN TESTICULAR LIPIDS CONTAINING LONG AND VERY LONG CHAIN POLYUNSATURATED FATTY ACIDS

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Therapeutic doses of radiation applied to the testis is known to result in oligospermia or aspermia. Differentiating spermatogonia are killed by apoptosis, resulting in depletion of cells in more advanced stages of spermatogenesis. In this work adult rats were irradiated locally by 6.5 Gy of X-rays produced by a linear accelerator to study the effects of irradiation on testicular lipids, focusing on those that contain long and very long chain (VLC, C24-C32) polyunsaturated fatty acids (PUFA). The testicular weight decreased with time due to a substantial reduction of cells of the germ line. Six weeks after irradiation, sphingomyelin and ceramide were reduced, completely loosing their VLCPUFA. A galactoglycolipid typical of germ cells, seminolipid, was undetectable. There was also a marked decrease of the major glycerophospholipids, including diacyl- and plasmenyl- subclasses, as well as the neutral glycerides, all showing marked depletion of their major PUFA, mainly 22:5n-6, followed by 20:4n-6. The amount of cholesterol esters increased considerably, mostly due to an accumulation of 22:5n-6, the VLCPUFA typical of this lipid augmenting to a lesser extent. The results suggest that the cholesterol esters accumulate in the somatic cells remaining after irradiation, whereas seminolipid, the sphingolipids and glycerophospholipids decrease as a result of germ cell depletion.

LP-P3.

TESTICULAR LIPIDS WITH LONG AND VERY LONG CHAIN POLYUNSATURARED FATTY ACIDS IN RATS TREATED WITH DOXORUBICIN

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Doxorubicin (DXR) is an anticancer drug that induces apoptosis of spermatogonia, which in time leads to the involution of the seminiferous epitethelium. In previous work we analyzed the short-term effects of a single dose (9 mg/kg) of i.p. DXR on the lipids that contain long and very long chain (VLC) polyunsaturated fatty acids (PUFA) of rat testis. Here we studied the effects of smaller (3 mg/kg) but repeated (weekly, for 4 weeks) doses of the drug, and waited longer to observe the effects (9 weeks), since the spermatogenic cycle takes about 63 days in the rat. After 9 weeks, a marked depletion of all cells of the germinal line was noted in the testis, as well as a marked epididymal aspermia that was consistent with the complete infertility of the males. This loss was accompanied by a marked decrease in sphingomyelin and ceramide, lipids that, in addition, had lost their characteristic VLCPUFA. There was also a substantial decrease in the amount of the major glycerophospholipids, that after 9 weeks had markedly lower amounts of their major PUFA, 20:4n-6 and especially 22:5n-6. The only testicular lipids whose content increased several fold with testicular involution were the cholesterol esters. These lipids accumulated substantial proportions of PUFA (at first 22:5n-6, later on also 20:4), suggesting that in normal conditions they could play a role as a reserve of PUFA in the seminiferous tubules.

LP-P4.

GLYCEROL-3-PHOSPHATE ACYLTRANSFERASE ACTIVITY SUBCELLULAR LOCALIZATION

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Two isoforms of glycerol-3-phosphate acyltransferase (GPAT) activity have been historically described in rat liver: a NEM-sensitive form located in the ER (mcGPAT) and a NEM-resistant form in the outer mitochondrial membrane (mtGPAT1). A second NEM-sensitive form was recently described in mitochondria of nule-mtGPAT1 mice.
Rat liver was fractionated by differential centrifugation, microsomes (Mc) and crude mitochondria (cMt) were obtained. Further purification of cMt in a Percoll self-forming gradient yielded highly-purified mitochondria (pMt), and two fractions of mitochondria-associated membranes, MAM1 and MAM2. About 20% of the NEM-resistant activity of the cMt fraction was recovered in the MAM1 fraction and the presence of the protein was corroborated by its reactivity to a polyclonal antibody raised against the full-length-recombinant protein. Contamination of the MAM1 fraction with outer mitochondrial membranes was discarded by measuring marker enzyme activities and probing against an outer mitochondrial membrane (VDAC).
We concluded that NEM-resistant GPAT is also localized in MAM fraction, a specialized region of the endoplasmic reticulum.

LP-P5,
PROTECTIVE EFFECT OF MELATONIN AGAINST ADRIAMYCIN INDUCED LIPID PEROXIDATION IN RAT LIVER: GENE EXPRESSION PROFILE ANALYSIS

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Adriamycin (ADR), an anthracyclinic antibiotic frequently used in quimioterapeutic treatments is highly toxic; it inhibits protein synthesis and provokes lipid peroxidation effects. Melatonin has recently been shown to have high antioxidative properties. We tested if melatonin is able to neutralize the oxidative damage induced by a single dose (7.5 mg/kg, i.p.) of ADR preceded (1 day) and followed (7 days) by a (6.0 mg/kg, i.p.) of melatonin. ADR treatment decrease the PUFAs C22:6 n-3 and C20:4 n-6 in rat liver mitochondria . When the treatment of adriamycin was followed by melatonin, the decrease in these fatty acids was not observed. A significant decrease in lipid peroxidation (CL assay) was observed after administration of ADR, which was restored to control values by post-treatment with melatonin. Gene expression profiles of ADR vs. ADR + melatonin treated rat livers indicated that both treatments induced significant changes. Quantitative real-time PCR analysis of 40 genes involved in oxidative stress revealed that CYP1B1 which is involved in electron transporter, cyclin-dependent kinase inhibitor 1A that possesses cyclin-dependent protein kinase inhibitor activity was induced at a more pronounced level in the ADR + melatonin treated samples than in the ADR treated ones. More genes having roles in heat-shock response were down-regulated in melatonin treated animals, such as hsp40 and hsp60 proteins. More studies are needed to reveal the effects of melatonin on ADR-induced oxidative damage in liver.

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LP-P6.
L-FABP SHUTTLES 20:4n-6 IN NUCLEAR LIPID POOLS

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Regulation of gene expression by fatty acids is an event that takes place inside the nuclei of eukaryotic cells. Fatty acids regulate the gene expression of enzymes involved in lipid and glucose metabolism, in adipogenesis process, and in cell differentiation. Due to their hydrophobic nature and poor water solubility, fatty acids move within the cells bound to L-FABP. Our aim was to study the role of L-FABP in the trafficking of nuclear fatty acids. Thus, nuclear 20:4n-6 pools were labeled by *in vitro* incubation of nuclei isolated from rat liver cells with [1-^{14}C]20:4n-6, in presence of ATP and CoA. Then, these control nuclei were reincubated without 20:4n-6, plus ATP, CoA and L-FABP. In control nuclei [1-^{14}C]20:4n-6 was incorporated into PL (54%), FFA (26%) and TG (20%). In PL it was esterified in PC (58%), PE (29%) and PI (13%). In reincubated nuclei we observed that 20:4n-6: 1)increased in the incubation medium and 2)in nuclear pools, decreased as FFA and increased in PI, as a function of L-FABP concentration. Reincubation conditions were repeated using BSA as a carrier protein control. In conclusion, L-FABP movilizes nuclear arachidonic acid, since it removes 20:4n-6 from nuclear pools and directs it to PI.

LP-P7.
INTERACTION OF DISCOIDAL APOAI LIPOPROTEIN COMPLEXES WITH CHO KI CELLS. INFLUENCE OF DISC SIZE AND CHOLESTEROL

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Apolipoprotein AI (apoAI) adopts different conformations in discoidal high density lipoprotein (HDL), depending on disc size and cholesterol (Ch) content. These alternative conformations, in special those of a central domain, seem to be quite important for determining the membrane affinity and Ch exchange rate between discs and lipid bilayers. This central domain is also responsible for triggering the mobilization of intracellular Ch pools toward the cell membrane (Gonzalez *et al.*, SAIB 2004). Thus, its alternative conformations could modulate these cellular responses. In order to test this possibility, we have reconstituted different sized apoAI discoidal complexes (rHDL) containing 1-palmitoyl-2-oleyl-phosphatidylcholine (POPC) or POPC and Ch, and compared their activity in promoting cellular Ch efflux and mobilization of de novo synthesized Ch toward the cell membrane. Although apoAI lipidation decreases its capacity to promote Ch efflux, this effect is independent of disc size and composition. On the contrary, mobilization of de novo synthesized Ch is strongly influenced by these factors, being the biggest Ch-containing particles entirely inactive to trigger this process.

LP-P8.
GANGLIOSIDE SYNTHETIC VARIATIONS IN THE TWO PHENOTYPES OF A HEPATIC STELLATE CELL LINE

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GRX cell line represents hepatic stellate cells and can be transformed from an actively proliferation myofibroblast phenotype into a fat-storing lipocyte phenotype. To evaluate in both phenotypes the contribution of the 3 ganglioside synthetic pathways, cells were preincubated with 5mM β-chloroalanine (SPT: serine palmitoyltransferase inhibitor), or with 25μM fumonisin B1 (CS: ceramide synthase inhibitor) and then radiolabeled with [U-^{14}C]galactose in the continued presence of inhibitors. Gangliosides were extracted, purified and analyzed by HPTLC. In myofibroblasts, complex gangliosides are mainly synthesized by recycling pathways while simple gangliosides use the de novo pathway. In lipocytes, de novo pathway has a lesser contribution and this is in agreement with the lower activity of the committed enzyme of sphingolipid synthesis (SPT activity) detected in this phenotype. It is suggested that the two phenotypes of GRX cell line have important differences in their glycosphingolid biosynthetic pathways and probably use distinct ceramide pools for these processes.

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LP-P9.

POSSIBLE ROLE OF SPHINGOSINE 1P (S1P) AS A SURVIVAL FACTOR IN MDCK CELLS SUBMITTED TO HYPERTONIC STRESS

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Renal papilla is the kidney zone that performs the final adjustment of urine composition. Structurally is constituted by tubular collecting ducts which have to survive and function in a non-favorable environment since are submitted to the highest renal interstitial tonicity. Sphingolipids constitute a lipid family with diverse relevant physiological actions such as constituting biomembranes (Sphingomyelin, SM), inducing cell differentiation (ceramide, Cer) or proliferation and survival (sphingosine 1P, S1P). In order to determine the contribution of these molecules to cell survival in hypertonic conditions, we studied sphingolipid metabolism in renal epithelial cells submitted to high NaCl concentration. Confluent-arrested MDCK cultures were grown in physiological or hypertonic media (150 or 250 mM NaCl, respectively) for 24 and 48 hs and ^{32}P-Pi and ^{14}C-palmitic acid (P), which monitors de novo synthesis, incorporation to SM, Cer and S1P was determined. Hypertonicity increased S1P de novo synthesis by 58 and 46%, while induced a slight increase (26%) or a decrease (39%) in ^{14}C-P incorporation to Cer after 24 and 48 hs, respectively. No changes were observed in radioactive SM after 24hs but after 48 hs of hypertonicity, SM labeling increased by 40% (^{14}C) and 92% (^{32}P) respectively. Considering that hypertonicity caused a dramatic drop in cell number, showing the remaining cells high viability, the above results suggest that cell survives to changes in media tonicity by inducing the synthesis of survival molecules such as S1P and dowregulating Cer production.

LP-P10.

IMPORTANCE OF SPHINGOSINE KINASE 1 IN MDCK CELLS PROLIFERATION AND SURVIVAL

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We have previously demonstrated that 24hrs treatment of subconfluent-MDCK cells with 25µM tDHS (D,L-threodihydro-sphingosine), an inhibitor of Sphingosine Kinase 1 (SK1), reduced cell number (32.5% of control) and viability (16.3%). Incubation with ^{32}P and ^{14}C-palmitic-acid, showed a reduction in sphingosine-1-phosphate (S1P) production (26% of radiolabelling SLs vs. 51% in the control) and Ceramide (Cer) accumulation (39% vs. 26%), respectively. In order to determine if the effect of tDHS treatment is due to S1P synthesis decrease or to Cer accumulation we carried out two strategies: 1)Inhibition of Cer synthesis by treatment with 50µM Fumonisine B1 (FB1), together with tDHS: this treatment promoted an attenuation in tDHS effect raising cell number and viability (65.7% and 53%) and decreasing Cer accumulation (11% of radiolabelling SLs). 2)Knockdown of SK1 expression, by transfecting MDCK cells with a SK1-specific siRNA: the treatment induced a)A reduction in SK1 expression (43% of control). b)Cell number reduction (66% of control) but with out loosing cell viability (97%). c)Reduction in total radiolabelled SLs and accumulation of radiolabelled sphingosine, in spite of no reduction in the percentage of incorporation of radioactive mark to S1P was obtained. Taken together these results indicate that pharmacological inhibition of SK1 with tDHS disturbed the sphingolipids-rheostat by accumulating of Cer and decreasing S1P production, affecting cell proliferation and viability. By other side, physiological block of SK1 by the specific siRNA, provoked a less unbalance in the rheostat, with out accumulation of Cer and affecting only cell proliferation.

LP-P11.

PHOSPHATIDIC ACID METABOLIZATION IN THE PRESENCE OF LYSOPHOSPHATIDIC ACID (LPA), CERAMIDE 1 PHOSPHATE (C-1-P) AND SPHINGOSINE 1 PHOSPHATE (S-1-P) IN CEREBRAL CORTEX SYNAPTOSOMES FROM ADULT AND AGED RATS

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We have reported the presence of phosphatidate phosphohydrolase, PAP2 isoform and the stimulation by aging of this activity in synaptosomes. PAP2 hydrolyzed not only phosphatidic acid (PA) but also other phosphorylated substrates such as LPA, C-1-P y S-1-P producing different intracellular mediators. The purpose of this work was to evaluated in synaptosomes the preference for the isoform of PAP2 by different concentrations of the alternative substrates and to analyzed the aging effect. Synaptosomes were prepared from adult (3 mo) and aged (26 mo) rats cerebral cortex. PAP2 was assayed using [^{3}H] PA as substrate. Diacylglycerol formed by PAP2 action was partially metabolized to monoacylglycerol and glycerol. The presence of PAP2b was observed by Western Blot. PAP2 from adult membranes diminished its activity by 20%, 36% and 14% with LPA, C-1-P and S-1-P, respectively. In aged membranes was observed a major competitive effect in the presence of C-1-P and S1P. A differential relation between DAG, MAG and glycerol was observed in the presence of the substrates. Our results are indicative of a differential preference of PAP2 by its alternative substrates in aged membranes respect to adult membranes.

LP-P12.

PGD2 MODULATES CYTIDYLYLTRANSFERASEa (CCTa) TRANSLOCATION TO NUCLEUS THROUGH PLD AND PLC PKC6MAPK ACTIVATION

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Phosphatidylcholine (PC) is a main lipid of biomembranes. We have previously demonstrated that renal papillary PC synthesis is regulated by endogenous-synthesized prostaglandin D_2 (PGD2) through PLD and PLC-PKC-ERK1/2 activation which seems to modulate nuclear CCTα. CCTα is the rate-limiting enzyme for PC biosynthesis and is regulated by reversible association with membrane lipids. In this work we studied CCT translocation to membrane lipids and its correlation with PC synthesis. CCT translocation was determined by westernblot analysis of different fraction (cytosol, nuclei and microsomes) with a polyclonal antibody against CCTα. This study was performed in the absence or in the presence of PGD_2, with or without the addition of PLD (1%ethanol, E), PKC (0.6µM cheleritrine, C), PLC (1µM U73122, U) inhibitors. 1min PGD_2 stimulates translocation to membrane (nuclei and microsomes), such an effect was blocked by E, C, U and also by U0126 (MEK inhibitor, M) correlating with PC synthesis. After 15min, CCT translocated from membrane to soluble fraction, according with lipid membrane reconstitution. This event was blocked by C, U, M and E correlated with PC synthesis. Our results indicate that PGD_2 stimulates CCT translocation to nuclear membrane and this event correlate with PC synthesis. Translocation involves PLD / PLC-PKC-ERK activation, and both pathways operate independently on CCT translocation and PC synthesis homeostasis.

LP-P13.

TRANSCRIPTIONAL REGULATION OF CTP: PHOSPHOCHOLINE CYTIDYLYLTRANSFERASE BETA

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In mammalian cells, two genes encode CTP:phosphocholine cytidylyltransferase: *ctα* y *ctβ*. CTα is the most abundant isoform while CTβ have been recently identified. In mouse, it is expressed as two different isoforms; CTβ2 y CTβ3. CTβ2 and CTβ3 differ in the N-terminal region: CTβ2 lacks the first exon, while CTβ3 excludes the second one. These enzymes are expressed in brain and gonads. The aim of the current work is to identify the mechanism that regulates the expression of each isoform. To analyze the promoter region we cloned by PCR two different fragments, one including the 5'UTR region of the *ctβ* gene (as a CTβ3 putative promoter) and the other correspondent to the 3' terminal of the first intron (as a CTβ2 putative promoter). The promoter activity was assayed using luciferase reporter construct transfected in Neuro 2A cell line. We observed that the expression of each isoform is driven by its own promoter. Using 5' deletion we identified the minimal promoter regions and by 5'RACE we mapped the start transcriptional points. To determine the role of each isoform in the neurite outgrowth and cell differentiation we analyzed the luciferase activity in cells treated with retinoic acid. After cell differentiation (neurite outgrowth) there is a clear stimulation in the expression of both isoform. This result suggests that CTβ could be involved in the biosynthesis of phosphatidylcholine during cell differentiation.

LP-P14.

POSSIBLE INVOLVEMENT OF CYCLOOXYGENASE-2 IN HYPERTONIC-INDUCED PHOSPHOLIPID SYNTHESIS

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Papillary cells function under physiological stress since they are submitted to the highest renal interstitial osmolality. We demonstrated that papillary tissue possesses an active phospholipid (PL) synthesis, which could act as a protective mechanism for cellular membranes in such an adverse environment. Recently, we have shown that in MDCK cultures, hypertonicity increases PL content and synthesis. Since cyclooxygenase-2 (COX2), reported as a survival protein, is involved in the maintenance of papillary PL synthesis, in the present work we explored the relationship between hypertonic-induced PL synthesis and COX-2 protein. For this purpose, confluent-arrested MDCK cultures were grown in physiological or hypertonic media (150 or 250 mM NaCl, respectively) for 24 and 48 hs, in the absence or in the presence of NS398 (specific COX-2 inhibitor). After treatment, PL synthesis and COX-2 protein levels were evaluated. Biosynthetic activity was increased by hypertonic media. Such an increase was not blocked by NS398 and was even higher than hypertonic condition alone suggesting that COX-2 activity is not involved in biosynthetic pathway. However, westernblot experiments revealed that COX-2 protein expression was higher in hypertonic cultures treated with NS398 respect to controls. These results suggest that COX-2 specific inhibitor upregulates COX-2 protein expression which could overpass NS398 inhibitory action. Hence, hypertonic increased PL synthesis could be related to the increased COX-2 activity contributing in this way to membrane homeostasis.

LP-P15.

DIACYLGLYCEROL GENERATION BY PHOSPHATIDYL CHOLINE BREAKDOWN CATALIZED BY A PHOSPHOLIPASE C FROM CENTRAL NERVOUS SYSTEM

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Diacylglycerol (DAG) derived from phosphatidylcholine (PC) acts as a lipid second messenger and it can be generated by the activation of phospholipase D (PLD) and phosphatidic acid phosphohydrolase type 2 (PAP2) pathway or by a phosphatidylcholine-specific phospholipase C (PC-PLC). Previously we had demonstrated the PC-PLC pathway in rat cerebral cortex synaptosomes (CC Syn). Our purpose was to characterize PC-PLC activity in central nervous system. Ethanol (a PLD activity marker) and D609 (a selective PC-PLC inhibitor) decreased DAG generation by 42% and 61%, respectively at 20 min incubation. These data demonstrate that both PLD/PAP2 pathway and PC-PLC contribute to DAG generation in CC Syn. It was also demonstrated that PC-PLC activity remained located mainly in the synaptosomal plasma membrane fraction. Western blot analysis with anti PC-PLC antibody showed a band of 66 kDa in CC Syn. In prelabeled synaptosomes with [^{3}H]glycerol DAG generation was stimulated by glutamate (5 mM). Our results indicate the presence of a novel DAG-generating pathway in CC synaptosomes, and its probable participation in oxidative processes induced by high glutamate concentrations.

LP-P16.

PHOSPHOLIPID PROFILE AND EXPRESSION OF ENZYMES INVOLVED IN LIPID METABOLISM IN RAT AORTA. EFFECT OF VITAMIN A DEFICIENCY

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Antioxidants are known to reduce cardiovascular events by preventing oxidative modification of low density lipoproteins. We have previously reported the prooxidative effect and increased triglycerides, cholesterol and phospholipid contents in aorta of vitamin A deficient-rats. Here, we evaluate the phospholipid profile and mRNA expression of regulatory enzymes of aorta lipid synthesis by RT-PCR. A group of Wistar male 21 d old rats were fed during three months with a free vitamin A diet (-A) and another one with the same diet plus 8 mg of retinol palmitate/kg of diet (+A). A group of -A rats was refed with control diet fifteen days before sacrifice. Individual phospholipids were separated by TLC using silica gel H plates and chloroform/methanol/water (65:25:4 by vol.) as solvent system before quantifying by Rousser method. Total RNA was isolated using TRIzol. Aorta of -A rats showed an increase of PC, PI+PS, and PE, a decrease of SM, and no change in PG. The mRNA of diacylglycerol acyltransferase-1, CTP: phosphocholine cytidylyltransferase-a, lipoproteinlipase and lectin-like oxidized LDL receptor- 1 increased in -A aortas in relation to +A. The restitution of vitamin A to the -A rats reverted all the changes observed. Thus, the vitamin A deficiency alters the aorta phospholipid pattern and expression of lipid regulatory enzymes.

LP-P17.

ANTI PE IgM ANTIBODIES ARE A UNIVERSAL TRAIT OF MAMMALS AND MAY REPRESENT AN INNATE IMMUNE MECHANISM

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Knowledge on phospholipids of plasma lipoproteins has long been acquired. For unknown reasons, the phosphatidylethanolamine (PE) content of these complexes is strikingly lower than that in cell membranes. On the other hand, it is also well known that PE is the main phospholipid in most enteric bacteria. We here report the universal presence of anti-PE IgM antibodies throughout mammals, as detected by an ELISA assay and confirmed by immunoblotting. By contrast, anti-phosphatidylcholine (PC) IgMs are less than half of those observed against PE, with the notable exception of mice. Presence of antibodies against sphingomyelin (SM), a major lipoprotein component, is almost negligible. Natural anti-phospholipid antibodies were confined to the IgM isotype in all cases. No anti-phospholipids IgGs were detected. The diversity of species in which anti-PE IgMs were observed, is an indication that their presence is a universal phenomenon that affects all mammals. We propose that these novel anti-PE IgMs, represent a common innate defense system against infection by gut resident bacteria, and that the low lipoprotein PE is an adaptation that allows the operation of these antibodies by preventing their binding to plasma components, and consequently their free availability.
Supported by ANPCyT and CONICET.

LP-P18.

BIOCHEMICAL CHARACTERIZATION OF PLASMA MEMBRANE-ENRICHED FRACTIONS AND LIPID MICRODOMAINS FROM *Bufo arenarum* OOCYTES

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The role of lipid microdomains ("rafts") in amphibian oocyte signal transduction pathways has not been elucidated to date. As a prerequisite to understand this involvement, it is necessary to characterize plasma membrane and lipid microdomains from full-grown *Bufo arenarum* oocytes. Plasma membrane-enriched fractions were obtained by centrifugation. Lipid microdomains were isolated either in the presence or in the absence of detergent (Triton X-100) by sucrose gradient centrifugation and characterized by Coomassie and Ponceau S staining as well as by Western-blotting. Lipids were analyzed by chromatography techniques. In plasma membrane preparations, phosphatidylcholine and phosphatidylethanolamine represented 68% of the total lipid content followed by cholesterol (9.90 nmol/mg prot.) and sphingomyelin (9.82 nmol/mg prot.). Density gradients and Western-blotting of detergent-free and detergent-treated membranes showed similar profiles. A distinctive band with apparent molecular weight of 66.2 kDa was observed only in low-density membrane fractions and was evidenced mainly in pellet fractions obtained from total plasma membrane compared to soluble fractions, suggesting its association with lipid rafts.

LP-P19.

ADVANCES IN THE CHARACTERIZATION OF A LIPOVITELLIN IN CRUSTACEAN

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In oviparous species, proteins and lipids are found in the vitellus; forming are lipoproteins called lipovitellins (LV). They are an important energy source for the embryo development and larvae growth and survival. We have previously isolated and characterized (mainly its lipid composition) the sole egg cytosolic lipovitellin in the freshwater shrimp *Macrobrachium borellii*. It has a native structure of 440 kDa, and two subunits of 94 and 112 kDa under dissociating conditions. In the present work we studied size, shape and structure of *M. borellii* LV using electronic microscopy, crosslinking, reagents, MALDI-TOF, circular dichroism, fluorescence and partial proteolysis. Results showed that LV has spheroidal morphology with an estimated size of 18.5 +/- 3.5 nm. The LV might contain in its structure two subunits of 94 kDa and another one of 112 kDa (more exposed to the aqueous medium); they are not linked by disulfide bonds. Using MALDI-TOF technique, 42 polypeptides were found to be homologous to a vitellogenin of a related species (*M. rosembergii*). Then, it was noted that this particle contains 35.7% a-helix, 16.6% β-sheet and 20% turns.

LP-P20.

FIRST EVIDENCE OF EXTRACELLULAR PHOSPHATIDYLINOSITOLPHOSPHATES INVOLVED IN PLANT DEFENSE RESPONSES

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Systemic acquired resistance (SAR) is a broad-spectrum resistance mechanism in plants that is activated in naive organs after exposure of another organ to a necrotizing pathogen. It has been reported that a lipid transfer protein (LTP) triggers SAR by translocating / transmitting a mobil signal, which could be a lipid molecule, from the infected tissue to distal organs of the plants. Since phospholipid molecules (PLs) are emerging as novel second messengers in plant defense signaling, the objective of this work was firstly to determine whether there are extracellular PLs, and secondly, if they could be involved in plant defense responses. To this aim, we analyzed cellular and extracellular PL profile of suspension tomato cells pre-labeled with [^{32}P] ortophosphate by thin layer chromatography. We detected PLs in the extracellular medium and their pattern was different from cell PL profile. Moreover we found that phosphatidylinositolphosphate (PIP) was the most abundant PL in the extracellular medium, and that treatment of suspension tomato cells with the pathogenic elicitor xylanase increased extracellular PIP levels substantially.
Finally, we demonstrate that exogenous addition of PIP triggers typical defense responses such as production of reactive oxygen species (ROS) and cell death, both in a dose dependent manner.
These indicate that extracellular PIPs are involved in plant defense responses, and suggest their putative role in plant signaling.

MI-P1.

INTEGRITY OF JNK PATHWAY IS REQUIRED TO PROTECT AGAINST CYTOTOXICITY TRIGGERED BY *Vibrio cholerae* EL TOR HAEMOLYSIN

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In previous studies we have shown that El Tor Haemolysin (ETH), a highly conserved pore forming toxin secreted by most *Vibrio cholerae* (VC) isolates, could be involved in the pathogenesis of VC strains lacking cholera toxin. Additionally, we demonstrated that this toxin induces intracellular calcium increase and apoptosis in human intestinal cells.
It is well established that several MAPK pathways senses cell injuries caused by different bacterial toxins, leading to a protective response or to cell damage. Preliminary results suggested the induction of c-Jun activation upon cell exposure to ETH. Hence, to study the involvement of the JNK pathway in response to this toxin, we exposed human intestinal CaCo-2 cells to different doses of wt or ETH-defective mutant VC culture supernatants, or to purified ETH, in the presence or absence of the specific JNK inhibitor SP600125. After different times post-exposition, cell survival was determined. JNK blockade lead to decreased cell survival upon exposure to the toxin. Furthermore, the same result was observed using *wt*-MEF (Mouse Embryo Fibroblasts) and their JNK-deficient derivative cells in similar experiments. Moreover, ETH induced rapid c-Jun phosphorylation, indicating that the JNK/c-Jun pathway is involved in cellular response to this toxin. Taken altogether, our results demonstrate that integrity of JNK pathway provides a cell protective response against cytotoxicity triggered by ETH.

MI-P2

***Brucella abortus* LUMAZINE SYNTHASE IS A NOVEL VIRULENCE FACTOR**

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Lumazine Synthase (LS) (E.C. 2.5.1.9) is the enzyme that catalyses the penultimate step in the pathway of riboflavin synthesis. Riboflavin is an essential cofactor and the precursor of coenzymes FMN and FAD. *In silico* analyses have revealed the presence of two genes coding for LS in the *B. abortus* S2308 genome: *ribH1* and *ribH2*. *ribH1* is arranged in an operon with four other genes, all of them coding for enzymes that participate in the pathway of riboflavin synthesis. In contrast, *ribH2* is not transcriptionally associated to other genes and has a unique regulatory element upstream. In order to determine if these proteins play any role in *B. abortus* virulence, we generated the insertional mutant *ribH2*::Gmr and the deletion mutant *ΔribH1*. Cell infection assays revealed no differences in intracellular replication between *ΔribH1* and the wild type strain. On the contrary, *ribH2*::Gmr was defective during early infection events. However, at later times p.i., similar levels of CFU were recovered from cells infected with the mutant and the wild type strains respectively. In spite of these, mice infection assays showed that *ribH2*::Gmr is very attenuated at 40 days p.i. These results suggest that RibH2 could be a virulence factor, having a function other than synthesis of riboflavin and that this function might be accomplished by RibH1.

MI-P3.

THE Cgt PROTEIN OF *Brucella abortus* REQUIRES AN INTACT MONONUCLEOTIDE BINDING DOMAIN TO FUNCTION IN TRANSPORT OF CYCLIC β-1,2-GLUCAN VIRULENCE FACTOR

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Brucella abortus Cgt protein responsible for the transport of cyclic β-1,2-glucan to the periplasm is required for intracellular replication and full expression of virulence (Roset *et al.,* 2004, Infect. Immun. 72: 2263-2271). The predicted membrane protein of 66 KDa, Cgt, has in the C-terminal domain all the conserved features of a typical ABC transporter such as the Walker site A (GxxGxGKS/T), the Walker site B (hhhhD), and the ABC signature, suggesting that this protein couple energy by NTP hidrolysis to transport the cyclic β-1,2-glucan. The Lys-374, found within the conserved Walker site A nucleotide binding motif of Cgt was changed to Ala by site-directed mutagenesis. The *cgt* gene altered in NTP-binding was unable to complement the *cgt* null mutant for either cyclic β-1,2-glucan transport or intracellular replication and virulence. These results demonstrated that an intact NTP-binding domain is critical for Cgt function in cyclic glucan transport and confirm that the cyclic β-1,2-glucan is necessary in the periplasm and/or extracellular to exert is action as virulence factor. On the other hand, the transport of the cyclic β-1,2-glucan to the periplasm was inhibited in wild type *B. abortus* after introducing a plasmid expressing a mutant *cgt* altered in the NTP-binding region. The dominant negative phenotype suggests that Cgt function as a multimer.

MI-P4.

IDENTIFICATION OF ACTIVE SITE RESIDUES OF THE INVERTING GLYCOSYLTRANSFERASE Cgs FROM *Brucella abortus*

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B. abortus cyclic glucan synthase (Cgs) is a 320-kD polytopic integral inner membrane protein responsible for the synthesis of cyclic β-1,2-glucan. To gain further insight into the protein domains essential for enzyme activity such as active site/s, we have compared the Cgs sequence to other glycosyltransferases, and we have identified the widely spaced D, DXD, E/D, (Q/R)XXRW motif that is highly conserved in all Cgs and in the active site of numerous glycosyltransferases. By site-directed mutagenesis and *in vitro* and *in vivo* activity assays, we have demonstrated that most of these residues are involved in the activity of Cgs. Cgs-(475-818) domain, where the D, DXD, D/E, (Q/R)XXRW motif was identified, may be implicated in [UDP-Glc:β-(1,2)oligosaccharide glucosyltransferase] activity being responsible for chain elongation during cyclic glucan synthesis. Furthermore, over-expression of inactive mutants results in wild type production of cyclic glucan when cells co-express the mutant form and the wild type form. Together, these results are compatible with a single addition model by which Cgs acts in the membrane as a monomeric enzyme, and uses the D, DXD, D/E, (Q/R)XXRW motif to form a single center for substrate binding and glycosyl transfer reaction.

MI-P5.

CONSTRUCTION AND CHARACTERIZATION OF A *Brucella abortus* MUTANT DEFECTIVE IN PHOSPHATYDILETHANOLAMINE

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Brucella membrane is composed by three major lipids: phosphatidylethanolamine(PE), Phosphatidylcholine and Ornithine lipid. In bacteria, synthesis of PE occurs by condensation of CDP-diacylglycerol with serine to yield phosphatidylserine (PS), in a reaction catalyzed by PS synthase (pss). A subsequent PS decarboxilation produces PE. We are interested in studying the role that these lipids could play in the bacteria-host cell interaction. We have identified in the *B. abortus* genome a candidate gene coding for pss. The putative pss gene was cloned and knocked-out and the corresponding *B. abortus* pss mutant was generated. The lipid profile of the mutant indicated that it is devoid of PE. Although the viability of this mutant was not affected, its growth in TSB medium was diminished. However, millimolar concentration of Mg^{2+} restored the normal growth phenotype. The mutant's capability to infect different *in vitro* cell cultures was severely affected.
All the factors mentioned above will contribute to a better understanding of *B. abortus* ability to avoid the host's immune defense and the establishment of a long-lasting infection.

MI-P6.

CHARACTERIZATION OF *Brucella abortus* RpoN: GENETIC COMPLEMENTATION OF *Sinorhizobium meliloti ntrA* MUTANT

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The alternative sigma factor σ^{54} (RpoN, NtrA) is required for transcription of a wide range of genes involved in diverse physiological functions. In *Sinorhizobium meliloti*, *ntr*A product is required for C_4-dicarboxylate transport and symbiotic nitrogen fixation. A gene with high homology to *S. meliloti ntr*A gene was identified in *Brucella abortus*. Analysis of the deduced amino acid sequence revealed that *B. abortus* σ^{54} has the characteristic domains and motifs commonly found in σ^{54} factors. We cloned the *rpoN* gene from the intracellular pathogen *B. abortus*. This gene was functional in *S. meliloti ntr*A mutant as was demonstrated by complementation assays, in which *rpo*N of *B. abortus* was able to restore growth in minimal media with succinic acid as a sole carbon source as well as the formation of functional nodules in alfalfa. To study the role of σ^{54} in *B. abortus* a mutant in the *rpo*N was constructed. In *B. abortus rpo*N is not essential for infection and multiplication in nonphagocytic or phagocytic cells, nor in the establishment of acute or chronic infection in mice. The mutation in the *rpo*N gene has no effect in osmotic, acidic and oxidative stress conditions analyzed, however the decrease of viability in rich and defined media suggest that σ^{54} is mainly involved in the survival of *B. abortus* under nutrient starvation conditions in stationary phase growth.

MI-P7.

THE *pha* GENE CLUSTER OF *Brucella abortus* INVOLVED IN pH ADAPTATION

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The operon *phaABCDEFG* in *Sinohizobium meliloti* code for a K^+ secretion system that is involved in the adaptation to pH changes and in symbiosis. In *B. abortus* a locus highly similar to pha operon of *S. meliloti* was identified. This cluster of genes is 6 kb and is predicted to encode six membrane-spanning proteins. In this study a polar mutant in *pha*A/B was constructed and characterized by *in vitro* and *in vivo* assays. Growth of the mutant was impaired in define medium at alkaline pH by concentration of added K^+ as low as 0.04 M. This phenotype was suppressed with 20 mM sodium acetate added. We studied the multiplication of the *Brucella* wild type and the mutant in professional and non-professional phagocytes. *B. abortus* 2308 *pha*A/B mutant showed differences in the adherence and/or invasiveness in HeLa and J774 cells and less ability to replicate intracellulary during the first hours after infection in HeLa cells. In mouse model the mutant resulted in a reduction of 0.88 and 0.89 Log CFU/spleen at 10 and 30 days post-infection respectively. The indexes of competition between both strains were 7.47 and 1.88 at 10 and 30 days post-infection respectively. These results suggest that the *pha* operon is important for the homeostatic capacity of pH and for K^+ resistance via an K^+/H^+ antiporter and might be necessary for adaptation to the intracellular lifestyle and virulence.

MI-P8.

IivA OF *Brucella* INTERACTS WITH PHOSPHOLIPIDS UNDERGOING A STRUCTURAL REARRANGEMENT

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We have previously identified the gene iivA of *Brucella* as a virulence factor. It encodes an 11 kDa basic protein of unknown structure and function which is highly conserved in bacteria. The sequence analysis predicts two coiled-coil regions encompassing the C and N terminal halves. Light scattering and cross-linking experiments of complete IivA and truncated forms of the C and N terminal coiled coil regions demonstrates that the protein self-associates in solution as a trimer by its C terminal region (Carrica M. *et al*., SAIB 2003). In this work, we demonstrate by circular dichroism (CD) that the C terminal region of IivA is α-helical, whereas the N terminal re- gion is mainly random coil. This result agrees with the increased susceptibility of this region to proteases as compared to the C terminal region. Phosphate anions and SDS produce a strong increa- se (40%) in the CD signal of the protein. Based on these results and the location of iivA in the genome of *Brucella*, we hypothesi- ze that IivA could interact with phospholipids. In this regard, we show that IivA interacts with phosphatidic acid (PA) by Lipid Overlay Assay. Moreover, we demonstrate by CD spectra and limited proteolysis that small unilaminar vesicle made of phosphatidylcholine and PA induce conformational changes and stabilize IivA. These data contribute to understand the bases of IivA function and the intimate mechanisms of the *Brucella* virulence.

MI-P9.
NITRIC OXIDE PRODUCTION IN BOVINE AND MURINE MACROPHAGES CELL LINES INOCULATED WITH *Brucella abortus*

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Nitric oxide (NO) have anti-*Brucella* activity in macrophage cells. Our objective was to compare the ability of different strains of *B. abortus* to induce NO production in the murine (J774) and bovine (BoMac) macrophages cell lines. Both cell lines were inoculated with $1.7x10^7$ CFU of *B. abortus* S19, RB51 (rough) and 2308 respectively, incubated for 2 hours, washed and added new media with gentamicin for 30'. to kill extracellular bacteria. All cells were lysed with deoxicholic acid 0.1% for 15'. Beside, both cell lines were also plated on coverslips and stained with acridine orange and Giemsa. CFU of the three strains were determined at times 0, 4, 24 and 48 hours. NO production was measure in the supernatant with Griess reagent kit. The multiplication of RB51 was declining with the time, in both macrophage cell lines, however, the smooth strain multiplication was increasing with the time. BoMac NO production was higher than J774 cells for all the strains. The maximum production of NO in BoMac was at 24 hs, while in J774 was at 48 hs. Although *B. abortus* LPS doesn't induce the NO production, NO level is higher in cells infected with the rough strain. Probably the luck of O-side chain of rough LPS has relevance in this behavior.

MI-P10.
A RECOMBINANT S19 *Brucella abortus* EXPRESSING A *Babesia bovis* ANTIGEN IS IMMUNOSTIMULATORY

Sabio y García J[1], Macedo G[2], Barsante M[2], Carvalho N[2], Carrica M[1], Farber M[1], Cravero S[1], Campos E[1], Costa S[2], Osvaldo R[1].
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Due to the strong cellular and humoral immune response that S19 *Brucella abortus* (live vaccine) elicits, it is an attractive vector for the delivery of heterologous antigens. The objective of the present study is to express antigens of pathogens that require the same type of immune response elicited by *Brucella* to control the desease they produce. S19 *B. abortus* expressing RAP1a (a conserved immunogenic antigen) of *Babesia bovis* were generated. *rap1a* was PCR-amplified as a complete version or without the sequence that encodes for its signal peptide. The amplicons were subcloned under different promoters and signal se-quences: *lacz, bp26* and *omp19* to study diverse subcelular localizations. BP26 is a peri-plasmic protein and OMP19 is associated to the outer membrane of *Brucella spp*. RAP1a as a fusion with the first aminoacids of either ß-galactosidase or OMP19 resulted in the expresion of RAP1 in association to the membrane of S19. Even though there was a relatively lower stability of the plasmids containing *rap1a*, compared to the empty plasmid, mice inoculated with S19pRAP or S19p19RAP developed specific immune responses to RAP1, being IgG2a the prominant subisotype of antibodies (analysed by ELISA). As a fusion protein with BP26, RAP1a was toxic. Therefore it can be concluded that the expression of RAP1 in *B. abortus* S19 is possible and immunostimulatory. Lymphocyte stimulation assays are being performed.

MI-P11.
IDENTIFICATION AND CHARACTERIZATION OF A NOVEL EXTRACELLULAR PHOSPHOLIPASE PRESENT IN CLINICAL ISOLATES OF *Enterobacter aerogenes*

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Enterobacter is an opportunistic pathogen associated to nosocomial infections, with increasing incidence in patients from intensive care units. Clinical isolates were phenotypically characterized and we selected an *E. aerogenes* strain that exhibited extra-cellular phospholipase activity. This activity was absent in strains from normal flora, suggesting that it might be a virulence factor. By phenotypic screening of a sub-genomic library we found a gene enconding for lipase activity. Sequence analysis revealed high homology to phospholipase A1 enconding genes, found only in *Serratia, Yersinia, Xanthomonas* and *Photorhabdus* (*E. aerogenes* has no available genome database). In parallel, by mini Tn5-random mutagenesis, we selected six phenotypically lipase null strains. Strikingly, analysis of the adjacent sequences to the insertion revealed homology to the *wecD, wecE, wecF* and *waaF* which are involved in the outer membrane glycolipid (ECA) and LPS biosynthesis. These mutants were not globally affected in the expression of exoproteins, suggesting that wec cluster and *waaF* gene products could be implicated in the expression, folding or secretion mechanism of the phospholipase.

MI-P12.
ALTERNATIVE SECRETION MECHANISM OF α-HEMOLYSIN OF *E. coli* BY MEMBRANE BLEBS

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α-Hemolysin is an important virulence factor produced by several strains of *Escherichia coli* involved in human extraintestinal diseases, like urinary tract infections, peritonitis, meningitis and septicemia. An operon composed by 4 genes (hlyA, B, C and D) are involved in synthesis of polypeptide, postraslational modification and secretion of the active toxin to extracellular media. The secretion apparatus comprises HlyB and HlyD and TolC.
However, Gram negative cells walls have a dynamics features: outer membrane vesicles (OMVs) are constantly being discharged from the surface of the cell during bacterial growth. This study shows the presence of HlyA on OMVs isolated from culture supernatans supplemented with cyclodextrin in order to enhance the levels of OMVs. OMVs characterized by electron microscopy are bilayered membranous structures, spherical with 50-250 nm diameter composed by phospholipid, lipopolysacharide and proteins. The presence of HlyA on OMV was detected by Western Inmunoblot and the hemolytic activity was tested by hemolytic assays employing sheep blood.OMVs could provide an alternative route for the delivery of HlyA and moreover an alternative mechanism of interaction between HlyA and target cells.

MI-P13.
DOES α-HEMOLYSIN OF *E. coli* FORM AN OLIGOMER STRUCTURE WHEN IT IS BOUND TO MEMBRANES?
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α-Hemolysin is an extracellular protein toxin (107 kDa) secreted by *Escherichia coli* that acts at the level of plasma membranes of target eukaryotic cells. Postraslational modification of the protein with fatty acids is required for all known cytotoxic activities which occurs at two internal lysine residues (K564 y K690).
This toxin promotes the formation of proteo-lipidic pores at lytic concentration rather than forming purely proteinaceous ionic channels.
We studied the interaction of this toxin with phospholipids membrane using artificial planar lipid membranes composed of asolectin. Addition of nanomolar concentrations of toxin resulted in an increase of bilayer conductance at a step concentration dependent fashion, suggesting that several toxin molecules could be involved in the conductive unit. To obtain conclusive information on the formation of the oligomer we used different cystein mutants of the toxin. These mutants were derivatized with fluorescent probes ALEXA-488 or ALEXA-546 (fluorescein and rhodamine derivatives) in order to study the formation of an oligomer on red blood cell membranes by Fluorescent Resonance Energy Transfer (FRET). It seems that the acyl chains may be involved in the oligomerization process.

MI-P14.
CHOLESTEROL DEPENDENT *Bordetella pertussis* ATTACHMENT TO HOST CELLS IS NOT LINKED TO THE BACTERIAL VIRULENT STATE
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B. pertussis (*Bp*) is a re-emerging human respiratory pathogen whose infectious process is not fully understood precluding the design of effective vaccines. The nature of bacterial attachment to host cells is often the key event in the outcome of the infection. The so-called adhesins of *Bp*, like Filamentous Hemagglutinin (FHA) or Cya, found critical for the binding activity of FHA, are expressed only in the virulent phase. However, we found the avirulent phase of Bp, regarded as important during early and late stages of infection, to be able to attach to respiratory cells. *In vivo* studies confirmed these findings. We here investigated the role of the cholesterol, a molecule involved in other persistent infectious processes, in the virulence-independent interaction of *Bp* with the host cells. Human respiratory epithelial cells (A549) treated with and without cyclodextrin were incubated with virulent and avirulent *Bp* wild type strain or two isogenic mutants deficient of either FHA or Cya. The synthesis of cholesterol *de novo* was inhibited by the addition of lovastatin. The results showed that the lack of cholesterol led to a significant decrease in bacterial attachment (about 75%) which proved independent of the virulent state or presence of FHA or Cya. Attachment inhibition studies with growing concentrations of cholesterol confirmed its involvement in the bacterial binding. Internalization and survival studies showed a relevant role of cholesterol in the intracellular trafficking. Whether this is a mechanism of attachment during transmission when bacteria is not yet expressing FHA or a way by which bacteria entering in the host in an avirulent state are directed to different compartments remains to be investigated.

MI-P15.
DIFFERENTIAL GROWTH PHASE-DEPENDENT REGULATION OF O ANTIGEN SYNTHESIS IN *Shigella flexneri*: ROLE OF THE RfaH ELONGATION FACTOR
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The O antigen (O Ag) is the most external structural component of lipopolysaccharide (LPS) of Gram negative bacteria. In *Shigella flexneri* the O Ag presents a bi-modal distribution or two preferred chain lengths (Short and Very long). In this work we studied the regulation of O antigen production during the bacterial growth. Analysis of the LPS in silver-stained polyacrylamide gels showed a phase-dependent regulation of the very long O Ag, which increased during the stationary phase of growth. On the contrary, the short O Ag distribution remained constant. This increase correlated with a higher expression of the elongation factor RfaH, as detected using a transcriptional fusion of the *rfaH* promoter to the *lacZ* reporter gene. RfaH positively regulates the transcription of the genes involved in the synthesis of the O Ag (*wba* operon). A *ΔrfaH* mutant did not synthesize very long O Ag and produced a lower amount of short O Ag during the entire bacterial growth. When the mutant was complemented with the intact *rfaH* gene in a multicopy plasmid, very long O Ag was produced at exponential phase. The results suggest that RfaH is involved in the differential growth-phase regulation of O Ag synthesis in *Shigella flexneri*.
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MI-P16.
MOLECULAR CHARACTERIZATION OF INVASIVE GENES OF *Shigella spp*.
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Shigella spp. is one of the main causative agents of bacterial diarrheas in children in Argentina. These microorganisms are able to invade enterocytes in colonic and rectal epithelia and to lyse intracellular phagocytic vacuoles, thus escaping into the cytoplasm where they multiply and then invade adjacent cells. The ability to invade is associated whit the presence of a megaplasmid (pINV ˜220 Kb) that encodes outer membrane proteins such as invasion plasmid antigens (Ipa). This family of proteins includes IpaA, IpaB, IpaC and IpaD proteins encoding by the ipaBCDA operon. In this work, a total of 100 isolates of *Shigella spp* were employed, 9 clinical isolates from a food borne infection and 91 clinical isolates of children with diarrhea. All isolates were typed by REP-PCR using BOXA1R and ERIC1R primers with boiled culture as template. Molecular detection of ipaBCDA operon was carried out by PCR using the ipaBCD-u and ipaBCD-d primers. Negative amplification of ipaBCDA operon from boiled cultures was confirmed using purified total DNA as template. The ipaBCDA operon was detected in 83 of the samples, characterized by a unique band of 600 bp. Most of the negative amplification isolates belong to *S. sonnei* fase II, although we found negative isolates from *S. sonnei* fase I and *S. flexneri*. Negative amplification was correlated whit the absence of plasmids and the lost of the invasive capacity of *Shigella* in cell cultures.

MI-P17.
THE *Salmonella enterica* RstA/RstB SYSTEM AND THE RpoS-DEPENDENT RESPONSE
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In *S. typhimurium* the PhoP/PhoQ two-component system governs the adaptation to Mg^{2+} limited-media, and controls essential pathogenic properties.
We determined that the expression of another two-component system, RstA/RstB is under Mg^{2+}-controlled, PhoP-dependent transcriptional regulation in *Salmonella*. We found that the expression of *narZ, spvA* and STM2689 is down-regulated and that the expression of STM2195 is up-regulated by RstA, when this regulator is over-expressed. Overproduction of this protein may be simulating its active form, in experimental conditions where its specific signal may be absent. We verified that the expression of RstA down-regulated genes is also dependent on RpoS, and that the expression of STM2196 (the only RstA regulated gene) is not dependent on this sigma factor. We showed that regulation of our mutants is not due to a transcriptional regulatory effect of RpoS on *rstA* and we also demostrated that *rpoS* is not under transcriptional control of RstA. Finally, we showed that RstA post-transcriptionally lowers RpoS cellular levels when RstA is overproduced.
In this way, RstA may be sensing an environmental stimuli that results in its own dependent genes activity regulation and also in the modulation of those RpoS- dependent. These results add a novel point of control in the complex RpoS regulation network.

MI-P18.
ROLE OF TWO COMPONENT SYSTEMS IN THERMAL REGULATION OF ANTHRAX TOXIN EXPRESSION
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Anthrax, a potentially fatal disease of animals and man, is caused by the Gram-positive endospore-forming bacterium *Bacillus anthracis*. Two large virulence-associated plasmids, pXO1 and pXO2, encode the components of the anthrax toxin, some of the genes required for spore germination, and genes involved in capsule synthesis. The anthrax toxin is a tri-partite molecule composed of the protective antigen, the lethal factor and the edema factor. The toxin genes, *pagA, cya* and *lef*, are coordinately regulated at the level of transcription and are induced by bicarbonate and increase in temperature, but the mechanism of thermal regulation remains unsolved.
In this study we examined the involvement of the *B. anthracis* genes BA5597-BA5598 in the temperature-dependent toxin expression. This putative two component system has high homology with *B. subtilis* DesK-DesR that regulates transcription of its sole desaturase, according to environmental temperature changes. Although the temperature regulation of *pagA* expression is not modified in BA5597-BA5598 mutants, the levels of toxin expression are reduced. We also analized the expression of *pagA* in a *B. subtilis* background using *pagA-lacZ* transcriptional fusions in the presence of the *B. anthracis* regulator AtxA. In mutants in the two component system *yvfT-yvfU*, which shares high homology with DesK and DesR, transcription of *pagA* is not affected.

MI-P19.
THE PROTEIN INTERACTION MAP OF P36 (ERP) FROM *Mycobacterium tuberculosis*
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The P36 gene (erp) of *Mycobacterium tuberculosis* encodes a secreted 36-kDa protein with a central domain containing several aminocidic PGLTS repeats. It has been demonstrated that P36 is a virulence-associated factor since the disruption of P36 gene impairs the growth of *M. bovis* and *M. tuberculosis* in mice (Berthet *et al*., 1998; Bigi *et al*., 2005). As a way to elucidate the function of P36, in a previous work we searched for P36-binding proteins by screening an *M. tuberculosis* DNA library with full-length P36 (bait) using a bacterial two-hybrid system. Two different putative ORFs were obtained whose products interact specifically with P36. These are Rv1417 and Rv2617c and encode for a possible conserved membrane protein and probable transmembrane protein respectively. Here we demonstrate the expression of these ORF during the *in vitro* culture of *M. tuberculosis* by RT-PCR. To further demonstrate the interaction between these proteins, we used the bacterial two hybrid system with Rv1417 and Rv2617c as baits. These preliminary results were confirmed by GST pull-down assay. In addition, the interaction between Rv1417 and Rv2617c, Rv1417 and Rv1417, Rv2617c and Rv2617c was also demonstrated. We also studied with the bacterial two hybrid system which domain of P36 is involved in the interaction with Rv1417 and Rv2627c. The two-hybrid system allows to start to decipher the possible biological role of proteins involved in virulence with no significant sequence similarities or obvious impact on phenotypes.

MI-P20.
EVOLUTION OF *Mycobacterium bovis* STRAINS IN ARGENTINA
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We have applied molecular typing techniques to *Mycobacterium bovis* strains from Argentina in order to explore the population structure and evolution of this pathogen. We have selected a subset of epidemiologically distinct strains from Argentina and applied three molecular techniques: spoligotyping, VNTR typing, and whole genome microarray analysis. The evolutive process accessed showed that argentinian *M. bovis* isolates would have evolved from an ancestral strain genetically similar to *M. bovis* BCG and the epidemiologically predominant strains are situated in the middle of this evolutive proccess. On the other hand strains that belong to minority and uniques patterns are situated at the end of this process. These results conducted us to try to identify the link between genotypic and phenotypic differences in the strains. This was performed by counting the CFUs after the infection of primary culture of bone marrow murine macrophages. The preliminary results showed that the CFUs counting was higher for the strains that have evolved recently respect of those that belong to the predominat pattern in Argentina and have been identify as ancient strains. At this moment *in vivo* whole genome microarrays expression analysis are being performed in order to identify the expression pattern differences between the analyzed strains.

MI-P21.
CHARACTERIZATION OF INVASIVE PNEUMOCOCCAL STRAINS THAT BELONG TO THE SPAIN 9V³ CLONAL COMPLEX

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The main objective of this work was to analyse, by different typing methods, the molecular epidemiology of strains of *S. pneumoniae* isolated from paediatric patients in Cordoba, Argentina. To this end, 10 β-lactam (βL) resistant and 10 βL susceptible capsulated clinical strains were characterized by multilocus sequence typing (MLST). Six βL resistant and four βL susceptible strains were included within Spain 9V³ clonal complex. The Spain 9V³ pneumococcal clone is one of approximately twenty international clones, and it is widely disseminated in European countries. The capsule biosynthesis is performed by enzymes that are codified by a cluster of capsular genes (*cps*), which are flanked by the *dexB* and *pbp1a* genes. This DNA region is a target for frequent capsular recombination and presents multiple divergences in its DNA sequence. We used the *cpsB* and *pbp1a* sequence analysis to characterize genotypically serotype-14 clinical strains, and to compare them with the Spain 9V³ clone. In our strains, the *cpsB* and *pbp1a* sequences were identical, but markedly different from those of European 9V³ variants, which present these genetic markers divergent from the original Spain 9V³ clone. The serotype 14 variants could be differentiated from the Spain 9V³ clone through the analysis of *cpsB* and *pbp1a* DNA sequences, but not by MLST. In conclusion, the Spain 9V³ clone dissemination in Cordoba present the same clonal origin, and indicate that this serotype 14 variant is genetically stable compared with the European 9V³ clones.

MI-P22.
THE MOLECULAR CHAPERONE ClpL IS INVOLVED IN THE ACID-STRESS INDUCED AUTOLYSIS OF *Streptococcus pneumoniae*

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ClpL belongs to the Hsp100 family of heat shock proteins. It carries out a chaperone function and probably is also involved in proteolysis, when it is associated with ClpP, removing damaged and denatured proteins. During studies of acid tolerance response in *S. pneumoniae*, we found that ClpL was highly induced at pH 5,6 (Cortes *et al.*, SAIB 2003). This pH value was also responsible for the autolysis observed in the wild type strain after 2 hours of incubation at 37ºC (Piñas *et al*., SAIB 2003). To evaluate the role of ClpL in acid-stress induced autolysis, we constructed a *clpL* mutant and studied its lytic phenotype when incubated at pH 5,6. We found that the *clpL* mutant did not undergo autolysis after 6 hours of incubation compared with the wild type strain. We further investigated the penicillin-induced autolysis of this mutant, and we observed lysis in both the mutant and the wild-type strains. These results suggest that ClpL is involved in the acid-induced autolysis and that its chaperone function is not essential for the activity of the major pneumococcal autolysin, LytA, since the *clpL* mutant could still lyse in the presence of penicillin. ClpL, acting as a chaperone, may be required to fold a protein that promotes the acid-induced autolysis, or to remove by proteolysis a protein that blocks this effect.

MI-P23.
MOLECULAR DIFFERENCES BETWEEN *Bordetella pertussis* CIRCULATING STRAINS AND THE VACCINE STRAIN USED FOR VACCINE PRODUCTION: IMPLICATION IN THE EFFICIENCY OF PERTUSSIS VACCINE INDUCED IMMUNITY AGAINST CURRENTLY CIRCULATING *B. pertussis* ISOLATES

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Pertussis is among the main infectious diseases worldwide. Although immunization has been used for decades, a resurgence of pertussis has been observed. This could be due to an antigenic divergence between vaccine and circulating strains. We obtained 48 *Bordetella pertussis* isolates from pediatric patients, which were different from the vaccine strain in their genotypes and main antigen sequences. The relevance of this divergence in protection was assessed with a respiratory model of *B. pertussis* infection in mice. Animals were vaccinated with pertussis vaccine or PBS, and challenged with sublethal doses of either vaccine strain or clinical isolates. In addition to the differences between vaccine-treated animals and PBS controls, a lower potential of immunity was induced by whole-cell pertussis vaccination to eliminate any of the clinical *B. pertussis* isolates from lungs ($p<0,001$). In immunoblot experiments employing whole cell lysates of clinical isolates as antigens and sera either from mice infected with different clinical isolates or from infected humans, clear differences in immunological patterns among the distinct clinical isolates were observed. These results were corroborated in 2D-PAGE; the differential spots are being identified by MALDI TOF.

MI-P24.
DETECTION AND IDENTIFICATION OF *Proteus mirabilis* OUTER MEMBRANE PROTEINS EXPRESSED *IN VIVO* IN RAT INTRAPERITONEAL DIFUSSION CHAMBERS

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Proteus mirabilis (PM) is an opportunistic pathogen that causes urinary tract infections, specially associated with catheterized patients and abnormalities in the urinary tract. Recently the genome sequence of PM was completed at the Sanger Centre (UK), and annotation is in progress. Our purpose was to detect and identify outer membrane proteins (OMP) expressed *in vivo* in intraperitoneal diffusion chambers (IPC) in the rat, using the genomic data available. The profiles of three PM strains were studied. Bacteria were also grown on iron replete and iron depleted media, and all OMP fractions were analyzed by SDS-PAGE. Iron restriction induced OMPs (IROMPs) were detected in bacteria grown *in vivo* and in iron depleted media. A differentially expressed OMP of about 97 kDa was detected only in the profiles of *in vivo* grown PM. An open reading frame (ORF) database was generated from PM genome data and then a database of tryptic digested ORFs obtained. A band from the IROMPs, with approx. 64 kDa, was obtained and analyzed by MALDI-TOF MS. The peptide mass spectrum of the 64 kDa OMP was searched against the PM digest database, and identified as an ORF on the PM genome. The hit showed homology to iron transport membrane receptors from several bacterial species. These results encourages the use of the described approach to identify other *in vivo* induced proteins, including the 97 kDa OMP detected only in the IPC.

MI-P25.

ANALYSIS OF THE ENDOGENOUS EXPRESSION OF *Pseudomonas aeruginosa* MutS, USING A HIS-TAG

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Escherichia coli MutS and MutL proteins play a crucial role in the methyl-directed mismatch repair pathway, very-short-patch repair, transcription-coupled nucleotide excision repair, and the prevention of the homeologous recombination; playing an important role in genetic stability. It has been shown that the expression of these proteins in *E. coli* can be modulated by cell physiology and differentiation, which would let cells to regulate their potential to evolve. Nothing is known about the regulation of the expression of these proteins in *P. aeruginosa*, a very versatile bacterium. In order to analyze the expression of *P. aeruginosa* MutS, we generated a mutant strain in which the endogenous *mutS* gene was replaced by a new copy having 18 extra nucleotides that codify for six histidine residues before the stop codon. In this way the endogenous MutS protein is synthesized having six histidine residues at the C-terminal region which let us to identify it using antibodies directed against the his-tag, or purify and concentrate it using metal chelation chromatography. Using this strain we observed, as described for *E. coli*, that the level of expression of *P. aeruginosa* MutS in cultures grown exponentially is higher than in stationary phase. These results suggest that regulation of MMRS proteins could be a common mechanism used by prokaryotes to modulate adaptive processes.

MI-P26.

HIGH FREQUENCY OF QUORUM SENSING DEFICIENT (*lasR*) MUTANTS EMERGE FROM A *Pseudomonas aeruginosa mutS* STRAIN

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Pseudomonas aeruginosa is an extraordinary versatile species involved in severe and often fatal infection in Cystic Fibrosis (CF) patients. A regulatory network termed quorum sensing (QS) contributes to the virulence of *P. aeruginosa* as a pathogen. In such regulatory system, *N*-acylhomoserine lactone signals regulate the expression of several hundreds of genes, via the transcriptional regulator LasR and the subordinate regulator RhlR. We recently found that mismatch repair disruption in *P. aeruginosa* determines the reproducible emergence of two morphotypical variants, mS1 and mS2 (Microbiology, 150:1327-1338, 2004). By incubation of *P. aeruginosa mutS* mS1 variants in aerated LB medium we observed that mS1 is the precursor of mS2. Moreover, after 500 h of incubation in such conditions, 70% of the cells diverged into mS2. Notably, mS2 displays differences in virulence traits that may stem from mutations in major QS regulators. By PCR amplification and DNA sequence of several QS genes, this work shows that mS2 variants are defective for LasR function due to independent point mutations in the *lasR* gene, suggesting that the inactivation of such gene could be one of the main responsible for mS2 diversification. Moreover, we determine that a non-functional LasR would confer a selective advantage under the tested conditions, since the viability in late stationary phase was significantly higher for mS2. Our results offer additional evidences of the high relevance of mutators phenotype in the bacterial adaptive evolution.

MI-P27.

HYPERMUTABILITY DUE TO *mutS* DEFICIENCY INCREASES THE FREQUENCY BUT REDUCES THE SPECTRUM OF *mucA* MUTATIONS LEADING TO MUCOIDY IN *Pseudomonas aeruginosa*

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P. aeruginosa is an opportunistic pathogen causing respiratory infections in Cystic Fibrosis (CF) patients. This bacterium colonizes the CF respiratory track growing as biofilms where mucoid, alginate-overproducing variants, emerge leading to chronic infection and a poor prognosis for the CF patients. In *P. aeruginosa* CF isolates, conversion to mucoidy is usually caused by a wide spectrum of mutations in the *mucA* gene (84%), of which 25% consists of a G deletion within a homopolymeric run of G (*mucA22*). Additionally, a high percentage (20%) of hypermutators isolated from CF has been reported. Here we show that hypermutability due to *mutS* disruption is sufficient to alter not only the frequency, but also the nature of *mucA* mutations. Studying the emergence of mucoids in isogenic wild type and mutS deficient populations of the *P. aeruginosa* PAO1, we observed a 37 fold increase in the frequency of mucoids that spontaneously emerged from the *mutS* mutant. In addition, 85% of them harbored mutations within the *mucA* gene. However, whereas at least 15 different types of *mucA* mutations were observed in non-mutator mucoid isolates, most of *mucA* mutations in the hypermutable mucoids are grouped in two types of mutations, 64% of them being *mucA22*. Our results give rise another possible explanation for the high frequency of mutators present in CF isolates and suggest that hypermutation could be one of the drivers for the conversion to mucoidy, a critical state for *P. aeruginosa* adaptability within the CF lung.

MI-P28.

REDUCTION OF THE MUTATION RATE AFTER OVEREXPRESSION OF MutS OR MutL IN *Escherichia coli*

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E. coli MutS and MutL proteins participate in the methyl-directed mismatch repair system (MMRS), the very-short-.patch repair, the transcription-coupled nucleotide excision repair, and in the prevention of the homeologous recombination. In the MMRS, upon binding of MutH to the MutSL-DNA complex, the endonuclease activity of MutH is activated, and cleaves DNA 5` to the Dam hemimethylated -GATC- sequence in the unmethylated strand, directing repair to this strand. It has been shown using different experimental systems that overexpression of MutS or MutL is able to reduce the mutation rate in different bacteria. However, the mechanism(s) responsible for this effect is not known. It has been reported that this effect is not the result of an effect on the GO (8-Oxoguanine) repair system or on the RecD and RecG pathway. Here we show that an effect in the reduction of the mutation rate, after overexpression of MutS or MutL, is also produced in a dam mutant strain of *E. coli*. As the MMRS is non functional in this strain, our results indicate that the MMRS is probably not involved in the reduction of the mutation generated by overexpression of MutS or MutL. Experiments in order to determine the mechanism(s) responsible for this effect are under development.

MI-P29.
ANTIOXIDANT RESPONSE OF *Xanthomonas axonopodis* PV. CITRI TO HYDROGEN PEROXIDE
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Xanthomonas axonopodis pv. citri (Xac) is the phytopathogen responsible for citrus canker. During invasion of host plant tissues, Xac is exposed to plant-produced hydrogen peroxide (H_2O_2), which is toxic to the pathogen. We studied the response of Xac to H_2O_2 and its possible participation in the interaction with plants. We evaluated the H_2O_2 resistance of early exponential Xac cultures and found that there is a decrease in viability with concentrations higher to 1 mM. Cultures pre-treated with sub-lethal concentrations of the oxidant showed an adaptive response that led to a higher resistance to lethal concentrations. These treatments produced an increase in the catalase activity. We also analysed this enzyme in different growth phases and observed that it was higher in stationary-phase compared to early-exponential phase. This was consistent with the capacity to resist H_2O_2. To investigate the possible participation of catalases in the interaction with host plants we determined the levels of this enzyme in Xac cultures grown in standard media and in XVM2, suspected to mimic the environment of plant intercellular spaces. We found that the catalase activity was higher in XVM2 compared to the other media, as well as the survival to H_2O_2. Our results indicate that catalase profile in Xac is different to that observed in other *Xanthomonas*, which show higher activity in early-exponential phase. In addition, catalase induction in XVM2 suggests a possible role of this enzyme in plant-pathogen interactions allowing the bacteria to overcome the oxidative stress associated to invasion.

MI-P30.
XANTHAN GUM ARE INVOLVED IN BIOFILM FORMATION AND PATHOGENICITY IN *Xanthomonas axonopodis* pv. *citri (Xac)-citrus limón* INTERACTION
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The plant pathogen *Xac* is responsible for the canker disease affecting citrus plants throughout the world. Here we show the role of xanthan gum in biofilm formation and, the pathogenicity of the bacterium. The polymerisation and export of xanthan are directed by the gum gene cluster (12 genes, *gumB* to *gumM*). We obtained a Xac mutant trough a cassette insertion at the *gumB* gene, which resulted in a mutant defective in xanthan synthesis. We studied the *in vitro* biofilm formation of the mutant and of the wild type bacterium by tagging the cells with a constitutive GFP containing plasmid and confocal fluorescence microscopy. This experiment showed that the wild type bacteria formed bacterial populations enclosed in a matrix attached to the chamber, whereas the xanthan defective mutant simply attached to the chamber bottom in a incoherent fashion. In addition, this mutant showed a significant impairment in growth and virulence in lemon leaves. This result allows us to speculate that the xanthan is neccesary for the bacterial biofilm formation and pathogenicity. Since biofilm formation has been implicated in various plant-pathogen interactions, the loss of xanthan mutant fitness could be linked to the inability to produce a functional biofilm.

MI-P31.
EXPRESSION OF AN *Agrobacterium tumefaciens* C58 OPERON UNDER SALT STRESS CONDITIONS
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Agrobacterium tumefaciens C58 is a a-proteobacterium of the *Rhizobiaceae* family. Regarding salt tolerance, *A. tumefaciens* has been divided into three taxonomic groups, biotype I y III are tolerant to 2 % of NaCl, while biotype II is not. The *A. tumefaciens* strain C58 used in this work belongs to biotype I. Sequence analysis of *A. tumefaciens* C58 genome revealed the existence of two orfs that codify for a Glycosiltransferase Domain (GTD), and a PhosphoHydrolase Domain (PHD), characteristic of the enzymes related to sucrose biosynthesis in cyanobacteria and plants. As the orfs are in the genome one next to the other, with an overlapping of four nucleotides, we studied the possibility that they could be members of an operon structure. Northern Blot assays allowed us to confirm the existence of a transcript of the size of the two genes expressed together. In spite of the similarity of the sequences under study with the enzymes related to the sucrose metabolism, no sucrose was ever detectable in *A. tumefaciens* C58 cells. Expression analysis assays carried out in this work, showed that the operon is translated only over salt stress conditions, with the maximum expression level after three hours of salt stress. However, whether this operon could be responsible for the biosynthesis of a protective osmolyte is still unknown and under study.
Supported by ANPCyT, UNMdP, CONICET and FIBA.

MI-P32.
NITRIC OXIDE PRODUCTION BY *Azospirillum brasilense* UNDER OXIC CONDITIONS
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Azospirillum is a plant growth-promoting rhizobacteria that produces nitric oxide (NO) by nitrate dissimilation pathway under anaerobic respiration. In plants, NO is involved in many physiological processes, so we were interested in studying the NO production by Az in order to correlate it with growth promoting activity of the bacteria. *A. brasilense* Sp245 (wt), the auxin-deficient mutant Faj009 and the periplasmic nitrate reductase mutant Faj164 were grown in agitated OAB medium with NH_4^+ or NO_3^- as N source, at 32ºC; 15 mM arginine was added when indicated. NO production was quantified by electron paramagnetic resonance (EPR) in pellets of bacteria grown 11 (middle log phase) and 16 h (end of log phase). In NH_4^+ supplemented medium both wt and Faj009 strains produced NO (in a range of 4 to 5 nmol.g^{-1} of bacteria) only at the end of log phase. NO production in the Faj164 mutant was detected earlier (11 h) and in higher amounts (9 nmol.g^{-1} bacteria). The addition of arginine (substrate for NO synthase) to medium with NH_4^+ increased the amount of NO in all strains, being of ca. 4 times higher in Faj164 than in the other strains. When wt and Faj009 strains were grown with NO_3^-, NO production were increased *ca*.100%, but not in the periplasmic nitrate reductase mutant. These results support an NO production by *Az* in a metabolic pathway that does not involve anaerobic nitrate respiration and also suggest the presence of a NO synthase.
CMF is a fellow from ANPCyT.

MI-P33.
STRESS TOLERANCE, BIOCONTROL AND SURVIVAL IN WHEAT RHIZOSPHERE OF *Pseudomonas spp.* SF 10B
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Pseudomonas spp. 10b was isolated from wheat roots and demonstrated to be PGPR, producing siderophore and indole acetic acid (IAA) and being able to solubilize phosphate. A spontaneous rifampicin resistant mutant (*Pseudomonas* spp. SF10bR), with similar viability than its wild type, was obtained. Wheat seedling were grown in pots with fertilized and non fertilized soil and inoculated with the spontaneous mutant, to a density of 10^8 cfu per plant. Fifteen, thirty and sixty days post-inoculation, bacteria were recovered from rhizosphere in both soils, but the number of viable cells was higher in non fertilized than in fertilized soil and it was maintained along the time. On the other hand, bacteria stayed in non fertilized buck soil until 30 days post-inoculation. The recount in endorhizosphere was below the resolution limits (10^3 cfu/ml). The same occurred in fertilized buck soil. The results indicated that the strain could survive better in soil with less nitrogen, perhaps because of low competition with native microorganisms.
Pseudomonas spp. SF10b is able to tolerate up to 750 mM NaCl and it grows at 30ºC and 37ºC but not at 42ºC. Under stress conditions, the viability is similar than in normal conditions in exponential phase, but diminishes in the stationary phase.
On the other hand, we studied the ability of *Pseudomonas* to inhibit *in vitro* phytopathogenic fungi: *Fusarium graminearum*, *F. verticiloides, F. solani, F. proliferatum, Sclerotinia sclerotiorum*, and *S. minor*.

MI-P34.
EFFECT OF OXYGEN AND LIGHT ON THE EXPRESSION OF THE *puf* OPERON IN PHOTOSYNTHETIC BRADYRHIZOBIA
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Photosynthesis gene expression in an indigenous *Bradyrhizobium* strain was assessed. The expression of the *puf* operon, which encodes most of the apoproteins of light-harvesting and reaction center complexes, was estimated by the accumulation of its mRNA and bacteriochlorophyll (BChl) production.
Bradyrhizobium sp. C7T1 strain was grown on modified MAG broth at 30ºC in a 5L Bio-Flo III fermentor under aerobic, semiaerobic or microaerobic condition (21%, 3% and 0.5% oxygen, respectively), under continuous light or light-dark regimes. Total RNA was prepared from the cell samples by hot-phenol method. BChl and carotenoids were measured after extraction with acetone:methanol. Accumulation of BChl was only observed in the presence of oxygen and was enhanced under semiaerobic o microaerobic conditions reaching values up to 60% higher, but it was abolished under continuous light growth conditions independently of oxygen concentration. The expression of the *puf* operon was induced by the decrease of oxygen tension, similarly to what was observed for the accumulation of BChl *a*. However, the *puf* operon shows a strong expression in cells grown under continuous white light illumination. This clear discrepancy between this result and the absence of photosynthetic activity observed under the same light condition indicate and additional post-transcriptional regulation of photosynthesis genes by light.

MI-P35.
GLUTATHIONE BIOSYNTHESIS, IN *Rhizobium tropici*, IS NECESSARY FOR EFFICIENT SYMBIOSIS WITH THE LEGUME *Phaseoulus vulgaris*
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In order to establish an efficient symbiosis, nitrogen fixing rhizobia must overcome the host defense reaction against invasion. One of the early events consists of an oxidative burst, which leads to a local increase in the O_2^- and H_2O_2 levels in the infected zones. Once differentiated into bacteroids within the nodule cells, protection against oxidative stress is also important, since the high rate of respiration necessary to supply energy for the nitrogen fixing process generates different reactive oxygen species (ROS) which must be kept low to avoid irreversible damage of the highly O_2-sensitive enzyme nitrogenase. As nodule senescence takes place, an increase in ROS is observed. Plant glutathione (or its analogue, homoglutathione) has been implied in protection against ROS during nodulation, but little is known about the importance of bacterial glutathione in symbiosis. We had previously demonstrated that *R. tropici gsh*B mutants produce, in symbiosis with *Phaseolus vulagris* nodules that became old prematurely. Also, this mutant is out-competed by the wild type in co-inoculations studies. We have now constructed *gsh*B and wild type strains carrying *gsh*B-gus transcriptional fusions and used them to monitor the expression of this gene during nodulation. We have also used microcopy to reveal accumulation of O_2^- and H_2O_2 in developing nodules.

MI-P36.
ISOLATION AND CHARACTERIZATION OF SALT SENSITIVE Tn5-MUTANTS OF *Ochrobactrum sp.11a*
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Soil salinity is an enormous problem for agriculture. The use of traditional breeding and plant genetic engineering with production of transgenic plants, plus the use of plant growth-promoting bacteria may be useful in developing strategies to facilitate plant growth in saline soils.
We have isolated two native strains from saline soil of Córdoba province that belong to *Ochrobactrum* genus with PGPR ability. They were designed *Ochrobactrum sp. 11a* y *Ochrobactrum sp. 3b*. To advance on the genetic of salinity tolerance, we have generated a collection of Tn5-B21 mutants and screened the collection in order to select those clones showing a salt sensitive phenotype. Selected clones failed to grow on GMS supplemented with 300mM NaCl. Some of these sensitive mutants showed a lag phase higher up than 40 h. In addition, in these collection thirty four transconjugants displayed differential expression of β-galactosidase activity in salt presence compared to the expression in the same medium without salt. On the other hand, we have found that both strains were able to promote wheat growth under salinity conditions. Previously, we determined this ability employing maize in the same conditions. The inoculation of wheat with the *Ochrobactrum sp. 11a* significantly ($p< 0.05$) increased the shoot dry weight about 42% as compared to the control under salinity conditions. In wheat seedlings inoculated with *Ochrobactrum sp. 3b* we found increments in the shoot dry weight, under the same conditions.

MI-P37.
EVALUATION OF INDIGENOUS PGPR BACTERIA FOR THE FORMULATION OF A MULTIPLE STRAIN SOYBEAN INOCULANT.
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We based our primary screening for bacterial isolates with plant growth promoting properties by dual culture method for antifungal activities against phytopathogen fungus. In order to select the most fitted isolates we developed an index concerning different properties. Antifungal and antibacterial activities were assayed by dual culture. Chemotaxis and growth stimulation by seeds and seedlings exudates was assessed. ß-hemolysis was determined, related to the presence of biosurfactants and biofilm formation. Hydrolytic enzymes activities and phosphorus solubilization were detected on plate assays. The presence of sequences associated with the synthesis of secondary metabolites with specific primers for lipopeptides, and several gram negative antibiotics were screened. We selected six isolates, two sporulating isolates whose antifungal and antibacterial activities would be related to the production of surfactin and some other lipopeptide. These isolates are positively stimulated by plant exudates and presents positive chemotaxis. Of the three phosphorus solubilizing pseudomonads, two of them showed sequences related to the synthesis of pyrrolnitrin with no negative interaction with a *Bradyrhizobium japonicum* commercial strain.

MI-P38.
PGPR ABILITY AND INDUCTION OF DEFENSIVE ENZYMES IN TOMATO BY RHIZOBACTERIAS
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Objective: To study the PGPR capacity of *Pseudomonas* and *Chryseobacterium* in tomato and to analyze enzymes involved in the plant defense.
Materials and methods: Surface sterilized tomato seeds were germinated and transferred to pots with vermiculite: peat. Seedlings were inoculated with native *Pseudomonas* spp. SF 4c, *Pseudomonas* spp. SF10b and *Chryseobacterium balustinum* CECT 5399 to 5 and 20 days. Shoot and root dry weight, shoot diameter and height were measured two weeks after the second inoculation. Results were analyzed by ANOVA at $p<0.05$.
Six days after reinoculation, ethylene, salicylic acid, and defensive enzymes of the plant [peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL)] were measured.
Results: Tomato plants inoculated with *Pseudomonas* spp. SF 4c, *Pseudomonas* spp. 10b and *C. balustinum* CECT 5399 were higher and presented an increment of the root and shoot biomass.
There were not increases of PO and PPO activities in plants inoculated with PGPR, compared with the non-inoculated ones. However, PAL activity was stimulated in roots of plant inoculated with *Pseudomonas* spp SF 4c and the production of ethylene was higher in this case too. Salicylic acid was not found in roots nor in inoculated neither in control plants.

MI-P39.
IDENTIFICATION AND CHARACTERIZATION OF A SUCROSE PHOSPHATE SYNTHASE FROM *Synechococcus* sp. PCC 7942
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The salinity tolerance of the freshwater cyanobacterium *Synechococcus* sp. PCC 7942 is determined by its ability for osmotic adjustment and the presence of sucrose as osmolyte. Sucrose is synthesized in a two-step pathway involving sucrose-phosphate synthase (SPS) and sucrose-phosphate phosphatase (SPP), and hydrolyzed by invertase. The present study describes the first isolation of a SPS encoding gene from a *Synechococcus*, particularlly, the PCC 7942 strain, and an expression analysis of the gene in the wild type strain and mutant strains. The SPS gene encodes a 88 kDa polypeptide, which is 47% and 45% identical to *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7002 SPS respectively. Both of them are bidomainal SPSs composed by a Glucosyl-Transferase Domain (GTD) and a Phospho-Hydrolase Domain (PHD). Additionally, sequence analysis of the *Synechococcus* sp. PCC 7942 genome, revealed that it has no other ORF encoding a putative PHD. This characteristic suggests that SPS from *Synechococcus* is likely to be a bifuntional enzyme. The fact that the SPS encoding gene was not expressed in mutants impaired in transcriptional factor NtcA or in signal protein PII indicates that sucrose metabolism may be coordinated with nitrogen assimilation through some or both proteins.
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MI-P40.
CAROTENOIDS CONTENT AND SUNLIGHT SUSCEPIBILITY IN *Rhodococcus sp.*
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An environmental pink pigmented bacterium was isolated and identified as *Rhodococcus sp*. Pigmentation mutants were obtained by chemical mutagenesis. Pigments present in the wild type strain (RMB90), in a pale yellow mutant (RMB91) and in two mutants exhibiting increased pigmentation (RMB92 and RMB93), were extracted with chloroform-methanol and analyzed by reverse phase HPLC. Survival of these strains after exposure to sunlight and ultraviolet radiation from artificial sources was studied under different physiological and irradiation conditions.
The ability of RMB91 to survive sunlight exposure was reduced with respect to that of RMB90. Resistance was similar in both strains when bacteria grew in the presence of a caratenoid synthesis inhibitor, which had no effect on survival of RMB91. Reduced sunlight resistance in RMB91 was also observed during irradiations under N2. Using artificial radiation sources, non pigmented bacteria were less resistant to UVA, but not to UVB or UVC. Lethal effects of sunlight and UVA on RMB92 and RMB93 were increased with respect to the wild type strain.
Carotenoids protect *Rhodococcus sp* against deleterious effects of sunlight. In non-photosynthetic bacteria studied to date, photo-protection by carotenoids was dependent on $[O_2]$. This is not the case with *Rhodococcus sp* RMB90, suggesting the occurrence of a different mechanism for protection. UVA radiation seems to play a key role in photo-damage.

MI-P41.
QUANTIFICATION, CHARACTERIZATION AND PROBIOTIC POTENTIAL OF CANINE VAGINAL LACTIC ACID BACTERIA

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Vaginal lactic acid bacteria (LAB) play a regulatory and protective role in several animal species. The aim of this work was to quantify and characterize LAB in vagina of bitches and to evaluate the probiotic potential of different selected isolates.
Samples (n=41) were obtained by gentle vaginal swabbing while clinical parameters were recorded. LAB and lactobacilli were quantified using MRS and Rogosa agar respectively. Selected isolates were characterized by cell morphology and biochemical routine tests. Evaluation of probiotic potential included adhesion to epithelial vaginal cells (microscopy counts) and antimicrobial activity (spot test against *E. coli*, *P. mirabilis* and *S. aureus*). Identification was done sequencing a 16S rRNA gene fragment.
LAB and lactobacilli counts were diverse (maximum of 10^8 and 10^6 CFU/sample) and were not correlated with any clinical parameter. Most of the isolates showed antimicrobial activity and presented variable adhesion values with a maximum of 123 bacteria per vaginal cell. In general, isolates were classified as *Lactobacillus* spp, *Enterococcus* spp and also *Bacillus* spp.
Despite the different clinical situations the studied flora was very stable and different isolates showed promising properties as probiotic candidates.

MI-P42.
CodY AND DtpT ARE INVOLVED IN THE MODULATION OF *prtP* DURING OSMOTIC STRESS IN *Lactobacillus casei*

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Components of the proteolytic system of Lactic Acid Bacteria (LAB) play an important role in the development of flavor in fermented food. Moreover addition of salt is commonly used in several industrial processes using these bacteria. We have demonstrated the role of peptides in the adaptation to high salt in *Lactobacillus casei*; the transcription activity of the *prtP* gene (codifying for the major proteolytic activity) was repressed by peptides but this repression failed in NaCl (Piuri *et al.,* 2003, *JAM* 95:372). The expression of PrtP has been linked to the peptide and oligopeptide transport systems *dpp, dtp* and *opp*. In this work we analyzed the behavior of a *dtpT* mutant, codifying for one of the peptide transporters present in the membrane of *Lb. casei*, during growth in high salt media. This mutant showed an osmosensitive phenotype and peptides preferentially transported by this protein failed to improve the growth of this bacterium in high salted medium. We verified the presence of the pleiotropic regulator CodY in this bacteria. A direct interaction between this regulator and the *prtP* promoter was shown by using *in vitro* electrophoretic mobility shift assays and Chromatin immunoprecipitation (ChIP-to-chip) experiments. These results suggest that CodY could mediate the lost of repression observed in a NaCl containing medium, even in the presence of peptides consistent with a decreased level of intracellular branched-chain aminoacids (BCAA).

MI-P43.
COMPARATIVE STUDIES OF DIFFERENT PROMOTER REGIONS OF *Lactococcus lactis* INTENDED FOR THE EXPRESSION OF HETEROLOGOUS PROTEINS

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There is an increasing demand for expression systems that allow the production of proteins of interest in the field of foods and health. In this sense, *Lactococcus lactis* has been used for the production of heterologous proteins in bioreactors, in fermented food products or directly in the tract of animals. In order to develop an effective system of expression for heterologous proteins in *L. lactis*, in the present work we have carried out transcriptional studies using different promoters regions coming from genes involved in the citrate degradation pathway and other metabolic routes of *L. lactis*. To compare the activity of these promoters, the vector pAK80 was used, which allows to make transcriptional fusions to *lacLM* reporter gene in *L. lactis*, and then ß-galactosidase activity was evaluated. The results of these assays demonstrated that the PcitM promoter possesses the highest levels of transcriptional activity, followed by the Pals and PaldB promoters. In a later step, PcitM promoter was used to express the viral antigen VP8. The expression analysis of VP8 trough Western blot allowed to prove its overexpression under acidic conditions.

MI-P44.
ON THE MECHANISM OF TELLURITE TOXICITY IN BACTERIA

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Tellurite, an acid anion of tellurium, is toxic for most bacteria, especially Gram negatives. However, several microorganisms exhibit natural resistance to potassium tellurite (K_2TeO_3,Tel^R phenotype). Since the genetic and biochemical bases underlying such resistance is rather diverse, we have used the thermotolerant, Gram positive, plasmidless, bacterium *Geobacillus stearothermophilus* V as an experimental model to study bacterial resistance to K_2TeO_3.
Our strategy was the isolation and characterization of *G. stearothermophilus* V genes that confer tellurite resistance when expressed in *E. coli*. Gene libraries of this organism constructed using high copy number cloning vectors were introduced into K_2TeO_3-sensitive *E. coli* cells and transformants were selected for the acquisition of a Amp^RTel^R phenotype. Evidence will be presented that demonstrated that potassium tellurite seems to exert its toxicity through the establishment of an oxidative stress inside the cell.
Supported by Fondecyt grant # 1030234 to CV.

MI-P45.

CONTRIBUTION OF THE *zwf* AND *fpr* GENES TO OXIDATIVE STRESS TOLERANCE IN *Escherichia coli*

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Glucose 6-phosphate dehydrogenase (G6PDH) and ferredoxin-NADP(H) reductase (FPR), encoded by the *zwf* and *fpr* genes, respectively, are committed members of the SoxRS regulatory system involved in superoxide resistance in *Escherichia coli*. Exposure of *E. coli* cells to superoxide led to rapid accumulation of G6PDH, while FPR induction was delayed. Bacteria over-expressing G6PDH displayed a protracted *soxRS* response, whereas FPR build-up had the opposite effect. Inactivation of any of the two genes resulted in enhanced sensitivity to MV killing relative to the wild-type strain. Accumulation of G6PDH over wild-type levels had no effect on MV tolerance, while FPR over-expression led to augmented survival. Recovery of damaged iron-sulphur clusters of hydro-lyases after oxidative challenge was faster and more extensive in transformed bacteria expressing FPR than in wild-type cells, indicating that the reductase contributed to this repair pathway *in vivo*. Reactivation could also be accomplished in a defined system containing both enzymes, their substrates and FPR acceptors ferredoxin or flavodoxin. G6PDH and FPR could act concertedly during the *soxRS* response to deliver reducing equivalents from carbohydrates, via $NADP^+$, to critical repair pathways of the stressed cell.

MI-P46.

TOXICITY FOR HYDROPEOXIDE IN *Escherichia coli* WITH DIFFERENTIAL EXPRESSION OF THE NADH DEHYDROGENASE-2

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The respiratory chain of *Escherichia coli* (*E. coli*) contains two NADH dehydrogenases. In the presence of its substrate, NDH-1 is able to generate a proton gradient whereas NDH-2 does not, partially uncoupling the respiratory chain. Our group demonstrated that: a) NDH-2 is a Cu(II) reductase; b) mutants in NDH-2 grow with more difficulty that the wild type strains, in media with high copper concentration and deficient in this metal; c) NDH-2 is damaged when membranes of *E. coli* were incubated in presence of copper and peroxides. To further investigate the participation of NDH-2 in the oxidative stress, we measured the oxygen consumption, after incubation with an organic peroxide (*t*-BOOH), by cells that over-express or lack the NDH-2. The results show that both mutants were more sensitive to peroxides damage than the wild-type strains. The damage was magnified in NDH-1 deficient strain. Therefore in controlled conditions, the presence of NDH-2 is positive for the cell because the enzyme would act as scavenger of free radicals avoiding damage on other components of the respiratory chain. In strains with excessive activity the scavenger capacity could be overcome by the oxidative capacity.

MI-P47.

EFFECTORS THAT MODIFY THE EXPRESSION OF NADH DEHYDROGENASE-2 GENE: INVOLVEMENT IN COPPER RESISTANCE IN *Escherichia coli*

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The respiratory NADH dehydrogenase-2 (NDH-2) of *Escherichia coli* is a cupric-reductase, coded by *ndh* gene. In rich medium and MT minimal medium, *ndh* is expressed only in exponential phase of growth due to the negative regulation of the global transcription factor FNR. However, we observed that in M9 minimal medium, the maximum level of expression was reached in early stationary phase and it was maintained in later stationary phase, even for 48 hs. The determinations were done by using the reporter gene of ß-galactosidase under the *ndh* promoter and also by the NDH-2 activity of a wild-type strain. Based on those data, we cross-checked each differential component in the growth media (M9/ MT) and we determined that phosphate and chloride concentrations define the behavior. The action of these effectors is independent of FNR regulation, as we saw using a *fnr*$^-$ strain. Moreover, the expression in stationary phase enhances the resistance against toxic concentration of copper. In conclusion, the changes in the expression pattern of NDH-2 due to specific components in the culture medium are associated to different copper sensibility, supporting our idea that NDH-2 is involved in the metal homeostasis.

MI-P48.

PRODUCTION AND CHARACTERIZATION OF A PIGMENT SYNTHESIZED BY *Escherichia coli* CELLS EXPOSED TO HIGH COPPER CONCENTRATIONS

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Studies carried out in our laboratory showed that different *Escherichia coli* strains produce a pink-brownish pigment when they were grown, aerobically or anaerobically, with high copper concentrations. The pigment was present either in cells or in the growth media (liquid or solid).Its production was detected during the whole growth curve, increasing in stationary phase. All strains tested produced the pigment, except for the *ubiA*$^-$ strain, which is defective in ubiquinone synthesis. Addition of *p*-hydroxibenzoate to the growth medium, which allowed mutants to synthesize ubiquinone, restores the pigment production. On the other hand, we partially purified the coloured compound by gel filtration chromatography (Sephadex G25) as a high molecular weigh fraction, which was chromatographied by strong anionic exchange chromatography (Q Sepharose fast flow). The pigment's fraction is negatively charged at pH7 and it shows an absorption peak at 370nm, and a luminescence at 475nm when it was excited at 370nm. It seemed to be associated to proteins and Cu^{1+}. The pigment could have a protective effect in cells under oxidative stress conditions caused by high copper level.

MI-P49.
SEARCH OF CHROMOSOMAL GENES INVOLVED IN MICROCIN J25 PRODUCTION

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Microcin J25 (MccJ25) is a plasmid-encoded, 21-amino-acid, antibacterial peptide produced by *Escherichia coli*. We have developed a two-plasmid system for identifying putative chromosomal genes involved in microcin synthesis or regulation. One of the plasmid bears the structural gene, *mcjA*, and two genes *mcjB* and *mcjC*, required for microcin maturation. This construction is lethal for cells since it lacks the immunity gene. However, it is viable in the presence of the other plasmid, a temperature-sensitive replicon that carries the MccJ25 immunity gene *mcjD* cloned. We transformed an *E. coli* host with both plasmids. Double transformants were viable at 30ºC and produced MccJ25. When shifted to 42ºC, the thermosensitive immunity plasmid was lost and, under this condition, the only cells expected to survive were those having a chromosomal or a plasmid mutation lowering or abolishing microcin production. Several candidate clones were isolated. After discarding plasmid mutants, one chromosomal mutant was selected for further study. The mutation abolished microcin production. It was mapped to the *lrp* gene, encoding an *E. coli* global transcriptional regulator. Most likely, the *lrp* gene product acts as a negative regulator at the transcription level. Currently, we are analyzing other mutants obtained by using this two-plasmid system, in the hope of identifying genes whose mutation perturb MccJ25 maturation.

MI-P50.
THE PLASMID pTUC200, ENCODING MICROCIN J25, HAVE UNUSUAL PROPERTIES

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Plasmid pTUC200 is a microcin J25 (MccJ25)-producing pBR322 derivative carrying a fragment from the wild-type MccJ25 plasmid, pTUC100. This fragment includes a copy of the insertion sequence IS1294 and the replicon of pTUC100. Plasmid pTUC200 has two interesting properties. First, it suffers spontaneous deletions at a low proportion. Second, it was stably maintained in a TolC$^-$ *E. coli* strain. Note that other microcin-producing plasmids are lethal for TolC$^-$ cells, since they cannot export the microcin produced. Regarding the deletion phenomenon, although it occurred spontaneously, its frequency was greatly increased in Tn5-carrying pTUC200, or when the transposon was present *in trans*. The nucleotide sequences of deletion junctions were determined for 4 deletants. All of them had lost a fragment containing the pTUC100 replicon. Deletions seem not to be the result of homologous recombination. One of the deletions end points is invariable and corresponds to the right end of IS1294. This suggests that the deletions are a result of IS1294 intramolecular transposition. We have as yet no explanation for the increase in deletion frequency in the presence of Tn5. As for the stability of pTUC200 in TolC$^-$ strains, genetic experiments showed that this is due to the prevalence of the pTUC100 low-copy-number replicon over that of the vector pBR322. This is unusual, since when two different replicons are fused it is the high-copy-number one which prevails. The resulting low expression of MccJ25 would maintain the intracellular antibiotic concentration below a toxic level.

MI-P51.
MICROCIN J25 ACTIVITY IN A MURINE SYSTEMIC INFECTION MODEL

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Microcin J25 (MccJ25) is a plasmid-encoded antibiotic consisting of 21 amino acid residues. This study was performed to determine the MccJ25 activity in an *in vivo Salmonella* Newport infection model. Two groups of BALB/c mice were inoculated with 10^6 bacteria. Two hours after infection, one group was treated with MccJ25, whereas the other was kept as a control. Two schemes of MccJ25 treatment were followed: i) Intraperitoneal injection of 18 mg/kg every 24 h during 146 h; and ii) the same dose every 4 hs during 28 h. Upon completion of the treatments, the mice were killed and the livers and spleens were removed to determine the colony forming units (CFU) per organ. The statistical significance of the differences between the numbers of viable organism in the organs of treated and untreated mice was determined. Colony counts decreased significantly (p<0,0001) in livers and spleens of treated group with the two treatments. The pharmacokinetic of MccJ25 was studied to determine the MccJ25 permanence in plasma. The mice were injected with 0,2; 0,5; 1 or 2 mg of antibiotic and blood samples were taken at various times after drug administration (5, 10, 15, 30, 60, 120, 180, and 240 min). MccJ25 reached the maximum concentration in plasma at 30 min. Microcin was still detectable 4 to 6 h after administration, depending of the dose injected. The present results open the way to study *in vivo* the therapeutic action of MccJ25.

MI-P52.
THE *yojI* GENE, ENCODING A MccJ25 PUMP, IS POSITIVELY REGULATED BY THE LEUCINE-RESPONSIVE REGULATORY PROTEIN (Lrp)

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We observed a greater sensitivity to MccJ25 when the Leu concentration in the medium was increased. Intracellular amount of the global transcriptional regulator Lrp inversely depends on Leu concentration. An *lrp* null mutant, RO64, did not show the Leu effect on sensitivity and was hypersusceptible to MccJ25. We found two potential Lrp binding sites (one of these identical to the consensus TTTATTCtNaAT) just upstream of *yojI*, a chromosomal gene which codes for a MccJ25 pump. To prove that Lrp is a positive regulator of *yojI* transcription, we transformed *lrp*$^+$ and *lrp*$^-$ strains with the plasmids pUCLO5 and pTrcHyojI both carrying *yojI*, in the first under control of native promoter and in the second, under control of *trc* promoter. Lrp$^+$ transformants displayed complete resistance to MccJ25 with both plasmids, while the Lrp$^-$ strain became resistant only with pTrcHyojI. To confirm the influence of Lrp on the expression of *yojI*, a *lacZ* gene fusion was constructed and mapped by restriction analysis. The expression level of the *yojI-lacZ* fusion in *lrp*$^+$ and *lrp*$^-$ strains was growth-phase-dependent. It was 10-fold higher in *lrp*$^+$ than in *lrp*$^-$ strains in stationary-phase. During exponential growth a low expression level was detected in *lrp*$^+$ strain indicating a stationary-phase induction of *yojI*. We conclude that an increased Leu concentration reduces *yojI* transcription through a reduction of Lrp levels. This, in turn, reduces MccJ25 export.

MI-P53.

MULTIDRUG RESISTANCE PUMP AcrAB-TolC IS REQUIRED FOR HIGH-LEVEL TetA-MEDIATED TETRACYCLINE RESISTANCE IN *Escherichia coli*

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Tetracycline (Tc) is an antibiotic which inhibits bacterial growth through the inhibition of protein synthesis. One mechanism of resistance among pathogenic strains is mediated by Tc efflux pumps which maintain its intracellular concentration at a low level. We observed that *Escherichia coli tolC* mutations severely reduced the high-level resistance to tetracycline afforded by Tn*10*- and plasmid-encoded TetA pumps. The reduction in the MIC was, in all cases, reverted by plasmid pAX629 carrying wild type *tolC* gene. The phenotype of Tc sensitivity was also seen with a Tc derivative, chlortetracycline (CTc). The influence of a *tolC* mutation on the expression of a *tetA*::*lacZ* gene fusion was examined. The expression of the fusion in the TolC$^-$ strain was similar to that in the TolC$^+$ parent strain, suggesting that the effect of *tolC* mutations on Tc resistance was not due to a down-regulation of the *tetA* gene. A defect in Tc efflux in the *tolC*::Tn*10* mutant was found by measuring the amount of TC accumulated. We demonstrated that mutational loss of the AcrAB multidrug efflux transporter had the same effect on the high-level resistance to Tc; therefore, it seems that *tolC* mutations act through inactivation of the AcrAB system These results are compatible with the hypothesis that the AcrAB pump is an important component in the development of high levels of resistance to Tc, perhaps by working in combination with TetA.

MI-P54.

THE ANTIBIOTIC CIPROFLOXACIN PRODUCES SUPEROXIDE IN *Escherichia coli* EVEN IN THE ABSENCE OF IRRADIATION

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Studies of most fluoroquinolones have reported that they cause phototoxicity, that involves reactions forming both singlet oxygen and superoxide anion ($O_2\$^-$), upon exposure to sunligth or UV irradiation. In this study we examined the formation light-independent of $O_2\$^-$ in *Escherichia coli* mediated by the fluoroquinolone ciprofloxacin.
We employed a semiquantitative method as indicator of intracelullar levels of $O_2\$^-$ *in vivo*, on the basis of the inactivation of aconitase by $O_2\$^-$. Several enzymes, including aconitase and 6P-gluconate dehydratase, contain [4Fe-4S] centers prone to $O_2\$^-$oxidation. The results indicated that a fraction (~30%), was inactivated with a very low dose of antibiotic in the absence of illumination. In this condition, the level of $O_2\$^-$ increased almost four times and up to ten fold, under UV irradiation. We could also demonstrate activation of the response to $O_2\$^-$ mediated by the *soxRS* regulon and dose-dependent increases in superoxide dismutase activity. We detected $O_2\$^-$ production with the tetrazolium dye XTT, upon UV irradiation, but failed to detect it in the absence of ligth, probably because this method is less sensitive than enzyme inactivation. Oxidative stress is a side-effect of these drugs and can induce cytotoxic reactions in cells.

MI-P55.

HIGHER CYTOTOXICITY OF METHICILLIN-RESISTANT *Staphylococcus aureus* (MRSA) EPIDEMIC CLONES RELATED TO THE PRODUCTION OF α-TOXIN

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Infections caused by MRSA are increasingly involved as main health compromise in worldwide for hospitalized patients. Some MRSA clones have the ability to spread easier than others within the hospital environment and thereby are frequently implicated in outbreaks. The specific phenotypic and genetic factors contributing to the epidemic behavior of this bacterium have not been identified until present. We examined the invasion and intracellular survival of both epidemic and sporadic strains of *Staphylococcus aureus* within epithelial and macrophages cell lines as an essential step to study their involvement in the epidemic capacity. Both, epidemic and sporadic *S. aureus* strains showed similar intracellular growth during the first 60 min. of infection. Besides to survive within the eukaryotic cells, all epidemic strains but not the sporadic ones induced a significant necrotic-like cytotoxicity following 12 h. of infection. This effect correlates with the production of the α-toxin identified as a major excreted protein involved in a drastic reduction of cell viability. Thus, *S. aureus* can persist in clinical setting through biofilm formation for which the α-toxin is required. In conclusion, the higher cytotoxicity evidenced in this study reveal a biological feature of these bacteria explaining why some strains can persist in the environment and disseminate easier that others, conferring the epidemic behavior.

MI-P56.

HIGH-LEVEL RESISTANCE TO MECILLINAM PRODUCED BY INACTIVATION OF SOLUBLE LYTIC TRANSGLYCOSYLASE IN *Salmonella enterica* SEROVAR TYPHIMURIUM

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Mecillinam is a β-lactam antibiotic highly specific for the penicillin-binding protein 2 (PBP 2) of Enterobacteria, which is involved in control of bacterial shape and cell division.
By screening for high-level mecillinam resistant derivatives of a low-level resistant strain (*cysB403 galE1922 relA21*::Tn*10*) of *Salmonella enterica* serovar Typhimurium, a MudJ insertion in the gene for soluble lytic transglycosylase (*slt*) was isolated. This insertion (*slt-1*::MudJ) increased the resistance to mecillinam of *cysB* and *cysE* strains (MIC: about 20-40 μg ml^{-1}) to a strikingly high level (MIC: 160 μg ml-1). As in *Escherichia coli* K-12, the *slt* mutation slightly increased the sensitivity of the wild type and of several strains that carried mutations which did not increase mecillinam resistance. All the strains acquired spherical cell shape when treated with mecillinam. The effect of *slt-1*::MudJ was limited to mecillinam since response to several other antibiotics was not altered by the insertion. The results presented in this paper demonstrate that soluble lytic transglycosylase performs an important role in the response to mecillinam which only becomes evident when failure of CysB/CysE function causes medium-level resistance. The results also suggest that soluble lytic transglycosylase interacts with and is partially inhibited by normal lipopolysaccharide.

MI-P57.

HSP70 CYTOSOLIC CHAPERONES INVOLVED IN THE SECRETION OF METALLO-ß-LACTAMASES IN GRAM-NEGATIVE BACTERIA

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In gram-negative bacteria, periplasmic proteins are synthesized in the cytoplasm as precursors and directed to the general secretory pathway (Sec) for their secretion. Whether and how the main cytoplasmic chaperones participate in this process is still obscure. We studied here the putative cooperations of the DnaK, GroE, and TF systems with the Sec pathway in *E. coli* by genetic procedures, using as a model the metallo-ß-lactamase (MßL) GOB from the gram-negative pathogen *Elizabethkingia meningoseptica*.
The coding GOB sequence was cloned and expressed in *E. coli* mutants in the different chaperone systems, and their capability to secrete an active MßL enzyme was analyzed by a plate dilution assay. Mutants in SecA or SecYEG showed very poor MßL activity in comparison to wt strains, indicating fundamental roles of the Sec pathway in proper GOB secretion. In turn, mutants in cytoplasmic chaperones including DnaK, DnaJ and Trigger Factor (TF) were also deficient in MßL secretion, indicating their importance in this process. The use SecA-TF double mutants provided further evidence for synergistic effects between these chaperones in GOB secretion. The overall data further highlights the existence of complex interactions between the Sec machinery with Hsp70/DnaK cytoplasmic chaperones and functional analogs in the secretion of MßLs in gram-negative bacteria.

MI-P58.

CarO, AN OUTER MEMBRANE PROTEIN OF *Acinetobacter baumannii* INVOLVED IN CARBAPENEM RESISTANCE, IS A PORIN FOR BASIC AMINO ACIDS

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The gram-negative aerobic pathogen *A. baumannii* is responsible for a large percentage of nosocomial infections, including pneumonia, bacteremia, skin, wounds, and urinary tract infections. Some strains are resistant to multiple antimicrobials, having successfully resisted attempts of eradication with ß-lactams, aminoglycosides, cephalosporins, and even fluoroquinolones.
Resistance to the ß-lactams carbapenems in multidrug resistant *A. baumannii* of local hospitals was associated with the loss of a 29 kDa outer membrane protein, CarO (Mussi *et al*., 2005). To determine the physiological function of CarO, we analyzed the growth capabilities of *ΔcarO* mutants on different carbon sources, including carbohydrates, carboxylic acids, and natural amino acids. These mutants were unable to grow only in L-ornithine. Transport experiments with [^{14}C]-Orn in whole cells and spheroplasts confirmed that the mutants were unable to import this amino acid. Competition experiments showed that Arg and His also interact with CarO, inhibiting [^{14}C]-Orn transport. We conclude that CarO represents an outer membrane channel for basic amino acids in *A. baumannii*.
Mussi M. A., Limansky A. S. and Viale A. M. Antimicrob. Agents Chemother. 49(4): 1432-1440.

MI-P59.

EXPOSURE TO SUBINHIBITORY CONCENTRATIONS OF PENICILIN INCREASES OPTOCHIN RESISTANT PHENOTIPE IN *Streptococcus pneumoniae*

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Optochin is an antibiotic utilized only for *in vitro* identification of pneumococcal isolates. Point mutations in *atpC* gene, which codify for a subunit of F_0F_1 ATPase, confer resistance to optochin (opt^R), and complicate the clinical diagnostic. Previously, we have characterized nine opt^R invasive strains, describing different *atpC* mutations. In this work, our aim was to analyse the putative causes of optochin resistance in *S. pneumoniae*. It's known that different stresses (hydrogen peroxide, antibiotics, etc) can increase the mutation rates in bacteria. In this sense, we exposed a wild-type strain to subinhibitory concentrations of different antibiotics, and we studied their impact on generation of opt^R strains. Remarkably, only penicillin was able to increase the optochin resistance rate. The penicillin-induced opt^R mutants were characterized and we found a wide range of *atpC* mutations that we could not find in clinical isolates. Probably, some *atpC* mutations alter the pneumococcal pathogenesis and they are lost during the infection process. To verify this hypothesis, we inoculated C57B mice with a pool of penicillin-induced opt^R mutants, and bacterial cells were recovered from liver. The *atpC* mutations were identified in 20 opt^R mutants selected at random, showing that most *in vivo*-selected mutants are identical to the opt^R mutants isolated from invasive diseases. We suggest that the mutation-rate increase due to penicillin exposition could be an adaptive strategy that we evidenced with the optochin resistance model in *S. pneumoniae*.

MI-P60.

INVOLMENT OF* pbpE IN THE OSMOADAPTATION OF *Bacillus subtilis

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B. subtilis cultures submitted to an osmotic stress increased its cell wall. Its structure was essentially due to the activity of several penicillin binding proteins (PBP). PBP4* is an endopeptidase involved in the peptidoglycan (PG) synthesis, coded by the *pbpE* gene. *pbpE-lacZ* fusions were studied. The wild type strain showed an increased transcriptional activity during stationary phase (5x) and an upper induction in a high salt medium (25x). In order to discriminate if this effect was due to the elevated osmolarity and/or to sporulation, cultures in sporulation repressed media were studied. The same level of induction was observed, discarding a sporulation effect. Moreover, the *ΔpbpE* strain was much more osmosensitive than the wild type strain. In addition, no defects in sporulation were observed in this mutant, suggesting that PBP4* might play a role in the osmotolerance only. Whole cells and walls from the wild type and *ΔpbpE* strains grown in low and high salt conditions were prepared and susceptibility to mutanolysin (a muropeptidase) studied. Whole cells obtained from high salt medium, showed an increased lytic sensitivity for both strains. Their purified cell-walls showed a higher resistance. Biochemical analysis of the PG, to examine the pentapeptide composition and the degree of crosslinking, were undertaken to study the role of PBP4* in high salt growth.

MI-P61.
EXPRESIÓN OF SURFACE LAYER PROTEINS OF *Bacillus sphaericus* DURING OSMOTIC STRESS
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Surface layers (S-layers) are crystalline arrays composed of a unique protein or glycoprotein with molecular mass ranging from 40 to 200 kDa. S-layers constitute the outermost structure of the cell envelope in numerous organisms of the domains Bacteria and Archaea. Isolated S-layer subunits can recrystallize into regular arrays on solid supports, liquid-surface interfaces, lipid films and liposomes. Besides the wide information concerning their genetic and structure, the knowledge of their biological functions is scarce. In order to establish whether these proteins were involved in the osmotic stress adaptation, *Bacillus sphaericus* strains were cultured in media containing or not a high NaCl concentration. As the S-layer proteins are not covalently linked to each other nor to the cell wall, we could disintegrated them into monomers by adding a denaturing agent such as guanidine hydrochloride; this technique allow us to recover almost all the S-layer protein. The yield obtained from NaCl containing cultures was higher than that of control. However we cannot discriminate if this result was due to a loosely attached S-layer or to a higher expression. No significant differences were observed for the expression of S-layer protein in whole cell extracts from both cultural conditions, although different MW proteins were detected by western blot from the NaCl condition, suggesting the use of a second promoter during the osmotic stress. Transcriptional analyses are currently undertaken in order to clarify it.

MI-P62.
QUORUM SENSING, COMMUNITY FORMATIONS AND THE IDENTITY OF *Bacillus subtilis*
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Many species of bacteria are highly social organisms with complicated cellular communication systems. This allows the cell to respond to different environmental stimuli. Co-ordinate expression of cellular components ensures adaptation, colonization and survival at the ecological niche. Nowadays, different communications signals are known. One of this, the borated pheromone (AI-2) has been proposed to serve as a "universal" signal for inter-species communication, because AI-2 is produced by a large number of bacteria with different genetic and physiological behaviours. Even though *lux*S, coding for the AI-2 synthetase, is present in *B. subtilis*, it has not been previously reported the role of AI-2 in this bacterium.
In this work we demonstrate that *B. subtilis lux*S gene is essential for the autoinducer-2 (AI-2) synthesis. This gene was transcripted al low levels in the wild type strain, but an isogenic *lux*S mutant strain showed upregulation of *lux*S expression. We observe that *lux*S gene expression was under the transcriptional control of the master sporulation regulator *spo0A*. Additionally, we observe that the ability of *lux*S mutant strain to form multicellular communities structures such as biofilms and fruiting bodies were significantly impaired. Besides we observe that the *lux*S mutant strain affected the antibiotic production of *Streptomyces coelicolor*. We show that *B. subtilis* AI-2 would be a play a key role as morphogen molecule and a signal for intra-specific identification.

MI-P63.
RELEVANCE OF TRANSCRIPTION FACTOR σ^B DURING GERNINATION AND POST-GEMINATION STRESS ADAPTATION IN *Bacillus subtilis*
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Spore development and stress resistance in the Gram-positive paradigm *Bacillus subtilis* are governed by the master transcription factors Spo0A and σ^B respectively. *B. subtilis* is a soil bacterium, and hence temperature changes would constitute a common environmental stress. We showed in previous reports that the coding gene for the regulatory protein σ^B is dramatically induced after a temperature downshift from 37°C to 20°C, and stills active belong to T_o. Loss of σ^B reduces stationary-phase viability of cold-adapted cells 10 to 15-fold. Here, we showed that *sigB* is important after the spore is formed, because a mutation in this gene reduces the germination capacity of spores in alcohol stressing germination medium. Moreover, it was observed a delay in the outgrowth for the *sigB* mutant when we compared it with the wild type spores under same conditions. Besides, we found a decrease in growth rate when the *sigB* mutant cells were stressed whit ethanol at 20ºC during the exponential growth phase. On the other hand, we tested the resistance of spores at different stress conditions: heat, UV-C light, organic solvents and acid. We found that the survival to UV-C radiation of *sigB* mutant's spores was considerable low than wild type's. Take in consideration the relevance of *sigB* in germination and spore resistance; we are engaged to determinate the role and regulation of σ^B during sporulation and germination in *B. subtilis*.

MI-P64
THERMAL INACTIVATION ANALYSIS OF EXTRACELLULAR PROTEASES OBTAINED FROM A SUB-ANTARCTIC *Colwellia* ISOLATE
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High activity at low temperature along with a limited thermal stability characterize cold-active enzymes, also named psychrophilic enzymes. Thermostability is one of the main features that determine the technological usefulness of such enzymes. In this study, the thermal stability of extracellular proteases produced by the sub-Antarctic bacterium *Colwellia* sp. IE1-3 was described by thermal kinetic and thermodynamic apparent parameters. Protease activity was measured using casein as substrate; the remaining activities at 25°C, after incubation of the enzyme extract without substrate in the temperature range 20-50ºC, were determined. The kinetic of thermal inactivation was predicted with an irreversible first order deactivation model. k_{in} values were calculated from the ln (v/v_0) vs. time, activation energy (Ea) from Arrhenius plot, and thermodynamic activation parameters from the transition state theory. Over the studied temperature range Ea_{in}, ΔH_{in}^* and ΔS_{in}^* were almost constant with values of 181.5 kJ mol^{-1}, 178.9 kJ mol^{-1} and 0.27 kJ $mol^{-1}K^{-1}$, while ΔG_{in}^* was between 99.3-91.2 kJ mol^{-1}. *Colwellia* sp. IE1-3 proteases showed higher thermosensitivity than most of the bacterial enzymes, and the ΔG_{in}^* value obtained was comparable to those of the few psychrophilic proteases characterized to date.

MI-P65.
METABOLIC REGULATION AND SECONDARY REACTIONS OF *E. coli* MALIC ENZYMES
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Malic enzyme (ME) catalyses the oxidative decarboxylation of malate to yield pyruvate, CO_2 and NAD(P)H. Two isoforms of ME have been identified by sequence homology in *E. coli*: *sfc*A (or *mae*A) and *mae*B (or *ypf*F). After cloning in an expression vector and over-expressing both ME isoforms, the purified recombinant enzymes were characterized, presenting several distinct kinetic and structural properties. In the present work, secondary reactions and metabolic regulation of the recombinant ME were analyzed. The pyruvate carboxylase activity was tested and the Km for pyruvate was obtained for both enzymes. These values indicate that this activity may not be important *in vivo*. In addition, the partition for oxaloacetate and the reduction of pyruvate were also analyzed. Interestingly, both enzymes were modulated by several metabolites. In the case of MaeB, it was strongly activated by glucose-6P and aspartate, although there was a minor activation due to oxaloacetate and glutamate. acetil-CoA, fumarate and fructose-6P inhibited this enzyme. SfcA was strongly inhibited by oxaloacetate and activated by aspartate. These results indicate that both enzymes may fulfill different metabolic roles *in vivo* and that their activity is highly regulated by different key metabolic compounds.

MI-P66.
ASSOCIATION BETWEEN MOBILE GENETIC ELEMENTS AND phb GENES IN *Pseudomonas extremoaustralis*
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Genes responsible for the synthesis of polyhydroxybutyrate (PHB) in *Pseudomonas extremoaustralis*, a new sp. isolated from a temporary pond in Antarctica, were cloned and analyzed. This species shows high stress resistance in association with high (up to 70 % of its cell dry weight) PHB production. It accumulates PHB using octanoate but not glucose as a carbon source. A PHB polymerase gene (*phbC*) was found downstream from genes coding for a beta-ketothiolase (*phbA*) and an acetoacetyl-coenzyme A reductase (*phbB*). The *phbC* gene from this strain was introduced into *Ralstonia eutropha* PHB-4 (*phbC*-negative mutant), restoring the PHB phenotype. Genes encoding putative insertion sequences, introns, membrane transporters and plasmidic proteins, were found downstream of the *phb* gene cluster, resembling genomic islands. The association between mobile genetic elements and phb genes suggests that horizontal transfer could be an adaptive response of species inhabiting extreme environments.

MI-P67.
CLONING OF *phnA1*-LIKE GENES OF POLYCYCLIC AROMATIC HYDROCARBON-DEGRADING BACTERIA FROM MARINE SEDIMENTS
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It has been suggested that bacteria belonging to the genus *Cycloclasticus* play an important role in the biodegradation of polycyclic aromatic hydrocarbons (PAHs) in the marine environment. One of the catabolic genes identified in these organisms is *phnA1*, which encodes for the alpha subunit of the iron-sulfur protein of the initial PAH-dioxygenase. In the present work, we analyzed the presence of *phnA1*-like genes in sediments of Ushuaia Bay, Tierra del Fuego, Argentine, using culture-independent techniques. Total DNA was extracted from intertidal surficial marine sediments. A primer set was designed to target the *phn*A1 gene from *Cycloclasticus* sp. A5 (AB102786) and close relatives (AF093000, AF092998, AF053737), and used to amplify *phnA1*-like genes from the extracted DNA. PCR products were cloned and sequenced. The analyzed clones showed similarities at the nucleotide level of 99.1 to 99.8% with *phn*A1-like genes identified to date. These findings broaden the known distribution of these genes to cold sediments of Ushuaia Bay.
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MI-P68.
THE BACTERIAL SMTR GII INTRON RECONGNIZES THE *attC* GENE CASSETTE ATTACHMENT SITE
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Background. Antibiotic resistant gene cassettes are mobile elements from the genome formed by a structural gene and a palindromic region called *attC* site. A group II intron is inserted in an integron gene cassette at the junction of the structural gene and its *attC* site. Analysis of the *S. marcescens* GII intron (Smtr) has shown that this intron is capable of invading different antibiotic gene cassettes. Methods. Double transformation of an intron-containing plasmid and 9 different target site-containing plasmids were inserted into *E.coli* JM109 ($RecA^-$) and JM107 ($RecA^+$). Positive events of the intron insertion was tested by PCR. Phylogenic analysis was done with the ClustalW (V1.86) software and secondary structures were analyzed with the mFOLD (V3.1) software. Results. Positive results have shown insertion at the consensus region TA/ACAA, while the negative targets presented a modification at the ACAA region. The phylogeny analysis of bacterial GII introns reverse transcriptases revealed an cluster for the Smtr-like introns that correlates with the DNA region where they home. Secondary structure analysis shows a stem loop for the *attC* sites different from other target sites. Conclusion. The Smtr GII intron can be inserted in any antibiotic resistant gene cassettes that has the TA/ACAA target site corresponding to the junction point between the structural gene and its *attC*, which provides the secondary structure for the target recognition. Smtr-like introns present a different lineage of evolution from other bacteria introns.

MI-P69.

PREDOMINANCE OF INCN PLASMIDS IN TOTAL COMMUNITY DNA ISOLATED FROM HIGH ALTITUDE ENVIRONMENTS IN THE ARGENTINEAN ANDEAN WETLANDS

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This study describes the diversity of environmental plasmids, from total DNA bacterial community samples, collected from ten different Andean wetlands locales (3660 to 4600 m altitude)

It was conducted by using PCR amplifications and DNA-DNA hybridization with specific oligonucleotides and DNA-probes, based on the incompatibility and replication (*inc/rep*) regions of a number of well-characterized plasmid incompatibility groups (IncQ, IncP, IncN and IncW). No amplifications were obtained with IncP or IncW oligonucleotides, however, PCR and southern hybridization analysis revealed the prevalence of IncN specific sequences in 50% of the sampled places that included water and sediments. IncQ specific sequences were amplified only from one environment. The general amplification pattern of the main plasmid group (IncN) in these environments differs from plasmids isolated in other environments including sea, water columns, sediment samples, soil samples, pig manure slurries, Antarctic natural assemblages and sugar beet. To our knowledge, this is the first report of plasmid diversity in such high altitude lake-environments.

MI-P70.

BACTERIA ISOLATED FROM PRISTINE HIGH ALTITUDE ENVIRONMENTS IN THE ARGENTINEAN ANDEAN WETLANDS: PLASMID PROFILE AND MULTIPLE ANTIBIOTIC RESISTANCE

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Andean wetlands, placed in the North-Western Argentine at 4,600 m altitude, are attractive for both, environmental and biotechnology studies. Most of these wetlands are completely remote and inaccessible, having a high salinity and metal contents, a wide range of daily temperature changes, and an important intensity of solar UV-B radiation.

Bacteria isolated from these environments were identified by 16S rDNA sequence and resulted in Gram-positive colored bacteria. Interesting features, to our knowledge never reported so far from bacteria isolates from these pristine high altitude lake-environments, such as similar plasmids profiles and multiple antibiotic resistances are the focus of this work.

At least two plasmids were found in all isolates studied by using modifications of the alkaline lysis method. Their preliminary characterization in this work includes size, incompatibility group through PCR, genetic transference to suitable hosts by transformation and conjugation, and studies of possible relationships of them with antibiotic resistances.

MI-P71.

RESPONSE OF *Acinetobacter jonhsonii* TO ULTRAVIOLET-B RADIATION

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Acinetobacter jonhsonii is a γ-proteobacteria isolated from water of Laguna Azul, a high altitude wetland (4554 m above sea level) from the North Western Argentinean Andes. The molecular and biological consequences of UV-B radiation were investigated under laboratory condition.

Methods: *A. jonhsonii* was exposed to artificial UV-B radiation. After the exposition the damage in the DNA, measured as cyclobutane pyirimidine dimers was quantificated using an inmuno dot blot technique. The number of colony forming units (CFU) were also determined. The presence of error-prone DNA repair system was study by measuring changes in the numbers of rifamycin-resistance colonies. The changes in extracellular and total protein composition were evaluated by SDS-PAGE.

Results: *A. jonhsonii* exhibited little DNA damage and an important resistance was observed with 48 % survival inhibition after UV-B irradiation. A 3.4 times increase in mutation frequency was found. No changes in protein composition were found.

MI-P72.

PRELIMINARY STUDIES OF NAPHTHALENE-DEGRADING BACTERIA ISOLATED FROM MUELLE STORNI SEDIMENTS

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In order to isolate naphthalene-degrading bacteria from sediments of a highly contaminated site with polyaromatic hydrocarbons, three successive enrichment cultures were made in a synthetic marine media with 0.5 % naphthalene as a sole carbon and energy source. Growth was performed with agitation in darkness at 25ºC and 15ºC. After incubation, several colonies were isolated on agar plates at the different enrichment conditions. DGGE procedure was applied in order to compare the resulting biodiversity of culturable bacteria and ARDRA method to select potentially different species of bacteria for further identification.

In a culture-independent approach, total DNA was purified from the same sediments, and alpha subunits of initial PAH-dioxygenase genes were amplified using a degenerate primer set, cloned and sequenced. All analyzed clones in the library were closely related to phnAc genes from the phenanthrene-degraders *Burkholderia* sp. Ch1-1, Ch3-5, Cs1-4 and Eh1-1 (AY367784-7; 99.5 to 99.8% similarity at the nucleotide level) and *Alcaligenes faecalis* AFK2 (AB024945; 95.7-95.9% similarity). The presence of these catabolic genes was also search on the isolated bacteria.

MI-P73.
CALCULATION OF MAXIMAL GROWTH RATES OF *E. coli* IN MIXTURES OF SUGARS, PERFORMING A FLUX BALANCE ANALYSIS OF ITS METABOLIC NETWORK

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There is experimental evidence that microbial cells utilize many carbon sources simultaneously under conditions of low concentrations, in contrast with what is suggested by the principle of diauxic growth. In addition, for growth with two sugars under carbon-limited conditions, lines of constant growth rate, drawn as a function of their concentrations, result in straight lines. This indicates that the sugars have an additive effect on growth.
Flux Balance Analysis (FBA) is a structural *in silico* systems biology approach that allows to calculate the maximum growth rate that a genome-scale reconstructed metabolic network would produce under constraints of flux balance, irreversibility/ reversibility and transport fluxes. Performing an FBA, the contour plots for the growth rate of *E. coli* were computed for several pairs of sugars. The curves obtained at low inputs of sugars are, for all practical purposes, straight lines, in agreement with the above mentioned experiments. This result suggests that, at low concentrations of sugars, the bacteria behaves as if it utilized the maximal capabilities of its network.
Finally, we built a model including only a few reactions that, when analyzed with FBA, also reproduces the straight lines.
This work is supported by CSIC, Universidad de la República, Montevideo and PEDECIBA, Montevideo.

MI-P74.
STREPTOMYCES SP BIODEGRADATION ACTIVITY ON PROPERTIES OF POLYHYDROXYALKANOATES

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Polyhydroxyalkanoates (PHA) are of great interest since their properties are similar to those of conventional thermoplastics with the advantage of their complete biodegradation. In the present work we studied the capacity of isolated *Streptomyces sp*. to degrade PHB-co-HV in mineral salt media (MSM) and measured the physico-chemical properties of the degraded samples. Degradation and changes in the molecular weight were measured by weight lost and intrinsic viscosimetry, respectively. Degraded samples of PHB-co-HV lost 45% of the initial weight after 45 days of incubation in the inoculated MSM with *Streptomyces sp*. No significative changes in the mean molecular weight were observed.
The surface of the degraded samples, analyzed by SEM, shows few big pores at the beginning of degradation. As the activity of the microorganisms increases a great number of pores, 10 times smaller than the ones first observed, appeared. *Streptomyces sp*. degrades the biopolymer randomly on all over the sample. Crystallinity and mechanical behaviour of the degraded samples were measured by X ray diffraction and tensile tests at constant strain rate, respectively. The ultimate tensile strength of degraded samples decreased up to 64% of the control samples after 30 days with no significative changes in the crystallinity. Extracellular protein was measured by the method of Bradford and the activity of PHA-depolymerase was determined by the clear zone method.

MI-P75
GENE ADAPTATION TO EXTREME ENVIRONMENTS

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This work is oriented to the study of gene adaptation to extreme conditions, such as the hydrothermal system located in Copahue, Neuquén, Argentina. The organisms living there develop under two pressure selection conditions: the high temperature of thermal water and the strong impact of ultraviolet (UV) radiation.
Several microorganisms found in this region were isolated and different colonies resistant to UV radiation were selected, a *Geobacillus thermoleovorans* strain identified through 16S RNA sequence, being the most remarkable. A gene library was prepared out of this strain with UV sensitive bacteria BH200 (uvrA::Tn10). A number of clons were isolated by means of UV selection, the most outstanding being a gene carrier able to codify for the guanosine monophosphate synthetase enzyme (GMPs). The suitability of said enzyme was proved by means of additional assays performed on ght1 bacteria (guaA26::Tn 10) which lacked the enzyme.
A transcript of 1100 pb was detected through Northern Blot. The result was consistent with that obtained for the mapping of the starting transcription site. The cloned GMPs produces an increase in growth speed and a greater biomass in BH200 bacteria.

MI-P76.
STUDY OF CHEMOTAXIS IN HALOARCHAEA

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Microorganisms belonging to the haloarchaeal group need high salt concentrations (3-4 M NaCl) for growth. Although chemotaxis has been investigated in detail in eubacteria (*E. coli*), the information about the chemotactic responses in haloarchaea is still scarce and most studies have been carried out in H*bt.salinarum*. The aim of this work was to perform a preliminary study of chemotaxis in haloarchaea. Haloarchaeal strains *Haloarcula hispanica*, *Halorubrum saccharovorum* and *Natronomonas pharaonis* were used in this study. We analyzed the chemotactic behavior of these microorganisms to different compounds using Chemical In Plug assay (CIP). These strains showed similar pattern of chemotactic responses against amino acids and organic acids except for carbohydrates; both *Har. hispanica* and *Hrr.saccharovorum* displayed a positive chemotactic response towards carbohydrates. Membrane and cytosolic fractions of haloarchaea were probed by Westernblotting using antibodies raised against peptides corresponding to receptor proteins from *Hbt.salinarum*. The results showed that the haloarchaeal strains used in this study have several proteins which are antigenically related to the Htrs of *Hbt.salinarum*.
This work was supported by EMBO, IUBMB, Conicet.

MI-P77.

STRUCTURAL HOMOLOGY WITH UBIQUITIN IN THE HALOALKALIPHILIC ARCHAEA *Natrialba magadii*

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Ubiquitin, a small protein that remains highly conserved among eukaryotes, has been not found in prokaryotes. Recently, several reports have been presented that associate the tertiary structure of ubiquitin with those of other proteins from eukaryotes and prokaryotes. The amino acid sequences of these proteins share very low identity. Indeed, they exhibit both fold and function similar to that of ubiquitin.
We have previously found that several haloalkaliphilic archaea contain proteins that react with antibodies against ubiquitin. Following these studies we have obtained in *Natrialba magadii* a PCR product of 400bp, which has an open reading frame homologous with the ubiquitin related proteins of the ThiS family, ThiS, MoaD and Urm1. The polypeptide encoded by this sequence, denoted as P400, displayed similar structural features to those of ubiquitin and related proteins. The alignment of these proteins indicates the preservation of several structurally and functionally significant amino acid residues.
Supported by UNMdP, CONICET and Fundación Antorchas.

MI-P78.

IDENTIFICATION AND CHARACTERIZATION OF UBIQUITIN-LIKE PROTEINS IN HALOARCHAEA

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Ubiquitin is a small protein, whose structure is very conserved among eucaryotes. It is involved in the mechanism of protein degradation. There is not genomic evidence supporting the presence of ubiquitin in prokaryotes. However, although their sequences are different, certain archaeal proteins show structural and functional homology with ubiquitin.
In this work we report the finding of proteins that react with an anti-ubiquitin antibody in different genera of halophilic archaea as analyzed by Western blot. Like ubiquitin, some of these proteins resulted heat-stable and reacted with the antibody after heating for 1 hour at 86°C. For a further characterization, the cell protein extracts were subjected to 2D SDS-PAGE. The subsequent immunoblot assay of the gels revealed many spots. These proteins are actually being further purified for sequencing. The obtained sequences will allow to establish whether they have either sequential or conformational homology, or both, with ubiquitin and related proteins.
Supported by UNMdP, CONICET and Fundación Antorchas.

MI-P79.

CHARACTERIZATION OF THE PROTEOLYTIC SYSTEMS EXPRESSED IN THE STATIONARY GROWTH PHASE IN THE HALOALKALIPHILIC ARCHAEON *Natrialba magadii*

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Proteolysis plays a key role in any cell and it has been poorly investigated in haloarchaea. The proteases NEP (extracellular), NSP (intracellular) and the endogenous protease inhibitor NSI were purified and characterized at the biochemical level from the haloalkaliphilic archaeon *Natrialba magadii* by our research group. The aim of this research was to characterize the proteolytic systems produced by *Nab. magadii* during the stationary growth phase. Azocaseinolytic activity due to NEP and NSP were detected in cultures that reached high optical densities ($OD_{600}$$2,5). In these cultures the production of biofilms was evident. Comparison of the V_e of NEP and NSP indicated that they did not correspond to the same protein. Cell extracts of cultures with OD_{600}≤2,5 produced NEP but not NSP, and the development of biofilms was not observed. However, high levels ($1,2x10^4$ UF/mg) of hydrolytic activity against Ala-Ala-Phe-MCA and NSI inhibitory activity were detected. Degradation of Ala-Ala-Phe-MCA was due to two different intracellular proteases: a metalloprotease previously denoted as NMP and a novel protease insensitive to various types of protease inhibitors which was named by us NXP. Partial characterization of NMP y NXP was carried out. The Mr of NXP was ~210 kDa as determined by gel filtration chromatography. Both NXP and NMP degraded fluorogenic chymiotrypsin substrates with Phe in the P1 site while trypsin substrates and azocasein were not hydrolysed. Besides, NXP efficiently degraded N-Succ-Leu-Tyr-MCA. NMP and NXP activities were not stimulated by ATP and were inactivated at 68°C 20 min.

MI-P80.

ACTIVITY AND STABILITY OF AN EXTRACELLULAR PROTEASE FROM THE HALOALKALIPHILIC ARCHAEON *Natrialba magadii* IN ORGANIC SOLVENTS

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The haloalkaliphilic archaeon *Nab. magadii* produces an extracellular protease at the end of exponential growth phase denoted as NEP for *Natrialba magadii* Extracellular Protease. This enzyme has the maximum activity for azocasein hydrolysis at 1.5 M NaCl and pH 8 at 45ºC. As salt has the effect of reducing water activity, this protease might be stable and active in aqueous/organic solvent and/or organic solvent media. The aim of this study was to analyze the activity and stability of NEP in the presence of different organic solvents. The azocaseinolytic activity was measured in solutions with decreasing salt concentrations (1.5 - 0.15 M NaCl) in the presence or absence of 30% (v/v) dimethylsulfoxide (DMSO) or dimethylformamide (DMF). The enzyme was partially purified from culture media harvested in the stationary phase (OD_{600}>1.5). In the presence of DMSO the proteolytic activity was at least 2-fold higher than the control (without DMSO) for salt concentrations lower than 1.5 M NaCl. However, DMF had a negative effect on the hydrolytic NEP activity. The stability of NEP was greatly enhanced by addition of DMSO even at salt concentrations as low as 0.15 M NaCl. These results show that NEP is a potential tool for biotechnological processes that involve biocatalysis in organic media.
Supported by grants from UNMDP and CONICET.

MI-P81.
PICOEUKARYOTIC DIVERSITY IN ARGENTINEAN SEA COSTAL SITE STUDIED BY MOLECULAR APPROACHES

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Marine picoeukaryotes (unicellular less than 3µm), are found throughout the marine photic zone worldwide and play fundamental roles in marine ecosystems. This assemblage is composed of chlorophyll-containing cells (phytoplankton) and heterotrophic cells, that have a pivotal role in both primary production and microbial loop, respectively. We studied the diversity of picoeukaryotes of samples collected from surface water at a coastal fixed station EPEA (38º28'S-57º41'W). Sequences of 18S rDNA environmental picoeukaryotes were retrieved and molecular fingerprinting techniques (RFLP and DGGE) were used to analyze the seasonal changes in the taxonomic composition. Genetic libraries were constructed and the clones that produced different RFLP pattern were sequenced. Phylogenetic tree constructed with ribosomal minilibraries revealed that organisms of the Prasynophyceae class may be dominant during autumn, including a member of clade VII, a new taxonomic group recently described. Organisms of the Stramenopiles, Dinophyceae, and Dictyochophyceae groups have been identified from spring samples. Diversity indices (Shannon and Simpson) and dendrograms (based on Euclidean distances) from DGGE and RFLP techniques revealed the annual diversity of the picoeukaryote assemblages.
Supported by PICT12233, and UNMdP grants.

MI-P82.
IS OXIDATIVE STRESS A COMMON MECHANISM OF STRESS AND STRESS RESPONSE IN BAKER'S YEAST?

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Industrial baker's yeast cells are exposed to simultaneous multiple stresses during their use in different commercial products, as in the manufacture of frozen doughs. It was postulated that oxidative stress represents a common mechanism of stress and stress response in these yeasts when submitted to different types of stresses. To test this hypothesis, two industrial baker's yeast strains one osmotolerant (O) and the other osmosensitive (NO), were frozen (F) (-20ºC, 24 h) and thawed (T) (25ºC), and the oxidative response was studied. After exposition of O and NO to F/T, preincubated with the ROS-specific stain 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA), a relative intensity of fluorescense 4 times lower was found for extracts of O and NO cells, when compared to fluorescence produced by H_2O_2 (0.1 mM) treatment. Moreover, preincubation of cells with ROS scavengers as ascorbic acid (10-100 mM) or β-carotene (25 µM), did not prevent cell viability loss after F/T treatment. On the other hand, SOD and catalase activities did not changed significantly in cell extracts after F/T. The lack of relationship between cell death induced by F/T and ROS production was confirmed by epifluorescence microscopy of yeast cells double stained with DCFH-DA and propidium iodide. Our results seem to indicate that although ROS as well as antioxidative responses are elicited after F/T, no cuantitative relationship could be established between F/T and oxidative stresses.

MI-P83.
CAROTENOID PIGMENTS ENHANCE PHOTOPROTECTION IN THE YEAST *Rhodotorula mucilaginosa*

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We analyze the relationship between carotenoid pigments and UV-B resistance in strains of the yeast *Rh. mucilaginosa*.
Carotenoid and phytoene (a carotenoid precursor) production as well as the cell survival to UV-B exposure were studied using a set of 13 strains both type strain and Patagonian native isolates. Albino and hyper pigmented mutants of one selected strain were obtained after UV-B mutagenesis and characterized by molecular (PCR-fingerprinting) and conventional techniques. These strains were studied as described above and compared with T type. Data from the first screening were submitted to principal component analysis showing a strong relationship between total carotenoids concentration and UV-B survival, while phytoene concentration did not co-relate with these two variables (carotenoids and resistance). In the second assay the albino mutant presented similar UV-B survivorship than the parental strain while the hyper pigmented (300% increase in total carotenoid) showed a consistent upgraded survival (250%).
These results support the idea that enhanced carotenoid concentration in pigmented yeasts could be responsible for survivorship and play a role as UV-B resistance mechanism.

MI-P84.
SUBSTRATE AFFINITY PATTERNS SHOWING BY LIPASE AND ESTERASE ACTIVITIES FROM *Aspergillus niger* MYA 135

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Introduction. Lipases (EC 3.1.1.3) are produced by various microorganisms either alone or together with esterases (EC 3.1.1.1). Lipases and esterases are biotechnologically important enzymes that catalyze the hydrolysis and synthesis of a wide array of esters. The explosive growth of biocatalytics process has led to an increasing demand for simple substrate affinity detection. In this work we analyzed substrate affinity patterns showing by extracellular lipase and esterase activities from *Aspergillus niger* MYA 135. Methods. Proteins were separated by native polyacrylamide gel electrophoresis. Lipases and esterases activities were detected using 1.3 mM of a-naphtyl derivates of acetate, propionate, butyrate, caproate, caprylate, caprate, laurate, myristate, palmitate or estereate as substrate. Released naphthol was coupled with 1mM Fast Blue to give a colored product. Reactions were carried out at 37°C in shaken plates containing 0.1M phosphate buffer (pH7). Both the substrate affinity and the number of band were analyzed. Results and conclusions. Supernatant of *A. niger* developed in mineral medium plus olive oil, exhibited the following substrate affinity patterns: 4 bands with acetate, propionate, butyrate or caproate; 2 bands with caprylate; and only 1 band with caprate, laurate, myristate, palmitate or estereate.
This work were supported by grant PIP 2052/00 CONICET and CABBIO 2000 cod 012.

MI-P85.
EFFECT OF CULTURE CONDITIONS ON BRANCHING DEGREE IN *Aspergillus niger* MYA 135
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Introduction. Filamentous fungi grow by hyphal extension and branching through processes that are regulated in a way that is still not completely understood. Many genes and physiological mechanisms are involved in a process of morphogenesis. In this work, we quantified hyphal morphology of *Aspergillus niger* MYA 135 obtained under different culture conditions. Methods. The effect of modifications in the environmental conditions on hyphal morphology was tested by changing either the temperature of incubation or the initial pH of the medium as well as by the addition of 0.5 g/l $CaCl_2$ or 0.1 g/l $FeCl_3$. The morphological parameter, hyphal growth unit length (L_{hgu}), defined as the total hyphal length (main hypha plus branches) divided by the number of tips, was measured. The morphology observed when growing the strain at 30°C in the basic fermentation medium was considered to be the control. Results and conclusions. The influence of either CaCl2 (39±3 µm) or initial pH 3 (39±2 µm) as well as incubation temperature of 40°C (39±3 µm) on branching degree was almost the same. Comparing with the reference cultivation (60±5 µm) there was a higher level of branching, according for the decrease of L_{hgu}. An increase of L_{hgu} value owing to either the addition of $FeCl_3$ (70±3 µm) or the initial pH 7 (68±4 µm) was also observed. Our findings show an induction of a given morphological growth pattern by permissible modification of a culture's environmental conditions.
This work was supported by grant 693/04 CONICET.

MI-P86.
EXPRESSION OF CAPSID POLYPEPTIDE cDNA OF THE *Bc*V1 VIRUS FROM *Botrytis cinerea* CCg378 IN *Escherichia coli* AND *Saccharomyces cerevisiae*
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The fungal strain *Botrytis cinerea* CCg378 is infected by a mycovirus named *Bc*V1, which has as genome two dsRNA molecules, dsRNA-1 and dsRNA-2. Bioinformatic analysis showed that both segments have only one open reading frame (ORF). The ORF from segment dsRNA-2 (2219 bp) would encode for a polypeptide of 70 kDa. This polypeptide sequence showed high similarity with the capsid proteins from viruses of *Fusarium poae* (*Fp*V) and *Atkinsonella hypoxylon* (*Ah*V). In order to verify the bioinformatic predictions and to determine if the assembly of the viral capsid depend of host factors, the cDNA obtained from dsRNA-2 was cloned in the bacterial and yeast expression vectors, pET-21d(+) and pYES2, respectively. Neither protein fractions of induced cultures from *E. coli* BL21(DE3) nor *E. coli* JM109(DE3) showed the presence of an overexpressed polypeptide of 70 kDa. In a similar way, induced cultures of *S. cerevisiae* 3443 did not overexpress a polypeptide of 70 kDa. The lack of overexpression of the expected polypeptide could be due to the formation of secondary structures in the 5' end of transcripts obtained from the recombinant vectors, the presence of rare codons for *E. coli* in the ORF of the cloned segment, the action of the yeast proteases or other possibilities which will be further analyzed and discussed in this work.

MI-P87.
AMPLIFICATION BY RT-PCR OF CYTOCHROME P-450 GENE SEGMENTS FROM ALKANE-GROWN *Beauveria bassiana*
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Entomopathogenic fungi are able to degrade insect cuticular hydrocarbons and to utilize them for energy production and incorporation into cellular components. The first oxidation round is presumably carried out by a cytochrome P-450 monooxigenase, in analogy to yeast systems. In order to detect the expression of this enzyme in alkane-grown *Beauveria bassiana*, degenerate primers were designed using the CODEHOP (consensus-degenerate hybrid oligonucleotide primer) algorithm. The Blockmaker program was employed to generate similar amino acids blocks of fifteen selected P-450 alkane hydroxilase proteins (P-450alk) of the yeast *Candida tropicalis, C. apicola, C. maltosa*, and *Yarrowia lipolytica*. Three candidate primer pairs were selected to use in RT-PCR experiments, and two cDNA segments of 474 and 547 bp were amplified, cloned in a pGEM T Easy vector, and sequenced. After alignments of these segments in the LALIGN program, a high percentage of identity with eukariotic cytochrome P-450´s was found. This is the first report on a putative P-450alk of filamentous fungi. Further studies in order to obtain the full sequence and to analyze the differential expression of these genes will be addressed.

MI-P88.
CHARACTERIZATION OF THE ACYL-COA OXIDASE IN *Beauveria bassiana*
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The entomophatogenic fungi *Beauveria bassiana* has the ability to metabolize hydrocarbons, characteristic components of the external lipid layer of the insect cuticle. Alkane-growth induction enhances fungal ability to kill its insect host. After the initial oxidation steps [hydrocarbon 6alcohol 6very long chain fatty acid (VLCFA)], the first enzyme involved in VLCFA oxidation in peroxisomes is the acyl-CoA oxidase (ACO). ACO activity was measured by spectrophotometry in the P_{20000g} fraction of glucose-grown (FS_0) and *n*-alkane grown cultures (FS_{alk}) employing acyl-CoAs of 18 to 24 carbons as substrates. A significant increment in the activity was observed in FS_{alk} as compared to that of controls (FS_0). Tetracosane-grown cultures showed the highest activity with lignoceroyl CoA. The reaction conditions were optimized employing lignoceroyl-CoA as substrate. A variable lag phase was observed when the activity was measured as a function of time. In the presence of 3- amino-1,2,4-triazole (AT), to prevent H_2O_2 consumption by endogenous catalase, the lag phase became shorter and disappeared when AT concentratios was raised from 40 to 200 mM, thus enhancing acyl-CoA oxidation. Enzyme activity was maximal on the presence of 240 ug peroxidase, 0.08 % Triton X-100 and 36 µM BSA. The apparent Km for lignoceroy CoA was 2.5 µM, the pH optimum was 7. The enzyme was fully stable after 2- h preincubation at pH 7-10.

MI-P89.
THIOREDOXIN REDUCTASE FROM *Entamoeba histolytica* HK9. MOLECULAR CLONING AND FUNCTIONAL CHARACTERIZATION

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Entamoeba histolytica, an intestinal protozoan, is the causative agent of amoebiasis. The parasite usually lives and multiplies within the human gut, under reduced oxygen. During tissue invasion, *E. histolytica* is exposed to elevated amounts (highly toxic) of reactive oxygen species (ROS). Pathways for ROS detoxification in this organism is controversial. It has been proposed cystein as a main intracellular thiol and one of the compounds responsible for the maintenance of redox balance. In this work we present the cloning of the gene (*trxr*) coding for thioredoxin reductase (TRXR), from *E. histolytica* genomic DNA. The gene was cloned into the vector pRSET-A and the construction obtained was used to transform competent cells of *Escherichia coli* BL21(DE3). The recombinant protein was purified and functionally characterized by its ability to catalyze the NADPH ($S_{0.5}$= 1.3 µM; n_H= 3.7) dependent reduction of both, 5,5´-dithiobis-(2-nitrobenzoic) acid (DTNB) ($S_{0.5}$= 1.4 mM; n_H= 1.2), and TRX from *E. coli* ($S_{0.5}$= 5.6 mM; n_H= 1.6). Our results support the occurrence of a TRXR/TRX system in *E. histolytica*, as a principal component of the parasite redox metabolism.
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MI-P90.
EFFECT OF POLYAMINE BIOSYNTHESIS INHIBITORS ON GROWTH AND PATHOGENESIS OF *Ustilago maydis*

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Polyamines (putrescine, spermidine and spermine) are ubiquitous polycationic compounds that have demonstrated to be essential for growth and development of all organisms. Inhibitors of its biosynthesis have largely been tested on its capability to prevent fungal plant diseases. In this work, we evaluated the effect of well-known polyamine biosynthesis inhibitors on growth, differentiation and pathogenesis of *Ustilago maydis*, the causal agent of the corn smut disease. This fungus shows a dimorphic transition during its life cycle, that is, grows saprophytically in a yeast-like form but become filamentous when two compatible cells fuse at plant surface, a process that implies conjugative tube formation. All the inhibitors demonstrated to affect vegetative growth as well as pH-induced dimorphic transition. Interestingly, the inhibitors also inhibited conjugative tube formation but mating of compatible cells and mycelial formation was not necessarily prevented. Finally, application of inhibitors before inoculation of *Arabidopsis thaliana* seedlings demonstrated to favor plant infection, suggesting an effect of inhibitors on plant polyamine metabolism. Posible implications and potential use of inhibitors to prevent fungal plant diseases are discussed.

MI-P91.
METALLOCARBOXYPEPTIDASES (MCPs) OF *Trypanosoma cruzi*: EXPRESSION AND CHARACTERIZATION

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The genome of *Trypanosoma cruzi*, the causative agent of Chagas Disease, encodes two MCPs of the M32 family, with 64% of identity between them: TcCP-1 and TcCP-2. These enzymes belong to a new family of peptidases whose members had been found so far exclusively in prokaryotes. This makes them possible candidates as targets for chemotherapy. Both full length *TcCP-1* and *TcCP-2* genes were expressed in *E. coli* as catalytically active polyhistidine-tagged recombinant enzymes. Despite their homology, purified TcCPs displayed marked differences. TcCP-1 acted optimally at pH 6,2 on furylacryloyl(FA)-Ala-Lys with a *Km* of 166 µM. Activity against N-carbobenzoxy-Ala-X (ZAX) substrates reveled a P1' preference for basic and some neutral C-terminal residues. Contrary, TcCP-2 preferred aromatic and alifatic residues at this position. The *Km* value for FA-Phe-Phe at pH 7,6 was 24 µM. Therefore, the specificity of both MCPs are complementary.
Western blot analysis also revealed a different pattern of expression for both enzymes. Whereas TcCP-1 is present in all life cycle stages of *T. cruzi*, TcCP-2 is mainly expressed in the insect vector ones. Indirect immunofluorescence staining suggested that both proteins are localized in the parasite cytosol.

MI-P92.
IDENTIFICATION OF QUINONE OXIDOREDUCTASE ACTIVITIES PRESENT IN *Trypanosoma cruzi*

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The cytotoxic effects that several lipophilic *o*-naphtoquinones such as β-lapachone present, were attributed to the formation of "reactive oxygen species"(ROS) by the one or two electron reduction of the quinone to semiquinone or hydroquinone respectively catalyzed by the "quinone oxidoreductases" (QOR). We have shown the presence in epimastigotes of *T.cruzi* of several such activities followed by the reduction of 9,10 phenanthrenquinone in the presence of either NADH or NADPH. In some cases these activities were associated to well known enzymes as trypanothione reductase and the "old yellow enzyme". We have also purified a "hypothetical protein" annotated in the genomic data base as a member of the family of D-isomer specific 2-hydroxyacid dehydrogenases, not detected before in trypanosomatids, that presented a NADH dependent QOR activity; and a p52 protein identified as a thiol: disulphide oxidoreductase which exhibits a remarcable QOR activity with the o-naphtoquinones β-lapachone, mansonone F, compound CG 10248 and menadione, which induce oxidative stress, as substrate. We have also detected in the *T.cruzi* genome the presence of a gene encoding a protein with high homology with the ζ-crystallin, a protein with QOR activity which we have cloned and expressed.

MI-P93.
HISTONE ACETYLATION IN *Trypanosoma cruzi*
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Genetic expression in higher eukaryotes is controlled at the beginning of transcription and is greatly influenced by chromatin structure. Lysine acetylation is one of the major modifications on histones tails and has been shown to upregulate transcription.
We analyzed *In vivo* acetylation of *T. cruzi* histones by Western blot using specific antibodies against acetylated histones H3 or H4. In both cases positive signal was obtained suggesting the presence of this regulatory mechanism in *T. cruzi*. Searching in *T. cruzi* genome database (TIGR) we found two sequences corresponding to histone acetyltransferases belonging to the MYST family (TcHAT1 and TcHAT2) and two histone deacetylases (TcSir2 and TcHDAC). In addition an acetyltransferase involved in transcription elongation (ELP3) was found.
All sequences were amplified by PCR, subcloned in pET22b+ vector and expressed in bacteria. After purification, fusion proteins were evaluated for activity. Acetyltransferases from the MYST family show activity only when associated in a major complex but recombinant ELP3 was active as a monomer. Here we present the first evidence for epigenetic control in *Trypanosoma cruzi*.

MI-P94.
POLY(ADP-RIBOSYL)ATION IN TRYPANOSOMATIDS
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The poly(ADP-ribosyl)ation is a posttranslational modification were an ADP-ribose branched polymer is attached to proteins. This polymer is synthesized by the enzyme poly(ADP-ribose)polymerase (PARP) from the substrate NAD^+. Two members of the PARP family are activated upon DNA damage PARP-1 and PARP-2. Searching *Trypanosoma cruzi* genome databases we found an ORF encoding for a PARP-2 homologue, which was cloned and expressed in Codon Plus BL 21(DE3) pLys E cells by using pET-Tc-PARP recombinant vector. We performed Southern, Northern and Western studies which reported a single copy gene; an mRNA of about 1.8 kb and a 65 kDa protein. A nuclear and kinetoplastic localization in epimastigotes was ascertained by IFI. The *Tc*-PARP was purified and it´s activity was found to be dependent on nicked DNA concentration but didn´t exhibit metal ion requirement. *Tc*-PARP was inhibited by nicotinamide, 3 aminobenzamide, theophylline and thymidine, as well as by Mn^{2+}, Ni^{+}, Zn^{2+} and ATP. We demonstrated an attachment of poly(ADP-ribose) chains, synthesized by *Tc*-PARP, to it-self. The *Tc*-PARP activity decreased due to automodification, and it was recovered after removing ADP-ribose polymer from the protein by alkaline treatment, thus suggesting a negative regulation by automodification. We have also found the catabolic counterpart, poly(ADP-ribose)glycohydrolase (PARG) in *T. cruzi* genome databases, suggesting an analogy with the known poly(ADP-ribose) metabolism in higher eukaryotes.

MI-P95.
THE TRANSKETOLASE (TKT) FROM *Trypanosoma cruzi*. CLONING, EXPRESSION AND CHARACTERIZATION
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Trypanosoma cruzi causes Chagas disease. TKT is a key enzyme in the non-oxidative branch of the pentose phosphate pathway that transfers a two-carbon glycoaldehyde unit from xylulose 5-P to ribose 5-P or erythrose 4-P, generating glyceraldehyde 3-P and sedoheptulose 7-P or fructose 6-P. This enzyme plays critical roles in the provision of phosphorylated carbohydrate intermediates in the cell. The analysis of protein sequence revealed that the C-terminus of the protein contains a type-1 peroxisome targeting signal (VHL), suggesting a possible glycosomal subcellular localization. Digitonin extraction experiments showed that TKT was predominantly cytosolic, although a significant component of the total activity was particulate. TKT is active in all biological stages of the *T. cruzi* CL Brener clone. We have cloned a gen of 2019 bp encoding the TKT, 66,7 % identical to the *Leishmania mexicana* TKT, and expressed it in *E. coli* as an N-terminal His6-tagged protein. We have determined the optimum pH and kinetic parameters (Km and Vmax). Western Blot experiments using specific rabbit polyclonal antisera showed that TKT is expressed in the four major stages of the parasite (more in the insect stages). Indirect immunofluorescence (epimastigote stage) confirms the dual localization suggested by digitonin experiments.

MI-P96.
TRANSIENT EXPRESSION OF THE NS5A PROTEIN OF HEPATITIS C VIRUS (HCV) IN HeLa CELLS
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Introduction: It is uncertain whether the HCV NS5A protein up- or down-regulates the expression of anti-apoptotic proteins, as well as if it plays any role in the cellular immortalization event.
Objective: To investigate the effect of transiently transfected HCV NS5A protein in the expression of cell cycle regulators.
Material and methods: pDEST12.2-NS5A recombinant vector was transfected in HeLa cells using Lipofectamine 2000. Appropriate controls were used. Cells were studied 24 hours after transfection. The expression of NS5A, c-Myc, cIAP1, cIAP2, Bcl-X_L / X_S, Tankyrase (up-regulator of telomerase) and Gankyrin was measured by *Western blot*. Primary specific mono- and polyclonal antibodies as well as secondary polyclonal antibodies were used throughout, and revealed by chemiluminescence using the highly sensitive ECL Plus system.
Results: The expression of NS5A was detected in transiently transfected cells. No differences were recorded between such cells and controls regarding the expression of cellular proteins involved in cell cycle regulation or immortalization.
Conclusions: HeLa cells transiently transfected with HCV NS5A protein do not show detectable changes in the level of expression of the studied proteins. Apoptotic stimuli are being investigated to further evaluate the participation of this protein in apoptosis regulation using a stable inducible expression system.

MI-P97.
GB VIRUS C (GBV-C) GENOMIC RNA IS PRESENT IN SPECIFIC LYMPHOCYTIC CELLS *IN VIVO*
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Introduction: GBV-C -also known as Hepatitis G virus- is a human virus whose replication cell target has not been identified.
Objective: To investigate GBV-C cell tropism in peripheral blood cells from a viremic blood donor.
Material and methods: Mononuclear and polymorphonuclear cells were obtained by standard procedures using Ficoll/Triyosom centrifugation and Dextran sedimentation. Monocytes were isolated by adherence to plastic and lymphoid subsets were purified using specific monoclonal antibodies and magnetic beads.
RNA was extracted from cellular pellets and 1 mg was seeded for RT-Nested PCR for both genomic [+] and anti-genomic [-] strands by using primers specific for the 5' NCR of the virus. Amplicons were analyzed by agarose gel electrophoresis and Southern hybridization with a ^{32}P-labeled internal oligoprobe.
Results: GBV-C genomic RNA was consistently detected in B cells but not in monocytes, CD4 [+] T cells, polymorphonuclear cells, nor in the last wash from any cell population. CD8 [+] T cells proved positive in 2 of 4 assays. The anti-genomic GBV-C RNA (replicative form) was undetectable in all cell populations using a highly strand-specific reverse transcriptase (*Tth* DNA pol).
Conclusions: GBV-C viral RNA is present in B and probably in CD8 [+] T cells *in vivo*, as shown for the first time in a natural infection.

MI-P98.
DEVELOPMENT OF MICROBIAL BIOSENSORS FOR BOD AND OTHER WATER-QUALITY RELATED PARAMETERS
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Environmental biosensors are a growing area of application, despite the still small participation on the worldwide biosensor market, where clinical glucose sensors explain close to 90% of the total sales. We developed microbial based biosensors, by coupling or immobilized a single microbial strain or a mixed community in close contact with two different transducers, a potentiometric carbon dioxide electrode or a thermistor. These two ways to following metabolic activity of the immobilized cells allow us the estimation of BOD5 in less than a half an hour; BOD calibration was made using two commonly used standards, a glucose-glutamic acid solution (GGA) and OECD solution. The biosensor was used in batch experiments or as detector in a FIA system, where the electrode was inserted in a house-made cell, in a typical set-up, by using a peristaltic pump and a low pressure 6 ways injector valve.
The use of lyophilized microbial material allows us high standardization of the microbial compositions and physiological behavior between different biosensor membranes; the correlation between our data (BODst, short term BOD) and the standard BOD (BOD5) was good when standard solutions are compared, but when real samples are measured the biosensor behavior was strongly dependent of waste-water composition.

MI-P99.
IDENTIFICATION OF *Streptococcus sp* AND *Enterococcus* SPECIES ISOLATED FROM BOVINE MASTITIS IN ARGENTINA BY RFLP
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Bovine mastitis is an infectious disease that affects both the quality and the quantity of milk production. Different studies carried out in Argentina by physiological and biochemical methods showed that *S. agalactiae, S. dysgalactiae, S. uberis, S. bovis* and *E. faecalis* species are frequently isolated from bovine mastitis. Molecular biological techniques have been proposed for the more accurate identification of the various species of genus *Streptococcus* and *Enterococcus*. The aim of this work was to identify to the species level 51 strains isolated from bovine mastitis by RFLP analysis of 16S DNAr. Twelve ATCC strains were used in this study. The strains were isolated from 6 herds located on the central-east region of Argentina. Our results showed that 35,3% of the strains were *S. dysgalactiae*, 19,6% *E. faecalis*, 13,7% *S. agalactiae*, 11,8% *S. uberis*, 9,8% *S. salivarius*, 5,9% *S. bovis*, 1,9% *S. equinus*, and 1,9% *A. viridans*. This PCR system could detect eight bacterial species from bovine mastitis.

MI-P100.
REGULATION OF CITRATE METABOLISM IN *Enterococcus faecalis* ATCC29212
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Citrate fermentation is an important industrial feature of Lactic Acid Bacteria. In addition, citrate metabolization has been described in pathogenic and opportunistic bacteria such *Klebsiella pneumoniae* and Enterococcus faecalis. The latter is involved in traditional raw milk cheese manufacture, contributing in the ripening process and in aroma development. Volatile compounds such as acetaldehyde, diacetyl, and acetoin, produced during cheese ripening as a consequence of metabolization of citrate, contribute to flavor.
In this report we characterized the expression profile of the *cit* locus and protein-DNA interactions involved in the locus regulation. Transcriptional analysis of the cit locus revealed two divergent operons, *citHO* and *oadDBcitCDEFXoadAcitMG*. The transcriptional initiation start sites of these two operons were mapped by primer extension. The transcriptional patterns show activation of both operons by citrate and strong catabolite repression in response to glucose addition to the growth medium. *In vitro* transcription and band shift experiments show that CitO, a member of the GntR family, is a new positive regulator involved in the regulation of the citrate fermentation in bacteria. In addition, we identified a *cre* site (catabolite response element) located in the promoter region of *citHO*, our results suggest that CcpA binds to the *cre* site diminishing the expression of the citrate transporter CitH and the transcriptional factor CitO.

MG-P1.

MOLECULAR CHARACTERIZATION OF THE SELENOCYSTEINE SYNTHESIS PATHWAY IN KINETOPLASTIDA

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The study of the translation processes attracts the interest of several groups due to its central role in the general metabolism of the cell, in particular the study of the amino acid selenocystein and the pirrolysine. The selenocystein amino acid represents the main biological form of the element selenium. The proteins involved in this pathway are: Selenocystein Sinthase (SELA), only described in eubacteria, Selenocystein Elongation Factor (SELB and EFSec), Selenophosphate Synthetase (SELD) a specific tRNA (tRNAsec - SELC) and a Selenocysteine Insertion Sequence (SECIS). In the attempt to identify to selenocysteine synthesis pathway in Kinetoplastidae we performed a search for SECIS-containing selenoproteins in *Trypanosoma brucei*. Two candidates were identified and are being characterized. SELB, SELD and tRNAsec (SELC) coding genes were identified from searches to the GENEDB (http://www.genedb.org/) database. To validate this candidate gene as participating in the selenocysteine synthesis and incorporation pathway as well as to determine the genes importance for the parasite metabolism we are undertaking their molecular characterization trough RNA interference experiments.
The SelC transcript has been characterized by cDNA synthesis using a specific oligonucleotide. The SelC gene has been cloned and sequenced to confirm its identity. The 1179 pb long SelD coding gene was PCR amplified from *Trypanosoma brucei* genomic DNA and cloned into the RNAi vector pZJM for transfection into *T. brucei* cells.

MG-P2.

MOLECULAR STUDIES OF SELENOPHOSPHATE SYNTHETASE (SEL D) OF *Leishmania major*

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The translation processes play a central role in the general metabolism of the cell. In particular the pathway of synthesis and incorporation of the amino acids selenocysteine and the pirrolysine, that results in the expansion of the genetic code to a total of 22 amino acids. Selenocysteine represents the main biological form of the element selenium incorporated into selenoproteins at an in phase termination codon (UGA). In *Escherichia coli* the main proteins involved are: Selenocysteine Synthase (SelA), Specialized Elongation Factor (SelB or EFSec), Selenophosphate Synthetase (SelD), a specific tRNA (tRNAsec - SelC) and a Selenocysteine Insertion Sequence (SECIS). The protein SelD, subject of this work, catalyses the formation of selenophosphate from selenite and ATP. Recent studies have verified the presence of the gene *selD* in *Leishamania* consists of 1197 base pairs that code for a protein of 399 amino acids with a molecular mass of 43 kDa. The *L. major* selD gene was PCR amplified and cloned into the expression vector pET28a. An effective expression and purification protocol were established for the recombinant protein. The recombinant SelD protein was characterized by Dynamic Scattering of Light (DLS) and native gel electrophoresis to determine its oligomeration state. This two independent techniques revealed a molecular mass of 84,3 kDa and 87 kDa, respectively, consistent with a homodimeric protein. Screening of crystallization conditions is under way.

MG-P3.

HAIRPIN AND HAMMERHEAD RIBOZYMES DIMINISH THE ACTIVITY OF MITOCHONDRIAL ALDEHYDE DEHYDROGENASE (ALDH2) IN RAT HEPATOMA CELLS: TOWARDS GENE THERAPY FOR ALCOHOLISM

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Aversion therapy for alcoholism may be achieved by reducing the activity of mitochondrial aldehyde dehydrogenase (ALDH2), an alcohol detoxifying enzyme. Disulfiram, an effective but very toxic drug, is clinically used to inactivate ALDH2. Alternatively, gene therapy strategies may prove useful to silence the *ALDH2* gene. Ribozymes are capable of diminishing protein synthesis by occupation and/or degradation of mRNA. Hairpin and hammerhead ribozymes directed to the same target within rat ALDH2 mRNA were tested in rat hepatoma cells in culture.
H4?II-E-C3 cells were lipofected with plasmids carrying ribozyme genes driven by a CMV promoter. The ALDH2 activity in cell extracts was measured spectrophotometrically. Antisense effects were assessed with control ribozyme genes.
The hairpin ribozyme provided 42% reduction of the ALDH2 activity, 18% being due to an antisense effect, while the hammerhead ribozyme afforded a reduction of 20% fully accountable by antisense effects. Thus, the hairpin ribozyme directed to nts 1553-1571 of the rat ALDH2 mRNA is a good candidate for *in vivo* experiments geared towards developing genetic drugs for alcoholism.
Beca U. de Chile PG06504/ Iniciativa Cientifica Milenio P99-031F/ FONDECYT 1040555.

MG-P4.

TRANSCRIPTIONAL REGULATION OF StarD7 GENE PROMOTER

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We have previously reported the cloning and characterization of a novel gene up-regulated in the choriocarcinoma JEG-3 cell line. This gene, denominated StarD7, encodes a protein that belongs to the StAR-related lipid transfer proteins involved in intracellular lipid transport pathways. In addition, we demonstrated that purified overexpressed StarD7 protein interacts differentially with phospholipid monolayers. To initiate studies of the *cis*- and *trans*-acting factors that are important for transcriptional regulation of StarD7 gene expression, we isolated the human StarD7 gene promoter from genomic DNA by PCR amplification. Transcriptional activity of several 5´-flanking regions of the StarD7 promoter driving the expression of the luciferase reporter gen was analyzed in Cos-7 and JEG-3 cells by transient transfection assays. Promoter activity was observed in different constructs containing from nt +157 up to nt -1525 of the genomic StarD7 sequence. The 5´regulatory region containing from nt -121 to nt -673 activated StarD7 and SV40 promoter transcription in JEG-3 cells. Instead, the construct nt -1525 to nt -673 repressed both promoters. Taken together, these data suggest that transcriptional positive and negative regulatory factors present in JEG-3 cells could specifically regulate StarD7 promoter by interacting with the regions previously mentioned.
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MG-P5.
CHARACTERIZATION OF THE PROOPIOMELANOCORTIN (*POMCa*) PROMOTER OF THE TELEOST FISH *TETRAODON NIGROVIRIDIS*

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The proopiomelanocortin prohormone (*POMC*) is produced mainly in the pituitary gland and brain, giving rise to several bioactive peptides including ACTH, α-MSH and β-endorphin. We recently reported that teleosts possess two paralogue *POMC* genes, called *POMCα* and *POMCβ*. The genome of the pufferfish *Tetraodon nigroviridis* is compressed 8-fold in relation to that of mammals, making it an attractive model to study regulatory elements. To characterize the Tetraodon *POMCα* promoter, we generated transgenic mice harbouring 900 bp of the promoter upstream of the *lacZ* gene. We observed that the Tetraodon promoter is able to drive transgene expression to melanotrophs and - in adrenalectomized mice- corticotrophs of the pituitary of transgenic mice. Experiments in corticotrophic AtT20 cells showed that the Tetraodon promoter is induced by the hormone CRH and the transcription factors Tpit and Pitx1, similarly to mammalian *POMC* promoters. Phylogenetic footprinting revealed a 20-bp sequence highly conserved between different species which, when deleted, led to a reduction of near 60% in promoter activity in AtT20 cells but not in CHO cells. Thus, the regulatory elements and transcription factors needed for *POMC* pituitary expression have been remarkably conserved in the hundreds of millions of years that separate teleost fishes and mammals.

MG-P6.
MUTATIONAL SCREENING OF APC GENE IN CHILEAN FAMILIES WITH FAMILIAL ADENOMATOUS POLYPOSIS

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Familial Adenomatous Polyposis (FAP) is a hereditary variant of colorectal cancer clinically well-defined. FAP is characterized by polyposis in the colon and variable extracolonic manifestation. The major genetic factor for FAP families is the presence of mutations in the APC gene, a tumor suppressor gene. Until now, no genetic studies have characterized patients with this disease in Chile. For this reason we proposed to study the presence of germline mutation in the APC gene in FAP families. Fourteen families affected by FAP were selected. All exons and intron-exon boundaries of the gene were analyzed using combination technique of Single Strand Conformation Polymorphism, Protein Truncation Test and sequencing. We found changes in twelve of the fourteen families. These mutations were: c.3632_3633insA in two families, c.3927_3931delAAAGA in two families, and c.790C>T, c.2486_2487delCA, c.3498T>G, c.3597_3598insA, c.3783_3784delTT, c.3916G>T, c.3920T>A (I1307K), c.3941_3942delG, all in one family. Nine of the ten mutations lead to premature translation termination. Of all changes identified six constitute novel germline mutation. This is the first mutational report of Chilean FAP patients. The six novel mutations contribute to the world genetic database.
FONDECYT 1040827.

MG-P7.
Y-CHROMOSOME STR GENOTYPING OF TWO SAMPLES FROM AMERINDIAN AND ADMIXED CHILEAN POPULATIONS

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Chile was colonized with men coming from two regions of Spain: Castilla and Andalucìa. The mixture with native women fixed the mtDNA haplotypes so, the genetic maternal lineage imprint of Amerindians is present in today's population. However, only a 32 % of Amerindian Y chromosome haplotypes have been identified in the same population using molecular markers other than STR. So, with the purpose to obtain the allelic frequency of 8 Y-STR (DYS19, DYS389_I, DYS389_II, DYS390, DYS391, DYS392, DYS393 and DYS385) in two Chilean populations: 54 men from Santiago, and 71 Aymaras from Arica were screened. Total DNA was prepared from peripheral blood lymphocytes and amplified by PCR with 8 STR specific primers followed by high resolution polyacrilamide gel electrophoresis and silver stained. Allelic and haplotype frequencies were determined by simple counting. Gene diversity (H) and exact test for population differentiation were calculated with the program TFGPA. There are 53 haplotypes seen once in the population of Santiago, and 69 individual haplotypes seen in the Aymara sample. The discrimination power of each locus was 98 % for Santiago and 97 % for Aymara. There is significant genetic differentiation between the two populations ($p<0.0001$) based on the diversity index 0.0989. For the locus DYS385 in Santiago, 26 haplotypes were observed. The genotype 11,14 was the most common, (11,1%), followed by genotype 12,14 (9.3%). In the Aymara population, 27 haplotypes were observed. The most common genotype was 15, 20 (14,1%) followed by 14,18 (12.7%). This is the first analysis done in Chile with the minimal haplotype of STR loci in two important cities.

MG-P8.
INVOLVEMENT OF THE ATM PROTEIN KINASE IN FAMILIAL BREAST CANCER: MUTATIONAL SCREENING

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The major genetic factor for high risk of breast cancer in families is the presence of mutations in the BRCA1 or the BRCA2 gene. In a previous study in 54 Chilean families, we found that only 20% carried mutations in one of these genes. It has been proposed that other low penetrance genes, like ATM and CHEK2, both components of the ATM-dependent signaling pathway, as BRCA1 and BRCA2 may explain part of the hereditary predisposition to breast cancer. For ATM, it has been shown that female heterozygous carriers for mutations in this gene have an increased risk of breast cancer. We performed a screening of the ATM gene in 42 non BRCA1 or BRCA2 patients from our previous study. We found 6 heterozygous missense mutations: D1853N, D1853V, F858L, F582L, D758Y, S707P. The D1853N variant allele was significantly associated with an increased risk of developing breast cancer (allele D vs allele N OR 2.8, 95% CI 3.52 2.22, $P<0.05$). The two missense mutations D1853V and F582L were found in 1/42 and 3/42 patients respectively but not in 75 controls. The missense mutation S707P has been associated to breast cancer in other populations. These results provide evidence that the variants D1853N, D1853V, F582L and S707P might be associated with increased familial breast cancer risk. It has been proposed that these aminoacid changes may affect association of ATM with other proteins acting in the same metabolic pathway, or turning ATM into a dominant negative function.
FONDECYT 1040779.

MG-P9.

GENETIC VARIANTS OF SEROTONIN TRANSPOR-TER AND 5-HT1B RECEPTOR GENES IN AYMARAS, HULLICHES AND CHILEAN ADMIXED POPULATION

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Substantial evidence suggest that dysfunction of brain serotonergic system is involved in the pathogenesis of many complex psychiatric diseases (PD). Accordingly, genes involved in serotonin biosynthesis, catabolism, and response, are strong candidates for a role in the genetic etiology of affective illness. The goal of this study is to document the range of allele frequency variation for genes important in human behavior, including serotonin transporter (5HTT) and receptor 5HT1B genes in Chilean admixed population (N=105), Aymaras (N=132) and Huilliches (N=90). Polymorphisms at these loci have not been previously studied in Chilean population. Serotonin transporter promoter polymorphism, SERTPR, was genotyped using PCR and 5HT1B G861C polymorphism was genotyped by PCR-RFLP, digesting with the restriction enzyme Hinc II. The genotype distributions were in accordance to Hardy-Weinberg equilibrium. Frequencies of SERTPR-s and 5HT1B 861C alleles, the ones associated to depression, are augmented in the three groups analyzed when they are compared to Caucasians. These differences may be related to the high rates of depression observed in Chilean population. Knowledge of this variation can be important for study design and data interpretation when individuals from various populations are research subjects.

MG-P10.

GENOME VARIATION INDUCED BY A CHANGE OF THE PLOIDY LEVEL IN PASPALUM NOTATUM

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Genetic and epigenetic changes accompany allopolyploid formation in several plant species. Similar changes have not yet been examined in autopolyploids. The aim of this work was to investigate the existence of early variation in genome sequence and methylation status taking place after autopolyploidization in *P. notatum*. The genetic structures of a diploid genotype and its autotetralploid derivative were compared by RAPD and AFLP markers, revealing ~9.5% of polymorphisms. The frequency of lost and gained bands suggest that sequence loss and/or insertion of repetitive sequences might be major forces of genome variation. Genomic Southern blots with/without methylation-sensitive restriction enzymes confirmed that at least some of the polymorphic loci were repetitive. Neither epigenetic modifications nor sequence elimination were observed between lines.

MG-P11.

ADMINISTRATION OF AN ADENOVIRUS EXPRESSING E7 FUSED TO CALRETICULIN INDUCES REGRESSION OF HPV16-ASSOCIATED TUMORS

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Human papillomavirus (HPV) infection has been established as causal agent of cervical cancer. Vaccines targeting the oncogenic proteins E6 and E7 of HPV-16 and 18 are the focus of current vaccines development. Calreticulin (CRT), a Ca2+-binding protein located in the endoplasmic reticulum (ER), associates with peptides delivered into the ER by transporters involved in antigen processing and with MHC class I-ß2 microglobulin molecules to aid in antigen presentation. Previous studies have shown that exogenous administration of CRT complexed with peptides in vitro can elicit a peptide-specific CD8+ T cell response. In this study a replication-deficient adenovirus expressing CRT/E7 (Ad-CRE/E7) was constructed to enhance the immunological efficiency of HPV-16 E7 antigen against TC-1 tumor, an animal model of cervical cancer. Cells infected with the Ad-CRT/E7 were shown to produce and address the protein CRT-E7 to the ER. The antitumor effect induced by administration of Ad-CRT/E7 was compared against an adenovirus expressing E7. Measurements of tumor size indicate that indeed calreticulin-E7 plays a significant role to protect mice against tumor development than what resulted from E7 alone.

PL-P1.

USE OF TRANSFORMED TOBACCO PLANTS WITH ENDOGENOUSLY ALTERED POLYAMINE POOLS, FOR STUDYING THE REGULATION OF ARBUSCULAR MYCORRHIZAL INFECTION

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A number of regulatory mechanisms of plant defence response have been described during the establishment of the arbuscular mycorrhizal (AM) In this work, we tested an experimental design including the use of transformed tobacco plants with endogenously altered polyamine pools, for studying the putative role of these polycations on the regulation of AM infection. Transgenic tobacco plants containing the oat arginine decarboxylase (ADC) gene under the control of a tetracycline-inducible promoter, mycorrhized or not with *Glomus intraradices* were used. Tetracycline induction decreased free spermine levels in non-mycorrhized plants transformed with the ADC gene, and increased putrescine pools in mycorrhized plants transformed with the ADC gene. There were no differences in mycorrhization parameters between control plants induced or not with tetracycline, whereas transformed plants, showed slightly, higher levels of mycorrhizal root length, entry points and vesicles due to tetracycline induction. However the absence of any significant correlation between shoot polyamine levels and mycorrhizal parameters suggests that these levels may be not directly involved in the regulation of fungal growth within the roots. Results are discussed regarding the improvement of experimental design.

PL-P2.

CHARACTERIZATION OF TRANSGENIC PLANTS WITH DIFFERENT LEVELS OF FERREDOXIN

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Ferredoxins (Fd) are a group of low molecular-weight proteins which have Fe-S centres. Plant-type Fds, that have one 2Fe-2S cluster per molecule, acts as a soluble electronic transporters between Photosystem I and ferredoxin-NADPH reductase (FNR) during the light phase of photosynthesis, to generate NADPH needed for CO_2 fixation. Moreover, there are other Fd-depedent enzymes, in which the reducing equivalents are utilized for nitrogen and sulphur assimilation and for nitrogen fixation. We have developed transgenic plants with different levels of ferredoxin. Plants with reduced levels of this protein were obtained using *antisense* technology and silencing using RNAsi. These plants showed variegated distribution of chlorophyll in the leaf, reduced CO_2 fixation rates and lower tolerance to moderate oxidative conditions. Arrested growth, pale green leaves and incapability to generate offspring were observed in seedlings with extreme phenotypes generated by RNAsi silencing. On the other side, plants with higher than physiological levels of ferredoxin did not show striking differences when were compared with wild-type ones. All these results allow us to suggest that a small reduction in the levels of Fd would lead to drastic alterations in the growth and development of plants.

PL-P3.

STUDY OF THE 1α,25(OH)$_2$D$_3$ BIOSYNTHETIC ROUTE IN *SOLANUM GLAUCOPHYLLUM*

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Solanum glaucophyllum produces 1α,25(OH)$_2$D$_3$ and related vitamin D$_3$ metabolites. Sterol biosynthesis in Angiosperms occurs trough cyclization of 2,3-oxidosqualene which renders cycloartenol. Thereafter, introduction of an alkyl group takes place at carbon 24 which is absent in vitamin D$_3$. *Sterol C24-methyltransferase 1 (SMT1)* is involved in this substitution and generates a branch in steroid metabolism, one pathway yielding 24-alkyl substituted sterols whereas the other route presumably mediates the synthesis of vitamin D$_3$. Our work focuses in elucidating the metabolic pathway implicated in 1α,25(OH)$_2$D$_3$ biosynthesis in *S. glaucophyllum*. Down-regulation of SMT-1 activity by incubation of cultured *S.g.* cells with sitosterol diverted carbon flux into 1α,25(OH)$_2$D$_3$ formation. Supplementation of medium with either 7-dehydrocholesterol or vitamin D$_3$ also increased 1α,25(OH)$_2$D$_3$ levels. Studies on the tissue and subcellular distribution of 25(OH)D$_3$-1α-hydroxylase activity, using [^{3}H]25OHD$_3$ as substrate and chromatographic isolation of the product, indicated that the enzyme is mainly localized in leaves, associated to mitochondria. Kinetic characterization of the 25(OH)D$_3$-1α-hydroxylase revealed normal Michaelis-Menten behaviour, with a Km of 11.4 ± 2,4 μM respect to 25(OH)D$_3$ and a Vmax of 1976 ± 89.72 pg/min.

PL-P4.

CHARACTERIZATION OF PHOSPHOENOLPYRUVATE CARBOXYLASE IN SPECIES OF FAMILY CHENOPODIACEAE HAVING SINGLE-CELL C4 PHOTOSYNTHESIS

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Recently, the paradigm that Kranz anatomy is required for functioning of C_4 photosynthesis in terrestrial plants has been broken. It was shown that *Bienertia sinuspersici* and *Borsczowia aralocaspica* (Chenopodiaceae) perform photosynthesis through unique single cell systems and they have C_4/CAM carbon isotope composition. Phosphoenolpyruvate carboxylase (PEPC) catalyses the first committed step of C_4 photosynthesis. Although this ubiquitous enzyme is also present in low levels in C_3 species, the form involved in the C_4 cycle has distinct kinetic and regulatory properties. The goal of this work was to characterize the PEPC present in both single-cell species and to compare with those present in other Chenopodiaceae C_3 and C_4 species.
In both species, PEPC is a tetramer of four subunits each with a molecular mass of about 100 kDa. In addition, native isoelectrofocusing and western blot using specific antibodies shown that the enzyme is phosphorylated during the day, as occurs in Kranz C_4-type PEPCs. This regulatory phosphorylation is responsible for changes in the kinetic parameters and sensitivity to malate during the day and night periods in agreement with a C_4-type PEPC. Thus, analysis of the kinetic and regulatory properties of PEPC in *B. sinusperisci* and *B. aralocaspica* supports the conclusion that C_4 photosynthesis is taking place in these species.

PL-P5.

BIOCHEMICAL CHARACTERIZATION OF STARCH SYNTHASE III FROM *ARABIDOPSIS THALIANA*

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Glycogen and starch are the major energy storage compounds in all living organisms. The metabolic pathways leading to their synthesis involve the action of several enzymes, among which glycogen (GS) or starch synthases (SS) catalyze the elongation of the alpha-1,4-glucans in each polymer. At least the existence of five isoforms was described in *Arabidopsis thaliana*; it has been reported that one of that isoforms (SSIII) has a regulatory function in the synthesis of transient starch. We carried out the cloning, expression in *E. coli* and characterization of the recombinant full length SSIII and a truncated form (named SSIII catalytic domain, SSIIICD) from *A. thaliana*. Kinetic analysis *in vitro* showed that both proteins were active and suggested a possible regulatory role of the N-terminal region. Fold class assignment methods and homology modeling resulted in a strong global similarity with *A. tumefaciens* GS, with ADP-binding residues fully conserved; remarkably, some ligand binding residues showed a significant evolutionary divergence. Indeed, we found that SSIIICD could complement an *A. tumefaciens* null mutant lacking GS, suggesting that the truncated isoform, like bacterial GS, could initiate glycogen synthesis *in vivo*.

PL-P6.

A NOVEL γ-CARBONIC ANDHYDRASE FROM *Arabidopsis thaliana* INVOLVED IN RETROGRADE REGULATION

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A novel group of 5 γ-Carbonic anhydrases (AtγCAs)has been recently identified in *Arabidopsis thaliana*. These proteins are targeted to mitochondria, and they are specifically located bounded mainly to the Complex I (NADH-ubiquinone oxidorreductase) of the mitochondrial electron transport chain. Proteomic studies of *atγca2* and *atγca3* plants revealed that complex I levels are diminished about 80% and 10%, respectively. Cell cultures show lower growth and respiration rates. Phenotipically, mutants plants are indistinguishable from wild type plants. Interestingly, AtγCA2 present several transcription factor features and is highly expressed in flowers.

In this work, we study mRNA levels of several nuclear genes encoding mitochondrial proteins by RT-PCR in different backgrounds (mutant, double mutant and AtγCA2 overexpressing plants). Results suggest that AtγCA2 could regulate gene expression of nuclear complex I genes mainly in flowers. Hence, the lower levels of complex I could be attributed to the specific function of AtγCA2 as a transcription factor involved in retrograde regulation.

PL-P7.

***SATIVAIN*, A PROTEOLYTIC ENZYME FROM GARLIC BULBS, SHOWS HOMOLOGY WITH PLANT LECTINS**

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We described previously the presence of a protein with cysteine-like peptidase behavior from tissue culture of garlic (*allium sativum*) and from bulbs extracts of white garlic (*Allium sativum* var. Inco 283). The enzyme, named *sativain*, was then purified from bulb tissues and characterized by anion-exchange and gel filtration chromatography followed by affinity chromatography on Concanavalin A resin. Homogeneity of the protein was confirmed by SDS-PAGE and isoelectrofocusing. An isoelectric point of 4.6 and a molecular mass of 26 kDa were found for the dimeric enzyme. The N-terminal sequence of sativain was performed and after comparison with the N-terminus of other 30 plant cysteine peptidases no similarities were found with them, while a great deal of sequence homology, 100 and 70% respectively, to ASA I (*Allium sativum* agglutinin I) and with a lectin fragment of *Allium scalonium* was observed. In addition, its sequence showed, between others, 50% similarities with cDNA-deduced internal fragment sequences from *Gossipium hirsutum* cysteine peptidase, egg cystatin, putative cysteine peptidase from *Oryza sativa*. Our results indicate that the proteolytic enzyme from garlic may be related to plant lectins. The possibility that garlic lectin possesses proteolytic activity in addition to its agglutinin function is underway.

PL-P8.

CHLOROPHYLL DEGRADATION IN *Arabidopsis thaliana*: DETECTION AND CHARACTERIZATION OF Mg-DECHELATASE ACTIVITY

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The enzymic release of Mg^{2+} from chlorophyllide, in the catabolic pathway of chlorophyll, has been demonstrated in higher plants as well as in algae, but detailed knowledge about the properties of Mg-dechelatase is scarce. MDS activity was detected in *Arabidopsis thaliana* leaves. Enzyme activity was determined by measuring the increase in absorbance at 686 nm, the wavelength at which the Mg^{2+} release is detected. The enzyme showed an apparent Km of 150 nM. The optimal temperature was found at 55°C and the optimal pH around 7.0. The activity decreased considerably after heat treatment (100°C, 10 minutes), although certain heat stability was detected. Variations in MDS activity during dark induced senescence in detached leaves were also determined. Activity increased constantly until reaching a maximum in day 4, after which it began decreasing. Purification through selective precipitation with ammonium sulfate was performed and the 40-90% fraction showed a considerably higher MDS activity than the crude extract.

PL-P9.

METABOLIC CHANGES INDUCED BY HIGH NADP-ME ACTIVITY IN *ARABIDOPSIS THALIANA* CHLOROLASTS

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Arabidopsis thaliana (Columbia-0 ecotype) was transformed with a vector containing the maize chloroplastic NADP-malic enzyme (NADP-ME) cDNA under the control of 35SCaMV promoter. This enzyme catalyzes the oxidative decarboxylation of malate, generating pyruvate, CO_2 and NADPH. The line with the highest expression level showed a 30-fold increase in NADP-ME activity. When grown under different light intensities, morphological differences between wild type and transgenic lines were evident. Chlorophyll content as well as photosynthetic performance indicators like Fv/Fm and ΦPSII were significantly lower for transgenic lines. CO_2 assimilation rates measured at increasing light intensities were also different in transgenic lines in comparison with wild type. On the other hand, sugar levels fluctuation during the day-night cycle also varied in plants expressing maize NADP-ME. These results suggest that the presence of a highly active NADP-ME in chloroplasts of *A thaliana*, could have important effects on the reductive power balance of this organelle, diminishing the efficiency of the overall photosynthetic process and affecting carbohydrate metabolism. These transgenic plants are a useful tool to better understand the regulation of plastid redox poise and related metabolisms.

PL-P10.
KINETIC AND STRUCTURAL CHARACTERIZATION OF NADP-MALIC ENZYME FAMILY FROM *ARABIDOPSIS THALIANA*

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The *A. thaliana* genome contains four genes encoding putative NADP-malic enzymes (ME1-4). This enzyme catalyzes the oxidative decarboxylation of L-malate to form pyruvate, CO_2 and NADPH. ME4 is localized to plastids, whereas the other three do not possess any organellar targeting sequence. The cDNAs of each isoform were expressed in *E. coli* and the purified recombinant proteins used for kinetic and structural studies. All the *A. thaliana* isozymes showed NADP-ME activity, but with significant differences in kcat values, affinities for both substrates and pH optimum suggesting different biological roles for each protein. On the other hand, ME4 exists in equilibrium of active dimers and tetramers, while the cytosolic counterparts are present as hexamers or octamers. All of them showed different native isoelectric points with the same pI under denaturing conditions, probably due to a change in the net charge in the native enzymes due to oligomerization. Unexpectedly, ME3 y ME4 exhibited inhibition by malate a pH 7, a particular feature of the enzyme in C_4 metabolism, as well as a fast reverse reaction (pyruvate carboxylation). The recombinant proteins were also active with NAD, although the specific activities were lower than the values obtained in the presence of NADP. In addition, the response of the purified enzymes to several key compounds involved in distinct metabolisms was also tested suggesting that some members of the AtNADP-ME family may be subject to metabolite control *in vivo*.

PL-P11.
IDENTIFICATION OF DOMAINS IMPLICATED IN TETRAMERIZATION AND MALATE INHIBITION OF MAIZE C_4 NADP-MALIC ENZYME BY ANALYSIS OF CHIMERICAL PROTEINS

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C_4 photosynthetic NADP-malic enzyme has evolved from non-C_4 isoforms during evolution and gained unique kinetic and structural properties. In order to identify the domains responsible for the structural and kinetic differences between maize C_4- and non-C_4 NADP-ME several reciprocal enzyme chimeras between these isoforms were constructed and analysed. By this approach, a region between aminoacids 102 and 247 was found to be implicated in the oligomerization state, being responsible for the tetrameric state of the C_4 NADP-ME. In this way, the strategy for oligomerization of these NADP-ME isoforms differs markedly from the one that is present in non-plant NADP-ME crystallized until present. On the other hand, the region from aminoacid 248 to the carboxyl terminal of the protein was implicated in the inhibition by high malate concentrations at pH 7.0 of the C_4 isoform. The inhibition pattern of the C_4-NADP-ME and some of the chimeras suggested an allosteric site responsible for such inhibition. Based on structural analysis, two sites are proposed as potential targets for malate allosteric binding. In this way, this inhibition may be important for the regulation of the C_4 isoform *in vivo*; being fully active when photosynthesis is in progress.

PL-P12.
STRUCTURAL AND KINETIC CHARACTERIZATION OF A PHOSPHOENOLPYRUVATE CARBOXYLASE (PEPC) FROM CITRUS FRUIT

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The increase in PEPC activity and protein level in frost-exposed Valencia orange fruit (*Citrus sinensis* L. Osbeck) has been reported before. In this work, the kinetic and structural properties of PEPC were analyzed in both control and frost-damaged fruit. In common with most other plant PEPCs, the enzyme showed lower K_m(PEP) at pH 8 than pH 7, being these values approximately 50% lower in frozen fruits respect to controls. The enzyme was modulated by several metabolites and the effects were generally more pronounced at pH 7 than 8. At pH 7, control fruit PEPC was activated by glucose-6-phosphate and potently inhibited by malate (with a low I_{50} of 8 µM), aspartate, glutamate and succinate. In contrast, frost-damaged fruit PEPC showed much lower sensitivity to these effectors with a pronounced increase in I_{50} for malate (125-fold). Citrus fruit PEPC appears to be an oligomeric complex with a mass estimated in 500-600 kDa, composed of 110-kDa subunits and unusual 74-Da polypeptides that were observed both in immunoblots of denaturing crude extracts and silver stained SDS-pages of PEPC activity bands excised from non-denaturing gels. These results suggest that citrus fruit PEPC is different from common plant PEPCs in its structural properties, while the different sensitivity to malate could indicate changes in the phosphorylation state in control and frost-damaged fruit PEPC.

PL-P13.
PROTEIN BEHAVIOR STUDIES DURING GRAIN WHEAT DEVELOPMENT

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The gluten proteins of wheat flour - monomeric and polymeric proteins - are recognised as the most important components governing bread-making quality. The ratio of SDS-soluble and SDS-insoluble proteins in mature grain is a useful criteria to evaluate the final use of flour. The aim of this work is to analyse the influence of environment and genotype on this ratio and follow protein accumulation rates during grain filling. Protein accumulation was measured by size exclusion HPLC and evaluated by multistacking SDS-PAGE at different stages of grain development for two wheat varieties. Preliminary results showed that, for both varieties, the accumulation protein rate varied between four main groups observed, indicating differential regulation of protein biosynthesis. The synthesis of albumins/globulins remain constant during the grain filling, confirming that these are metabolic or structural proteins. The gliadins and SDS-soluble polymeric proteins have different behavior during grain filling depending on genotype. The fourth group observed, mainly SDS-soluble and less than 15Kda, represent the higher percentage observed in early stages and then dramatically decreased. On the other hand, the protein composition analysed by electrophoresis allowed to follow the polymerisation rate of glutenin subunits.

PL-P14.

CHARACTERIZATION AND PARTIAL CLONING OF ARGINASE FROM *Pleurotus ostreatus*

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Arginase (EC 3.5.3.1) catalyzes the hydrolysis of arginine to ornitine and urea. The enzyme is widely distributed in living organisms and plays several important biological functions, including urea genesis and regulation of cellular arginine levels. At present, the best characterized are the mammalian and some bacterial and yeast arginases. However, there is no information about the enzyme from the fungi species *Pleurotus*, which we are characterizing in view of their potential use for waste degradation and as a nutritional source of proteins. In this study, the arginase activity was found to depend on the stage of development of *P. ostreatus*; significant arginase activity was detected in carpophores, but not in mycelia. The enzyme was purified and kinetically characterized. The specific activity was 1,8 μmoles of urea/mg protein/min and the *Km* for arginine was 4,7 mM. The enzyme was activated by low concentrations of Mn^{2+} y Ni^{2+} and completely inactivated for Zn^{2+}. The inhibitory actions of products and substrate analogs was also examined. By sequence analysis and RT-PCR experiments using total RNA of *P. ostreatus* carpophores, we have amplified a fragment of 650 base pairs, which most probably correspond to the arginase gene.

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PL-P15.

PURIFICATION AND CHARACTERIZATION OF AN ELASTASE KUNITZ-TYPE INHIBITOR FROM *CAESALPINIA ECHINATA* (PAU-BRASIL) SEEDS

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Proteolytic enzymes, involved in several physiological processes, are maintained under tight control, mainly by inactivation by inhibitors. In plants, some serine proteinase inhibitors are particularly abundant and used as tools in studies of animal enzymes. *Caesalpinia echinata* (pau-brasil) belongs to the Leguminosae family from where we have already identified human plasma kallikrein and cathepsin B inhibitors. The aim of this study was to purify and characterize an elastase inhibitor isolated from *Caesalpinia echinata* seeds. An initial saline extraction of ground seeds and acetone precipitation were followed by protein purification using an ion-exchange chromatography, a gel filtration and a reversed phase chromatography. All purification steps were followed by electrophoresis in polyacrilamide gel (SDS-PAGE). The inhibitory activity was evaluated on chromogenic substrate hydrolysis by elastase. Protein N-terminal sequence was determined by Edman degradation. The one chain aminoacid inhibitor (23 kDa) presented $Ki = 24 \times 10^{-9}$ M for elastase and the N-terminal sequence indicated that the inhibitor belongs to the Kunitz family.

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PL-P16.

20S PROTEASOME AND PROTEIN OXIDATION IN MAIZE (*Zea mays* L.) LEAVES TREATED WITH CADMIUM

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Cadmium produces carbonylation of proteins. In order to test the possible involvement of the 20S proteasome on the degradation of oxidised proteins, we investigated in maize (*Zea mays* L.) leaf segments the effect of different cadmium concentration on activities, protein abundance and oxidation of the 20S proteasome. We also investigated the accumulation of carbonylated and ubiquitinated proteins. With a low Cd concentration (50 μM $CdCl_2$), abundance of 20S proteasome protein remained similar to control, while trypsin- and PGPH-like activities were significantly increased. This increments in 20S proteasome activities were probably caused by the enhanced in 20S proteasome oxidation and was responsible for the degradation of oxidised proteins. When leaf segments were treated with higher cadmium concentration (100 and 200 μM $CdCl_2$), 20S proteasome activity and protein abundance decreased, with a concomitant accumulation of carbonylated and ubiquitinated proteins. These results suggest that the 20S proteasome could be involved in secondary antioxidative defences, causing degradation of oxidised proteins in plant cells under oxidative stress generated by cadmium.

PL-P17.

SUNFLOWER DEVELOPMENT IS ALTERED BY A LONG-TERM CADMIUM AND COPPER TREATMENT

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In the last years, human activities have contributed to the environmental pollution by allowing the uncontrolled increment of waste products in the atmosphere, soil and water. Cadmium is not essential for plant growth and it is phytotoxic even at very low concentrations and copper is an essential element for normal plant growth but toxic for plants at high concentrations.

The aim of this work was to evaluate the effect of different concentrations (0.1, 0.5 and 1 mM) of Cd and Cu on sunflower growth and development during 16 days. Metal accumulation, shoot and root growth, relative water content (RWC), chlorophyll content, proline content and alterations in leaf morphology were evaluated. Sunflower roots accumulated a significantly great amount of metals than shoots. The magnitude of Cd accumulation in sunflower roots and shoots was significantly higher than that of Cu. However, a different pattern of accumulation was observed between both metals. Cadmium and copper at the higher concentrations produced an inhibition of shoot and root growth and a reduction of RWC. However, sunflower plants treated with 1 mM Cd were capable of preserving the pigment level over the controls up to day 10, when chlorophyll started to decrease, but still maintaining a higher level than that observed with 0.1 or 0.5 mM Cd. This result could be explained by the different cell organization of parenchymas and the greater number of chloroplasts observed on histological sections of cotyledons (10 days) and leaves (16 days) of Cd 1mM treated plants.

PL-P18.
LIGHT-DEPENDENCE OF CADMIUM AND PARAQUAT EFFECT ON CATALASE IN SUNFLOWER DISCS

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Catalase (CAT, EC 1.11.1.6) is involved in detoxification of H_2O_2 generated by environmental stresses, as well as by physiological conditions. In sunflower (*Helianthus annuus* L.), 8 isoforms of CAT, products of 4 different genes, were identified: *CATA1* and *CATA2* code for matrix CATs, whereas *CATA3* and *CATA4* code for core CATs. The effect of different oxidative conditions, generated by Cd^{2+}, H_2O_2 and paraquat (PQ), on CAT transcripts, isoforms and activity were studied. CAT activity decreased 30% respect to control, after 8 h of 300 µM $CdCl_2$ treatment under light conditions and this effect was avoided when 10 mM $MnCl_2$ was supplied with Cd^{2+} in the incubation medium. Native PAGE staining revealed no differences in CAT isoenzymes. The transcript analysis of *CATA3* showed an increase of 2.9 times over the control. In order to compare Cd^{2+} stress with other oxidative stress conditions, PQ or H_2O_2 were assayed. PQ increased *CATA3* expression 2.2 times (over the control) after 2 h of incubation under light. However, no changes were observed when H_2O_2.was used. The qualitative pattern of carbonylated proteins showed that Cd^{2+} increased oxidation of proteins with molecular weight higher than 45 kDa. Cd^{2+} and PQ induced ROS formation in the chloroplasts, evidenced with 2´,7´-dichlorofluorescein diacetate, and this effect was reverted by ascorbate. Considering the results detailed above, we can conclude that light is necessary for Cd^{2+} and PQ effect on *CATA3* transcript. Neverthless, the decrease observed on CAT activity was due to a an oxidative modification produced by the metal on the CAT protein.

PL-P19.
NADPH OXIDASE INVOLVEMENT IN Cd-INDUCED OXIDATIVE STRESS IN SUNFLOWER LEAF DISCS

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A rapid generation of reactive oxygen species (ROS), mainly superoxide anion ($O_2^{\cdot-}$) and H_2O_2 are considered to be a component of the intracellular response to biotic and abiotic stress. The likely source of ROS is a NADPH dependent oxidase. The objective of this work was to elucidate which type of ROS mediates the signaling pathways involved in cadmium toxicity in sunflower leaf discs.treated with 0.5 mM $CdCl_2$ as well as the role of a plant NADPH oxidase in ROS formation. *In vivo* superoxide anion formation was analyzed using NBT. H_2O_2 evolution was studied with DAB and xylenol orange. NADPH-dependent oxidase activity was measured using the SOD-inhibited reduction of NBT.
NADPH-dependent oxidase activity was inhibited by 0.5 mM Cd in the cytosolic fraction (to 27% of the C). This decay was neither reversed by $LaCl_3$ nor by $CaCl_2$, while EGTA showed a variable behavior. The addition of DPI, a NADPH dependent oxidase inhibitor, to the reaction medium decreased oxidase activity by over 60% whereas NaN3 (a haem protein inhibitor) had no significant effect on superoxide generation. Hydrogen peroxide formation was inhibited by Cd 0.5 mM by 40% respect to the controls and this inhibition was reverted by both $LaCl_3$ and $CaCl_2$ EGTA did not avoid Cd-induced enzyme inhibition. Our result suggest that superoxide and H_2O_2 generation in sunflower leaf discs is regulated in Cd-treated sunflower leaf discs by the inhibition of NADPH-dependent oxidase activity and the role of calcium in ROS formation seemed to be related to H_2O_2 evolution.

PL-P20.
GLUTAMINE AND GLUTAMATE SYNTHETASE ARE MODIFIED BY OXIDATION IN NODULES OF SOYBEAN PLANTS TREATED WITH CADMIUM

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Cadmium causes oxidative damage and hence affects nitrogen assimilation. In the present work we tested the relationship between the inactivation of the enzymes involved in nitrogen assimilation pathway (glutamine synthetase (GS)/ glutamate synthase (GOGAT)) and the protein oxidation in nodules of soybean (*Glycine max* L.) plants under Cd^{2+} stress. Therefore, the effect of Cd^{2+} and reduced gluthatione (GSH) on GS and GOGAT activities, and protein abundance and oxidation were analyzed. Under the metal treatment, aminoacids oxidative modification occurred, evidenced by the accumulation of carbonylated proteins, especially those of high molecular weight. When Cd^{2+} was present in the nutrient solution, although a decrease in GS and GOGAT activities was observed (17% and 52%, respectively, compared to controls), the protein abundance of both enzymes remained similar to control nodules. When GSH was added together with Cd^{2+} in the nutrient medium, it protected the nodule against cadmium induced oxidative damage, maintaining GS and GOGAT activities close to control values. These results allows us to conclude that the inactivation of the nitrogen assimilation pathway by cadmium in soybean nodules is due to an increment in GS and GOGAT oxidation that can be prevented by the soluble antioxidant GSH.

PL-P21.
HEME OXYGENASE ACTIVITY AND SIGNALING IN SOYBEAN PLANTS

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We have previously demonstrated that the induction of heme oxygenase-1 plays a protective role for soybean plants against oxidative stress. Here, we have investigated for the first time the possible signal transduction pathways involved in heme oxygenase-1 induction in leaves of soybean plants. Treatment with 200 µM Cd during 48 h increased 87% thiobarbituric acid reactive substances, whereas GSH decreased 70%, respect to controls. These effects were prevented by preincubation with diphenyleneiodonium (DPI, NADPH oxidase inhibitor), cantharidin (protein phosphatases inhibitor), LaCl3 (calcium channel blocker) and [1,2,4]oxadiazolo-[4,3-a]quinoxalin-1-one (ODQ, guanylate cyclase inhibitor). 200 mM Cd produced increased concentrations and in situ accumulation of H_2O_2 and $O_2^{\cdot-}$, which were again prevented by DPI, cantharidin, $LaCl_3$ and ODQ. Moreover, Cd- induced heme oxygenase-1 activity was also totally or partially abolished by pretreatment with the different inhibitors. These results clearly demonstrated that the signal transduction pathways involved in oxidative stress, triggered by cadmium ions were similar to those implicated in heme oxygenase-1 induction, and supported the proposal of a close relationship between oxidative stress generation and heme oxygenase induction in higher plants.

PL-P22.

CADMIUM TOLERANCE IN TRANSGENIC *ARABIDOPSIS THALIANA* OVEREXPRESSING *BRASSICA NAPUS* THIOREDOXIN *F*

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Thioredoxins (Trx) are ubiquitous small proteins (ca. 12 kDa) involved in the regulation of the thiol-disulphide status of target enzymes. In higher plants chloroplasts, two types of Trx (*m* and *f*) would participate not only in the modulation of chloroplast enzymes but also in the response to oxidative stress. Heavy metals are among the highly toxic compounds for cells whose action would rely on mechanisms of oxidative stress. In this context, the Cd regulation of Trx gene expression and the inhibition of Trxs activities by Cd was reported in *Chlamydomonas reinhartii* and *E. coli* but there was little information about the relationship bettween Trxs and heavy metals in higher plants. To analyze this alternative, we constructed, via *Agrobacterium* transformation, transgenic *Arabidopsis thaliana* plants overexpressing *Brassica napus* Trx-*f* and subsequently evaluated the action of Cd over germination, initial and mature growth. No differences were found between normal and transgenic seeds in the percentage of germination. At variance, the rate of radicule growth in transgenic lines was much higher than in the wild type counterpart. Notably, mature plants of the former (50d) accumulated in green tissues higher levels of Cd that the latter upon exposition for 5d to 1mM Cd in the hydroponic solution. The response of mature transgenic lines overexpressing Trx-f was phenotypically different from the normal counterpart. Although the underlying mechanisms are still unknown, these results are the first experimental indication of a link between the leaf Trx system and the oxidative stress elicited by heavy metals.

PL-P23.

ALUMINIUM INCREASES THE EXPRESSION OF *Arabidopsis thaliana* GENES CODING FOR CHLOROPLAST THIOREDOXINS

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Thioredoxins (Trx) are small multifunctional redox active proteins widely if not universally distributed among living organisms. In higher plants, two types of chloroplast isoforms, Trx-*f* and Trx-*m*, are functional in the regulation of processes associated to the Benson-Calvin cycle for photosynthetic CO_2 assimilation. Recent evidence support the idea that Trxs also participate in cellular mechanisms for circumventing the deletereous effects of stress conditions. Therefore, in the present study with *Arabidopsis thaliana*, we analized the effects of toxic levels of Al on the expression of genes coding for Trx-*f* and Trx-*m*. By means of semi-quantitave RT-PCR we observed that, in plants grown under hydroponic conditions, the higher the aluminium in the external solution the higher the expression levels of both the *AtTRXm* and *AtTRXf* genes. In parallel, we (i) estimated the uptake of Al by Atomic Absorption Spectrophotometry and (ii) controlled the physiological status of plants via the chlorophyll content.. Our results suggest that chloroplast Trxs play an important role in the tolerance to aluminium toxicity in *A.thaliana*. If so, the action of Trxs could be similar to sulfhydryl-bearing proteins that complex toxic metals, e.g. phytochelatins.

PL-P24.

CLONING OF TWO PUTATIVE POLYGALACTURONASE GENES AND ANALYSIS OF THEIR EXPRESSION DURING RIPENING OF STRAWBERRY CULTIVARS WITH CONTRASTING FRUIT FIRMNESS

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During fruit ripening, cell wall polymers suffer modifications as a consequence of the coordinated action of a range of enzymes. Polygalacturonases (PGs) are hydrolases involved in pectin degradation during ripening of fleshy fruits, as strawberry.In order to obtain the full length sequence corresponding to partial PG clones previously isolated from strawberry fruits, a RACE reaction was performed with the BD Smart™ RACE cDNA Amplification Kit (Clontech). In the present work, we report the cloning of two putative polygalacturonase full length genes: *FaPG* and *PGX8*, from strawberry fruits of Camarosa cultivar. *PgX8* has a deletion of 85 bp and a possible frame shift that would produce an inactive protein. By semiquantitative RT-PCR we have analyzed the expression pattern of both genes on three strawberry cultivars with contrasting fruit firmness. Our results show that PGs expression is different in each cultivar. The expression of *PGX8* is higher in the firm than in the soft cultivars, while the opposite occurs in the case of *FaPG*.

PL-P25.

HORMONE INFLUENCE ON THE EXPRESSION OF STRAWBERRY EXPANSIN GENES

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Since strawberry is a non climateric fruit, ethylene is not the major hormone in the control of the ripening process. It is thought that the decline in auxin production towards the end of the growing stages marks the onset of ripening in strawberry. Other hormones such as ABA and giberellins have been detected in straberry fruits and they could have an influence in strawberry ripening. Expansins are cell wall proteins which are able to increase cell wall elongation rate and have been related to the process of fruit softening. Hormonal regulation of these proteins in strawberry had not been studied until now, except for FaEXP2 whose expression level in fruit was not influenced by ethylene or auxin treatment. In order to characterize the possible hormone influence in the expression of expansin genes, we analyzed the efect of ethylene, NAA, GA3, ABA and deachened fruits (achenes are the source of endogenous auxin) in the expression of a set of expansin genes in strawberry (*FaExp1, FaExp2, FaExp4, FaExp5* and *FaExp6*).

PL-P26.

α-ARABINOFURANOSIDASE (α-ARA) IN STRAWBERRY FRUIT: ENZYME ACTIVITY AND EXPRESSION OF RELATED GENES IN CULTIVARS WITH CONTRASTING FIRMNESS

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Fruit softening is accompanied by cell wall modification leading to solubilization and depolimerization of its components. Cell wall disassembly is mediated by several proteins. Arabinose, is one of the most abundant neutral sugar in strawberry and it is also the one showing the largest decrease during ripening. α-ARA catalyzes the hydrolysis of arabinose residues present in pectin and hemicellulose side chains. In this work, we report for the first time the presence of α-ARA in strawberry and the cloning of three putative α-ARA encoding genes. We analysed α-ARA activity during ripening of strawberry cultivars with contrasting fruit firmess. The softest cultivar showed higher activity levels and the activity increased throughout ripening. A partial purified sample was analyzed in a Sephacryl S100 column and a peak corresponding to 70 KDa was found. We screened a ripe fruit cDNA library using degenerate primers and found three putative α-ARA full length clones. Semi quantitative RT-PCRs were carried out to investigate the expression of these genes during fruit ripening and in different tissues.

PL-P27.

PATHWAYS OF GLUTAMATE PRODUCTION IN TOMATO FRUITS RIPENING

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In tomato fruits (*Solanum lycopersicum*) the content of free glutamate (Glu) increases markedly during ripening, in all the varieties analysed up to now, becoming the most abundant free amino acid. Sources of Glu in tomato fruits could be the phloem system or the *in-situ* enzymatic production. Glutamine synthetase and Glu synthase decreased with the ripening process while Glu dehydrogenase (GDH) is induced. However, the increment in GDH activity cannot explain the rise in fruit Glu content. To explore the possible sources of Glu, we used tomato fruits of cv Micro-Tom, a dwarf variety. We followed enzyme activities of proteases as well as Glu transpeptidases. We observed mark increases in the proteolityc activity during fruit ripening using azo-casein as substrate. However, when using endogenous proteins as substrate the highest protease activity was observed in green fruit. A rise in the (-glutamyl transpeptidase activity was also noted in red fruits. Other sources of Glu will be also discussed, including the contribution of the GABA shunt enzymes. Results indicate that the Glu increase during ripening could be the consequence of the induction of multiple enzymatic activities of the fruit pericarp, and the endogenous substrates are currently being investigated.

PL-P28.

CLONING AND EXPRESSION OF A ß-XYLOSIDASE (*FaXyl1*) FROM *FRAGARIA X ANANASSA*

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Strawberry is a non-climateric fleshy fruit, which softens quickly and has short post-harvest life. The process is associated with an increment of pectin solubility and a reduction of the content of hemicelluloses. In this work, we cloned the full-lenght cDNA encoding a putative ß-xylosidase (*FaXyl1*) from *Fragaria X ananassa*. The analysis of the predicted protein showed that *FaXyl1* is closely related to other ß-xylosidases from higher plants. Product of *FaXyl1* contains a predicted signal peptide and seems to be targeted to the extracellular matrix. The presence of several potential N-glycosylation sites in the amino acid sequence is in accordance with the extracellular location. Recombinant protein over-expressed in *E. coli* showed ß-xylosidase activity against the artificial substrate p-nitrophenyl ß-D-xilopyranoside. As other ß-xylosidases, the enzyme obtained from strawberry, showed a relatively high thermal stability. Western blot analysis of the corresponding protein was performed in strawberry cultivars with different softening rates. Softest cultivar showed a higher FaXyl1 protein accumulation during ripening. A possible COOH-terminal processing of the primary translation product is discussed.

PL-P29.

INFLUENCE OF UV-C IRRADIATION ON EXPANSIN AND PECTIN-METHYLESTERASE GENE EXPRESSION IN STRAWBERRY FRUIT

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The exposure to UV-C delays fruit softening, one of the main factors determining fruit postharvest life. This delay in softening may be caused by changes in the activities of enzymes and proteins involved in cell wall disassembly. Expansins are cell wall proteins involved in fruit softening, while pectin-methylesterases (PME) are cell wall enzymes related to pectin demethylation, and their activity is affected by heating, another physical treatment based on abiotic stress. We analyzed *FaPME1* gene expression in irradiated strawberry fruits and also mesured PME activity after treatment. An increase in PME activity immediately after the treatment was found. The expression of *FaEXP2, FaEXP4* and *FaEXP5* genes was analyzed in the same fruit samples, as well as expansin protein acumulation. For these three genes we found a decrease in expression 4 h after treatment and an increase after 24 h. The decrease in gene expression after treatment correlated with a diminution of expansins.

PL-P30.
BENZYLAMINOPURINE (BAP) MODIFIES THE SOURCE-SINK RELATION IN WHEAT BY INHIBITION OF SUGAR AND AMINO ACIDS EXPORT TO PHLOEM

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Nitrogen (N) deficiency stimulates protein degradation and N remobilization from old to young leaves. When N deficiency was imposed to wheat plants (N-plants), there was a fast decrease in protein and amino acids and an increase in sugar concentrations in the third fully expanded leaf. In the phloem exudates there was a decrease in amino acids but not in sugar concentrations. After four days of N starvation, the expanding leaves showed a lower protein and chlorophyll content than in the N+ plants. Glutamine synthetase (GS1), but not GS2 and glutamate dehydrogenase (GDH) activities increased in the N-plants. However, when plants were supplied with 20 μM BAP, soluble proteins and sugars content increased in the third leaf, while soluble sugars and amino acids concentration decreased in the phloem exudates in both conditions. In expanding leaves a decrease in proteins and chlorophyll, but not in amino acids and sugars could be observed with or without N supply. BAP addition also increased GS1 but not GS2 and GDH activity. These results suggest that BAP affects the source/sink relationship in the plant by decreasing the amino acids and sugars phloem loading, and that GS1 is involved in amino acids cycling within the cell.

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PL-P31.
INTERACTION OF THE PLANT HOMEODOMAIN PROTEIN WUSCHEL WITH DNA

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Wuschel is an *Arabidopsis thaliana* transcription factor containing a compact homeodomain (HD), giving its own name to the family that it belongs to. This HD presents extra amino acid residues in two sites of the molecule, which may result in an atypical structure and have unpredictable effects on the interaction with DNA. Wuschel has been characterized as a key regulator of meristem differentiation, and apparently plays a critic role in the maintenance of embryonic cell identity. In this study, the HD of Wuschel was expressed as a fusion with GST from *Schistosoma japonicum* and purified by affinity chromatography. The binding sequence, CTCAG, has been determined by selecting binding sites from random-sequence oligonucleotides through several rounds of binding and amplification (SELEX). *In vitro* DNA binding, followed by electrophoretic mobility shift assays, indicated that this HD has a higher affinity for the SELEX resulting sequence than for the TAAT core. TAAT was previously proposed to be the target sequence of Wuschel in the second intron of the floral homeotic gene *AGAMOUS*, by analogy with typical HD binding sequences. Nevertheless, the sequence bound *in vitro* with highest affinity (CTCAG) is also present in the same intronic region, suggesting that an alternative interaction is actually taking place in the transcriptional regulation of the gene *AGAMOUS*.

PL-P32.
DETERMINATION OF THE DNA BINDING PROPERTIES OF HAT3.1, A PLANT PROTEIN WITH A NON-CANONICAL HOMEODOMAIN

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HAT3.1 is an *Arabidopsis* homeodomain protein characterized by an N-terminal domain known as the PHD-finger. The similarity between this motif and the RING-finger domain raises the possibility that HAT3.1 may be involved in either transcriptional activation, recombination or DNA repair.
The HAT3.1 homeodomain (HD) differs substantially from those in other HD proteins identified so far, even in positions which are almost invariant or highly conserved. In the present work, we demonstrate that the HAT3.1 HD is able to specifically bind DNA and identify its target sequence by the random binding site selection assay (SELEX). The consensus sequence identified after twelve rounds of binding and amplification differs markedly from those recognized by other HDs. The unusual DNA binding characterisitics of HAT3.1 may be explained by the particular amino acid composition of the HD. Accordingly, several site-directed mutants (H51N, H51A, K50Q and K50A) were constructed to study their DNA binding affinity and specificitty compared with the wild-type protein. In addition, footprinting and missing nucleoside experiments using hidroxyl radical cleavage of DNA were used to obtain a more detailed picture of the binding of the HAT3.1 HD to its target site.

PL-P33.
REDOX REGULATION AND DNA-BINDING SPECIFICITY OF THE CLASS III HD-ZIP PROTEIN ATHB-9

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The homeodomain (HD) is a 61-amino acid motif present in several eukaryotic transcription factors. It folds into a characteristic three-helix structure that binds DNA. HD-Zip proteins constitute a plant-specific HD family. Class III HD-Zip proteins present different HD architecture and binding properties than other HD-Zip proteins. In this work, we study the behavior of the class III HD-Zip domain from Athb-9, which was expressed as a fusion with GST from *Schistosoma japonicum* and purified by affinity chromatography. Since sequence analysis revealed the presence of conserved cysteines in the HD of all HD-Zip III proteins, we have tested the effect of oxidants and reductants on DNA binding and quaternary structure of Athb-9. Electrophoretic mobility shift assays and non-reducing polyacrylamide gel electrophoresis under different redox conditions indicated that the protein is activated in the presence of reductants and the formation of interchain disulfide bonds between adjacent monomers seems to be related with a decrease in DNA binding. The thioredoxin system was able to promote full *in vitro* activation of the protein, suggesting an *in vivo* regulatory mechanism. Finally, site-directed mutagenesis studies indicated that HD-Cys 54 would be important for the DNA-binding specificity displayed by Athb-9. A mutant with alanine in this position was able to bind DNA sequences not recognized by the wild-type protein. A model for the Athb-9/DNA interaction is proposed.

PL-P34.
MOLECULAR CHARACTERIZATION OF THE PROMOTER REGION OF *HAHB-4*, A MEMBER OF THE SUNFLOWER HD-ZIP FAMILY

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Regulation of gene expression in plants occurs through several mechanisms. Taking as model *Hahb-4*, a member of the sunflower HD-Zip I subfamily, we have informed in a previous work that the promoter region of this gene directs tissue/organ specific gus expression in *Arabidopsis thaliana* transgenic plants as well as shows induction by drought, NaCl or ABA stimuli. In this presentation we report that *Hahb-4* is regulated by dark/light conditions in sunflower at the transcriptional level. To gain insight into the segments/boxes responsible of each type of expression, we have obtained constructions with different deletions and point-mutations, as well as chimeras, including different segments of *Hahb-4* promoter. Analysis of the stable transgenic plants obtained allowed us to locate dark inducible responsible sequences between -319 and -419. Otherwise, sequences that direct expression in lateral root initiation are located downstream -419 bp, while the segments directing vascular central root expression are present between -1070 and -1131 bp
Together, the results indicate that regulation of *Hahb-4* expression can be associated to a cis element located around -350 bp in the case of dark and around -800 for root vascular cylinder. Conservation and divergence of some recognized cis elements in arabidopsis and sunflower will be presented and discussed.

PL-P35.
TRANSCRIPTIONAL NETWORKS REGULATED BY THE SUNFLOWER HAHB-4 TRANSCRIPTION FACTOR TO IMPROVE WATER STRESS TOLERANCE

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Hahb-4 is a member of the sunflower family of HD-Zip transcription factors. We have previously described that it is regulated by water availability and ABA, as well as the obtaining and characterization of *Arabidopsis thaliana* transgenic plants that overexpress this gene. Transgenic plants present a characteristic phenotype including a high drought tolerance.
In order to investigate the molecular mechanisms involved in the water stress tolerance conferred, we have performed microarray analysis using RNA obtained from overexpressing *Hahb-4* and wild-type plants under normal or water stress conditions. More than three thousand genes have changed their expression levels comparing the different samples. We have grouped them according to their behaviour. Possible direct targets have been confirmed by real-time RT-PCR. We have noted that otherwise a good number of senescence avoidance involved genes are induced a counterpart of chloroplast biogenesis involved ones are repressed. Accordingly, we have done physiological measurements supporting these molecular observations.
We can conclude that the observed water stress tolerance may be due to the observed changes in several processes including chloroplast biogenesis, senescence and oxidative stress response.

PL-P36.
CONTROL OF BIOLOGICAL PATHWAYS BY microRNAs IN PLANTS

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MicroRNAs (miRNAs) have been recently recognized as a novel mechanism to regulate gene expression. They are small RNAs of 18 to 21 nucleotides in length that recognize partially complementary sequences in target mRNAs and guide them to cleavage or translational arrest. They are encoded as long precursors with fold?back structures by small gene families. In plants, they have been implicated in many key processes such as development and stress responses.
Currently, we are characterizing several plant miRNAs. To reach this aim, the miRNAs were overexpressed in *Arabidopsis thaliana* by transformation with the corresponding precursor under the control of a strong constitutive promoter. Also, plants were transformed with target genes containing silent mutations that make them resistant to the miRNA. Finally, the macroscopical and molecular phenotypes of these transgenic plants are evaluated to get insights into the regulated biological pathways. The role of several miRNAs in plant development will be discussed.

PL-P37.
***HAHB-1*, A SUNFLOWER HD-ZIP TRANSCRIPTION FACTOR, NEGATIVELY REGULATES DEVELOPMENTAL RATE IN RESPONSE TO OSMOTIC STRESS**

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Sunflower *Hahb-1* has been isolated from a stem cDNA library. This 1195 bp cDNA encodes a 323 amino acid protein that belongs to subfamily I of HD-Zip transcription factors. It has been suggested, and experimentally demonstrated, that members of this family are involved in developmental processes related to environmental conditions. Northern analysis done with RNA isolated from sunflower tissues/organs indicate that this gene is primarily expressed in stems and this expression is induced by treatments with ethylene. Continuous ilumination also positively affects *Hahb-1* transcript levels. We have obtained transgenic *Arabidopsis thaliana* plants that express this gene under the control of the 35S CaMV promoter. Transformed plants have shorter roots and present a delay in developmental rate and finish the life cycle taller than wild type plants. Treatments with gibberellins or ethylene reverse this developmental rate delay. In addition, transgenic plants are more sensitive to osmotic stress than wild type ones. The observations done led us to propose that this gene is a negative developmental rate regulator in response to osmotic stress and it is involved in gibberellins and ethylene mediated signal transduction pathways.

PL-P38.

ANALYSIS OF THE PROMOTER REGIONS OF GENES ENCODING ARABIDOPSIS CYTOCHROME C OXIDASE SUBUNITS 6a AND 6b

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Cytochrome c oxidase (COX) is a multimeric complex composed of several subunits, three of them encoded by the mitochondrial genome and the rest encoded by the nuclear genome. In plants, there are at least 4 subunits encoded in the nuclear genome: COX5b, COX5c, COX6a and COX6b. In this work, we analyzed the expression patterns driven by the promoter regions of the Arabidopsis genes *COX6a, COX6b1, COX6b2* and *COX6b3*. To perform this analysis, we transformed plants with the gus gene under the control of the respective sequences and analyzed GUS enzymatic activity by histochemical assays. *COX6b1* promoter sequences directed GUS activity in cotyledon and leaf veins and hydathodes, trichomes, pollen grains, the stigma and in developing seeds. In *COX6b2* lines, expression was detected in roots, cotyledon and leaf veins, hypocotyl vascular bundle, and in the shoot apical meristem and pollen grains. Plant lines that contained *COX6b3* promoter sequences produced similar expression patterns than *COX6b2* lines. Notably, plants that contained the *COX6a* promoter/*gus* fusion, showed GUS activity only in pollen grains. The results indicate that, while all genes show expression in pollen, *COX6b* promoters have evolved to produce differential expression patterns in other parts of the plant.

PL-P39.

ANALYSIS OF THE *CYTC-2* PROMOTER FROM *ARABIDOPSIS THALIANA*

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Mitochondrial biogenesis involves the expression of genes located in the nucleus and within the organelle. To analyze the molecular mechanisms involved in the expression of plant respiratory chain components involved in cytochrome c-dependent respiration, we have characterized the promoter region of the *Arabidopsis cytc-2* (At4g10040) gene, one of the two genes encoding cytochrome c. Plants transformed with *cytc-2* promoter/gus fusions showed expression in vascular tissues of cotyledons, leaves, roots and hypocotyls, and also in anthers. Quantitative measurements in extracts prepared from different organs suggested that expression of *cytc-2* is higher in leaves than in flowers and roots. The analysis of progressive upstream deletions showed that a deletion down to nucleotide -256, that excludes two copies of the site II element (TGGGCC/T), produces a general decrease in GUS expression levels, while a further deletion down to nucleotide -140 completely abolished expression. The site II element was found to be essential for *cytc-1* gene expression, together with a *telo* box motif (AAACCCTA). A site-directed mutation in a G-box (CACGTG) element present at -172 of the *cytc-2* promoter produced a strong decrease in expression. These observations suggest that site II and G-box elements, among others, are implicated in *cytc-2* gene expression. Accordingly, *cytc* promoters would be composed of common and gene-specific regulatory elements that produce differential expression patterns.

PL-P40.

CHARACTERIZATION OF THE PROMOTER REGION OF A CYTOCHROME C GENE FROM SUNFLOWER

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Cytochorme c is a small nuclear encoded hemoprotein that participates in mitochondrial electron transport chain. In a previous work we have reported that at least two genes encoding this protein are present in the sunflower genome. Northern blot analysis and *in situ* hybridization have indicated that cyt c is primarily expressed in flowers and roots.

An 856 bp DNA fragment corresponding to cyt *c* promoter region has been isolated by inverse PCR and its sequence determined.

In silico analysis reveals putative binding sites for transcription factors and a Telo box motif. To gain insight into the segments/boxes responsible of its expression, we have cloned the entire and serial deletions of this cyt *c* promoter region fused to the *gus* reporter gene, and transformed *Arabidopsis thaliana* plants with them. Histochemical analysis of transgenic plants indicate that the whole fragment directs expression in anthers, root tips, nascent lateral roots and vascular tissue from hypocotyls and roots and includes a repressor binding site. Shorter fragments direct expression also in leaves vascular tissues, cotyledons and sepals while pollinic sacs staining disappeares.

The results indicate that tissue specific expression is directed by this promoter. We were able to detect regions responsible for this expression. Conservation and divergence between species of active cis elements will be presented and discussed.

PL-P41.

MOLECULAR CLONING OF *Stcdpk1* GENE

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In plants, calcium-dependent calmodulin-independent protein kinases (CDPKs) are key intermediates in calcium-mediated signalling, that couple changes in Ca^{2+} levels to a specific response. These enzymes have a unique structure consisting of an aminoterminal catalytic domain fused to a carboxyterminal calmodulin-like domain (CLD) with four EF hands Ca^{2+} binding sites, and require only micromolar concentrations of free Ca^{2+} for their activity (Harmon *et al*., 1986; Harper *et al*., 1991; Roberts and Harmon, 1992). During potato tuberization, dormancy and sprouting of potato tubers, StCDPK1 (GenBank Database accession numbers AF115406) is differentially expressed

In order to clone the *Stcdpk1* gene, a CLONTECH genomic library was screened using the full-length StCDPK1 cDNA as probe. One of the positive clones contained the 3' end of the gene. Based on this sequence and on StCDPK1 coding sequence a PCR approach was used to amplify the complete gene. Stcdpk1 spans a 6290 pb region from the ATG to the polyadenylation consensus. The gene comprises 8 exons (E1:715 bp; E2:144 bp; E3: 153 bp; E4:116 bp; E5:168 bp; E7: 128 bp; E7: 100 bp; E8:73 bp) and 7 introns (I1:918 bp; I2: 97 bp; I3: 2301 bp; I4:779 bp; I5: I197 bp; I6: 82 bp and I7:79 bp). E1, E2 and E3 encode the protein kinase domain while E4 encodes the junction region and the CLD is encoded by 4 small exons. Most CDPKs from Arabidopsis share this structure but with smaller intronic regions. Mapping of *Stcdpk1* gene in the potato genome is currently being performed by SSCP analysis.

PL-P42.
ISOLATION OF AN ACTIVE PISTIL COMPONENT RESPONSIBLE FOR LePRK2 DEPHOSPHORYLATION
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LePRK2 is a receptor kinase located at the plasma membrane of tomato pollen grains. In mature pollen, it forms a high molecular weight complex together with LePRK1 (another pollen receptor kinase) and other still unknown proteins. In this complex, LePRK2 is phosphorylated. When incubated with exudates from pistils, LePRK2 is specifically dephosphorylated and the high molecular weight complex is disrupted completely. Previously, we showed that the active pistil component responsible for these two phenomena has a molecular weight of 3-10 kDa, is heat stable, partially resistant to protease activity, resistant to acid treatments but not to base hydrolysis. We decided to isolate that pistil component using a combination of different chromatographic methods (cationic and anionic exchange resins and reverse phase chromatography) and solvent extractions (chloroform-methanol extraction and acetone precipitation). In the last step of our designed purification protocol, LePRK2 dephosphorylation activity coelutes with a single peak from anionic exchange chromatography (MonoQ) at ~100 mM ammonium bicarbonate. Further studies of ^{13}C-NMR and mass spectroscopy will be used to determine the structure and molecular weight of this component. For the understanding of LePRKs complex signal transduction during pollen tube growth, it will be essential to identify this pistil component.

PL-P43.
FUNCTIONAL ANALYSIS OF THE EXTRACELLULAR DOMAINS OF POLLEN RECEPTOR KINASES LePRK1 AND LePRK2 FROM TOMATO
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LePRK1 and LePRK2 are two pollen-specific receptor kinases from *Solanum lycopersicon* (tomato) that are localized at the plasma membrane of the pollen tube. Both LePRK1 and LePRK2 can be co-immunoprecipitated from pollen or when expressed together in yeast. The addition of tomato or tobacco style extracts specifically disrupts this interaction. We constructed three types of cytoplasmic deletion constructs for both LePRK1 and LePRK2. All of them lacked the extracellular N-terminal domain (ECD) and bore different portions of the cytoplasmic domains (CD). Each deletion construct of one receptor was properly co-expressed in yeast with the corresponding full-length construct of the other receptor. Results obtained by co-immunoprecipitation assays showed that the kinase domain of LePRK2 is necessary for the interaction with LePRK1. We also made two constructs that had the ECD and lacked totally or partially the CDs. Co-immunoprecipitation assays showed that the ECD of each receptor are sufficient for their interaction. Contrary to the interaction between full-length LePRK1 and LePRK2, tobacco style extracts did not disrupt the interaction between any of the ECD deletion constructs and the corresponding full-length. All these results suggest that the LePRK2 kinase domain is responsible for the interaction and lead us to propose that the ECDs, after binding to the style ligand, activate the CDs transducing the signal through complex dissociation.

PL-P44.
INVOLVEMENT OF AN HETEROTRIMERIC G PROTEIN IN CRYPTOCHROME MEDIATED RESPONSES DURING *ARABIDOPSIS* SEEDLING DEVELOPMENT
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Plants as immotile organisms are able to sense and interpret the light environment adjusting their growth and development, leading to sophisticated light-signaling networks. In *Arabidopsis thaliana* red light/far red light is perceived by five phytochromes (phyA through phyE), and blue light by two cryptochromes (cry1 and cry2). Arabidopsis has a single gene encoding for the alpha subunit of the heterotrimeric G protein (GPA). Pharmacological studies and experiments with transgenic Arabidopsis overexpressing GPA suggested that the heterotrimeric G protein is involved in red, far red and blue light regulation of Arabidopsis seedling development. However literature is controversial in this regard. Studies using *gpa* mutants do not support this idea at least for the red light/far red light signaling pathway. Here we combine a biochemical, molecular and genetic approach to analyzed this issue in detail using *phyA, phyB, cry1* and *cry2* single mutants. The *cry1* mutant showed significantly less GTP binding compared to the WT, even in darkness. GPA transcript levels detected by RT-PCR were normal in the *cry1* mutant precluding a transcriptional control. To study a translational regulation we are conducting western blots using a G protein antibody. Pull-down experiments, using *in vitro* synthesized proteins, are used to study interaction between CRY1 and GPA. This study will open new insights in light signal transduction mediated by heterotrimeric G proteins and will contribute to our understanding of plant cell signaling.

PL-P45.
DETECTION OF PHOSPHOLIPIDS IN APOPLASTIC FLUID OF SUNFLOWER SEEDS
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The apoplast of plants is an interconnected liquid layer surrounding the cells that transports water, micronutrients, amino acids, hormones, signaling molecules, RNA and proteins, serving as a conduct for the delivery of information containing macromolecules. Recent indirect evidences suggests the existence of putative lipid signals involved in long distance signaling upon stress. However, current knowledge on lipids molecules acting as signals is limited to intracellular events. The aim of this work was to determine the presence of phospholipids (PLs) potentially involved in signaling mechanisms in the seed apoplastic fluids. The extracellular fluid (EF) from sunflower seeds was obtained by vacuum-infiltration and centrifugation. Western blot analyses indicate the absence of the intracellular marker glyceraldehide-3P-deshydrogenase (GAPDH) in the EF, indicating no significant intracellular contamination. Lipid fraction from the EF was submitted to thin layer chromatography (TLC) analysis showing the presence of several lipid spots. PLs were further identified by electrospray ionization mass spectrometry (ESI-MS). The analysis pointed out that phosphatidylinositol (PI), phosphatidic acid (PA) and phosphatidylglycerol were the most abundant components. Lysophosphatidyl glycerol, lysophosphatidyl choline, lysophosphatidylethanolamine, phosphatidylcholine and phosphatidylethanolamine were also detected, but as minor components of seed EF. This pattern is clearly different from that obtained by ESI-MS of total seed extracts. The presence in apoplastic fluids of PLs such as PI and PA, which are known to participate in intracellular signaling events, suggest that they may also have a role as long-distance signals in plants. Experiments are in progress to confirm this hypothesis.

PL-P46.

WOUNDING INCREASES SALT TOLERANCE IN TOMATO PLANTS. EVIDENCE ON THE PARTICIPATION OF LeCDPK1 IN CROSS -TOLERANCE SIGNALING

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Cross-tolerance is the phenomenon by which a plant resistance to a stress results in resistance to another form of stress. In tomato plants, it has previously been shown that salt stress causes the activation of wound-related genes. However, very little is known about how different stresses interact with one another, and about which are the signaling components that interrelate the responses triggered by different stress types. In the present work we demonstrate that mechanical wounding increases salt stress tolerance in tomato plants (*Solanum lycopersicon*) through a mechanism that involves the signaling peptide systemin and the subsequent synthesis of JA. We also provide data indicating that calmodulin-like activities are necessary for the downstream signaling events that lead to cross-tolerance between wounding and salt stress. Finally, we gathered evidence supporting the hypothesis that LeCDPK1, a Ca^{2+}-dependent protein kinase from tomato previously described in our lab, participates in this cross-tolerance mechanism interrelating the signaling responses to wounding and salt stress.
Supported by CONICET, FONCyT and UBA.

PL-P47.

STRUCTURAL AND KINETIC CHARACTERIZATION OF A PHOSPHOENOLPYRUVATE CARBOXYLASE (PEPC) FROM CITRUS FRUIT

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The increase in PEPC activity and protein level in frost-exposed Valencia orange fruit (*Citrus sinensis* L. Osbeck) has been reported before. In this work, the kinetic and structural properties of PEPC were analyzed in both control and frost-damaged fruit. In common with most other plant PEPCs, the enzyme showed lower K_m(PEP) at pH 8 than pH 7, being these values approximately 50% lower in frozen fruits respect to controls. The enzyme was modulated by several metabolites and the effects were generally more pronounced at pH 7 than 8. At pH 7, control fruit PEPC was activated by glucose-6-phosphate and potently inhibited by malate (with a low I_{50} of 8 µM), aspartate, glutamate and succinate. In contrast, frost-damaged fruit PEPC showed much lower sensitivity to these effectors with a pronounced increase in I50 for malate (125-fold). Citrus fruit PEPC appears to be an oligomeric complex with a mass estimated in 500-600 kDa, composed of 110-kDa subunits and unusual 74-Da polypeptides that were observed both in immunoblots of denaturing crude extracts and silver stained SDS-pages of PEPC activity bands excised from non-denaturing gels. These results suggest that citrus fruit PEPC is different from common plant PEPCs in its structural properties, while the different sensitivity to malate could indicate changes in the phosphorylation state in control and frost-damaged fruit PEPC.

PL-P48.

ATMBF1 GENES MODULATE THE SEED THERMOTOLERANCE AND GERMINATION RESPONSES AGAINST OXIDATIVE STRESS CONDITIONS

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Multiprotein bridging factor 1 (MBF1) is a transcriptional co-activator that mediates transcriptional activation by bridging between an activator and the TATA-box binding protein. To better understand the roles of MBF1 genes during the response against oxidative stress and thermotolerance, we carried out the analysis of different *Arabidopsis thaliana* MBF1 mutant lines: T-DNA insertion mutants (SALK and Syngenta collections) and transgenic over-expressing lines for the three *At*MBF1 genes (a, b or c). Plants homozygous for the T-DNA insertion in *At*MBF1c and the triple mutant (*At*MBF1abc), were treated with methyl viologen or heat shock. Seeds showed a delay in the germination rate compared to the wild type after treatments. However, the over-expressing lines (35S::*At*MBF1 b and c) showed a higher germination rate than the wild type seeds under the same stress conditions. All these findings suggest that *At*MBFs genes may modulate the response to oxidative stress and heat-induced oxidative damage during germination by a still unkown mechanism.
Supported by ANPCyT, CONICET, UNMDP.

PL-P49.

A NEUTRAL/ALKALINE INVERTASE ISOFORM FROM WHEAT IS INDUCED BY COLD, SALT AND OSMOTIC STRESSES

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Neutral/Alkaline-Invertases (N/A-Inv) are cytosolic sucrose-hydrolysing enzymes with pH optima in the range 6.8-8.0. They have been identified in plants and cyanobacteria but their role is not known yet. It was suggested they regulate the entry of sucrose into different cytosolic pathways and more recently were reported as involved in stress responses in plants. In the present work we compare the chromatographic pattern (on DEAE-sephacel) of N/A-Inv of protein extracts from leaves of control and stressed (cold, salt and osmotic) wheat seedling. The results showed that in mature leaves, at least, a novel N/A-Inv isoform is induced in response to cold, salt and osmotic stresses. On the other hand, from wheat EST database a full-length sequence coding for a putative N/A-Inv, (*ta-N/A-inv*), was identified. We cloned this coding sequence by RT-PCR and expressed it in *Escherichia coli* as a 6xHis fusion protein using pRSETA vector. The recombinant protein had the expected molecular weight (/ 60 kDa) and was immunodetected by antibodies anti-cyanobacterial N/A-Inv. We studied the expression pattern of *ta-N/A-inv* in different organs and in response to environmental stresses. The results presented in this work report the identification of the first N/A-Inv from wheat and its expression pattern. We conclude that the expression of *ta-N/A-inv* is differentially regulated in different organs and in response to environmental stresses. In conclusion, we suggest that N/A-Inv might be involved in stress responses exerted in plants by contributing to the regulation of carbon partitioning in different organs.
Supported by ANCPyT, CONICET, UNMdP and FIBA.

PL-P50

EXPRESSION AND CHARACTERIZATION OF A CALCIUM-DEPENDENT PROTEIN KINASE FROM WHEAT

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Calcium-dependent protein kinases (CDPKs) include an extensive group of signaling molecules that mediate many processes in plants, such as growth, development and responses to external stimuli. We have previously identified in the wheat genome an ORF encoding a putative CDPK (*Tacdpk1*) that is up-regulated by sucrose (Suc) and possibly involved in sugar signaling. The aim of this work was the expression of *Tacdpk1* in an heterolog system (*E. coli*) and its characterization. For this purpose the full length encoding sequence (1828 bp) was obtained by RT-PCR from the RNA extracted from wheat leaves treated with Suc. The deduced protein had the typical structural features of the CDPK family and high similarity with other CDPKs (>70%). The *Tacdpk1* was expressed in *E. coli* as a 6xHis fusion protein using the pRSETA vector. The recombinant protein was in the soluble fraction and had the expected size (/58 kDa). Immunoassays with antibodies against a conserved region of CDPKs confirmed the identity of the heterolog protein. Expression analyses of *Tacdpk1* by semiquantitative RT-PCR were carried out in leaves and roots under cold, salt stress and hormones treatments. In conclusion, in this work we expressed the first CDPK from wheat and gave more evidences for its possible role on signaling.
Supported by ANPCyT, CONICET, UNMdP and FIBA.

PL-P51.

HEME OXYGENASE BEHAVIOR IN ULTRAVIOLET-B IRRADIATED SOYBEAN PLANTS

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Ultraviolet-B (UV-B) radiation has a negative impact on plant cells, and leads to the generation of reactive oxygen species (ROS). Heme oxygenase (HO) plays a protective role against oxidative stress in mammals, but little is known about this issue in plants. Here, we report for the first time the response of HO in leaves of soybean plants subjected to UV-B radiation. HO activity, protein and gene expression, as well as stress markers were evaluated. Under lower UV-B doses (7.5 and 15 kJ m^{-2}), the production of thiobarbituric acid reactive substances (TBARS) remained unaltered, while quantitative RT-PCR revealed that HO and catalase (CAT) transcripts were increased 40% and 20% after 8h, respectively. Treatment with 30 kJ m^{-2} brought about a 90% enhancement in TBARS indicating that an oxidative burst occurred, and a down-regulation in gene expression was observed. Immunoblot analysis showed a 4.3 and 3.7-fold increase in HO protein after irradiation with 7.5 and 15 kJ m^{-2}, respectively. HO and CAT enzymes activities were enhanced at these doses but diminished at 30 kJ m^{-2} UV-B. These results indicate that the upregulation of HO and CAT genes at the lower doses occurred as a signal of cell protection against oxidative damage. On the other hand, irradiation with 30 kJ m^{-2} overcome the cellular antioxidant capacity and repressed the response as a result of ROS overproduction.

PL-P52.

A NOVEL SELECTION MARKER ALLOWS PLANTS TO TOLERATE OSMOTIC STRESS

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In nature, accumulation of trehalose acts as a mechanism of drought resistance. Trehalase activity maintains low levels of this sugar in plants. In order to induce trehalose accumulation a trehalase cDNA was cloned and used to generate transgenic tobacco plants that express a trehalase RNA antisense under the regulation of 35S and rd29A promoters. These transgenic plants showed a decrease in trehalase activity and, surprisingly, they grew *in vitro* in the absence of sucrose, may be as a result of increased photosynthetic activity. Consequently we tested if expression of trehalase RNA antisense could be used as a marker to select transgenic plants in the absence of a carbon source. In fact, transgenic tobacco plants were obtained from explants expressing trehalase RNA antisense in sucrose-free medium. In addition, these transgenic plants showed a tolerance to high osmotic stress *in vitro* compared to wild type controls. Therefore this trehalase RNA antisense could be use as an innocuous selectable marker that at the same time might confer stress tolerance and better photosynthetic activity.

PL-P53.

UNDERSTANDING THE NITRIC OXIDE PARTICIPATION IN THE AUXIN SIGNALING PATHWAY

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Nitric oxide (NO) is a signaling molecule in plants that has a proven cross talk with the growth regulator auxin. However, it is still unknown how NO could be acting in the auxin induced response. On the other hand, auxin effects are partially mediated by its ability to regulate gene expression controlling the abundance of transcriptional repressor proteins of the Aux/IAA family. These proteins are degraded via proteasome by binding to the TIR1 protein, wich is a component of the SCF^{TIR1} ubiquitin-ligase complex.
In this work, we demonstrated that exogenous indole-acetic acid (IAA) application increased NO levels in Arabidopsis seedlings roots, detected by an NO specific fluorescent probe. *In vivo* treatments of transgenic Arabidopsis seedlings indicated that the scavenging of the endogenous NO inhibited the promoter activity of the synthetic BA3 auxin-response element. The simultaneous addition of IAA and NO donors overinduced the activity of the BA3 promoter, despite of that NO donors applied alone fail to induce its activity. Additionally, GST-pull down assays indicated the auxin-mediated interaction between Aux/IAA17 and TIR1 proteins is NO-dependent. These results suggest that NO is necessary for the activity of the BA3 auxin-response element, probably by modulating the Aux/IAA protein stability.
Supported by UNMdP, CONICET and ANPCyT.

PL-P54.

NITRIC OXIDE: POSSIBLE SOURCES AND EFFECT ON LIPIDS OF SOYBEAN LEAVES CHLOROPLASTS

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Since nitrite has been postulated as a possible source of nitric oxide, nitrite-dependent NO generation was assessed in chloroplasts by EPR using MGD-Fe (II) as spin trap. The generation of NO was dependent of NaNO2 concentration in the incubation medium up to 4 mM. Chloroplasts incubated with 1 mM nitrite showed a NO production of 2.7±0.8 nmol min^{-1} mg^{-1} prot. The $(MGD)_2$-Fe(II)-NO complex was not detectable when chloroplasts were previously boiled. Inhibition of photosynthetic electron flow by DCMU resulted in a lower rate (0.89±0.04 nmol min^{-1} mg^{-1} prot) of NO generation in illuminated chloroplasts. Isolated stroma showed a lower rate of NO generation as compared to that obtained with tylakoids, suggesting that tylakoids are the main fraction involved in nitrite-dependent NO generation. The effect of NO and peroxynitrite on chloroplast membranes oxidation was evaluated as carbon-centered radicals assesed by EPR, employing POBN as spin trap. Lipid radical production decreased by 26%, 30% and 35% in chloroplasts exposed to 100, 250 and 500 µM GSNO, respectively. However, incubation in the presence of 100 µM peroxynitrite lead to an increase in lipid-derived radicals (27%). Taken as a whole, these data suggests that i) nitrite is a possible source of NO in soybean chloroplast, and ii) lipids could be either protected or damaged according to the reactive nitrogen species (NO or peroxynitrite) generated.

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PL-P55.

NITRIC OXIDE AND H_2O_2 DURING DEVELOPMENT AND SENESCENCE OF SOYBEAN COTYLEDONS

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The aim of this work was to characterize the role of nitric oxide and H_2O_2 during the development and senescence of soybean cotyledons. Chlorophyll, soluble proteins content and electrolyte leakage were used as markers of cotyledon senescence. Chlorophyll content that reached a maximum value of 0.5 µg mg FW^{-1} at day 10 of development started to decrease after 20 days. Both, fresh weight and soluble protein significantly increased at day 7 of development. Nitric oxide (NO) content and nitrite-dependent NO generation assessed by EPR showed a maximum at day 5 of development. H_2O_2 generation in intact cotyledons was assesed employing DCFH/HRP in the presence of DTPA to minimize metal catalysis. No significant differences were observed on DCF-fluorescence during the initial 15 days of development, however an increase of DCF-fluorescence was observed at day 19 of development in agreement with a raise in electrolite leakage.

NO could be related to the mechanism wich promote the growth of the cotyledon during the first days of development, and a possible source of NO generation seems to be related with nitrite availability. Oppositely, reactive oxygen species, probably H_2O_2, could be a part of a complex signaling pathways that triggers the starting of senescence and posterior falling of the cotyledons.

PL-P56

NITRIC OXIDE INDUCES CELLULOSE SYNTHESIS IN TOMATO

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The formation and differentiation of the cell wall plays a key role in plant morphogenesis. Cellulose is the most abundant polysaccharide in plants and provides the framework for all plant cell walls. Although its abundance and simple chemistry, cellulose synthesis remains a poorly understood process. Recently, it has been shown that cell wall development is promoted by H_2O_2 and hydroxyl radicals, indicating that redox chemistry may function as a signal regulating cellulose synthase subunits assembly and function. Since, nitric oxide (NO) plays a role in cell redox homeostasis the objective was to analyze the effect of NO on cellulose synthesis. Tomato (*Lycopersicon esculentum*) germinated seeds were grown in water or 200 µM of the NO donor sodium nitroprussiate (SNP) for 5 d. Total cellulose content was 20 % higher in SNP treated seedlings. Additionally, we measured the incorporation of ^{14}C-glucose into the nitric/acetic acid-insoluble cell wall fraction of 2-day-old tomato seedlings treated with water or with increasing concentrations (50-300 µM) of SNP for 24 h. SNP is able to induce cellulose synthesis in a dose dependent manner. The treatment with the herbicide 2,6-dichlorobenzonitrile (DCB) inhibited 30 % cellulose synthesis and interestingly, SNP treatment reverted that inhibition. These results suggest a novel role of NO in plant cell wall synthesis. Semiquantitative RT-PCR analysis are undertaken to study the NO effect on the expression of cellulose synthase genes.

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PL-P57.

NITRIC OXIDE MEDIATES PLANT ROOT CELL RESPONSES TO IRON DEPRIVATION

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Iron (Fe) is an essential nutrient required by plants, but changes in soil availability may limit Fe acquisition by roots. It is not known how root cells sense or signal the changes that occur after the onset of Fe deficiency. In tomato plants, nitric oxide (NO) production shows a significant increase in roots within 6 h after Fe deprivation. NO accumulated in a discrete region of roots that has been shown to be active in Fe uptake and translocation. Moreover, NO is mainly produced in the root epidermis, where genes responsible of Fe uptake are expressed. Suppression of endogenous NO by treatment with an NO scavenger prevented the up-regulation of high-affinity Fe-uptake genes that are normally induced by Fe deficiency. Pharmacological experiments show that the most probable source of NO is the enzyme nitrate reductase, since its inhibition blocked NO production and Fe-deficiency induced gene expression. Application of NO was not sufficient to induce Fe uptake-related genes in roots grown under Fe-plenty conditions. However, exogenous NO stimulateed gene induction produced by Fe-deficiency. Other root responses to Fe deficiency, like increase of ferric reductase activity and root hair formation are also mediated by NO. These results support that NO production is an early root response to Fe deficiency that modulates gene expression and physiological adaptations in roots related to Fe uptake.

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PL-P58.
FEATURES OF PROLINE DEGRADATION IN PLANT-PATHOGEN INTERACTIONS
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Incompatible plant-pathogen interactions trigger a known type of programmed cell death (PCD), the Hypersensitive Response (HR), at the infection sites. The biochemical signals promoting PCD in HR remains to be investigated. We recently described net alterations of the L-Proline (Pro) metabolism in HR. In addition, accumulation of Δ^1-Pyrroline-5-carboxilate (P5C), an intermediate of Pro degradation, was proposed to induce PCD. Hence, we analyzed the putative effects of P5C along HR development. We used *Arabidopsis thaliana* cell suspension cultures infected with *Pseudomonas syringae* strains to produce or not HR (incompatible and compatible interactions, respectively). We evaluated by RT-PCR the transcriptional regulation of the genes implicated in Pro catabolism, Pro-dehydrogenase (*ProDH*) and P5C-dehydrogenase (*P5CDH*), along infection. Rapid induction of *ProDH* and a repression of P5CDH were only observed in incompatible interactions. The uncoupled expression of these genes is suspected to cause P5C accumulation. We found that these transcriptional changes parallel the second phase of the oxidative burst and the alkaline pH shift, and they precede cell death. Interestingly, pre-treatment of cells with a specific ProDH inhibitor abolishes pathogen-induced cell death. Taking together these results suggest that P5C is a key component of HR during plant-pathogen incompatible interactions.

PL-P59.
SALICILIC ACID AND JASMONIC ACID SIGNALING THE *ARABIDOPSIS-ERYSIPHE* INTERACTION
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We study the *Arabidopsis thaliana* responses induced by the attack of the virulent fungal pathogen *Erysiphe cichoracearum*. Although this pathogen leads to development of plant disease, it is expected that host defenses become induced under this interaction. Using microarray expression profiling, we previously characterized plant genes differentially expressed at the stage of haustorium establishment. We here analyze how the classical modulators of host defenses, salicylic acid (SA) and jasmonic acid (JA), contribute to signal plant responses at this stage. For that purpose the expression profiles of infected wild type, *npr-1* (impaired in SA pathway) and *jar-1* mutants (insensitive to JA) were compared. A Principal Component Analysis was applied to exclude genes insensitive to SA or JA defining four different groups of genes with different expression patterns in wild type, *npr-1* and *jar-1* plants. Looking for common regulatory elements in each of these gene groups, we found conserved modules of 6 to 12-mer on their promoter regions. Finally, we analyzed the effect of exogenous SA or JA and their mutually inhibitory capacity in the expression levels of these genes.

PL-P60.
FUNGAL ELICITOR INDUCES HYPERSENSITIVE RESPONSE AND SYSTEMIC OXIDATIVE BURST IN STRAWBERRY PLANTS
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The recognition of a defense elicitor stimulates an oxidative burst generating reactive oxygen species (ROS) that induce defense genes and localized cell death restricted to the lesion zone. This hypersensitive response (HR) is accompanied by the development of resistance to virulent pathogens. The aim of this study was to analyze in strawberry plants, the production of two reactive oxygen species (O_2^- and H_2O_2) and HR in response to a fungal elicitor obtained from an avirulent strain of *Colletotrichum fragariae*. The extract containing the elicitor (20 µl) was infiltrated on the abaxial surface of one leaflet per plant of the cultivar Pájaro. Results indicate that two hours after the application of the elicitor, ROS species such as O_2^- and H_2O_2 accumulate in the inoculated leaflet and not inoculated proximal and distal tissues, suggesting the induction of a systemic cellular response. Two days after the infiltration we have observed the formation of necrotic lesions restricted to the infiltrated zone, that closely resemble the HR. A strong epifluorescence located in cells surrounding the necrotic lesion, was also observed, indicating the accumulation of compounds derived from the phenylpropanoid pathway, highly stimulated during the HR. Plant cells recognizing the elicitor induced a cascade of short and long distance signals that are currently being under study in our laboratory.

PL-P61.
CHARACTERIZATION OF *Citrus sinensis* TYPE 1 MITOCHONDRIAL ALTERNATIVE OXIDASE
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Mitochondrial alternative oxidase (AOX) is a quinol oxidase that catalizes cyanide-resistant reduction of O_2 to water without translocation of protons. It is encoded by a nuclear gene family classified in two subfamilies: AOX1 and AOX2/3. AOX1 is known for its induction in biotic and abiotic stress stimuli. Our objective is to characterize the AOX type 1 in *Citrus sinensis*. Using a 440 bp region belonging to orange AOX1 as a probe, a *Southern Blot* with *Eco*RI enzyme was carried out. Two alleles (2.1 and 5.6 kbp) were detected. A sub-library was constructed using 2.1 kbp fragments in pBluescriptKS+ vector and screened with the same probe, detecting two positives clones which were sequenced. A 354 bp sequence belonged to promoter region and cis-acting elements were detected *in-silico*. The most relevant are TCA-Box (Salicilic Acid Response), WRKY (associated with pathogen defence), and PR-Box (Pathogen Response). The other 1749 bp completely cover the coding sequence (four exons and three introns), 5'UTR, and part of 3'UTR. Two highly conserved cysteine residues which are involved in homo-dimerization by S-S bond formation and two regions EXXH corresponding to the active site were found. Phylogenetic tree grouped AOX clone with dicots AOX1. *C. sinensis* may posses two AOX1 alleles, being one of them characterized. Its putative stress participation will be analyzed.

PL-P62.

PARTICIPATION OF *Xanthomonas axonopodis* pv. *citri* EPS IN CITRUS CANKER AND IN NON-HOST PLANTS RESPONSES

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Xanthomonas axonopodis pv. *citri* (*Xac*) causes citrus canker, a serious disease that results in important losses. The extracellular polysaccharide (EPS) produced by xanthomonads is termed xanthan and many roles have been suggested for the EPS in plant-pathogen interaction. A cluster of 12 genes are involved in the biosynthesis and secretion of xanthan, named *gumB-gumM* in *X. campestris* pv. *campestris*. We have characterized the participation of xanthan in the interaction with host and non-host plants. We isolated a region of the *gum* cluster of *Xac* and constructed mutants in the *gumD* gene, using a suicide vector transferred by biparental mating. For disease symptoms assays, bacterial suspensions were infiltrated into leaves of host plant orange (*Citrus sinensis*) and into leaves of non-host plants cotton, tomato and tobacco. No differences were observed in lesions and bacterial growth curves in wild type (WT) and mutant *Xac* inoculated host plant leaves. Also, epiphytic survival was investigated and no differences between inoculations with both strains were observed. The infiltration of WT and mutant Xac in non-host plants induced in both cases hypersensitive responses. The results suggest that xanthan may not be indispensable for *Xac* interactions with host and non-host plants.

PL-P63.

CYTOTOXIC ACTIVIY OF POTATO ASPARTIC PROTEINASES COULD BE INVOLVE THIORREDOXIN REDUCTASE INTERACTION

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Potato aspartic proteinases (*St*APs) have in their sequence a domain named "Plant Specific Insert" (PSI) which has a high homology with saposin like proteins (SAPLIPs). This family of proteins are able to permeabilize cell membranes via interaction with lipids and/ or thioredoxin reductase (Trx). Previous reports have shown that the cytotoxic activity of one of these proteins, NK-lysin, would be regulated via Trx interaction.

We have previously reported the direct relationship between cytotoxic activity of *St*APs on *Fusarium solani* and the capacity of these proteases to produce cell membrane permeabilization mediated by binding to the cell surface. In this work we show that the cytotoxic activity and the capacity of binding to cell surface of spores and hyphae of *F. solani* were highly dependent on the integrity of the disulfide bonds, because these activities were inhibited when StAPs were treated with dithiotreitol prior to incubation with fungal structures. The amounts of DTT assayed were selected taking into account the reported DTT concentrations able to inhibit the cytotoxic activity of NK-lysin (0.1-0.5 mM). Results obtained showed that DTT affects the StAPs cytotoxic activity and surface binding capacity in a dose-dependent form. These results encouraged us to search for interaction of antimicrobial plant proteins and peptides with disulfide bridges essential to their folding, with plant and/or fungal Trx by analogy with mammalian cell systems.

PL-P64.

EFFECT OF TUNICAMYCIN ON POTATO ASPARTIC PROTEINASES SECRETION

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Apparently, the effect of glycosylation is protein specific. Plant aspartic proteinases (AP) contain two or more consensus N-glycosylation sites; however, the importance of those is not well understood. We have previously reported that the effect of the glycosylation of StAP (*Solanum tuberosum* Aspartic Proteinases) is important for their antimicrobial activity, because it would increase the capacity to induce membrane permeabilization. In this work, we investigated the effects of tunicamycin on the secretion of *St*AP1 and *St*AP3 to the apoplast.

To inhibit glycosylation, potato tubers and leaves were treated during 12, 24 y 48 hours with or without 0.5mg/ml of tunicamycin. After treatment, total proteins were analysed by SDS-PAGE and, potato aspartic proteinases by Western blot. The results showed that the treatment with tunicamycin did not affect the protein pattern of tubers and leaves. Aspartic proteases were induced by wounding; however, when tubers and leaves were treated with the inhibitor, the basal level of those proteases were not modified. On the other hand, also we observed that in tubers treated during 24 or 48 hr with tunicamycin, a protease inhibitors present in the apoplast were not secreted, suggesting that glycosylation would be involved in the secretion these pathogenesis related proteins.

PL-P65.

DEFENSE MECHANISMS INDUCED BY BABA IN MULTIGENIC RESISTANCE POTATO CULTIVARS. SYSTEMIC RESPONSE AGAINST *PHYTOPHTHORA* AND *FUSARIUM*

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The aim of this work was to characterize the biochemical mechanisms by which BABA (ß-amino-butyric acid) increases resistance in potato against phytopathogens by promoting a systemic acquired resistance. Two potato cultivars, with different level of horizontal resistance to *P. infestans*, were analyzed. BABA applications were carried out at different times during plant growth and defense response were analyzed in post harvest tubers. BABA application did not affect tuber harvest yield. Moreover, in our greenhouse conditions, the number and weight of tuber recovered from BABA treated plants increased respect to control plants. The levels of phytoalexins increased in *Phytophthora* infected tubers, and this increase was higher in tubers from BABA treated plants. In addition, the expression of an aspartic protease also increased in tubers from treated plants, in both cultivars. In potato-*Fusarium* interaction the accumulation of a fungal serine protease was lower in infected tubers from treated plants respect to infected but non treated tubers. This decrease correlates with a higher plant aspartic protease expression in the same system. It is also necessary to evaluate the durability of this defense response in tuber during storage and to know the optimal time of BABA application to obtain a effective SAR.

PL-P66.
INCREASE OF THE RESISTANCE TO LATE BLIGHT BY BIOCOMPATIBLE COMPOUNDS

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In Argentina, more than 80% of the cultivated potato varieties are susceptible to late blight. This means that most potato agroecosystems are dominated by large patches of genetically homogeneous crop, which favored rapid development of the disease. Therefore, large amounts of fungicides are needed to control it, resulting in high costs of production, environmental contamination and the risk of developing pathogen resistance. All these factors have emphasized the need for an alternative chemical for integrated crop management. Phosphites, differing in its mode of action from fungicides, could be such an alternative. Hence, the present study was to determine the effect of Potassium and Calcium Phosphite (KPhi and CaPhi) on the control of late blight. Plants of cvs. Shepody and Kennebec (grown under greenhouse conditions), were sprayed with doses of 2 lt/ha of KPhi and CaPhi or water, six weeks after emergence. Five, eleven, fourteen and twenty days after these treatments, leaves were cut and inoculated with *P. infestans*. At the first time (5 days after treatment), the leaves were processed for biochemical analysis. The expression and activity of basic chitinases and ß1-3 glucanases were increased by CaPhi treatment in both cultivars, but only in cv. Kennebec with KPh. High level of protection against *P. infestans* was observed until fourteen days of treatment with KPhi in both cultivars and lower with CaPhi. These protections decreased at 20 days after treatment. It is also necessary to evaluate the application times and rates for the control of potato tuber infections.

PL-P67.
POTATO POLYGALACTURONASE INHIBITOR PROTEIN (PGIP) INHIBITS A POLYGALACTURONASE FROM *RHIZOCTONIA SOLANI* AG - 3

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Rhizoctonia solani AG-3, a fungal pathogen of potato, produces polygalacturonases (PGs) when grown *in vitro* on pectin as carbon source. These enzymes are produced by fungal pathogens during early plant infection and are believed to be important pathogenicity factors. We have previously reported the purification of an extracellular endo-polygalacturonase (endoPG) from *Rhizoctonia solani* AG3. The molecular mass of the enzyme was estimated to be 40 kDa.

Here, we report that this enzyme is inhibited by a potato polygalacturonase- inhibiting protein (PGIP). Moreover, potato PGIP had low inhibitory activity against PG from *A. niger*, indicating specificity towards the pathogen enzyme.

Results obtained shown that the levels of PGIP are increased in sprout in response to infection with *Rhizoctonia solani* AG-3. This results suggest a possible role of potato PGIP in defense against *Rhizoctonia solani* AG3.

PL-P68.
STUDY OF THE INCOMPATIBLE INTERACTION BETWEEN *Colletotrichum fragariae* AND THE CV. PAJARO OF STRAWBERRY

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We have previously reported that the cv Pájaro of strawberry presented a strong incompatible interaction with an avirulent isolate of *C. fragariae*. Macroscopic observations of leaves showed that, whereas a virulent isolate of *C. acutatum* used as control provoked a severe plant damage 10 days after the infection, the avirulent strain produced no damage, suggesting that a defense response was induced without the development of a hypersensitive response (HR). The aim of this work was to analyze microscopic events involved during the incompatible interaction and to investigate possible defense mechanisms supporting experimental observations. Microscopic studies showed that during the incompatible interaction, plant cells and leaf cuticle remain intact in contrast to the compatible interaction. Other conspicuous anatomic features were observed suggesting that plants undergo into a defense response. Molecular studies let us detect the expression of a glutathion S transferase that showed a 34% identity with a tomato GST/GPX Bax inhibitor and by using DAPI (4',6-diamidino-2-phenylindole) we have not detected PCD, suggesting that the induction of HR may be repressed by the BI-GST/GPX like protein detected in strawberry. This observation suggests that the ethylene defense signal pathway may be implicated.

PL-P69.
WHEAT DEFENSE AGAINST THE DISEASE CAUSED BY *SEPTORIA TRITICI*. THE DEGRADATION OF SPECIFIC PROTEINS OF CONIDIOSPORES BY LEAF EXTRACELLULAR PROTEASES

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The intercellular fluid of wheat leaf contains a serine protease activity. It changes according to the resistance of cultivars to the attack of the pathogenic fungus *Septoria tritici*. Thus, 10-12 days after the inoculation of plants with *S. tritici*, it increases 4 times in the resistant cv. Pigüé and decreases lightly in the susceptible cv. Isla Verde. We observed that the conidiospores of *S. tritici* do not germinate when are incubated during 24 h with the apoplastic serine protease activity. These results suggested that the aforementioned activity participates in the defense of wheat plants against the attack of *S. tritici*. Even though there are numerous families of proteins that present antifungal activity, their mechanisms of action are scarcely known. In order to advance in the knowledge of the mechanism that determines the antifungal capability of the protease activity, its action on the proteins of S. tritici conidiospores was examined. By means of SDS-PAGE, there were recognized four degraded proteins whose relative molecular masses were 86.4, 59.8, 44.4, and 37.2 kDa. The subsequent identification of these proteins would constitute a significant advance to comprehend the defense mechanism of wheat plants against *S. tritici*.

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PL-P70.

RESISTANCE GENES ANALOGS STUDIES ON *SOLANUM COMMERSONII* AND ASSOCIATION WITH *RALSTONIA SOLANACERUM*

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S. commersonii is a native potato from Uruguay and the region, it is attractive for breeding by confer resistance to Ralstonia solanacearum, one of the most world devastating bacterial disease. Several studies report the presence of common motif between proteins encoded resistance genes (R genes) from different plant species.
In this work degenerated primers were constructed based on consensus sequence from conserved domains of proteins encoded by R-genes. Thus, Tir, NBS, kinase and WRKY motif were used for design primers, also considering the potato codon usage. Resistant and susceptible S. commersonii genotypes were used to identify and characterize resistance genes analogs (RGAs) by PCR and CAPS approach. Nested-PCR appears to confirm the presence of gene structurally similar to the gene *RRS-1*, reported in Arabidopsis which confers resistance to Ralstonia.
Specifics primers will be designed based on sequence information of commersonii RGA-amplicons that will be used in a segregant resistant x susceptible progeny.

PL-P71.

EFFECT OF TUNICAMYCIN ON POTATO ASPARTIC PROTEINASES SECRETION

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Apparently, the effect of glycosylation is protein specific. Plant aspartic proteinases (AP) contain two or more consensus N-glycosylation sites; however, the importance of those is not well understood. We have previously reported that the effect of the glycosylation of *St*AP (*Solanum tuberosum* Aspartic Proteinases) is important for their antimicrobial activity, because it would increase the capacity to induce membrane permeabilization. In this work, we investigated the effects of tunicamycin on the secretion of *St*AP1 and *St*AP3 to the apoplast.
To inhibit glycosylation, potato tubers and leaves were treated during 12, 24 y 48 hours with or without 0.5mg/ml of tunicamycin. After treatment, total proteins were analysed by SDS-PAGE and, potato aspartic proteinases by Western blot. The results showed that the treatment with tunicamycin did not affect the protein pattern of tubers and leaves. Aspartic proteases were induced by wounding; however, when tubers and leaves were treated with the inhibitor, the basal level of those proteases were not modified. On the other hand, also we observed that in tubers treated during 24 or 48 hr with tunicamycin, a protease inhibitors present in the apoplast were not secreted, suggesting that glycosylation would be involved in the secretion these pathogenesis related proteins.

PL-P72.

PROLINE EFFECTS ON SECONDARY METABOLIC PATHWAYS IN *RUBIA TINCTORUM* CELL SUSPENSIONS

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Proline biosynthesis in plants stimulates the pentose phosphate pathway which results in an increasing carbon flux through the shikimate pathway. Shikimate pathway supplies carbon structures for many secondary metabolic pathways in plants. Chorismate, which is the end product of the shikimate pathway, becomes the branch point for the synthesis of phenylpropanoid and anthraquinones (AQs) in Rubia tinctorum secondary metabolism. We tested the effect of proline addition in plant suspension cultures of R. tinctorum in order to study the competition between the two above mentioned secondary metabolic pathways. Suspension cultures were treated with proline at different concentrations 0.25, 5 and 25 mM. Low proline level (0.25mM) produced an increase on AQs (50%) while high levels of proline (5 and 25 mM) showed a significant decrease on AQs accumulation (35% an 50%) and an increase in phenolics concentration. The increase on phenolic acids content was preceded by an induction on PAL activity. Glucose-6-phosphate dehydrogenase was induced during stationary phase of the growth curve. Suspension cultures treated with proline 5 y 25 mM showed lower levels of Peroxidase activities than the control treatment.

PL-P73.

INTERACTION STUDIES BETWEEN FIVE MITOCHONDRIAL γ-CARBONIC ANDHYDRASES FROM *ARABIDOPSIS THALIANA*

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A novel group of 5 γ-Carbonic anhydrases (γ- CA1, γ- CA2, γ- CA3, γ- CAL1 y γ- CAL2), has been recently identified in *Arabidopsis thaliana*. These proteins are targeted to mitochondria, and they are specifically located bounded mainly to the Complex I (NADH-ubiquinone oxidorreductase) of the mitochondrial electron transport chain. The γ-Carbonic anhydrase proteins containing left-handed parallel beta helix (LßH) domain display imperfect tandem repeated copies of hexapeptide sequence characterized as [LIV]-[GAED]-X2-[STAV]-X. This domain is involved in protein-protein interaction. There is only one example fully characterized of γ- CA from *Methanosarcina thermophila*, named CAM. Cristallografic studies of this protein shown a quaternary homotrimeric structure coordinating a Zinc ion. In the present work we postulate a plant CA complex by *in vivo* Yeast two hybrid assays.

PL-P74.
MOLECULAR CLONING, EXPRESSION AND PURIFICATION OF *Arabidopsis thaliana* FRATAXIN HOMOLOG (AtFH)

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It has been proposed that frataxin, a nuclear encoded mitochondrial protein, participates in Fe-S cluster assembly, mitochondrial energy metabolism, respiration and iron homeostasis, but its precise function remains elusive. This protein is highly conserved from bacteria to mammals and plants without major structural changes, specially at the C-terminal domain, suggesting that it could play similar function in all these organisms. We recently described *AtFH*, a plant gene with significant homology to other members of the frataxin family. The plant frataxin domain was expressed in *Escherichia coli* BL21-CodonPlus (DE3)-RIL cells and purified by one-step Ni-chelating chromatography. The protein was determined to be 98% pure and migrates as a single protein band of about 15 kDa as assessed by SDS-PAGE. *Arabidopsis* null mutants deficient in AtFH expression showed higher rate of CO_2 fixation respect to wt. Results also showed that this protein is escential for full-activity of succinate dehydrogenase (SDH) and aconitase, two mitochondrial iron-sulphur containing enzymes.
Results are in agreement with the involvement of AtFH in Fe-S cluster assembly in plant mitochondria.

PL-P75.
CLONING AND EXPRESSION OF MutS AND MutL COMPLEXES FROM *Arabidopsis thaliana*

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The mismatch repair (MMR) system is critical for maintaining the overall integrity of the genetic material, and the basic features of this system have been highly conserved during evolution. Proteins unique to the MMR system are known as "Mut" proteins and were originally identified in prokaryotic organisms, where their loss enhances the accumulation of DNA replication errors and results in a mutator phenotype. Plants face unique obstacles to long term genetic integrity. They lack reserved germ lines: gametes arise from meristem cells that have already divided many times. For this reason, plant somatic genome-maintenance activities must be as efficient as eukaryotic counterparts, perhaps more so. The aim of this study was to characterize the proteins involved in the initial step of the plant MMR pathway, MutSα, MutSγ and MutLα. MLH1 and PMS1 were obtained by reverse transcription followed by PCR using specific primers. The cDNAs fragments as well as those of MSH2, MSH6 and MSH7 were cloned in compatible expression vectors. It remains to be investigated gene and protein expressions in mitotic (root, meristem), meiotic (flower) and non-mitotic/non-meiotic tissues (leaf). In addition, functional analyses of these *A. thaliana* protein complexes are being performed in yeast using sensitive yeast reporter systems.

PL-P76.
FUNCTIONAL GENOMICS: TOWARDS THE ANALYSIS OF A POTATO DEFENSOME

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One important disease of the potato tubers (*Solanum tuberosum*) is the dry rot caused by *Fusarium* species. Previously in our lab, potato tubers inoculated during 24 h with *F. eumartii* were used to generate a cDNA library. The differential screening of the library was performed using control and subtracted probes. This approach led to the isolation of 340 differentially expressed signals stored as phage suspensions. In order to isolate these cDNAs, we have optimized a PCR reaction, using universal primers that flank the inserts. The amplification products were analyzed by agarose gel electrophoresis, isolated, purified and then sequenced. Approximately 75% of the sequences showed similarity to previously described genes found in public databases. Genes involved in basic cellular processes, i.e., metabolism, protein synthesis, folding, cellular transport and DNA replication represented 41%. Genes involved in cell regulation processes, i.e., signal transduction and transcription represented 21%. Genes with stress related functions comprised 13% of the total cDNAs. The rest of the cDNAs (25%) represented putative proteins with unknown functions. This cDNA collection is a rich source of defense/stress-related and regulatory genes; once its analysis is completed, the cDNAs will be used to study their expression related to biotic and abiotic stresses by means of DNA macroarray.

PL-P77.
OXIDATIVE STRESS IN HUMIDIFIED AGED *SALIX NIGRA* SEEDS

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Previous studies on *Salix nigra* seeds suggest the oxidative damage as an important cause of aging, and its reversion by humidification at 100% relative humidity, to the activation of antioxidant defenses. To test this hypothesis, SOD, GSH, pigments, MDA, BLUE and oxidized proteins were measured during humidification at 15, 30, 60 and 120 min. As compared with control, aged seeds showed a remarkable increase in oxidative damages (MDA, BLUE and oxidized proteins). During humidification, those parameters showed a slight increase at 15 min followed by a progressive decrease. As far as antioxidant defenses (GSH and SOD), they increase during humidification. The lower oxidative stress detected at 30 and 60 min humidification correlated with increase of normal germination. Results seem to confirm the hypothesis in that oxidative damages would be the principal cause of aging and that antioxidant defense mechanisms are activated by humidification.

PL-P78.

SUPRESSION OF ENZYMATIC BROWNING IN POTATO PLANTS BY RNA INTERFERENCE

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Damage caused by post-harvest enzymatic browning is one of the main economic problems to potato producers and Food Industry. We have developed different transgenic potato plants which present Polyphenol oxidase (PPO) activity inhibition by using RNA interference technology.
In order to silence PPO expression, sense and antisense gene fragments of 400 bp from the 5' region of the potato *pot32* gene and an 840 bp spliceable spacer were ligated into a binary vector pZP200-HYG under the control of a constitutive promoter to generate a recombinant plasmid pJAPI. This plasmid was subsequently transformed into potato (*Solanum tuberosum*) var. spunta via *Agrobacterium tumefaciens* mediated tuber transformation.
Selection with hygromycine and PCR detection assays yielded over 30 different transgenic lines. RNA expression, PPO activity and phenotype were initially analyzed in 4 transgenic lines.
We show that PPO mRNA levels and PPO activity correlate with the reduction in enzymatic browning in these lines and no detrimental side effects have been detected so far.
This suppression which eliminates sensitivity to bruising could prevent huge losses occurring during the harvest (over 20%) and help avoid the use of various additives during the processing of potato derived commercial products.

PL-P79.

A NOVEL SELECTION MARKER ALLOWS PLANTS TO TOLERATE OSMOTIC STRESS

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In nature, accumulation of trehalose acts as a mechanism of drought resistance. Trehalase activity maintains low levels of this sugar in plants. In order to induce trehalose accumulation a trehalase cDNA was cloned and used to generate transgenic tobacco plants that express a trehalase RNA antisense under the regulation of 35S and rd29A promoters. These transgenic plants showed a decrease in trehalase activity and, surprisingly, they grew *in vitro* in the absence of sucrose, may be as a result of increased photosynthetic activity. Consequently we tested if expression of trehalase RNA antisense could be used as a marker to select transgenic plants in the absence of a carbon source. In fact, transgenic tobacco plants were obtained from explants expressing trehalase RNA antisense in sucrose-free medium. In addition, these transgenic plants showed a tolerance to high osmotic stress *in vitro* compared to wild type controls. Therefore this trehalase RNA antisense could be use as an innocuous selectable marker that at the same time might confer stress tolerance and better photosynthetic activity.

PT-P1.

A PROTEOMIC STUDY OF A FRESH ISOLATED AND A LONG-TERM CULTURED STRAIN OF PROTOZOAN PARASITE *Trichomonas vaginalis*

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Human infection by the protozoan pathogen *Trichomonas vaginalis* causes one of most common sexually transmitted diseases throughout the world. It emerges as an important cofactor in amplifying HIV transmission and is associated to cervical cancer, atypical pelvic inflammatory disease and infertility. The aim of this work was to identify proteins differentially expressed among a fresh isolate of *T. vaginalis* (FMV-1) and a long-term cultured parasite (FF28JT-Rio), one highly cytotoxic and other with low cytotoxicity to MDCK monolayers, respectively. $1x10^9$ parasite were lysed by freeze/thawing and proteins were precipitated with TCA and cold acetone wash. Pellets were dissolved in 9M Urea, 4% CHAPS, 60mM DTT and 0.2% ampholytes. IEF gel profiles were obtained in 4-7 pH gradients, 18 cm gel strips, 500 µg sample, 12% polyacrylamide gels and Coomassie blue staining. Peptides from in gel tryptic hydrolysis were collected, submitted to MS and MS/MS analysis (ABI 4700 MALDI-TOF-TOF) and searched at NCBI using MASCOT software. Mass spectrometry identified proteins from cytoske-leton, glycolytic pathway, heat shock, amino acid metabolism and anti-oxidative stress in both samples. Cysteine proteinases, HSP70 and lactate dehydrogenase isoenzyme were found differentially expressed between the strains.

PT-P2.

DIFFERENCIAL EXPRESSION OF PROTEINS FROM EPIMASTIGOTES OF *TRYPANOSOMA CRUZI* CULTIVATED IN DIFFERENT CARBON SOURCES USING PROTEOMIC ANALYSIS

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The hemoflagellate protozoan *Trypanosoma cruzi* is the etiological agent of Chagas disease. The illness continues to be an important public health problem in South America, and the occurrence of the protozoan and the respective insect vectors encloses an area from the south of the United States to the north of Argentina. The objective of this work is to analyze the proteomic expression of *T. cruzi* when using different carbon sources and to characterize key molecules in the metabolism of the parasite or in its interaction with the host. Initially, axenic cultures of epimastigote forms of *T. cruzi* (strain CL Brener) were used for the bi-dimensional electrophoresis assays from four culture conditions. Cells were lysed by freezing and thawing and proteins were precipitated with trichloroacetic acid. The samples were submitted to bi-dimensional electrophoresis, which separates proteins by isoelectric focusing (pH 5-8), and molecular weight. The gel patterns were analyzed using the PDQUEST program (Biorad). Our preliminary results show qualitative and quantitative differences at the protein expression level and some spots are being identified by mass spectrometry. This study is of considerable relevance for the development of new strategies of imunotherapy and drug design against this pathogen.
Support: PDTIS-FIOCRUZ, FAPERJ, CNPq.

PT-P3.

PROTEOMICS OF *Trypanosoma cruzi*: EXPRESSION, PURIFICATION AND PRELIMINARY STRUCTURAL STUDIES

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Trypanosoma cruzi is the etiologic agent of Chagas' disease. The objective of our work is the resolution by X-ray crystallography of the three-dimensional structure of *T. cruzi* proteins as a first step for rational drug design based on the structure. The development of new drugs is based in the search for protein targets in metabolic routes absent in the human host and common in trypanosomatids. The proteins studied at the present are: TcAK (arginine kinase), TcNDPK (nucleoside diphosphate kinase), TcFIP1-like (factor interacting with Pap1) and TcCPSF30 (cleavaje polyadenylation specificity factor). The plasmids are provided by the other members of the research network. They are over-expressed as a fusion protein with a N-terminal His-tag. They are purified to be used for crystallization and other structural assays. His-TcNDPK was over-expressed and purified in the native state with a high purity and a high concentration for primary crystallization assays. Work is being done on the over-expression of His-TcFIP1 and TcCPSF30. The structure of TcAK has been solved at 1.8 Å resolution. This work is being done within the framework of the *Chagas´ Disease Research Network*. The research network has established a common methodological matrix to study protein complexes of different biological functions.

PT-P4.

NEUROTOXINS FROM *Micrurus pyrrhochryptus* AS NEW TOOLS IN STRUCTURE-FUNCTION STUDIES OF NICOTINIC RECEPTORS

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Snake venoms contain enzymatic and non-enzymatic proteins, peptides and other small compounds, such as citrate, nucleosides and acetylcholine. Elapid snake venoms have shown to be sources of phospholipase A2, presynaptic and postsynaptic neurotoxins. Patients bitten show clinical symptoms leading to death due to muscle paralysis followed by respiratory arrest. These effects are the consequence of nicotinic receptors blockage. We work with *Micrurus pyrrhocryptus* venom; one of its major characteristics is a high complexity degree. That is why in a first step, proteins were resolved by molecular filtration; eleven fractions were obtained. In order to establish function-structure relationship, they were tested for phospholipase and myotoxic activities as well as for their capacity of binding the nicotinic acetylcholine receptor; creatine kinase assay and the level of indirect hemolysis indicate high phospholipase activity and dot assays confirmed the presence of postsynaptic neurotoxins. In order to purify the major components, protein mixtures from gel filtration were resolved by C18-RP-HPLC. Purified fractions were submitted to mass spectrometry and N-terminal microsequencing analysis and sequence alignment was performed. Results allowed the identification of several phospholipases, and short and long neurotoxins in which we are mainly interested in.

PT-P5.

MOLECULAR MARKERS IN CHILDREN PAROTID SALIVA. A PROTEOMIC APPROACH

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The "normal" protein pattern of physiological fluids can be used as reference for differential diagnosis of pathologies. Saliva proteome is composed by a number of proteins involved in the homeostasis of the oral cavity.
The main objectives of this work are: a) to describe the protein profile of parotid saliva of healthy children; b) to identify molecular markers for juvenile rheumatoid arthritis (JRA). Ten samples of parotid saliva of healthy children and ten samples of children with juvenile rheumatoid arthritis were collected with a Lashley cup following stimulation with a 2% citric acid solution.
After molecular exclusion chromatography, each fraction was submitted to mass spectrometry (MS). The fraction containing 10-23 kDa molecular species was analyzed by RP-HPLC. A further MS analysis as well as 2D SDS-PAGE allowed detection of a group of proteins that may be considered molecular markers of the "normal" children parotid saliva. Those samples of saliva from children with JRA were submitted to the same procedure. Similarities and differences observed should indicate that the method could be a new tool for the diagnosis of this disease.

PT-P6.

INFLUENCE OF DIETARY METHIONINE ON THE UBIQUITINATION OF MOUSE LIVER CYTOSOLIC PROTEINS

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Protein content decreases 50% in the liver of mice fed with a protein free diet. The ubiquitin-dependent system is responsible for the degradation of short-lived and abnormal proteins in eukaryotic cells. This work was designed to examine the effect of diets on the ubiquitination of cytosolic proteins *in vivo* and in isolated hepatocytes. Balb-c female mice were fed during 5 days with normal (23% of casein), protein free, and protein free containing Met, respectively. The isolated hepatocytes were incubated for 4h with a complete medium, minimal medium, and minimal medium supplemented with Met. The ubiquitin conjugates were analysed by Western blot with polyclonal antibodies. The results indicated that: a) the total protein content of undernourished hepatocytes decreases as observed *in vivo*; b) the main size range of ubiquitinated proteins *in vivo* and *in vitro* was 89000-18000 and 73000-34000, respectively; c) the level of some ubiquitinated proteins was modified by diets (while some increased others decreased); e) supplementation with Met caused a pattern of ubiquitinated proteins similar to that of controls. Then dietary Met can increase in both systems the stability of some proteins.
Supported by CONICET, UNLP and UNMdP.

ST-P1.
EVIDENCE OF FUNCTIONAL MODULATION OF MITOGENIC CASCADES BY PTH IN INTESTINAL CELLS: EFFECT OF AGEING

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In the present study we examined the role of PTH on members of the MAPK family as it relates to ageing by measuring hormone-induced changes in the activity of JNK 1/2 and p38 MAPK in enterocytes isolated from young (3 month-old) and aged (24 month-old) rats. Our results show that, PTH induces a transient activation of JNK1/2, with the greater response achieved at 2 min (+3 fold). The hormone also stimulates JNK1/2 tyrosine phosphorylation, in a dose-dependent fashion, being maximal at 10 nM. PTH-induced JNK1/2 phosphorylation was effectively suppressed by its selective inhibitor SP600125 (20 μM). Moreover, hormone-dependent tyrosine phosphorylation and activation of JNK1/2 was dependent on intracellular calcium, since the pretreatment of cells with BAPTA-AM (5 μM), an intracellular Ca^{2+} chelator, blocked PTH effects. With ageing, the response to PTH was signifincantly reduced. However, the amount of basal protein expression determined by Western blot analysis for JNK was not different in the enterocytes from young and aged rats. PTH does not stimulate p38 MAK in intestinal cells; furthermore, the hormone decreases, within 15 to 30 min, the basal phosphorylation and activity of p38 MAPK. PTH increased enterocyte DNA synthesis. The response is dose-dependent and decreases (-40%) with ageing. Of physiological significance, in agreement with the mitogenic role of the MAPK cascades, this effect was blocked by the specific inhibitors of ERK1/2 and JNK1/2. The results obtained in this work expand our knowledge on the mechanism of action of PTH in duodenal cells, revealing that activation of JNK1/2 and ERK1/2 is linked to PTH regulation of intestinal cell proliferation, and that this mechanism is impaired with ageing.

ST-P2.
17β-ESTRADIOL ABROGATES APOPTOSIS IN C2C12 MUSCLE CELL LINE THROUGH NON- CLASICAL ESTROGEN RECEPTORS

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The action of estrogen can be mediated by the classical nuclear estrogen receptor or through putative receptors with non-classical localization. There is evidence showing antiapototic effects of estradiol in various cell types. The present study was aimed to analyze these effects of 17β-estradiol in apoptotic murine skeletal muscle C2C12 cells. We demonstrated by DAPI staining, DNA laddering and Western blot assays using anti-PARP antibody, that etoposide (25 μg/ml), as well as H_2O_2 (1 mM), induced apoptosis which could be inhibited by estradiol. In contrast, the estradiol-BSA conjugate did not exert this protective effect. Apoptosis was not inhibited by estradiol using saponin-permeabilized C2C12 cells in presence of a monoclonal anti-ERα antibody against the ER estradiol binding domain. In view that immunocytochemistry and ligand binding studies indicate that the ER is mainly localized in mitochondria, and that 17β-estradiol treatment does not translocate ER to the nucleus, the antiapototic action of estradiol in C2C12 muscle cells may involve the activation of non-classically located estradiol receptors.

ST-P3.
AGE-RELATED ALTERATIONS OF 1,25(OH)$_2$-VITAMIN D$_3$ -DEPENDENT P38 MAPK ACTIVATION

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In intestinal cells, 1α,25(OH)$_2$-vitamin D$_3$ (1α,25(OH)$_2$D$_3$) regulates gene expression via the specific intracellular vitamin D receptor and induces fast non-transcriptional responses involving stimulation of transmembrane signal transduction pathways. We have previously shown that the hormone activates the mitogen-activated protein kinases ERK1/2 and p38 MAPK in rat intestinal cells (enterocytes). In the present study, we analyzed, for the first time, alterations in p38 MAPK response to 1α,25(OH)$_2$D$_3$ in rat enterocytes with ageing. In enterocytes from young rats, the hormone increased, in a time and dose-dependent fashion, the tyrosine phosphorylation and activity of p38 MAPK, with a maximun at 3 min (+2 fold). Basal levels of p38 MAPK tyrosine-phosphorylation were not significantly changed in old enterocytes, but the hormone response was greatly diminished (+1 fold at 2-3 min). p38 MAPK phosphorylation impairment in old animals was not related to significant changes of the kinase protein expression and do not explain the decreased response to 1α,25(OH)$_2$D$_3$. Extracellular Ca^{2+} chelation with EGTA, suppressed hormone activation of p38 MAPK in both, young and aged rats, demonstrating that extracellular Ca^{2+} is required for full activation of p38 MAPK in enterocytes stimulated with 1α,25(OH)$_2$D$_3$. Enterocytes exposure to the hormone also resulted in the rapid induction of c-Fos expression, peaking at 5 min (+3 fold), effect that was blocked by SB 203580, a specific inhibitor of p38 MAPK, and partially suppressed by the ERK1/2 inhibitor PD 98059. 1α,25(OH)$_2$D$_3$-induced c-Fos expression is lost in old animals. Impairment of 1α,25(OH)$_2$D$_3$ activation of p38 MAPK upon ageing results in abnormal hormone regulation of the c-Fos oncoprotein synthesis and thereby may affect intestinal cell function.

ST-P4
ROLE OF P2Y$_2$ RECEPTOR AND MECHANICAL STRESS-ACTIVATED Ca^{2+} INFLUX (SACI) IN ERK1/2 AND P38 MAPK STIMULATION BY ATP IN OSTEOBLASTS.

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We studied the modulation of intracellular Ca^{2+} concentration ($[Ca^{2+}]I$) and its relation to ERK1/2 and p38 MAPK activation by ATP in ROS 17/2.8 osteoblastic cells. ATP, UTP or ADP (10μM) similarly induced an increase in $[Ca^{2+}]i$ showing that more than one P2Y purinoreceptor subtype is expressed in these cells. Moreover, ATP and UTP, but not ADP, activated ERK1/2 and p38 MAPK suggesting a role for P2Y$_2$ receptors in the ATP action on MAPK. This effect was abolished by cell treatment with 2.5-5 μM Gd^{3+} or a free- Ca^{2+} buffer (plus 0.5 mM EGTA) but not by the intracellular Ca^{2+} chelator BAPTA or voltage dependent Ca^{2+} channels inhibitors (5 μM nifedipine and 5 μM verapamil). Confocal microscopy analysis suggested that purinergic-dependent $[Ca^{2+}]I$ rise was mainly nuclear and due to Ca^{2+} release from intracellular stores. Stimulation by mechanical stress activated a transient Ca^{2+} influx sensitive to Gd^{3+} (SACI) in cells pretreated with ATP or UTP but not ADP. Both ATP dependent -$[Ca^{2+}]I$ mobilization and -SACI were suppressed by suramin (purinergic antagonist) and neomycin (a PI-PLC inhibitor). The results suggest that P2Y$_2$ receptor stimulation by ATP in osteoblasts sensitizes mechanical stress activated Ca^{2+} channels involving PI-PLC activation and leading to calcium influx and a fast phosphorylation of ERK 1/2 and p38 MAPK.

ST-P5.
MODULATION OF ERK1/2 AND P38 MAPK SIGNALING PATHWAYS BY ATP IN OSTEOBLASTS

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This study investigated the modulation of ERK1/2 and p38 MAPK signaling pathways by ATP in a rat osteoblast-like osteosarcoma cell line (ROS17/2.8). We found that ERK1/2 was activated in a dose-dependent manner from 1 to 100 μM ATP, whereas p38 activation reached the maximum at 10 μM ATP. The time-response showed maximum levels of MAPK phosphorylation within 5 min of treatment with 10 μM ATP. This activation was almost completely blocked using neomycin (2.5 mM), an inhibitor of PI-PLC, Ro 318220 (1μM), a PKC inhibitor, and PP1 (50 μM), a potent and selective inhibitor of the Src-family of tyrosine kinases. In addition, ATP stimulated MKK3/6 and c-Src(tyr416) phosphorylation which were inhibited by Ro 318220, suggesting that PKC is an upstream mediator in the signaling cascade. Immunocytochemistry studies supported p38 MAPK activation by ATP and interestingly revealed a nuclear localization of this kinase and a translocation of c-Src into the nucleus. These results show that ATP stimulates the ERK1/2 and p38 MAPK pathways in osteoblasts involving as upstream mediators PI-PLC and PKC, and translocation to the nucleus of Src familiy kinases. In addition, this study demonstrates for the first time nuclear localization of p38.

ST-P6.
CALCITRIOL AND 17β-ESTRADIOL -DEPENDENT ACTIVATION OF MAPKS IN SKELETAL MUSCLE CELLS: ROLE OF ELK-1 AND CREB

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The mitogen activated protein kinases (MAPKs) have been classified into at least six subfamilies, among which ERK1/2, JNK1/2 and p38 MAPK are the most extensively studied. Whereas ERK1/2 is considered to respond to growth signals, JNK1/2 and p38 are activated by cellular stresses. In various cell types, calcitriol (1α,25-dihydroxy-vitamin D_3) and 17β-estradiol promote biological responses through activation of MAPK cascades. We have previously shown that calcitriol stimulates muscle cell proliferation via ERK1/2. In this work, using as experimental model the skeletal muscle cell line C2C12, we demonstrate that calcitriol and 17β-estradiol phosphorylate and activate ERK1/2 and p38 MAPK, in a time-dependent fashion. Maximal effects were seen at 90 and 30 min (ERK1/2) and at 60 and 15 min (p38 MAPK) for calcitriol and 17β-estradiol, respectively. Calcitriol and 17β-estradiol also induced the phosphorylation of CREB and Elk-1 transcription factors in an ERK1/2-dependent manner. Simultaneous addition of both hormones potentiated CREB phosphorylation. Of relevance, Elk-1 phosphorylation dependent on either hormone, was correlated with c-fos oncoprotein expression. These results demonstrate that the ERK1/2 and p38 MAPK signalling pathways play an important role in regulating immediate early genes in the skeletal muscle line C2C12.

ST-P7.
EFFECTS OF α_2-MACROGLOBULIN (α_2M) ON THE ACTIVATION OF INTRACELLULAR SIGNALING PATHWAYS USING CELL LINES WITH DIFFERENTIAL EXPRESSION OF α_2M RECEPTORS

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α_2-M is a broad specific plasma proteinase inhibitor. Upon binding to proteinases, it undergoes a major conformational change that exposes receptor recognition, which is named as (α_2M*). Two surface cell receptors have been proposed for α_2M*: LRP-1 and Grp78. Our results and other authors have demonstrated that α_2M* generate cellular proliferation and activate intracellular signaling pathways such as MAPK and PKB. However, the molecular mechanisms about the α_2M* receptors involved are unclear. In this work we investigated the surface cell receptor responsible to mediate the intracellular signaling pathways by α_2M* using cell lines that express constitutively and differentially both α_2M* receptors. With this propose, we used macrophage derived cell line, J774, which is LRP-1(+) and grp78(-), and the cell line Cho-K1 which is LRP-1(-) and grp78(+). On these cell lines we analyzed the down-stream effect of α_2M*, measuring ERK-MAPK and JNK-MAPK pathways by Western blotting. The main results obtained showed that α_2M* at different concentrations (7, 20, 60 and 180 nM) promoted in J774 and Cho-K1 a differential kinetics of ERK1/2 phosphorilation and C-jun activation. In conclusion, we demonstrated that α_2M* activates intracellular signaling pathways, which are mediated by LRP-1. In addition, this work constitutes the first evidence that LRP-1 can activate the JNK/MAPK pathways.

ST-P8.
INTRACELLULAR SIGNALING FOR ESTRADIOL INDUCED AXOGENESIS

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We have previously shown that 17-β-estradiol (E2) (and the plasma membrane-non permeating E2BSA) induces a sustained and strong phosphorylation of ERK (pERK) that is required for E2-induced axogenesis in cultured neurons from male fetuses. Moreover, the Ca^{2+} antagonist BAPTA and the PKC inhibitor Ro 32-0432 completely abolished the neuritogenic effect of E2. In order to investigate if PKC and intracellular Ca^{2+} were involved in MAPK-activation, after 2 DIV hypothalamic neurones were pre-treated for 1h with Ro 32-0432 or BAPTA-AM, pulsed for 15 min with E2 and harvested for Western blotting. Both treatments significantly reduced pERK, indicating the confluence of signals on the MAPK pathway. We asked whether the changes in pERK were significant enough to affect transcriptional activation. To examine this, we studied the level of CREB phosphorylation, a downstream transcription factor target of MAPK. E2 induced phosphorylation of CREB at Ser133 and the inhibition of MEK1/2 by UO126 completely blocked this effect. In summary, these results demonstrate that E2 induces axogenesis in male-hypothalamic neurons through activation of the MAPK pathway. Concurrently, calcium signalling through activation of PKC converges onto the same pathway. ERK1/2 is phosphorylated, triggering effects on gene transcription via CREB and regulation of the cytoskeletal machinery, both required to induce axon growth.

ST-P9.
REGULATION OF ALTERNATIVE SPLICING IN THE MAMMARY GLAND DIFFERENTIATION
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Alternative splicing generates distinct proteins from a single gene. The fibronectin (FN) gene provides a paradigmatic model for studying alternative splicing regulation. We have previously shown that growth factors (GF) secreted by mesenchymal cells up-regulate FN EDI and IIICS exon inclusion in mammary epithelial cells via PI 3-kinase and, on the contrary, a basement membrane-like extracellular matrix (BM) down-modulates the inclusion of these alternative regions. Using siRNAs and pharmacological inhibitors we showed that this effect is entirely dependent on the activation of JNK. Furthermore, the BM inhibits the effects of GF on alternative splicing, consistent with the opposite roles reported for PI 3-kinase and JNK. The effect of GF can be reproduced by over-expressing a constitutively active form of AKT, and the latter is inhibited by the over-expression of JNK.
On the other hand, treatment with BM triggers ERK dephosphorylation. We are currently investigating its involvement in FN splicing regulation.
These results strengthen our understanding of how extracellular timuli are converted into changes in splicing patterns.

ST-P10.
MAP KINASE PHOSPHATASE-1 GENE EXPRESSION INDUCED BY 8BR-CAMP IS INDEPENDENT OF ERK 1/2 ACTIVATION
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MAP kinase phosphatase-1 (MKP-1) is a dual activity phosphatase involved in the inactivation of MAP kinases (MAKPs). Several stimuli that activate MAKPs also trigger MKP-1 gene induction. We have already demonstrated that cAMP promotes MKP-1 induction in MA-10 Leydig cells. Since it is known that cAMP also promotes ERK1/2 activation in these cells, we aimed to determine if MAPKs mediate MKP-1 induction triggered by cAMP. For this purpose we studied the effect of the ERK1/2 inhibitor PD98059 on MKP-1 mRNA levels in MA-10 cells. Northern blot analysis revealed a notable increase of MKP-1 mRNA levels in cells exposed to 8Br-cAMP (1 h). This effect was not overcome by PD98059, even when this compound was effective to abrogate the ERK1/2 activation promoted by the analogue of the cyclic nucleotide. In addition, we determined that the effects of 8Br-cAMP on MKP-1 and ERK1/2 were both blunted by a PKA inhibitor, H89. Taken together, all these data suggest that in MA-10 cells the PKA activation prompts MKP-1 gene expression by an ERK1/2-independent mechanism.
Supported by CONICET, ANPCyT and UBA.

ST-P11.
ERYTHROPOIETIN NEUROPROTECTIVE EFFECT ON SH-SY5Y CELLS INDUCED TO APOPTOSIS BY TNF-ALPHA
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The human neuroblastoma SH-SY5Y cells are an excellent model for the study of neuroprotection induced by treatment with human recombinant erythropoietin (Epo). We investigated signaling pathways involved in this Epo effect in a model of apoptosis induced by tumor necrosis factor-alpha (TNF). Apoptosis of SH-SY5Y cells was developed by addition of 25 ng/ml TNF for 24 h and detected by typical morphological changes (Hoechst staining) and DNA laddering. Under this condition, a decrease in Epo receptor (EpoR) expression was observed at mRNA and protein levels, assayed by RT-PCR and Western blot, respectively. The pre-treatment for 12 h with 25 U/ml Epo attenuated the signs of apoptosis observed under the effect of TNF while the levels of EpoR expression was recovered. An increased expression of bcl-2 at mRNA and protein levels, but no changes in bcl-x, bax and c-Flip mRNA levels were also detected. An assay in the presence of the inhibitor wortmannin showed that the antiapoptotic effect of Epo was, at least in part, mediated by PI3K.
In conclusion, the results show an Epo neuroprotective capacity to antagonize TNF-induced apoptosis in SHSY5Y cells, action that is partially dependent on the modulation of EpoR and also associated with the increased expression of the antiapoptotic protein Bcl-2.

ST-P12.
INSULIN AND IGF-1 SIGNALING INVOLVES PI3K ACTIVATION IN CEREBRAL CORTEX SYNAPTIC ENDINGS
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Phosphatidylinositol 3-kinase (PI3K) phosphorylates the 3'OH position of the inositol ring of phosphoinositides. PI3K signaling is activated by a variety of extracellular stimuli and has been implicated in a wide range of cellular processes including cell cycle progression, cell growth, cell motility, cell adhesion and cell survival. We have previously demonstrated the presence of PI3K and its activation under oxidative stress conditions in cerebral cortex synaptic endings. The goal of this work was to study the involvement of PI3K in neurotrophic factor action in the synaptic terminal. For this purpose we evaluated the phosphorylation of poliphosphoinositides in synaptosomes incubated in the presence of either insulin (175 nM) or IGF-1 (15 nM) and 5 µCi [γ-^{32}P]ATP. Insulin increased phosphatidylinositol monophosphate (PIP) levels by 80% with respect to control conditions at 5 min incubation. On the other hand, IGF-1 stimulated PI kinase by 187% compared to the control. LY294002, a selective PI3K inhibitor, abolished PI kinase activation. Additionally, we demonstrated that both growth factors were able to increase Akt phosphorylation at serine 473 position. Our results demonstrate the involvement of PI3K/Akt pathway in insulin and IGF-1 signaling cascade in cerebral cortex synapses.

ST-P13.
ANGIOTENSIN II MODULATES INSULIN-INDUCED TYR-PHOSPHORYLATION OF IRS-4, IN RAT LIVER MEMBRANES

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Angiotensin II (Ang II), a major regulator of blood pressure, is also involved in the control of cellular proliferation and hypertrophy. As hypertension and Insulin (Ins) resistance are risk factors for cardiovascular diseases, Ang II and Insulin cross-talk may have an important role in hypertension development.
The effect of Ins on protein tyrosine phosphorylation was assayed in rat liver membrane preparations, a rich source of Ins receptors. Ins (10^{-7} M) induced tyr-phosphorylation of different proteins. We observed that Insulin consistently induced tyr-phosphorylation of a 160 kDa protein (pp160) with maximum effect between 1 and 3 min. The pp160 protein was identified by anti-IRS-4 but not by anti-IRS-1 antibody. Pre-stimulation with Ang II (10^{-7} M) diminishes tyr-phosphorylation level of pp160/IRS-4 in a dose-dependent manner. Okadaic acid, the Ser/Thr phosphatase inhibitor, increases the Ins-induced pp160 phosphorylation and prevents the inhibitory effect of Ang II prestimulation. Genistein, a tyrosine kinase inhibitor, diminishes tyr-phosphorylation level of IRS-4. PI3K inhibitors Wortmanin and LY294002, both increase tyr-phosphorylation of IRS-4, either in the presence of Ins alone or combined with Ang II. These results suggest that Ins and Ang II modulate IRS-4 tyr-phosphorylation in a PI3K-dependent manner.

ST-P14.
CALCIUM CHANNELS OF THE INOSITOL-1,4,5-TRISPHOSPHATE / RYANODINE RECEPTOR SUPERFAMILY IN *Trypanosoma cruzi* EPIMASTIGOTES FORMS

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Intracellular calcium plays a crucial role as a second messenger for the control of a variety of cellular process in *Trypanosoma cruzi*, such as differentiation, invasion into mammalian cells and adaptation to new environment of their different hosts. Channels involved in calcium release from intracellular store in the parasite has not been yet characterized. A search in protein database (BLATp) revealed the presences of proteins in *T. cruzi* with characteristics to IP_3/ryanodine receptor superfamily. By inmumoprecipitation and fluorescent confocal microscopy we observed an inmunoreactions with antibody anti-IP_3 type II receptor from humans. The treatment of the parasite loaded with fura2-AM with ryanodine evoked the calcium release in a fashion manner dependent of the concentration. Moreover, the addition of the caffeine, activator of ryanodine receptor, was also able to induce an increase of intracellular calcium. These results suggest that *T. cruzi* possess intracellular calcium release channel with similar properties of the IP_3/ryanodine receptor superfamily.

ST-P15.
ELECTRICAL STIMULATION ACTIVATES PI3K AND PLCγ1 IN SKELETAL MYOTUBES

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During tetanic stimulation of myotubes, a fast calcium signal related to excitation-contraction coupling can be seen. Seconds after stimulus ends, a slow calcium signal dependent on IP_3 and PLC (associated regulation of gene expression) is evident. PLC isoforms involved in this process are unknown. We show activation of both PI3K and PLCγ1 after electrical stimulus. Furthermore, we determined PIP3 mass increases peacking 40 seconds after stimulus ended (74 ± 16% over control, n=5). Blockage of PI3K by wortmanine completely inhibited both the IP3 rise and slow calcium signal. Moreover, tetanic stimulation induced PLCγ1 phosphorylation with kinetics consistent with IP_3 production and slow calcium signal. Immunohistochemistry for phosphorylated PLCγ1 show a perinuclear and cytoplasmic location. This phosphorylation was blocked by wortmanine. PI3Kγ shows a distribution consistent with location of t-tubules. These results suggest a possible participation of PI3Kγ in the activation of PLCγ1. This sequence appears to be part of the depolarization induced signaling pathway associated to calcium regulated adaptive responses in muscle cells.
Financed by FONDAP 151006.

ST-P16.
CALCIUM REQUIRES cAMP TO INDUCE ACROSOMAL EXOCYTOSIS THROUGH A PKA-INDEPENDENT, EPAC-MEDIATED PATHWAY

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Epac binds to and is activated by the second messenger cyclic adenosine 3´, 5´-monophosphate (cAMP). In sperm, various signaling pathways required to achieve egg-fertilizing ability depend on the intracellular rise of cAMP. Most of these processes were thought to be mediated by cAMP-dependent protein kinases (PKA). Here we report a new pathway for cAMP-induced acrosome reaction (AR) involving Epac. The AR is a specialized type of regulated exocytosis leading to a massive fusion between the outer acrosomal and the plasma membranes of sperm. Calcium is the paradigmatic trigger of regulated exocytosis and we show here that its effects on acrosomal release are fully mediated by cAMP. Calcium failed to trigger the AR when intracellular cAMP was depleted by an excess phosphodiesterase or when Epac was sequestered by specific blocking antibodies. The non-discriminating dibutyrylcAMP and the Epac-selective 8-pCPT-2Me-cAMP analogues triggered the AR in the absence of cytosolic calcium. This indicates that cAMP - via Epac activation - has the ability to drive the whole cascade of events necessary to bring exocytosis to completion, including tethering and docking of the acrosome to the plasma membrane, priming of the fusion machinery, mobilization of intravesicular calcium, and ultimately, bilayer mixing and fusion. cAMP-elicited exocytosis was sensitive to anti-alpha-SNAP, anti-NSF and anti-Rab3A antibodies, to intra-acrosomal calcium chelators, and botulinum toxins, but was resistant to PKA blockers. These experiments now identify Epac in human sperm and define its indispensable role in exocytosis.

ST-P17.

ADENYLYL CYCLASE ISOTYPE 7, PARTICIPATES IN *Xenopus laevis* OOCYTE MATURATION

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Xenopus laevis oocyte maturation is induced by the steroid hormone progesterone through a non genomic mechanism that implicates the inhibition of the effector system adenylyl cyclase (AC). Recently, it has been shown that the $G_{\beta\gamma}$ protein heterodimer is involved in *Xenopus* oocyte cell cycle arrest. Since AC is the proposed target for $G_{\beta\gamma}$action, we considered of importance to analize the function of the $G_{\beta\gamma}$ regulated AC7 isoform in oocyte maturation. Here we show that microinjection of AC7 mRNA caused inhibition of progesterone-induced oocyte maturation and on the contrary, depletion of AC7 by microinjection of siRNA, potentiated the maturation process. To confirm xAC7-$G_{\beta\gamma}$ regulation, we constructed a minigene encoding the AC-$G_{\beta\gamma}$ interacting region (xAC7pep) to block within the oocyte this interaction. We found that microinjection of xAC7pep accelerated progesterone-induced maturation, as did AC2pep minigene, indicating that AC peptides are probably blocking G?? interaction with oocyte endogenous xAC7. Direct physical interaction between $G_{\beta\gamma}$ and xAC7 was also demonstrated by the yeast two-hybrid system. From these results we can conclude, that a $G_{\beta\gamma}$-activated AC is playing an important role in the *Xenopus* oocyte, by maintaining it in meiotic arrest.

Proyecto FONDECYT Nº 1030806.

ST-P18.

COMPARTMENTALIZED CAMP-DEPENDENT SIGNALING IN *T. CRUZI* BY TWO DIFFERENT PHOSPHODIESTERASES

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Cyclic nucleotide-specific phosphodiesterases (PDEs) are key regulators of cAMP signaling pathways by controlling the spatial and temporal levels of intracellular cAMP. It has been proposed that compartmentalization of cAMP related enzymes is important for the regulation and specificity of cAMP signaling.

In the present work, we report the identification of two novel phosphodiesterases form *T. cruzi*, *Tc*PDE4 and *Tc*PDE-D.

Both were able to complement a heat-shock-sensitive yeast mutant deficient in phosphodiesterase genes.

*Tc*PDE4 presents three conserved domains, FYVE, phosphohydrolase and PDEaseI, shows the inhibition profile characteristic for PDE4 subfamily and is specific for cAMP with an intermediate Km value of about 20µM. This enzyme remained associated to membrane structures in recombinant yeast cells and confocal laser scanning microscopy of *T. cruzi* epimastigotes indicates that *Tc*PDE4 seems to be located in endosome-like vesicles, according to the presence of its FYVE domain. On the other hand, *Tc*PDE-D doesn't present other functional domains besides the catalytic domain. Finally, subcellular fractionation of *T. cruzi* epimastigotes followed by Western blot assays revealed that this enzyme is located mainly in the citoplasmatic fraction.

ST-P19.

DIMORPHIC SHIFT OF *CANDIDA ALBICANS* MUTANT STRAINS LACKING A *BCY1* ALLELE ENCODING THE REGULATORY SUBUNIT OF PKA

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We investigated the morphogenetic behavior of several *C. albicans* mutant strains bearing one or both *BCY1* alleles, coding for the PKA regulatory subunit (R) in a wild-type and in a *TPK2* null genetic background, as well as that of different mutants having one *BCY1* allele fused to GFP. Morphological changes were assessed in both, solid and liquid media, at 37°C. Strains bearing a unique *BCY1* allele, tagged or not, behaved similarly, displaying pseudohyphae and true hyphae; they also expressed a minor quantity of Bcy1p. In contrast, in strains having both *BCY1* alleles, irrespective of the GFP insertion, hyphal morphology was almost exclusive, indicating that the GFP insertion did not interfere with Bcy1p function. DE-52 column chromatography of soluble extracts of yeast cells from strains bearing one *BCY1* allele (normal and fused to GFP) and subsequent western blot analysis showed co-elution of Bcy1p and Bcy1p-GFP with phosphotransferase activity, indicating that interaction between regulatory and catalytic subunits was not impaired by the GFP tag. Modeling of *C. albicans* Bcy1p, using as templates the coordinates of the crystallized rat and bovine R subunits, revealed a perfect overlap of the three backbones, suggesting a similar tertiary structure.

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ST-P20.

MOLECULAR MECHANISM OF PROTEIN KINASE A PHOSPHORYLATION DURING TRANSITION FROM RESPIRATORY TO FERMENTATIVE METABOLISM IN *SACCHAROMYCES CEREVISIAE*

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In *S. cerevisiae*, the cAMP dependent protein kinase (PKA) has three partially redundant TPK1, TPK2 and TPK3 genes encoding the catalytic subunits. Glucose-dependent activation of PKA activity changes the phosphorylation state of its Tpk1. Strains carrying inactive Tpk1 isoform (*tpk1K116R* or *tpk1^{w1}*) were transformed with plasmids expressing Tpk2, Tpk3 or Tpk1-HA. A cAMP peak was triggered by glucose addition to glycerol-growing cells; during this peak we measured PKA activation *in situ* in permeabilized cells,PKA-dependent phenotypes and the phosphorylation state of *tpk1*, followed by native western blot. The phosphorylation state of the inactive Tpk1 molecule did not change upon PKA activation, remaining in a low phosphorylation state. The results suggest an intramolecular phosphorylation mechanism of Tpk1. Peptides were designed based on Tpk1 sequence in order to identify which aminoacid is phosphorylated in Tpk1. Eight peptides containing these sites were synthesized. *In situ* and *in vitro* assays using permeabilized cells or purified Tpk1TAP respectively, indicate that only one peptide (LLRKSQRFP) was substrate for the kinase, suggesting that S^{179} is a potential phosphorylation site in the whole Tpk1 protein.

ST-P21.
IDENTIFICATION OF PIN-TYPE PEPTIDYL-PROLYL ISOMERASE IN *Trypanosoma cruzi*

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Peptidyl-prolyl isomerases (PPIases) are an evolutionarily conserved group of proteins that support the *cis-trans* isomerization of the peptide bond preceding Pro residues. Prolyl isomerases include three major subfamilies; the cyclophilins, FK506 binding proteins (FKBPs) and the parvulins. Recently, we have identified in *T. cruzi*, two genes members of the parvulin family of the PPIases called TzPin1 and TzPin2. These Pin1-type PPIases in parasites have a distinctive catalytic domain but not N-terminal protein-protein binding domain (WW domain) or analogous module. As hPin1, the substrate specificity of the recombinant TzPin1 indicates a preference for acidic residues NH_2-terminal to the proline. To approach a functional assay, we used the temperature-sensitive YGD-*ts22* strain of *S. cerevisiae* known to be mutated in the ESS1 gene locus. The experiment showed that only the overexpression of TzPin1 was able to restore the function of ESS1 despite the lack of the WW domain. Western blot analysis using the TzPIN1 specific antiserum detected an 11kDa band in the cell extract from epimastigote stage. Pro isomerization has been seen to be important in key cellular function, and the discovery of a functional hPin1 homologous will shed light on the cellular implication of Pro isomerization in trypanosomatids.

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ST-P22.
THE CYTOSKELETON IN THE MODULATION OF NF-kB IN NEURONAL CELLS

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In neuronal cells, NF-kB participates in the transduction of synaptic stimuli into changes in gene expression. One effect of zinc deficiency is an alteration in the cytoskeleton. In this study, we examined if zinc deficiency-induced alterations in the cytoskeleton contribute to impairment in NF-kB nuclear translocation.Human neuroblastoma IMR-32 cells were incubated in control or zinc deficient (1.5Zn) media without or with 0.5 µM vinblastine (VB), 0.5 µM colchicine (Col) or 0.5 µM cytochalasin D (Cyt) for 24 h. Cells incubated with the cytoskeleton disrupting drugs showed impaired NF-kB nuclear translocation. A similar distribution was observed in the 1.5Zn cells. This altered nuclear translocation was accompanied by a lower transactivation of NF-kB-driven genes. Altered RelA distribution was observed in the 1.5Zn, VB, Col and Cyt cells. An impaired interaction among dynein, importin a and tubulin was observed in the 1.5Zn, VB and Col groups. Thus, zinc deficiency can result in a disruption of the cytoskeleton that impairs NF-kB nuclear translocation, with consequential reductions in the expression of NF-kB-dirven genes. We suggest the above as a mechanism underlying the brain pathologies associated with zinc deficiency.

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ST-P23.
FOCAL ADHESION AND STRESS FIBER FORMATION TRIGGERED BY THY-1 IN ASTROCYTES DEPENDS ON ITS INTERACTION WITH SYNDECAN-4

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Our reported data indicate that Thy-1 interacts with $\alpha v\beta_3$ integrin on astrocytes and stimulates astrocytes to form focal adhesions (FAs) and stress fibers (SFs) in a manner that is dependent on $\alpha v\beta_3$ integrin clustering and activation of the small GTPase RhoA. FAs mediate adhesion to a matrix and their formation not only involves integrins but also proteoglycans like syndecan-4. Interestingly, Thy-1 posssesses a heparin binding domain and direct interaction of Thy-1 with sulfated glycans has been reported. FA and SF formation through syndecan-4 in fibroblasts requires the activation of PKCα and RhoA. Thus, the hypothesis that Thy-1-stimulated FA and SF formation in astrocytes requires both $\alpha v\beta_3$ integrin and syndecan-4 receptors was tested. Astrocytes were found to express syndecan-4 mRNA and protein. Thy-1-induced RhoA activation was inhibited by heparin in a dose dependent manner. Additionally, preliminary results show that treatment with heparitinase or the PKCα inhibitor Gö 6976, decreased RhoA activation and FA and SF formation. The participation of PKCα in these events will be confirmed by transfecting with adenovirus constructs for wt and dominant-negative PKCα. Participation of syndecan-4 in the Thy-1-induced response will also be demonstrated using siRNA technology.

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ST-P24.
ATM/ATR ACTIVATION IS INVOLVED IN p19INK4d INDUCTION IN RESPONSE TO DNA DAMAGE BY MULTIPLE GENOTOXICS

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p19INK4d is a member of INK4, a family of proteins involved in cell cycle regulation causing CDK4/6 inhibition. Recently, this protein has been implicated in the cellular response evoked by UV-damaged DNA. The aim was to investigate the role of p19 in DNA damage response and to characterize the signal transduction pathways involved. SH-SY5Y neuroblastoma cells treated with the antitumoral drug *cis*platin or β-amyloid peptide cause a doses-dependent increase of p19 mRNA levels, as determined by Northern blot. p19-overexpressing cells treated with any of the aforementioned genotoxics displayed and enhanced DNA repair and were more resistant to apoptosis, as determined by unscheduled DNA synthesis and caspase-3 activity assays, respectively. Opposite effects were observed in p19-deficient cells. In WI38 human fibroblasts 5 mM caffeine, an ATM/ATR inhibitor, blocked *cis*platin-mediated p19 induction, although the basal expression remain unaltered Immunoprecipitation assays demonstrated that p19 was not only induced but phosphorylated in response to *cis*platin. The present results confirm a role of p19 in the response to DNA damage caused by several genotoxics and suggest the involvement of PI3 kinase-like proteins, ATM/ATR, in p19 induction/activation.

ST-P25.
EXTRACELLULAR INOSINE IN TUMOR NECROSIS FACTOR-ALPHA INDUCED NITRIC OXIDE PRODUCTION IN CULTURED SERTOLI CELLS

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Recent reports have described purinergic modulation of tumor necrosis factor-alpha (TNF-α) signaling in neutrophils and astrocytes. In Sertoli cells, both TNF-R 1 and TNF-R2 TNF-α receptors are present and this cytokines modulates many functions of these cells related to the maintenance of spermatogenesis. Sertoli cells express distinct purinoreceptors and previous work has shown that these cells secrete extracellular nucleotides and their metabolites. In this work, we studied the possible role of extracellular purines in TNF-α signaling in cultured Sertoli cells. This cytokine increased inosine concentration from 30 min to 6h with no effect at 24h. Both TNF-α and inosine increased nitrite accumulation and nitric oxide synthase activity. Erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA), an adenosine deaminase inhibitor, abolished the TNF-α induced inosine increase, nitrite accumulation and nitric oxide synthase activity. These results suggest that extracellular inosine acts as intermediary in TNF-α stimulated nitric oxide production in cultured Sertoli cells.
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ST-P26.
LPS TRANSACTIVATION OF MACROPHAGE 1-HYDROXYLASE: ROLE IN LOCAL CONTROL OF IMMUNE RESPONSES

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The present studies examined the mechanisms mediating lps in inducing 25-d 1-hydroxylase mrna expression, using the murine macrophage cell line raw 264.7 and luciferase reporters driven by the whole[-1651, +22] and minimal[-85+22] murine 1-hydroxylase promoters. We found direct transactivation of 1-hydroxylase by lps in a dose and time dependent manner, and lps+γifn synergism in the transactivation of both promoters. Lps induction of whole promoter activity was 5.4-fold higher than that of the minimal promoter. Experiments with inhibitors of pathways that are activated by lps in macrophages combined with truncation/deletion analysis and emsa assays confirmed a role for jnk-ap1 activation in the response to lps. Next, we examined the role of c/ebpß in lps induction of minimal promoter activity. The substitution of the consensus core of the c/ebpß site in the promoter, resulted in a 40% reduction of minimal promoter activity in response to lps. Nuclear extracts of raw cells treated with lps showed increased expression of c/ebpß in a dose dependent manner. Specific inhibition of pka-mediated c/ebpß phosphorylation, using h89, caused a marked reduction in the reponse to lps. Thus, lps directly transactivates macrophage 1-hydroxylase through c-jun and c/ebpß-mediated mechanisms.

ST-P27.
EXTRACELLULAR INOSINE MODULATION OF RAW 264.7 MACROPHAGES RESPONSE TO BACTERIAL WALL COMPONETS

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Macrophages activation is involved in the innate immune response to bacterial infections. Bacterial wall components such lipopolysacharide (lps), for gram negatives, and lipoteichoic acid (lta) and peptidoglycan (peg), for gram positives, are recognized by macrophages trough toll-like receptors, leading to inflammatory response. Inosine is an anti-inflammatory endogenous nucleoside and it is released into the extracellular space at times of cellular stress. In macrophages, this nucleoside was described to reduces some events related to inflammation, including cytokines and nitric oxide production. We evaluated extracellular inosine modulation of raw 264.7 macrophages response to lps, lta and peg. Lps, lta and peg stimulated nitrite accumulation in macrophages cultures. No effect of extracellular inosine was observed in lta and peg stimulated nitrite production. However, extracellular inosine abrogated lps induced nitrite and reactive oxygen species production. No effect of inosine was observed in lps increased metalloproteinase (mmp) 9 activity, although extracellular inosine alone increased mmp 9 activity. Our results show that extracellular inosine has distinct effects in the response of macrophages to gram negative or positive bacterial wall components.
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ST-P28.
NITRIC OXIDE AND PHOSPHATIDIC ACID SIGNALING DURING STOMATAL CLOSURE

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Nitric oxide (NO) and Phosphatidic acid (PA) are two emerging molecules in plant signaling. It has been reported that both NO and PA are produced in guard cells in response to the plant hormone abscisic acid (ABA) during stomatal closure. In addition, the absence of any of this two signaling components, partially blocks ABA induction of stomatal closure. Since NO and PA signaling pathways share some common components (i.e, ion channels, calcium, etc), we decided to study the occurrence of a cross talk between both pathways in guard cells. PA is generated via phospholipase D (PLD) or via phospholipase C (PLC) in concerted action with diacylglycerol kinase (DGK). With that aim, we treated *Vicia faba* epidermal strips with the NO donor SNAP in presence or absence of specific inhibitors of PA synthesis enzymes. Results showed that primary alcohols (inhibitors of PA formation via PLD activation) and PLC inhibitors (neomycine and U73122) blocked NO induction of stomatal closure. We have also set up a system to measure *in vivo* phospholipid production in *V.faba* guard cells. An increase in PA content during NO-induced stomatal closure was observed. These results suggest that, in *V. faba* guard cells, PA is required for NO-dependent stomatal closure.
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ST-P29.
PHOSPHOLIPID AND NITRIC OXIDE SIGNALING INVOLVED IN AUXIN-INDUCED ADVENTITIOUS ROOT FORMATION

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We have previously demonstrated that nitric oxide (NO), cGMP and MAP kinases are involved in the auxin response during adventitious root formation (ARF) in cucumber (*Cucumis sativus*). Additional evidence supported an involvement of Ca^{2+} and Ca^{2+}-dependent protein kinase activity during ARF (Pagnussat et al. Plant Physiol. 2003 132:1241-1248, 2004 135:279-286; Lanteri *et al*., unpublished). Since blockers of IP_3-regulated Ca^{2+} channels suppressed the ARF induced by either the auxin indole acetic acid (IAA) or NO, we hypothesized that the enzyme phospholipase C (PLC), which catalyses the formation of IP_3 and diacylglycerol (DAG), might be part of the signaling cascade that trigger ARF. In plants, DAG is subsequently converted to phosphatidic acid (PA) through the action of the enzyme DAG kinase. Therefore, we measured the *in vivo* levels of PA in response to NO and auxins. Primary roots of 7-days-old seedlings were removed, and cucumber explants were labeled with [$^{32}P_i$] and then treated with the NO donor SNAP or with IAA. Both PA and phosphatidylinositol phosphate accumulated within 10 minutes of treatment. This accumulation was dose dependent. Interestingly, these 10 minute-NO or auxin treatments were sufficient to induce ARF when measured 5 days later. Further investigations will be directed to elucidate the role of early responses leading to ARF.

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ST-P30.
INFILTRATION OF INFLAMMATORY CELLS PLAYS AN IMPORTANT ROLE IN MATRIX METALLOPROTEINASE EXPRESSION AND ACTIVATION IN THE HEART DURING SEPSIS

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Septicemia is an emerging pathological condition that involves among other effects, refractory hypotension and heart dysfunction. We have investigated the contribution of resident non-myocytic cells to heart alterations in wild type and NOS-2-KO mice under septic shock condition by LPS administration. These cells contribute to the rapid infiltration of additional inflammatory cells that enhance the onset of heart disease through the release of inflammatory mediators. Our data shows that early activation of resident monocytic cells plays a relevant role on the infiltration process, mainly of MHC II and CD11b positive cells determined by Western blot and immunohistochemistry. The infiltration was significantly impaired in NOS-2-KO mice or after pharmacological inhibition of NOS-2 or COX-2 since cardiomyocytes failed to express these enzymes. However, heart tissue and cardiomyocytes cells lines express and activate MMP-9 through mechanisms regulated, at least in part, by NO and PGs in an additive way, as was studied by real time PCR, Western blot, immunohistochemical and enzyme activity assays. These results provide a direct link between the inflammatory response in the and ECM remodelling by the MMPs released by the cardiomyocytes. These data suggest that the activation recruitment of inflammatory cells to heart is a major early event in the cardiac dysfunction promoted by septicemia and septic shock.

ST-P31.
STAT3, STAT5A AND STAT5B REGULATION IN HC11 MAMARY EPITHELIUM CELLS

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Signal transducer and activator of transcription (STATs)-mediated gene regulation has been implicated in cellular functions with relevance to a variety of processes. Stat proteins play a crucial role in normal and tumoral mammary epithelium. Stat5 is strongly activated towards the end of pregnancy and persists in an activated state until cessation of suckling. Contrary, Stat3 activation is hardly detectable during lactation, but is strongly induced at the onset of involution. The aim of our work is to study the regulation of the activation of Stat3 and Stats5 to determine if exists crosstalk between them. Here, we study the levels of p-Stat3, p-Stat5a and p-Stat5b in HC11 cells, treated with different hormones pretending the cellular context in the distincts phases of the mammary epithelium. We confirmed that LIF activates Stat3 and found that in this context, there is a decrease in the levels of p-Stat5b. The levels of p-Stat3 were not increased with prolactin treatment, that induced β-casein expression in competent cells. As in transient transfection assays, we determined that Stat5a, Stat5b and Stat5a/Stat5b heterodimer repressed *bcl-X* promoter 1 (P1), we determined the levels of transcription from P1 by RT-PCR in the different conditions. We did not find any difference respect to the basal control. This is not in accordance with the levels of the p-Stats analyzed, but this could reflect the presence of another factor/s regulating P1. We are now studying the levels of p-STATs in cells treated with both stimulus, lactogenic hormones and LIF.

ST-P32
β-LAPACHONE ACTIVATES A MRE11P-TEL1P G1/S CHECKPOINT IN BUDDING YEAST

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ß-lapachone is an anticancer agent that selectively induces cell death in several human cancer cells. The mechanism of ß-lapachone cytotoxicity remains unknown. Here we report that ß-lapachone was cytotoxic in the budding yeast *Saccharomyces cerevisiae*. ß-lapachone treatment delayed cell cycle progression at the G1/S transition and incremented phosphorylation of the Rad53p checkpoint kinase. These checkpoint responses were regulated by Mec1p and Tel1p kinases. Mec1p was required for Rad53p phosphorylation and cell survival following ß-lapachone treatment in asynchronous cultures, but not for the G1 delay. The *tel1*Δ mutation increased sensitivity to ß-lapachone in a *mec1* defective strain and compromised checkpoint responses in G1. Both Rad53p phosphorylation and G1 delay were fully dependent on a functional Mre11p-Rad50p-Xrs2p (*XMR*) complex, and mutants in the *XMR* complex were hypersensitive to ß-lapachone treatment. Finally, *XRS2* and *TEL1* worked epistatically regarding ß-lapachone sensitivity and Xrs2p was phosphorylated in a Tel1p dependent manner after ß-lapachone treatment. Taken together, these findings indicate that ß-lapachone activates a Mre11p-Tel1p checkpoint pathway in budding yeast. Given the conserved nature of the Mre11p-Tel1p pathway, these results suggest that activation of the Mre11-Tel1p checkpoint could be of significance for ß-lapachone antitumour activity.

ST-P33.
MUSCARINIC M4 RECEPTOR FUNCTION IN MEMORY CONSOLIDATION
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Specific cholinergic muscarinic agonists enhance while antagonists disrupt memory. There are five muscarinic receptor subtypes (MAChR) and more than one subtype is expressed in the same cell. M1, M3 and M5 MAChR usually interact with Gq proteins, while M2 and M4 subtypes mainly act through Gi proteins inhibiting Adenylyl Cyclase (AC). The role of each MAChR in learning and memory is poorly understood due to the lack of selective ligands. The selective muscarinic toxins (MTs), snake venom peptides, allowed gaining insight into the functions of different MAChR. We used MT3, an antagonist highly selective for M4 subtype, to inhibit ^{3}H-scopolamine binding to evaluate the proportion of M4 receptors in the rat brain and we found that M4 is predominant in the hippocampus. MT3 caused amnesia when injected in the hippocampus after training rats in different tasks. MT3 *in vitro* reversed the muscarinic inhibition of AC activity. The high proportion of M4 together with the positive effect on memory consolidation and the inhibitory action on AC, strongly support a positive modulatory function for this receptor in the hippocampus.

ST-P34.
THE INTRAMITOCHONDRIAL ARACHIDONIC ACID RELEASE IS NEEDED FOR CHOLESTEROL TRANSPORT
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We have previously described that the Arachidonic Acid- preferring Acyl-CoA synthetase 4 and the mitochondrial Acyl-CoA thioesterase I (Acot2) regulate, in a concerted mode, the intracellular levels of AA and the steroids production. Steroidogenic cells express Acot2 but also a cytosolic isoform (Acot1) which is 92.5% homologous to the mitochondrial enzyme. The aim of this study was to determinate the role of Acot1 in the steroidogenesis. For this purpose, we overexpressed Acot1 in the MA-10 Leydig cell line. Cell transfection with a plasmid containing the full sense Acot1 cDNA produced a clear increase in the Acot1 expression level as analyzed by immunocitochemistry and western blot. The Acot1 overexpression did not affect the cell viability as assessed by Trypan Blue exclusion method. The 22-OH cholesterol-sustained steroid synthesis in the Acot1 over expressing cells was not affected. A little decrease of steroid production (% of inhibition: 21.99 ± 2.2) was evidenced when we studied the effect of Acot1 overexpression on cAMP-stimulated steroidogenesis although Acot2 overexpression produced a significant increase in the levels of steroid synthesis (% of stimulation: 66.3 ± 6.1) both compared with mock-transfected cells.
From these results we conclude that only the isoform Acot2 participates in the steroid synthesis, hence, the intramitochondrial AA release is needed for cholesterol transport. Further studies have been made to determinate de role of Acot1 in these cells.

ST-P35.
INSULIN PROMOTES DIACYLGLYCEROL KINASE (DAGK) ACTIVATION BY DIFFERENT MECHANISMS IN RAT CEREBRAL CORTEX (CC) SYNAPTOSOMES
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Insulin action on DAGK was investigated in CC synaptosomes from adult (3-4 mo) rats. The roles of PIP_2 phospholipase C (PIP2-PLC) and PC phospholipase D (PC-PLD) coupled to phosphatidate phosphohydrolase type 2 (PAP2) in the DAGK activation by Insulin were investigated. Neomicyn, an inhibitor of PIP2-PLC; as well as ethanol, an inhibitor of phosphatidic acid (PA) formation by the transphosphatidyl reaction of PC-PLD and DL propranolol, an inhibitor of PAP2 were used in the present study. Insulin (0,1 μM) increased PA synthesis through [γ-^{32}P]ATP and endogenous DAG as co-substrate. This activated synthesis was strongly inhibited either by ethanol or DL propranolol. Insulin effect on PA synthesis was also observed in the presence of Neomicyn. When exogenous unsaturated (18:0-20:4) DAG was present, Insulin increased PA synthesis. However, this stimulatory effect was not observed when exogenous saturated (di-16:0) was present. Taken together these data indicate that the increased synthesis of PA by Insulin can be mediated by the activation of both, a PC-PLD/PAP2 pathway to provide DAG and a direct DAGK activation that is associated to the utilization of a 18:0-20:4 DAG specie. The stimulatory Insulin action on DAGK activity was abolished when R59022, a DAGK inhibitor, was present. Under this condition, an increased accumulation of PIP2 is observed, thus suggesting that this lipid is associated to DAGK activation by Insulin.

ST-P36.
T_2 RECEPTOR INDUCE TYR-PHOSPHORYLATION OF SHP-1 PHOSPHATASE IN PND15 RAT HINDBRAIN
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Ang II participates in cell growth control and organogenesis by interacting with its receptors. The aim of the present study was to analyze the signal transduction mediated by Ang II receptors in cerebellum and brainstem in postnatal day 15 (PND15) rats. We described that Ang II (10^{-7} M) elicits protein tyrosine dephosphorylation in hindbrain (PND15) membrane preparations. The selective AT_2 antagonist PD 123319 blocked Ang II-induced tyr-phosphorylation. The phosphatase inhibitor, Na_3VO_4 (2 mM), blocks Ang II effect, suggesting the coupling of the receptors to a protein tyr-phosphatase (PTPase). Inmunocomplexes obtained with anti-SHP-1 antibody and developed with anti-PY99 antibody showed that Ang II induces tyr-phosphorylation of SHP-1 within 5 min, in a dose-dependent form. In order to demonstrate the association of SHP-1 to AT_2 receptors we performed immunoprecipitation assays with anti-AT_2 antibody and developed with anti-SHP-1. We showed that immunocomplexes obtained with anti-AT_2 antibody contained SHP-1 phosphatase. Since AT_2 receptor has no intrinsic PTK activity, we assayed the presence of Src in the immunocomplexes. Src was present in Ang II stimulated immunocomplexes suggesting that Src is responsible of SHP-1 tyr-phosphorylation. These results suggest a potential role of AT_2 in growth and differentiation by activating PTPase SHP-1 and the possible involvement c-Src in SHP-1 activation.

ST-P37.
CHARACTERIZATION OF THE INTERACTION BETWEEN *BACILLUS SUBTILIS* DESK AND ITS COGNATE RESPONSE REGULATOR DESR
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The *des* gene of *Bacillus subtilis* codes for an acyl-lipid Δ5-desaturase, Δ5-Des, which introduces double bounds in membrane phospholipids. Its expression is strictly regulated in response to membrane fluidity changes, by the two-component system DesK/R, composed of a membrane associated kinase, DesK, and a soluble transcriptional regulator, DesR.
To give additional biochemical and biophysical support to this model, we studied the interaction between DesK and DesR by Far Western Blots. We found that purified DesR is capable of interact with the cytoplasmatic domain of DesK (DesKC) fixed to a nitrocellulose membrane. We also found a 66 kDa subunit protein that being recognized by immunopurified anti-DesR antibodies, interacts with DesKC *in vitro* and its level is increased in the double mutant YvfT/U. The *in vivo* analysis of the DesK/R and DesK/Δ5-Des interactions will be performed by double hybrid systems assays. In addition, in order to structurally characterize this interaction we are performing crystallization assays. Until now, we had obtained crystals of DesKCV188, a punctual mutant derived from DesKC. Moreover, we performed *in vivo* studies of the localization of DesK/R and Δ5-Des by fluorescence microscopy.

ST-P38.
NON-CLASSICAL LOCALIZATION OF 17β-ESTRADIOL BINDING PROTEINS IN C2C12 MUSCLE CELL LINE
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The classical isoform of the estrogen receptor (ERα) has been reported to localize almost exclusively in the nucleus. However, studies on non-genomic steroid effects have also suggested the existence of receptors residing at the cell surface. In this work we present biochemical and immunological data supporting extra-nuclear ERα localization in the C2C12 muscle cell line. Immunocytological studies revealed that estrogen binding proteins have mitochondrial and perinuclear localization. The immunoreactivity was estrogen-responsive since the hormone impeded the binding of a specific antibody against the steroid ligand domain of the ERα. These results were confirmed by the detection of estrogen binding sites using fluorescent estrogen-BSA conjugates and binding assays, in which tritiated estradiol could be partially displaced by different estrogen agonists and antagonists. Western blot detection and protein purification of ERα in subcellular fractions using estrogen-BSA in affinity chromatography and specific antibodies confirmed that ERα-like proteins could be expressed at the mitochondrial-microsomal level. The non-classical distribution of native pools of ERα-related immunoreactivity suggests an alternative mode of ERα localization/function in muscle cells.

BL-P1.
CENTRAL DEPRESSANT ACTION OF FLAVONOID GLYCOSIDES
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Anxiety, depression and insomnia are very common mental disorders; the efficacy of present treatments is frequently diminished by side effects. We have previously demonstrated that some flavonoids possess affinity for the central benzodiazepine binding site of the $GABA_A$ receptor, and exert anxiolytic but not depressant effects in rodents. Recently we identified in valerian extracts two flavonoid glycosides, hesperidin and linarin, with sedative and sleep-enhancing activities in mice. The aim of the present work was to establish the structure-activity relationship for several flavonoid glycosides using different behavioral assays: locomotor activity, hole board exploration and thiopental-induced sleeping time. The possible role of $GABA_A$ receptors in these effects was explored by flunitrazepam and TBOB binding experiments, picrotoxin antagonism *in vivo* and electrophysiological assays in oocytes expressing $GABA_A$ receptors.
The results suggest that flavonoid glycosides exert sedative and sleep-enhancing activities with a mechanism not involving $GABA_A$ system. Hesperidin was the most active compound assayed and any change of its structure decreases or suppresses activity.

BL-P2.
HIPPOCAMPAL CHANGES IN ERK 1/2 AFTER ADMINISTRATION OF HESPERIDIN, A BIOFLAVONOID WITH SEDATIVE-HYPNOTIC ACTIONS
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Inhibitory actions of flavonoids on kinase protein activity have been demonstrated. Hesperidin (HN), a flavanone glycoside isolated by us from *Valeriana wallichii* extracts, has sedative-hypnotic effects not related to the BDZ binding site in the GABAA-R. The aim of our work was to compare the sedative-hypnotic activity of HN in relation with its mechanism of action, in particular, its effects on intracellular protein kinases activity. Neo-HN, a structural analogue of HN, with less biological efficacy, was used as a control. After drug administrations and testing of their behavioural activity, mice were sacrificed and their hippocampi were dissected out and homogenized. Thereafter, 10% SDS-PAGE gels of protein extracts were performed and analyzed by western blot with pTyr, pERK1/2, ERK1/2 and pαCamKII antibodies. There was a significative reduction in phosporylation levels of ERK1/2 in subjects treated with HN and, to a lesser extent, in the group treated with neo-HN. No changes were detected regarding phosphorylation of αCaMKII. In conclusion, the sedative-hypnotic action of HN was associated with a decrease in ERK1/2 activity but not with αCaMKII activity. A less effective analogue of HN induced lower changes in phosporylation levels. These results suggest that intracellular signalling involving ERK1/2 could be responsible of the depressant action of HN.

BL-P3.

TOLERANCE TO SEDATIVE EFFECTS. AN ALTERNATIVE CHOICE, HESPERIDIN OR DIAZEPAM?

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Chronic treatment with benzodiazepines results in tolerance to their effects and the evidence points to different operating mechanisms. We have demonstrated that acute treatment with hesperidin (HN) or diazepam (DZ) has a dose-dependent response in the holeboard test. These compounds have similar efficacy and the co-administration of both drugs evidences a synergistic interaction *in vivo*.
In the present work we compared the behavioral effects of chronic treatment with DZ, HN and HN-DZ fixed-ratio combinations. Mice were given daily i.p. injections of the drugs, and were assayed in the locomotor activity and holeboard tests after 7 days of treatment. Results indicate that HN does not produce the tolerance effects clearly manifested by DZ. Otherwise, the synergistic effects of HN-DZ fixed-ratio combinations were also present in the chronic treatment. It suggests that flavonoids, besides being potentially valuable single drugs, may also be used with advantage in combination with benzodiazepines.

BL-P5.

MK-801 ADMINISTRATION IMPAIRS CONDITIONED DIAZEPAM DEPENDENCE

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We study the effects of the previous MK-801 administration on the development of anxiety, the concomitant increased hippocampal synaptic plasticity observed after chronic DZ administration and the over expression of mRNA NMDA - NR1 and NR2B, on hippocampal formation observed in the withdrawal to DZ. Male rats were divided into four groups according to the drug chronic treatment: DZ, VEH (vehicle), MK-DZ (MK-801-DZ) y MK-VEH (MK-801 -vehicle). 24 hours after the last injection, animals were tested in a plus maze and anxiety was assessed. The DZ group was divided in dependent group (DZ-D), and non dependent group (DZ-ND), which show the same anxiety levels that VEH group. Previous MK-801 impaired the anxiety observed in DZ-D group. We did not observe differences between MK-VEH and VEH. DZ-DEP group have a lower threshold to elicit LTP than DZ-NDEP, VEH and MK-VEH. In the MK-DZ group, LTP generation was not possible after 400 Hz of stimulation. DZ-DEP group showed an increase in the hybridization signals of NMDA-NR1 and NMDA-NR2B subunits respect to DZ-NDEP and VEH groups. No differences were observed between DZ-NDEP, MK-VEH, MK-DZ and VEH groups. These results show the reversion of anxiety developed by the chronic DZ administration, the concomitant increased hippocampal synaptic plasticity observed during withdrawal and the increased mRNA expression for the NMDA subunits by MK-801.

NR-P4.

INVOLVEMENT OF THE BRAIN ANGIOTENSIN II IN THE BEHAVIORAL SENSITIZATION INDUCED BY AMPHETAMINE

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Brain Angiotensin II (Ang II) participates in the regulation of fluid and electrolyte homeostasis. Through stimulation of the physiologically active AT1 receptors, Ang II controls the response of the HPA axis and the brain and peripheral sympathetic activity during stress. The stress response activate the same neuronal pathways that psychostimulant drugs like amphetamine (Amph). Several studies have been confirmed that stress increase the individual vulnerability to drug abuse autoadministration. Our purpose was to study the possible role of the brain Ang II in the locomotor sensitization induced by Amph. Male rats weighing 250-300 g were treated during 5 days with 3mg/kg candesartan cilexetil (AT1 antagonist) orally and 24h later received one injection of 5mg/kg Amph. Animals were tested 1 or 3 weeks later using a challenge of 0.5mg/kg of Amph and the locomotor activity was registered during 2 hours. We found that the locomotor sensitization induced by Amph was higher after 3 weeks of 5mg/kg Amph injection, this effect was attenuated by the blockade of the AT1 receptor. The antagonist treatment induced a slightly increase in the locomotor activity when tested 3 weeks later. No effect of the antagonist was found after 1 week injection. We also found that 5 days of the AT1 receptor antagonist decreased the noradrenergic activity in the locus coeruleus. These results suggest a possible role of brain Ang II in the sensitization induced by Amph.

BL-P6.

INVOLVEMENT OF 5-HT$_{1A}$ RECEPTORS IN THE VENTROLATERAL PERIAQUEDUCTAL GRAY MATTER IN THE REGULATION OF DEFENSIVE BEHAVIORS

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In the present study we investigated the role played by 5-HT$_{1A}$ receptors in the ventrolateral periaqueductal gray (VlPAG) in the regulation of the two defensive responses generated by the elevated T maze (ETM). Methods. Male *Wistar* rats were tested in the ETM after intra-VlPAG injection of the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT or the antagonist WAY-100635. The effects of these drugs in ETM were compared to those caused by them in the light/dark transition test. Results. In the ETM, intra-VlPAG injection of 8-OH-DPAT caused an anxiolytic effect. Surprisingly, WAY-100635 microinjected into VlPAG also caused this effect. In the light/dark transition test, both drugs caused anxiolytic effect. Conclusion. Our results showed that intra-VlPAG injection of 8-OH-DPAT and WAY-100635 affects the inhibitory avoidance response in the ETM, suggesting that 5-HT$_{1A}$ receptors in this area preferentially mediate defensive behaviors related to GAD. Interestingly, the microinjection of both 5-HT$_{1A}$ agonist and antagonist caused the same behavioral response in the two tests used. Given other data showing that 8-OH-DPAT has excitatory and inhibitory effects on VlPAG neurons, it remains to be investigated whether these drugs caused their effect by acting on different neuronal population.
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BL-P7.

STRESS EFFECT ON THE EXPRESION OF CDK5 AND P35 PROTEINS IN BRAIN AREAS OF THE AVERSIVE CIRCUIT

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Methods: Males Wistar rats (290-320 grs) were restrained during 1 hour or 30 minutes and immediately or 30 minutes after stress sacrificed for immunochemistry assay. Another group of animals, were cannulated in the lateral septum or the basolateral amygdala for the infusion of olomucine (40 nm/ul) a Cdk5 inhibitor; or isolomucine (40 nm/ul) an inactive analogous of olomucine; or vehicule 15 min before restraint. One day later, anxiety was measured in the elevated plus maze (EPM).
Results: A 1 h stress session increased the levels of Cdk5 and p35 in cingulate cortex (Cg), cortical amygdala (ACo) and dorsal lateral septum (LSD) whereas 30 minutes of restraint increased the levels of p35 in basal amygdala (BLA), dentate gyrus (DG) and the dorsomedial portion of periaqueductal grey substance (PAGDM). Also, restraint reduced the percentage of time spent in open arms of EPM and this decrease was normalized by olomucine, but not after isolomucine or vehicule, administered either in the lateral septum or in the basolateral amygdala.
Conclusions: Stress increases the expression of Cdk5 y p35 in brain areas involved in the processing of emotional information. This enhancement, at least in lateral septum and basolateral amygdala, could be functionally associated with the generation of emotional sensitization.

BL-P8.

REACQUISITION AND REINSTATEMENT OF CONDITIONED FEAR IN ETHANOL WITHDRAWN ANIMALS: EFFECT OF D-CYCLOSERINE

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Adults male Wistar rats treated with an ethanol-containing liquid diet (6% v/v) for 14 days were submitted to a fear conditioning paradigm (3 footshocks, 0.4mA, 3s, ITI: 30s) 3 days after the last consumption. Rats were then subjected to 4 extinction trials performed 24, 48, 72 and 96 h later by exposing the animals to the conditioned context and freezing was registered during each trial for 10 min. Immediately after the first extinction trial, half of the animals were injected with d-cycloserine (DCS, 5 mg/kg i.p- a dose that does not influence the extinction in control rats) and the other half with saline. The spontaneous recovery of learned fear was tested seven days after the last extinction trial. The next day, animals were submitted to a reacquisition (1 footshock, 0.4mA, 3s, in the conditioned context) or reinstatement (1 footshock, 0.4mA, 3s, in a new context) procedure and the freezing response was evaluated in the respective context 24 h later.
A comparable low level of fear behavior was detected in all animals 7 days after the last extinction trial indicating retention of extinction memory. Following reinstatement or reacquisition training ethanol withdrawn animals displayed higher levels of freezing than controls and this increase was prevented by DCS pretreatment. The enhanced sensitivity to the facilitatory effect of DCS in ethanol withdrawn animals may be mediated by adaptive changes in NMDA receptor induced by ethanol dependence.

BL-P9.

MONOTERPENES, GABA & BEHAVIOUR: FROM *IN VITRO* TO *IN VIVO*

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Monoterpenes, including (+)-borneol, (+)-menthol and thymol, are compounds present in a variety of plant essential oils including valerian (*Valeriana officinalis*) and lavender (*Lavandula officinalis*). (+)-Borneol and other monoterpenes produce mild sedation in mice when inhaled, suggesting actions at GABA receptors. Using two-electrode voltage clamp electrophysiology, monoterpenes were tested at various $GABA_A$ receptor subtypes, as well as Rho1 $GABA_C$ receptors mutated to expressed the $GABA_A$ barbiturate (rho1I307S) site and the low affinity benzodiazepine (rho1W328M/I307S) site. The tested monoterpenes were found to have efficacious GABA-modulatory activity at all tested receptor subtypes and mutants, except for the wild-type Rho1, suggesting monoterpenes may act at a variety of sites on the $GABA_A$ receptor, including the barbiturate and benzodiazepine sites. The modulatory responses of (+)-borneol are not inhibited by the benzodiazepine antagonist flumazenil, suggesting a novel mechanism of action. In animal behavioural studies, including the elevated plus maze and open field test, (+)-Borneol produced significant myorelaxation and a mild anxiolytic effect, supporting the compound's *in vitro* GABAergic effects. These electrophysiological and behavioural results indicate monoterpenes are promising lead compounds for future anxiolytic and myorelaxant drugs.

BL-P10.

HIPPOCAMPAL CHANGES IN TGF-β1 mRNA DO NOT CORRELATE WITH LEVELS OF ERK1/2 AND CREB IN AN ANIMAL MODEL OF DEPRESSION

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The ERK1/2-CREB transduction pathway is known to be impaired in major depressive patients, and improved after antidepressant treatment. This pathway can be modulated by TGF-β1, a cytokine with neuroprotective properties within the hippocampus. This work evaluates if TGF-β1 changes in an animal model of depression, and how it can relate to the ERK1/2-CREB pathway. Male Sprague-Dawley rats were subjected to 2,5h/day of restraint for 14 days, and were chronically injected i.p. with 10mg/kg of the antidepressant Desipramine (DMI). Restrained rats had several behavioural impairments which were prevented by the administration of DMI. Also, these animals had reduced hippocampal TGF-β1 mRNA, as shown by in situ hybridization. This reduction was prevented by DMI. As for ERK1/2 and CREB, western blot analysis of whole hippocampus showed an increased phosphorylation after restraint, effect prevented by DMI only for ERK1/2 but not for CREB. These results suggest a role for TGF-β1 in depression, acting as a neuroprotectant, but not modulating the ERK1/2-CREB pathway.
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BL-P11.
EFFECT OF ß NORADRENERGIC RECEPTORS IN THE BEHAVIOR INDUCED BY NEUROPEPTIDE GLUTAMIC ACID ISOLEUCINE AMIDE

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The neuropeptide glutamic acid isoleucine amide (NEI) injected intracerebroventricularly (icv) induced excessive grooming behavior (EGB) and motor activity (MA) through A-10 dopaminergic neurons as well as the noradrenergic system. We studied whether the last system is involved in the NEI induced these behaviors. Male Wistar rats weighing 250-300g were used. The rats were icv implanted. The resulted shown that any of the adrenergic antagonist *per se* in the doses used affect EGB and MA. Propranolol, a general ß adrenergic antagonist, injected icv previous to NEI, suppresses the behavior provoked by the peptide in a dose response manner. Metoprolol, a ß1 adrenergic antagonist, blocked also these behaviors. Whereas the prior icv injection of phentolamine, an α adrenergic antagonist, as well as ICI 118,55, a ß2 adrenergic antagonist, did not affect at any of the doses tested the studied behaviors induced by NEI. On the other hand, isoproterenol, a general ß agonist, as well as dobutamine, a ß1 agonist, behaved very similar to NEI. These data support the hypothesis of a relationship between NEI and the ß noradrenergic receptor, being specifically the ß1 receptor and similar to other endogenous peptides as neurotensin, cholecytokinin, sustance P and α-NSH; NEI could exert a neuromodulator effect in the CNS.

BL-P12.
ANXIETY-LIKE BEHAVIOR INDUCED BY IL-1beta IS MODULATED BY alpha-MSH THROUGH CENTRAL MELANOCORTIN-4 RECEPTOR

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The proinflammatory cytokine IL-1β influences neuroendocrine activity and produces other effects, including fever and behavioral changes such as anxiety. The melanocortin neuropeptides, such as alpha-melanocyte-stimulating hormone (α-MSH), antagonize many actions of IL-1, through specific melanocortin receptors (MC-R) in the central nervous system. The objective of our study was to establish the effect of MSH peptides on IL-1β-induced anxiety-like behavior and the melanocortin receptors involved. We evaluated the effects of intracerebroventricular administration of IL-1β (30 ng) and MC-R agonists: α-MSH, an MC3/MC4-R agonist (0.2 µg) or γ-MSH, an MC3-R agonist (2 µg) or HS014, an MC4-R antagonist (2 µg), on an elevated plus-maze test. Injection of IL-1β induced an anxiogenic-like response, as indicated by reduced open arms entries and time spent on open arms. The administration of α-MSH reversed IL-1β-induced anxiety with co-administration of HS014 inhibiting the effect of α-MSH. However, the associated treatment with γ-MSH did not affect the anxiety response to IL-1β. These data suggest that α-MSH, through central MC4-R can modulate the anxiety-like behavior induced by IL-1β. The finding that HS014 produced an increase in anxiety may indicate that endogenous α-MSH has an anxiolitic effect.

BL-P13.
HISTAMINE ENHANCES MEMORY CONSOLIDATION THROUGH A H2 RECEPTOR-DEPENDENT MECHANISM

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The participation of the hippocampal histaminergic system in memory consolidation remains controversial because, so far, this issue used has been analyzed using multi-trial learning tasks and *icv* infusions and, therefore, the studies lacked anatomical specificity and were unable to discriminate consolidation and retrieval related events. To get around this, we studied the role played by histamine in memory consolidation using the hippocampal-dependent, one-trial, inhibitory avoidance task (IA) in combination with stereotaxically-localized infusions. Rats with cannulae aimed to the CA1 region of the hippocampus were trained in IA and tested for retention 24 h later. When infused into CA1 immediately after training, but not later, histamine produced a dose-dependent promnesic effect without altering locomotion, exploratory behavior, anxiety or expression of the IA response. This effect was mimicked by the histamine N-methyltransferase inhibitor SKF91844 and the H2 receptor agonist dimaprit and was blocked by the H2 receptor antagonist ranitidine, but not by the H1 receptor antagonist pyrilamine, the H3 receptor antagonist, thioperamide or the NMDAr polyamine-binding site antagonist ifenprodil. By themselves, ranitidine, pyrilamine, thioperamide or ifenprodil did not affect memory consolidation. Our data suggest that, when given into CA1, histamine enhances memory consolidation through a H2 receptor-dependent mechanism but endogenous histamine does not participate in this process.

BL-P14.
ROLE OF GLUTAMATE NMDA RECEPTOR CONTAINING NR2B SUBUNIT IN MEMORY CONSOLIDATION IN THE HIPPOCAMPUS

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The NMDA glutamate receptor (NMDAR) is involved in activity-dependent synaptic plasticity and in related central functions like learning and memory. The NMDAR consists of NR1 and NR2, and sometimes NR3 subunits, NR1 being essential and always present. The hippocampus is crucial in consolidating different kinds of memories. To study the role of different NMDAR subtypes in memory processing, adult Wistar rats were stereotaxically cannulated in both hippocampus under anesthesia. MK801, a NMDAR non-selective antagonist, or ifenprodil, a NR2B subunit selective antagonist, were infused through the cannulae immediately after training in different behavioral tasks; the rats were tested for retention 24 h later. We have shown that NMDAR is involved in habituation to an open field and in an inhibitory avoidance task to a foot-shock. On the other hand, ifenprodil had no effect on both tasks. However, when an underthreshold foot-shock was applied, ifenprodil improved the performance compared to control rats, and did not appeared to learned or remember the task. These data suggest that the hippocampal NR2B subunit is a negative modulator on the formation of specific memories. Neither MK801 nor ifenprodil affected the performance in the object recognition test, suggesting that this task is independent from NMDAR of the dorsal hippocampus.

BL-P15.
EXTINCTION AND REACQUISITION OF FEAR MEMORY REQUIRE HIPPOCAMPAL p38MAPK ACTIVITY

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Evidences indicate that extinction involves learning of a new association rather than erasure of previously stored information. The MAPKs are involved in the induction of plastic mechanisms and are activated during learning of hippocampal-dependent, fear-motivated tasks. Here we analyzed the role of a MAPK family member, p38MAPK, in extinction and reacquisition of step-down inhibitory avoidance (IA) memory. Rats trained in IA were submitted to 5 daily extinction sessions during which the avoidance response was elicited in the absence of the unconditioned stimulus. Immediately after each session, animals received intra-CA1 infusions of vehicle, the p38MAPK inhibitor SB203580 or its inactive analog, SB202474. SB203580 blocked IA extinction which was otherwise evident in vehicle and SB202474-treated animals. SB203580 did not affect extinction when infused into CA1 180 min after the extinction sessions. If after the end of the extinction procedure animals are retrained, they reacquire the avoidance response. However, they failed to do so if received intra-CA1 PP2 immediately following retraining. Our results indicate that, like original learning, extinction and reacquisition of the IA response require hippocampal p38MAPK activity.

BL-P16
MORPHOLOGICAL AND BIOCHEMICAL CHANGES IN THE POSTNATAL RAT BRAIN IN AREAS RELATED TO LEARNING AND MEMORY

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We investigated the effect of the non-selective muscarinic antagonist scopolamine (i.p.) on learning in 22 days old Wistar rats. Although scopolamine has been classically reported as amnestic in adults, at this age scopolamine resulted facilitatory in an inhibitory avoidance task. We exclude the possibility of either an "anxiety" or "pain" effect using a T-maze and a tail-flick test, respectively. Then we studied structures putatively involved, like the hippocampus and the amygdala. Morphological changes were observed in paraformaldehyde-perfused brains stained with hematoxilin-eosine comparing adult and young animals aged 4, 22, 32, 42 days. Autoradiographic studies with ^{3}H-N-Methyl Scopolamine showed a decrease in the density of muscarinic sites in the amygdala at day 22 and 42 (optic densities 48.73 ± 3.92 and 34.75 ± 5.21, respectively), while the density in the hippocampus increased from 53.78 ± 2.04 to 59.01 ± 1.36.
It is suggestive that those changes in muscarinic sites which took place with a different sign in hippocampus and amygdala during development, are in correspondence with the observed behavioural changes.

BL-P17.
MUSCARINIC M4 RECEPTOR FUNCTION IN MEMORY CONSOLIDATION

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Specific cholinergic muscarinic agonists enhance while antagonists disrupt memory. There are five muscarinic receptor subtypes (MAChR) and more than one subtype is expressed in the same cell. M1, M3 and M5 MAChR usually interact with Gq proteins, while M2 and M4 subtypes mainly act through Gi proteins inhibiting Adenylyl Cyclase (AC). The role of each MAChR in learning and memory is poorly understood due to the lack of selective ligands. The selective muscarinic toxins (MTs), snake venom peptides, allowed gaining insight into the functions of different MAChR. We used MT3, an antagonist highly selective for M4 subtype, to inhibit ^{3}H-scopolamine binding to evaluate the proportion of M4 receptors in the rat brain and we found that M4 is predominant in the hippocampus. MT3 caused amnesia when injected in the hippocampus after training rats in different tasks. MT3 *in vitro* reversed the muscarinic inhibition of AC activity. The high proportion of M4 together with the positive effect on memory consolidation and the inhibitory action on AC, strongly support a positive modulatory function for this receptor in the hippocampus.

BL-P18.
EFFECT OF GHRELIN ON SHORT-TERM AND LONG-TERM MEMORY

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Ghrelin (Gr) is an orexigenic peptide produced by the stomach and The Central Nervous System. In a previous work we have shown that hippocampus (Hi), dorsal raphe nucleus and amygdala participate in the central effects of Gr (Carlini *et al.*, 2002; 2004) and that Gr increase long term memory (LTM) retention concomitantly with feeding behavior.
In the present work we analyzed the participation of Gr on the formation and retention of short-term memory (STM) and LTM using step down task in rats. Gr (0.03, 0.3, y 3.0 nmol/µl) was administrated in Hi or intracerebroventriculary (icv) 15 min pre training (memory formation) or immediately after training (memory retention). The animals were tested twice: 1 h and 24 hs after training in order to measure STM and LTM respectively. Hippocampal Gr administration did not modify STM formation; however LTM formation increased only with the dose of 3nmol/ul. Contrarily icv administration increased latency time when the rats were injected pre and pos-training in a dose related manner indicating that icv injection increased memory formation and memory retention (STM and LTM).
In conclusion, differences observed between icv and Hi treatment suggest that another structure would be necessary for the memory formation induced by GR.

BL-P19.
FLUOXETINE INHIBITED THE EFFECT OF GHRELIN UPON MEMORY RETENTION AND FOOD INTAKE
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Ghrelin (Gr) is a new orexigenic peptide. In a previous paper we have demonstrated that the Hippocampus (Hi), participates in the central effects of Gr, particularly on food intake and memory retention. It has been demonstrated that Gr inhibited *in vitro* serotonin release from rat hypotalamic synaptosomes.
In the present work we analyzed the participation of serotoninergic (5HT) neurotransmition in the increased food intake and memory retention induced by Gr using pharmacological and behavioral (step down test) approaches.
We have measured short and long term memory retention (STM, LTM) and food intake (1, 2, 4 and 24 hs post injection) in animals treated with Fluoxetine (FX) administrated intraperitoneatly (ip), a selective 5HT reuptake inhibitor, prior to hippocampal Gr (0.03, 0.3, y 3.0 nmol/µl) administration.
The treatment with FX alone decreased LTM and food intake at 1 h post treatment. FX also inhibited the effect on STM and LTM induced by Gr administration and the orexigenic effect of the peptide.
In conclusion, these results suggest that the appetite stimulating activity and the increase of STM and LTM induced by Gr could be mediated probably by a decrease in the 5-HT release induced by Gr.

BL-P20.
EFFECT OF NATURALLY SECRETED AMYLOID-BETA FIBRILS AND SYNTHETIC AMYLOID-BETA PEPTIDES IN AN INVERTEBRATE MEMORY MODEL
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Amyloid ß protein (Aß) fibrillogenesis is considered one of the crucial steps of Alzheimer's disease pathogenesis. Although several studies have reported data about structure and biological effects of fibrillar synthetic Aß, very little is known about endogenous neuronal amyloid fibrils and their biological effect on memory processes. Recently, it was proposed that memory deficits are caused by different stages of aggregation, particularly by oligomers and by different Aß peptides composition. Here we evaluated the effect of naturally secreted fibrils and different Aß peptides administration in the context-signal memory of the crab *Chasmagnathus*. We found a clear amnestic effect at very low doses of naturally secreted Aß fibrils, fibrillated synthetic $Aß_{1-42}$ and $Aß_{3-42}$ but, unexpectedly, no amnestic effect of $Aß_{1-40}$ was observed. Conversely, no amnestic effect was found when administered non-fibrillated oligomerized $Aß_{1-42}$ peptides. Effect of fibrills on ERK pathway was evaluated as well. Thus, effects on memory were only found in the case of fibrillated peptides administration.

BL-P21.
CHARACTERIZATION OF AMYLOID-BETA PRECUSOR PROTEIN HOMOLOGUE GENE IN THE CRAB CHASMAGNATHUS. PRELIMINARY STUDIES OF GENE EXPRESSION DURING MEMORY FORMATION
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The amyloid precursor protein (APP) family members are transmembrane glycoproteins. In humans, one of these members is the source of the amyloid peptide found in neuritic plaques of Alzheimer's disease patients and it is proposed as the cause of memory deficit and neurodegeneration in this disease. Members of this family are synaptic proteins involved in synaptogenesis and neural plasticity, both in vertebrates and invertebrates. Here we describe the cloning, sequencing and homology comparison with vertebrate and invertebrate species of partial copy DNA sequence of the third invertebrate orthologue member of this family, the crab *Chasmagnathus granulatus* amyloid-beta precursor protein like protein (chAPPL). In our laboratory was developed a contextual associative memory model in this specie, well characterized in behavioral and mechanistic features. Thus, cloning of this gene allows to developing studies of the role of this gene in memory processes in invertebrates. In a preliminary study we found chAPPL expression in different tissues and their induction during long-term memory formation.

BL-P22.
LEARNING RESULTS IN TWO PEAKS OF c-FOS AND HOMER-1a EXPRESSION IN THE HIPPOCAMPUS
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It is well known that early protein synthesis is required for long term memory (LTM) formation and consolidation. Using a one-trial Inhibitory Avoidance (IA) task, we have previously shown that inhibition of hippocampal protein synthesis, during a restricted time window near the time of training, impairs LTM. For this reason, we decided to study the expression of several plasticity-related proteins by western blot analysis in hippocampal homogenates. Results show an increase in c-Fos and Homer-1a protein levels, one hour -but not immediately- after IA training. Moreover, the levels of these two proteins are also increased 24 hours after training in the hippocampus. Therefore, we decided to study if these changes could be finding in other brain structures. Using immunohistochemistry techniques, our preliminary data revealed that learning-associated changes in c-Fos also occur in cerebral cortex. Thus, these results show time restricted and simultaneous modulation of two immediate early genes with different function (one is a transcription factor and the other belongs to the post-synaptic density). Although rapid induction of these genes is likely to be involved in structural and functional modification associated with LTM formation, little is known about the functional significance of late changes in activity-regulated genes like c-Fos and Homer-1a.

BL-P23.
MEMORY CONSOLIDATION PROMOTES THE EXPRESSION OF cFOS THROUGH A SRC AND ERK1/2-DEPENDENT MECHANISM

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Memory consolidation requires the occurrence of transient changes in synaptic efficacy followed by long-lasting modifications involving the expression of several plastic-related genes. Protein tyrosine phosphorylation plays an important role in synaptic plasticity. Particularly, the members of the Src family of non-receptor tyrosine kinases are currently under intense scrutiny given their enrichment in the post-synaptic synaptic terminal where they interact with the NMDAr. Using pharmacological and biochemical tools, we analyzed the participation of these kinases in the consolidation of the memory for a one-trial, step-down, inhibitory avoidance learning task (IA) in the rat. PP2, a specific inhibitor of the Src family, but not its inactive analog, PP3, dose-dependently blocked memory consolidation when given into the CA1 region of the dorsal hippocampus immediately after training rats in IA. IA training was accompanied by the rapid and reversible phosphorylation of Src at Tyr-416 and Tyr 215 and by the Src-dependent phosphorylation of the NMDAr subunit NR2B at Tyr-1472 in the CA1 region. Moreover, when given after IA training, PP2 blocked the learning-induced, rNMDA-dependent activation of the ERK1-2 pathway together with the ERK1/2 and rNMDA-dependent phosphorylation of CREB at Ser-133 and the expression of cFos. Our results indicate that Src, or other member of its family, is an upstream factor able to link the up-regulation of the NMDAr to the induction of gene expression needed for the definitive consolidation of the mnemonic trace.

BL-P24.
MEMORY CONSOLIDATION INDUCES NMDAr- AND CaMKII-DEPENDENT MODIFICATIONS IN AMPAr PROPERTIES

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The NMDAr-dependent activation of CaMKII is necessary for induction of the long-term potentiation of AMPAr-mediated responses in the CA1 region of the hippocampus, a putative model for learning and memory. We analyzed the interplay among NMDAr, CaMKII and AMPAr during consolidation of the memory for an inhibitory avoidance learning task (IA) in the rat. Bilateral intra-CA1 infusion of the NMDAr antagonist AP5 or of the CaMKII inhibitor KN-93 immediately after IA training hindered memory consolidation. Learning of the avoidance response induced the NMDAr-dependent translocation of αCaMKII to PSD-enriched fraction isolated from dorsal CA1 and the autophosphorylation of this kinase at Thr-286. IA training increased the quantity of GluR1 and GluR2/3 AMPAr subunits and the phosphorylation of GluR1 at Ser-831 but not at Ser-845 in CA1 PSDs. The intra-CA1 infusion of KN93 and AP5 blocked the increases in GluR1 and GluR2/3 levels and the phosphorylation of GluR1 brought on by IA training. Our data suggest that IA training promotes the learning-specific and NMDAr-dependent activation of CaMKII in the CA1 region of the dorsal hippocampus and that this activation is necessary for phosphorylation and translocation of AMPAr to the PSDs, similarly to what happens during LTP.

BL-P25.
PERSISTENCE OF A FEAR-MOTIVATED MEMORY IS DEPENDENT ON HIPPOCAMPAL PROTEIN SYNTHESIS DURING A RESTRICTED TIME WINDOW

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To the present time, memory research has been extensively and successfully devoted to the study of the mechanisms and brain circuits involved in long term memory formation. It is widely accepted that consolidation for several learning tasks is dependent upon hippocampal *de novo* protein synthesis. In this sense, it has been demonstrated that many learning tasks, including Inhibitory Avoidance (IA), require *de novo* protein synthesis during the first 6 hr after acquisition. However, the role of early hippocampal protein synthesis in memory persistence has never been addressed. Intra-hippocampal inhibition of protein synthesis by anisomycin caused amnesia for IA learning in a 7-day retention test, but not in a 2-day retention test in a time-dependent manner. A learning-dependent increase in the level of five hippocampal proteins that peaked at 24 hr was detected by immunoblot. Anisomycin infusion that caused a memory deficit at 7 days, prevented c-Fos, Homer-1a and Akt increases at 24 hr. Our results reveal that early and time-restricted protein synthesis in rat hippocampus is required after acquisition for persistence, but not formation of IA memory. This hippocampal mechanism for memory persistence may involve c-Fos and Homer-1a expression around 24 hr after acquisition.

BL-P26.
HIPPOCAMPAL MOLECULAR MECHANISMS ASOCIATED WITH SPATIAL FAMILIARITY

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We recently demonstrated that pCREB levels registered in the hippocampus of rats submitted to an extensive exploratory training in an open field (OF), were below to that observed in control animals. Such decrease does not correlate with memory retrieval or improvement in the formation of long-term memory of habituation. Instead, it is associated with the familiarity to the arena and its recognition.

To investigate the molecular pathways associated with this pCREB decrease, we study the hippocampal protein levels of several protein kinases and the enzymatic activity of phosphatases involved in CREB phosphorylation. We also examined the mRNA levels of several genes with CRE-dependent promoters. Results show a consistent decrease in PKMz protein amount (a constitutively active fragment of PKCz) measured in hippocampal homogenates and in the sub-cellular fraction that contains synaptics terminals. No changes in other protein kinase levels or protein phosphatases activities were found. RT-PCR analysis shows a decrease in Zif268 and an increase in Homer 1a, without changes in PKMz mRNA levels.

Results suggest that decrease in CREB phosphorylation, associated with spatial familiarity, may be due to a biochemical pathway involving PKMz and could in term modulate the expression of different genes.

BL-P27.
INHIBITION OF HIPPOCAMPAL PROTEIN SYNTHESIS AFTER RETRIEVAL HINDERS SPATIAL MEMORY
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Non-reinforced retrieval returns memory to a labile state. It is believed that the fate of such destabilized mnemonic trace depends on the occurrence of one out of two competing processes: extinction and reconsolidation. Here we show that in the rat, the repeated non-reinforced expression of spatial memory causes extinction which is unaffected by inhibition of protein synthesis within the CA1 region of the dorsal hippocampus. However, if the number of non-reinforced retrieval sessions is insufficient to induce long-lasting extinction, then a hippocampal protein synthesis-dependent reconsolidation process recovers the original memory. Inhibition of hippocampal protein synthesis after spatial reversal learning sessions impairs retention of both reversal and original spatial preferences suggesting that reversal learning involves reconsolidation rather than extinction of the original memory. In addition, when given systemically or into the CA1 region after non-reinforced retrieval, the partial NMDAr agonist cycloserine improves subsequent memory retention. Our results show the existence of a hippocampal protein synthesis dependent reconsolidation process that operates to recover retrieval-weakened memories from incomplete extinction and suggest that, as consolidation, reconsolidation can be not only blocked but also enhanced.

BL-P28.
SYNTHESIZING BIRD SONG
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In this work we present an electronic syrinx: an analogical integrator of the equations describing a model for sound production by oscine birds. The model depends on time varying parameters with clear biological interpretation: the air sac pressure and the tension of ventral syringeal muscles. We test the hypothesis that these physiological parameters can be reconstructed from the song.
In order to do so, we built two transducers. The input for these transducers is a song. The first one generates a signal that we use to reconstruct the bronchial pressure, while the second one allows us to reconstruct the syringeal tension (in both cases, for the time intervals where phonation takes place). By driving the electronic syrinx with the output of the transducers we generate synthetic song, and compare it with the original song. Important qualitative acoustic features are reproduced by the synthetic song.
These devices are especially useful to carry out altered feedback experiences, and applications as biomimetic resources are discussed.

NC-P1.
CALCIUM LEVELS MODULATE POST- TRANSLATIONAL ARGINYLATION
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Arginine can be posttranslationally incorporated by the enzyme arginyl-tRNA protein transferase into the NH2-terminus of soluble acceptor proteins. Among the arginylated proteins is calreticulina (CRT), a mayor intracellular calcium binding protein. Many cellular functions of CRT, such as chaperone and nuclear export activities are regulated by Ca^{+2} whose binding alters the conformation of CRT. Taking this into consideration, we studied if the posttranslational arginylation of CRT is also regulated by Ca^{+2}. *In vitro* we found that the incorporation of ^{14}C-arg is inhibited by Ca^{+2} whereas it is increased in the presence of the Ca^{+2} chelator EGTA. This is a specific effect on CRT since the arginylation of the other protein substrates was insensitive to calcium or EGTA. To address if arginylation of CRT is also modulated by Ca^{+2}, we induced intracellular Ca^{+2} depletion in cultured cells by treatment with a combination of SERCA pump-inhibitor Thapsigargin and the membrane-permeant Ca^{+2} chelator Bapta-AM. We found that intracellular Ca^{+2} depletion dramatically increased the amount of arginylated CRT (100% respect to the cells cultured in the presence of Ca^{+2}) as determined by immunocytochemistry assays. These results show that the posttranslational arginylation of CRT is regulated by Ca^{+2} levels, suggesting that a conformational change of CRT may be required for this modification to occur.

NC-P2.
CHANGES IN THE HYPOXIA INDUCIBLE FACTOR-1 STABILITY IN IRON DEFICIENT RATS SUBMITTED TO NEONATAL ISCHEMIA-HYPOXIA
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Hypoxia is controlled by the hypoxia inducible factor-1 (HIF-1). Its activity is regulated via stability regulation of its αsubunit. HIF-1α degradation is accomplished by a family of enzymes (PHDs), the activity of which depends on the availability of O2, 2-oxoglutarate and Fe2+. PHDs hydroxylate HIF-1α to facilitate its ubiquitination. Poly-ubiquitinated HIF-1α is then recognized and degraded by the 26S proteasome.
Aim: study changes in the stability of HIF1α mediated by the Ub-Proteasome system in the iron deficient animals submitted to ischemia-hypoxia treatments. Results: 1) The levels of HIF1α in the pups submitted to ischemia-hypoxia and in the iron deficient animals increases with reference to controls. 2) There is a significant decrease in the chymotryptic y tryptic activities evaluated in the soluble fraction of homogenates obtained from the brain of these animals 3) Although less significant, this decrease is also observed in animals submitted to isquemia or to isquemia-hypoxia. Conclussions: Changes in the activity of the Ub-proteasome system as well as the low levels of available Fe could be responsible for the increase in the levels of HIF-1α. Further experiments are in progress to confirm this hypothesis.

NC-P3.
ENHANCED EXPRESSION OF P35 IN SYNAPTOSOMAL FRACCTIONS OF STRIATUM, AFTER ACUTE AND CHRONIC D-AMPHETAMINE ADMINISTRATION

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The cellular and molecular mechanisms of sensitization in addictive process are still unclear. Chronic cocaine administration up-regulates the expression of cyclin-dependent kinase 5 (Cdk5) and its activator p35 in striatum through the induction of ΔfosB. It would be reasonable to consider that cdk5/p35 modulates the effects of psichostimulants administration; in which the increase of cdk5/p35 expression and activity would be directed to specific targets that regulate signaling pathway proteins which counterbalances the sensitized behaviors. Our goal is to obtain new and detailed behavioral and biochemical evidences about the participation of cdk5/ p35 in D-amphetamine sensitization process. We have found that acute and chronic D-Amphetamine treatment, in periadolescent rats, can induce cdk5 activity in striatum as well as the increase and translocation of its activator, p35, from cytosolic to membrane synaptosomal fraction. In addition, chronic D-Amphetamine treated p35 KO mice showed significantly reduced locomotor activity compared with WT mice. The presents results indicate that cdk5/ p35 signaling could participate in the in the acute and chronic D-Amphetamine-induced behavioral and cellular events during the development and expression of sensitization. The knowledge of cellular and molecular mechanisms in drugs abuse addiction would profit the development of more efficient therapies in drug addiction pathologies.

NC-P4.
DOPAMINE MODULATES MITOCHONDRIAL FUNCTION. INVOLVEMENT OF NITRIC OXIDE

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Dopamine is able to induce neurotoxic effects in several neurological and psychiatrics diseases. It has been postulated that dopamine may generate changes on mitochondrial function by inhibition of respiratory chain. The aim of this work was to determine the *in vitro* effects of dopamine on mitochondrial function and the possible involvement of nitric oxide (NO). Mouse brain mitochondria were isolated by differential centrifugation. Further mitochondrial purification was performed by Ficoll gradient. Mitochondrial function was evaluated through the measurements of: a) O_2 consumption in intact mitochondria and b) respiratory complexes activity in submitochondrial particles (SMP). No significant changes in O_2 consumption in state 4 were observed after dopamine incubation. Dopamine (5-10-15 mM) inhibited state 3 O_2 consumption in brain mitochondria by 19, 28 and 46% respectively. When mitochondria were incubated with 15 mM dopamine in the presence of the NOS inhibitor L-NNA (0.5 mM), state 3 respiratory rate was decreased by only 17%, showing that NO may play a role in respiratory chain inhibition by dopamine. Incubation of SMP with 1mM dopamine inhibited complex I activity by 20%. Complex IV was significantly inhibited by incubation of SMP with 1 mM dopamine, and the effect was partially prevented by L-NNA. Our results show that NO is involved in the mechanism of inhibition of the respiratory chain by dopamine.

NC-P5.
TRANSFERRIN ACTION ON THE EXPRESSION OF MYELIN BASIC PROTEIN INVOLVES FYN TYROSINE KINASE ACTIVITY

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We have previously shown that addition of apoTransferrin (aTf) to the culture medium accelerates the maturation of Oligodendroglial Cells (OL). An increase of Myelin Basic Protein (MBP) expression is involved in this aTF action. Preliminary results indicated that aTf treatment of OL cultures induces an increase in both Fyn Tyrosine Kinase (Fyn) activation and expression. Activation of the MBP gene by Fyn is important for myelination. In this work we examinaed if aTf action on MBP expression involves the Fyn signaling pathway. For this purpose OL primary cultures or N19 OL line cultures were treated with aTf and PP2, a Fyn inhibitor. We found a decreased MBP expression in aTf treated cells when PP2 was added to the medium. We also performed Gel Shift Assays using the sequence on the MBP gene promoter that is responsive to the Fyn signaling pathway. It was found that aTf treatment enhanced the binding to that sequence. Unexpectely, we also observed that in N19 cells, when aTf was present, PP2 treatment increased the protein binding to that sequence. These results suggest that aTf regulation of MBP expression involves the Fyn signaling pathway.

NC-P6.
PERMEABILLITY TRANSITION IN BRAIN CORTEX MITOCHONDRIA OCCURS WITH A DECREASED PRODUCTION OF NITRIC OXIDE

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Increased and sustained cytosolic Ca^{2+} concentrations are predominantly coped by mitochondria, which are able to accumulate this cation in high concentrations, in order to permit the signaling function of Ca^{2+}. In this way, mitochondria can protect neurons from deleterious effects of abnormal Ca^{2i} influx, which could lead to the activation of hydrolytic and degradation pathways associated with apoptosis and necrosis. In order to analyze the effect of Ca^{2+} loading in rat brain cortex mitochondria, permeability transition (MPT), respiratory function and mitochondrial membrane potential, were studied in association with the mitochondrial endogenous NO production. Energized organelles after induced MPT by addition of Ca^{2+} (200 nmol/mg protein), showed mitochondria depolarization (20%) and impaired respiratory function, with a 60% decrease in state 3 respiratory rate. These findings were accompanied by a 46% decrease in mitochondrial endogenous NO production. L-NNA pretreatment before Ca^{2+} loading, exerted 31% protection against swelling and did not change mitochondrial NO production. Meanwhile ADP addition shortly before C^{2+} loading, protected against swelling, it did not restore mitochondrial respiration, and did not change NO production as compared with Ca^{2+} loaded organelles. We can conclude that Ca^{2+} MPT in brain cortex mitochondria is associated with an impaired respiratory function, decreased transmembrane potential and a decreased mitochondrial endogenous NO production.

NC-P7.

SOMATIC KNOCKDOWN OF NURR1: AN APROACH TO EVALUATE ITS FUNCTION IN THE DOPAMINERGIC SYSTEM IN THE ADULT BRAIN

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Movements and emotions are basic features of vertebrate behavior and the midbrain dopaminergic system is the key controller of these activities. Nurr1 is an orphan nuclear receptor essential for the generation of dopaminergic neurons of the Substantia Nigra (SN) and Ventral Tegmental Area as demonstrated in the Nurr1 KO mice. Other and we have shown that Nurr1 can activate the expression of Tyrosine Hydroxylase (TH) in various cell lines and preliminary data indicate that Nurr1 can activate the transcription of c-ret, the receptor of GDNF. However, it is still unclear the mechanism that underlie these effects. To determine if Nurr1 is regulating TH transcription *in vivo* we have performed chromatin immunoprecipitation to detect the binding of Nurr1 to the TH promoter in rat SN. To further evaluate Nurr1 control of TH and c-ret expression *in vivo* in adult rat SN neurons, we have designed and delivered a ribozyme (Rz) against the mRNA of Nurr1 via Adeno-Associated Virus to create a somatic KO of Nurr1. By RT-PCR we have detected a decrease in Nurr1 expression in Rz-infected rats. Using this methodology we have also analyzed the expression of TH and the Dopamine Transporter. Up to date, this is the first approach to explore the physiological role of Nurr1 in the mesencephalic dopaminergic system.

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NC-P8.

EFFECTS OF MCH AND GNRH ON SOMATOLACTIN SECRETION FROM ORGAN-CULTURED PITUITARY OF THE CICHLID FISH *CICHLASOMA DIMERUS*

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Somatolactin (SL) is a hormone belonging to the GH/PRL family, produced in the pars intermedia of teleost pituitary. Its function is uncertain and there is very scarce information on the regulation of SL secretion. Two forms of SL were detected in *C.dimerus*: 32 kDa, a glycosylated form and 28 kDa, a deglycosylated faster migrating form. Our trials using double immunohystochemistry showed a close association between SL cells and both GnRH and MCH fibers. Analysis by immunoblots of *in vitro* culture of pituitaries showed that GnRH and MCH stimulated above the basal level the SL release in a dose-dependent manner. There was no difference in the rates of release between the two forms of SL. When the pituitaries were first stimulated with MCH, an increase in the SL release was observed. When MCH stimulus was withdrawn and after reaching the basal level of secretion again, a new increase in SL was observed with GnRH addition. The pituitaries that did not receive either GnRH or MCH stimulation showed a decrease in SL secretion on 2nd day of culture. These results demonstrated that MCH and GnRH stimulated the SL release. Like other pituitary hormones, it is likely that SL is under hypothalamic control and could be implicated in diverse areas including background adaptation and reproduction.

NC-P9

BRAIN DERIVED NEUROTROPHIC FACTOR, BDNF, IN THE CENTRAL NERVOUS SYSTEM OF *CICHLASOMA DIMERUS* (Teleostei, Perciformes)

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Brain derived neurotrophic factor, BDNF, is a member of the neurotrophin family. In teleosts, BDNF was detected in retina, neuromasts and olfatory epithelium but at the moment, there is not available information about the distribution of BDNF in teleosts nervous system. By immunohystochemistry we found BDNF expression in the central nervous system of *C. dimerus* along the optic nerve, retina, pineal, intermediate lobe, optic tectum, hypothalamus and neurohypohysis. These areas are related with photoreception and environment adaptation. Specificity of the antibody was confirmed by Western blot, obtaining a single band of 39 kDa similar to the previously reported molecular weight in other species. In *C. dimerus* BDNF was detected both in cytoplasm as well as in nuclei. Immunoblot analysis of subcellular fractions (nuclear and cytoplasmatic) confirmed the nuclear localization.
The BDNF role in the mature teleost nervous system is still poorly understood. In *C. dimerus* the distribution of BDNF suggests that it may be involved in background adaptation. On the other hand nuclear localization has been reported for other neurotrophins and growth factors but not for BDNF. In the nucleus, the function of BDNF is still not clear, but as well as in other species BDNF may autorregulate its own expression in *C. dimerus*.

NC-P10.

PARTICIPATION OF P53 FAMILY PROTEINS IN THE OLIGODENDROGLIAL DIFFERENTIATION. REGULATION BY THE UBIQUITIN-PROTEASOME SYSTEM

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We studied the possible relationship between ubiquitin-proteasome (UbP) system and oligodendroglial lineage development. Previously we demonstrated that addition of lactacystin (L), a specific proteasome inhibitor, at low concentrations, to primary cultures of oligodendrocytes (OLGcs) produced their withdrawal from the cell cycle and induced their differentiation. Additionally, we demonstrated in the N20.1 cell line, that a decrease in proteasome activity enhances MBP promoter activity by stabilization of Sp1 and p27. Recent studies demonstrate the participation of p53 proteins family in OLGcs maturation. Turnover of these proteins is regulated by the Ub-P system. Western blot analysis in the N19 cell line, suggest that p53 is present when the cells proliferate or at early stages of differentiation. Transfection of the N19 cell line by electroporation shows that GFP-p53 expression is increased when the cells proliferate and the treatment with lactacystin increases even more GFP-p53 levels. Our results would suggest that the differentiation observed in the presence of L could be mediated, at least in part, by p53 stabilization and its participation in the onset of the differentiation program.

NC-P11.
SCHWANN CELL (SC) DIFFERENTIATION IS AFFECTED BY THE REDOX STATE

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We have previously described that 70 ng/ml Fe^{3+} and holoTf were able to promote SC differentiation *in vitro* through the release of cAMP and CREB phosphorylation.The aim of the present study was to evaluate if changes in the intracellular redox state induced by the addition of free Fe^{3+} or holoTf are involved in this prodifferentiating effect.The content of intracellular oxidant species was measured using a 5 (or 6)-carboxy-2´7´-dichlorodihydrofluorescein diacetate probe (DCDCDHF), which crosses the membrane, and after oxidation, is converted into a fluorescent compound. DNA content was also evaluated. Results are expressed as the ratio DCDCDHF/propidium iodide fluorescence. The effect of a potent antioxidant as N acetyl cysteine on P_0 levels, a major protein of peripheral myelin was measured by Western blot. Our results demonstrate that 1 to 5 minutes after addition of Fe^{3+} o holoTf there is an increase in the intracellular content of oxidant species, that was not observed at 15 minutes. In coincidence with these results, cAMP release was observed at 9 minutes. N acetyl cysteine prevent the prodifferentiating effect of Fe^{3+} on SCs measured by the levels of P0. Further experiments are neccessary to understand the mechanism underlying in the prodifferentiating effect of Fe3+ and the involvement of the redox state in such an effect.

NC-P12.
DECREASE IN THE PROTEASOME ACTIVITY ENHANCE REMYELINATION IN CUPRIZONE INTOXICATED ANIMALS BY AN IMPROVEMENT IN MITOCHONDRIAL ACTIVITY

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Introduction: Addition of lactacystin to oligodendroglial cell cultures induces their exit from the cell cycle and their differentiation. Recently results in Swiss male mice undergoing a spontaneous remyelination after cuprizone-induced demyelination (CPZ) were intracraneally injected (ICI) in corpus callosum with lactacystin at day 5 of their remyelination period (RP) and evaluated 10 days after. We observed an increase in myelin deposition, total proteins, myelin galactolipids and in MBP isoforms expression related to control. Our results obtained in primary cultures of OLGcs suggest that CPZ induces demyelination by decreasing the activity of mitochondrial complexes in these cells, with a lost in energy production and an increase in the production of free radicals. Aims: Evaluate changes in the mitochondrial complexes activity (I, I/III and II/III) in mice brains during their CPZ intoxication and spontaneous remyelination. The response of CPZ intoxicated (D) and control animals (C) ICI at day 5 of RP with vehicle or lactacystin (CV, CL, DV and DL).Results: D show a decrease in the activity of complexes correlated to the demyelination process compared to C. During the RP, D enhance their mitochondrial activity. DL and CL complexes activity are enhanced in relation to DV and CV respectively. Conclusion: The enhance remyelination obtained by a decrease in the proteasome activity is due, at less in part, to the increase of activity of mitochondrial complexes.

NC-P13.
PRESENCE OF THE mRNA OF TRANSFERRIN (Tf) DURING THE PHYSIOLOGICAL MATURATION OF SCHWANN CELLS AND AFTER PHERIPERAL NERVE INJURY

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Tf is an iron carrier protein playing a key role in cell metabolic activity that is regarded as a growth, survival and differentiation factor. The aim of the present work was to evaluate the presence of the mRNA of Tf in the sciatic nerves and in isolated Schwann cells (SCs) during their physiological maturation and in the Wallerian degeneration. The presence of Tf mRNA was evaluated by Northern blot in sciatic nerves from 15 day old rats embryos (E15) and from 4 day old rats (P4). In SCs isolated from embryos and from crushed sciatic nerves the presence of the mRNA of Tf was evaluated by RT-PCR. The levels of myelin proteins such as MBP and P0, and of p75NTR a marker of immature and non myelinated SCs were studied. We demonstrate for the first time that the mRNA of Tf is present in sciatic nerve at E15 when the nerve is still immature, while it is absent in the postnatal period. The presence of the mRNA of MBP and P0 correlate inversely with the expression of the Tf mRNA. In crushed sciatic nerves Tf mRNA appears at 3 and 5 days while it´s absent at 7 and 14 days post injury. These results strongly suggest that Tf may play an important role at early stages of SCs maturation, as well as in the first days after sciatic nerve injury. Whether the modulation of Tf expression may be used therapeutically in peripheral neuropathies remaines to be studied.

NC-P14.
EFFECT OF GAP-43 ON CELL CYCLE PROGRESSION IN NIH3T3 CELLS STABLY TRANSFECTED

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The nervous sytem-specific GAP-43 is significantly up regulated in neurons and glia that are differentiating. It has also been shown that GAP-43 mRNA and protein expression are lost in select human and mouse glioma cell lines and that re expression of GAP-43 in deficient C6 glioma cells results in growth supression. We have demonstrated that cells expressing GAP-43 showed changes in cell morfology but no changes in cell adhesion. In this study we examined the influence of stable GAP-43 expression in NIH3T3 on cell proliferation. To investigate the effect of GAP-43 on cell cycle progression, cells were syncronized by serum deprivation and propidium iodide stained nuclei were analysed by flow cytometry at different times in culture in complete medium. BrdU incorporation at different times was also evaluated. On the basis of the results, we focused on the G1 phase of the cell cycle as the control point for GAP-43 overexpressing cells growth arrest. The expression of Cyclin E and Cdk2 was markedly time course decreased in. cells overexpressing GAP43 However, the level of CyclinD1 and Cdk4 was sligtly decreased. Although GAP-43 is primarily expressed in post-mitotic neurons, our results suggest that this protein may have a role in cell cycle regulation at early stages of neuronal and glial differentiation.

NC-P15.
EFFECT OF TRANSFERRIN ON HIPOMYELINATION INDUCED BY IRON DEFIFCIENCY

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Iron deficiency is associated with hypomyelination, characterized by a decrease in myelin lipids and proteins. We previously showed that apotransferrin (aTf) injected intracranially into normal (non-iron deficient) rats induces an early differentiation of oligodendroglial cells and an increased deposition of myelin. In this work, we used an iron deficient rat model to test if aTf could revert the hypomyelination induced by iron-deficiency. Iron deficient rats were intracranially injected at post-natal day (PND) 3 with either aTf or physiological solution. At PND 17, myelin was purified and its composition analyzed (protein, galactolipids, phospholipids and cholesterol). We found a moderate increase in the amount of proteins, galactolipids and phospholipids but not in cholesterol in aTf injected rats. These results suggest that our model of aTf injection could improve the myelin deficit induced by iron depletion.

NC-P16.
EFFECT OF MANIPULATION OF IRON TRANSPORT OR STORAGE ON MYELIN COMPOSITION USING TWO DIFFERENT ANIMAL MODELS

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To test the association between iron depletion and changes in myelin composition we introduce two genetically modifed animal models, each with a unique defect in iron metabolism. One of the animal models, the hypotransferrinemic (hpx) mouse, lacks the ability to make transferrin. The other animal model is a heterozygote for a null mutation in H-ferritin. In the hpx mice, concentration of total protein, phospholipids and galactolipids in isolated myelin was increased in comparison to controls. The myelin proteins CNPase and PLP and all the isoforms of MBP were significantly higher in hpx mice compared to control. The reliance of the hpx mice on transferrin injections for survival appears to impact on myelin composition. On the other hand, a decrease in H-ferritin expression was associated with a decrease in myelin proteins galactolipids and phospholipids, but only the decrease in PLP reached statistical significance among the individual proteins analyzed. Compromised iron storage capacity has a predominant effect on lipids in myelin. Overall, these results demonstrate how myelin composition can be affected by loss of iron homeostasis.

NC-P17.
QUINOLINIC ACID INDUCES CHANGES IN EXPRESSION OF COAT PROTEINS IN AREAS OF RAT BRAIN

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Clathrin- mediated endocytosis (CME) is the best-understood mechanism of macromolecule internalization. In brain, CME is required for recycling of membrane proteins after the release of neurotransmitters in the synapse. Clathrin coated vesicle (CCV) formation is triggered by recruitment of assembly proteins as adaptors AP-2 or AP180 onto membrane domains where different receptors are concentrated. Association of GABA (A) receptors and AP2 represents an important mechanism in the postsynaptic modulation of inhibitory synaptic transmission. Striatal quinolinic acid (QA) injections induce loss of GABAergic medium spiny projection neurons, mimicking Huntington disease. We measured expression of coat proteins in areas of rat brain .AP-2 (alpha 1 and 2 subunits) and AP-180 expression were studied by WB analysis by using specific monoclonal antibodies. We observed that QA induce a decrease of both subunits of AP-2 in striatum at 48 hr, whereas the proteins significantly increase in the cerebellum. Alpha 1 subunit was increased in hippocampus and no changes were observed in cortex or brainstem . AP180 expression was not modified. We concluded that QA alters selectively the transport via CCV in striatum and cerebellum, two main areas involved in the control of movement.

NC-P18.
DT-DIAPHORASE PREVENT THE AGGREGATION OF α-SYNUCLEIN INDUCED FOR SPECIES DERIVATED FROM OXIDATIVE METABOLISM OF DOPAMINE

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It has been observed that the α-synuclein can form an adduct with a product of the oxidation of the dopamine, inducing the aggregation of α-synuclein. The oxidation of the dopamine generates aminochrome, that can be reduced for many flavoenzymes that reduce quinones with one electron for time, generating a very reactive semiquinone, the leukoaminochrome-o-semiquinone (LSQ), which one is very toxic to the neuron. The DT-diaphorase, the only one reductase that reduce the quinones with two electron simultaneously, prevents the formation the radical LSQ and its toxicity. We postulate that LSQ can increase the aggregation of the α-synuclein and the DT-diaphorase prevents this aggregation. We incubate α-synuclein with pure aminochrome and aminochrome plus NADH cytochrome C reductase, in presence and absence of DT-diaphorase and analyze the aggregation of α-synuclein with western blot. The results show that aminochrome induce the aggregation of α-synuclein to low molecular weight structures and the DT-diaphorase prevent this aggregation. The reduction of the aminochrome with NADH cytochrome C reductase also prevent the aggregation of α-synuclein to low molecular weight structures, nevertheless induce the aggregation of α-synuclein to high molecular weight structures like fibrils.

NC-P19.
AMINOGLYCOSIDE OTOTOXICY IN TRANSGENIC MICE THAT OVER-EXPRESS THE NACHR ALPHA9 SUBUNIT
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Hearing impairment can be caused by a variety of factors including genetic, noise, ototoxic substances such as aminoglycosides, and aging. Hearing deficiencies are caused by loss of hair cells or spiral ganglion neurons and these changes are often permanent. Due to the importance of the use of aminoglycosides in the clinic, it is important to understand the mechanisms underlying the ototoxic process. It has been suggested that the activation of the efferent system that innervates outer hair cells (OHCs) exacerbates aminoglycoside-induced ototoxicity. Acetylcholine is the main neurotransmitter released at the efferent-OHC synapse and the receptor that mediates synaptic transmission is composed of α9 and α10 nicotinic cholinergic receptor (nAChR) subunits. To investigate the role of the efferent innervation in the ototoxic process we have treated a strain of mice that over-express the α9 subunit with kanamycin and compared the degree of damage to hair cell with that of wild-type mice. The percentage of OHCs found to be missing in the α9 over-expresser was significantly increased (79%, third row, middle turn) when compared to that of wild-type mice (26%, third row, middle turn). These results indicate that the α9 over-expresser is more sensitive than wild-type mice to kanamycin-induced ototoxicity.

NC-P20.
ACTIVATION OF *DE NOVO* SYNTHESIS OF CERAMIDE INDUCES PHOTORECEPTOR APOPTOSIS
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The precise mechanisms leading to photoreceptor death in the retina are still unknown. We investigated whether an increase in ceramide, a sphingolipid known to trigger apoptosis upon cellular stress, activates apoptosis in rat retina photoreceptors in culture. Paraquat (PQ)-induced oxidative stress led to the accumulation of newly synthesized [^{3}H]ceramide in photoreceptors, which almost doubled the amount found in controls 2 and 4 hs after PQ addition, but did not affect the amount of [^{3}H]sphingomyelin. Ceramide increase was parallel to the onset of photoreceptor apoptosis. We previously showed that inhibiting ceramide synthesis with fumonisin B1 (FB) prevented photoreceptor apoptosis upon PQ addition. To confirm those results and establish which pathway is involved in ceramide synthesis, we treated cultures with cycloserine, which blocks the first step in ceramide synthesis, 30 min before PQ treatment; this addition completely blocked PQ-induced photoreceptor apoptosis. FB addition at day 0 partially diminished photoreceptor apoptosis by day 6 *in vitro*, which otherwise occurred in the absence of photoreceptor trophic factors. These results suggest that an increase in ceramide levels triggers photoreceptor apoptosis in different situations of cellular stress, and that this increase arises in the stimulation of *de novo* synthesis of ceramide.

CH-P1.
CIRCADIAN REGULATION OF PHOSPHATIDYL CHOLINE BIOSYNTHESIS IN CULTURED FIBROBLASTS
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Recently, we have demonstrated that cultured fibroblasts oscillate in the biosynthesis of their phospholipids which is driven by an endogenous clock involving *per1* expression (Márquez *et al*. *Faseb J.* 2004). Phosphatidylcholine (PC) is the major lipid component in mammalian membranes. The synthesis of PC is controlled by a rate-limiting enzyme: CTP- Phosphocholine Cytidylyltransferase (CCT) regulated highly. Total cellular CCT activity is the sum of CCTα and CCTβ isoforms. Our results showed that the biosynthesis of PC exhibits a daily oscillation in culture of fibroblasts after synchronization with serum shock. In this culture, we observed that the CCT activity show a significant daily oscillation ($F= 2, 3301$; $P< 0.009647$); with maximum levels of activity at 6.5 h and 35h after serum shock. The period (τ) of oscillation is ~ 29 h, which is coincident with period (τ) previously observed in the 32P-phospholipid labelling of NIH 3T3 fibroblast. For immunocitochemistry we observed that CCTα, CCTβ2 y CCTβ3 isoforms maintain subcelular localization at different time of subjective day (CCTα is nuclear, and reticulum-cytoplasm for CCTβ1 and CCTβ2). The levels of expression of that isoforms changes substantially along the day. Our data suggest that the daily oscillation of CCT activity in NIH 3T3 fibroblast corresponds to the differential circadian regulation in some isoforms of CTP-Phosphocholine Cytidylyltransferase.

CH-P2.
LIGHT AND CIRCADIAN REGULATION OF MELATONIN SYNTHESIS IN CULTURED RETINAL GANGLION CELLS
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Melatonin is synthesized in the pineal gland and the retina of many vertebrate species. In the retina, melatonin appears to play a mayor role in the circadian regulation of retinal physiology. The melatonin synthesis is rhythmic and driven by an endogenous circadian clock, which is also regulated by the interaction of the environmental photic input and the circadian clock. Within the retina, many reports suggest that the melatonin synthesis takes place in photoreceptors (PRCs), however, we have recently shown that retinal ganglion cells from chicken embryos (eRGCs) also synthesize ^{3}H-melatonin under a circadian bases, indicating that they are autonomous circadian oscillators (Garbarino-Pico *et al*., 2004). Recently it was found that a subpopulation of mammalian RGCs is intrinsically photosensitive by depolarizing to light (Berson *et al*., 2002). In this work, we show that cultures of eRGCs synchronized to a 12:12 LD cycle for 3 days and released to DD, biosynthesized radiolabeled melatonin in a rhythmic manner with higher levels during the subjective day whereas light exposure significantly inhibits this synthesis. In order to investigate the potential pathway of phototransduction taking place in eRGCs, we used different effectors to modulate the light effect on melatonin synthesis. Preliminary data suggest that an invertebrate-like cascade may be acting in this photic pathway. The results indicate that chicken eRGCs are intrinsically photosensitive as seen in the regulation of melatonin synthesis by light.

DN-P1.
CELL CYCLE REGULATION BY TROPHIC FACTORS IN MULLER STEM CELLS
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Muller glial cells are eye stem cells and hence potentially able to regenerate the different neuronal cell types in the retina. However, their use for this purpose demands to understand the molecular signals that regulate their exit and/or reentry to the cell cycle, and the mechanisms that regulate differentiation into the different neuronal cell types occurring in the retina. We have previously shown that glial derived neurotrophic factor (GDNF) promoted cell cycle progression in Muller cells. We show here that several trophic factors could regulate cell cycle exit and re-entry. In addition to GDNF, basic fibroblast growth factor (bFGF) and insulin stimulated BrdU incorporation and promoted the expression of nestin, a marker of stem and proliferating cells in the retina, and *Pax6*, a master gene essential for eye development and, hence, probably required during regenerative processes of the retina. In close correspondence with the above results, these trophic factors down regulated the expression of p27, a cell cycle inhibitor. On the contrary, the lipid molecule docosahexaenoic acid (DHA) promoted cell cycle exit of glial cells, diminishing BrdU incorporation, and enhancing p27 expression. As a whole these results suggest that trophic factors regulate the exit or reentry to the cell cycle in Muller stem cells.

DN-P2.
ACTIVATION OF FETAL INSULIN-LIKE GROWTH FACTOR-1 RECEPTORS BY IGF-1 IS ESSENTIAL FOR THE ESTABLISHMENT OF NEURONAL POLARITY
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How a neuron becomes polarized remains an outstanding question. Recent studies suggest that in cultured hippocampal neurons selection of the future axon requires activation of phosphatidylinositol 3-kinase (PI3k) by growth factor receptor tyrosine kinases. Consistent with this, accumulation of active PI3k and its phospholipid product PIP3 at the growth cone of a minor neurite is crucial for the outgrowth of the future axon and the polarized distribution of mPar3 and mPar6, two proteins required for axon specification. We now provide evidence about the identity of the growth factor-tyrosine kinase receptor system involved in PI3k activation at the growth cone of the future axon. Our results indicate that activation of fetal IGF-1 receptor (containing the subunit βgc) by IGF-1 is essential for axon specification. Suppression of βgc-IGF-1 receptors by siRNA treatment prevents axon formation and the polarized distribution of PI3k-mPar3-mPar6. We also demonstrate that co-transfection of the suppressed cells with a fast-cycling cdc-42 cDNA (a downstream effector of the PI3k pathway involved in neuron polarization) recovers the phenotype inducing outgrowth of a single or several axons. Taken together, our results show that the activation of PI3k pathway by IGF-1 (and not other growth factors as BDNF) is essential for axonal outgrowth and the establishment of neuronal polarity.

DN-P3.
NEUROTROPHIC ROLE OF VEGF IN THE OLFACTORY EPITHELIUM OF *Bufo arenarum*
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Recently, Vascular Endothelial Growth Factor (VEGF) has been shown to be a neurotrophic factor in the peripheral and central nervous system. Co-expression of VEGF and its receptor Flk-1 in neurons was noted. In the Olfactory epithelium, neurons are replaced continuously by means of apoptosis, proliferation and differentiation processes. We have observed the presence of VEGF and Flk-1 in the olfactory system during development of the toad *Bufo arenarum*. VEGF co localizes with Flk-1 in mature neurons, however Flk-1 was also observed in the basal cells (stem) layer. Because VEGF promotes neuronal proliferation in other system, we examined the possibility that it also stimulates neuronal proliferation in the olfactory epithelium during larval development. Intracerebral administration of VEGF (2ng/µl) increased immunostaining of Flk-1 and BDNF. These increments were confirmed by Western blot. On the other hand, VEGF injection stimulated PCNA immunostaining and BrdU incorporation into immature basal cell and scattered cells in other layers of the epithelium. In addition, the treatment with green tea (0.5%), and VEGF inhibitor, caused and increase of the activated-caspase 3 immunostaining (apoptotic marker).
These evidences suggest that VEGF play an important role as a neurotrophic factor in the development of the olfactory epithelium in *Bufo arenarum*.

DN-P4.
ESTROGEN REGULATION OF NEUROTROPHIN RECEPTORS IN SYMPATHETIC NEURONS
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Target-derived nerve growth factor (NGF) is a key mediator for sympathetic axon growth throughout life. Actions of NGF are mediated by two neuronal receptors (TrkA and p75), although TrkA activation seems to account for most of the growth-promoting effects of NGF. We previously showed that estrogen increases the levels of NGF mRNA and protein in the rat uterus. However, despite this up-regulation, a decreased sympathetic uterine innervation is observed following estrogen treatment. We hypothesize that the lack of growth-responses of uterine sympathetic nerves to increased NGF could be related to changes in the neuronal responsiveness to neurotrophins. Using densitometric immunohistochemistry, we assessed the effects of chronic estrogen treatment to prepubertal rats on the levels of TrkA and p75 in uterine-projecting sympathetic neurons and sympathetic neurons of the superior cervical ganglion (SCG) of the paravertebral chain. The main findings were: (1) estrogen treatment had no effects on fluorescence intensity of TrkA and p75 in sympathetic neurons of the SCG; (2) estrogen decreased the levels of TrkA in uterine projecting-neurons without affecting levels of p75. Reductions in TrkA alters the ratio of p75 to TrkA and this change might be relevant in determining the response of uterine sympathetic neurons to NGF and other neurotrophins (i.e. BDNF), contributing to the inhibitory effects of estrogen on uterine sympathetic nerves.

DN-P5.
CRX IS NECESSARY BUT NOT SUFFICIENT TO GENERATE FULLY DIFFERENTIATED PHOTORECEPTORS
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Pax6 is necessary for eye development and Crx for the generation of photoreceptor cells. To understand the role of Crx on photoreceptor differentiation we studied its expression *in vivo* and *in vitro* during rat eye development along with that of Pax6, as well as the effects of docosahexaenoic acid (DHA), a photoreceptor trophic factor. Progenitor cells labeled with BrdU *in vivo* expressed Pax6 during retina differentiation at every embryonic stage analyzed. On the contrary, Crx was expressed at embryonic day 20 and only in postmitotic photoreceptors, preceding the onset of opsin expression. Similar results were found *in vitro*, when retinal cells were cultured in chemically defined media, pulsed with BrdU and fixed at different time points. Progenitor cells in culture initially expressed Pax6 and nestin, even during mitosis. Pax6 and nestin expression were downregulated and Crx upregulated as progenitors left cell cycle. However, only a small percentage of Crx-positive cells also expressed opsin. Interestingly, addition of DHA promoted neurite elongation, outer segment development and opsin expression. Hence, Crx is necessary to initiate photoreceptor differentiation, but not sufficient for photoreceptor development, and DHA is required to promote further differentiation in these cells.DN-P5.

DN-P6.
RETINAL PIGMENT EPITHELIAL CELLS DIRECT SPATIAL ORIENTATION, SURVIVAL AND DIFFERENTIATION OF PHOTORECEPTORS
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We have previously shown that retinal pigment epithelial cells (ARPE-19) partially prevented photoreceptor apoptosis in neuron-ARPE-19 cocultures lacking photoreceptor trophic factors. Addition of photoreceptor trophic factors, docoxahexaenoic acid (DHA) and glial cell-derived neurotrophic factor (GDNF) to cocultures showed no additive protective effect. Epithelial cells and DHA enhanced photoreceptor differentiation, stimulating the development of apical processes and opsin correct localization in these processes. DHA increased opsin expression in cocultures, while epithelial cells did not. We have previosuly described that epithelial cells in cocultures promote a rapid spatial reorganization, which ends with the apical face of epithelial cells exposed to neurons, as occurs *in vivo*. Addition of a metalloproteinase inhibitor (TIMP-1) blocked the ability of epithelial cells to separate neurons from their substrate, without affecting the outgrowth of lamellipodia, suggesting that metalloproteinases are involved in the reorganization process. These results suggest that epithelial cells are crucial for photoreceptor development and orientation, and thus essential for developing the final retina structure.

DN-P7.
PATTERN OF EXPRESSION AND ACTIVITY OF UPA SYSTEM DURING LAMINAR ORGANIZATION OF OPTIC LOBE
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The extracellular matrix (ECM) remodeling plays an important role in cell migration, neurite outgrowth and tissue remodeling in the development of nervous system. Urokinase-type plasminogen activator (uPA) and its receptor (uPAR) trigger ECM degradation, cell adhesion and cell migration. We investigated the expression and activity of uPA and uPAR in the developing chick optic lobe. This structure shows a cortical organization that develops following a cephalo-caudal gradient axis (c-cGA). Embryos of different ages were used to perform immunohistochemical and biochemical studies. Results show that two successive waves of uPA activity extend along the c-cGA. The first one correlates with a massive neuronal migration and the second one coincides with neurite outgrowth and synapse formation. Immunohistochemical studies show a complex pattern of uPA and uPAR expression that involves different cell types (glia and neurons) and specific layers along the lamination process. These results suggest that the uPA system plays a central role in ECM remodeling and morphogenetic events.
This work was supported by grants from UBACYT and CONICET, Argentina.

DN-P8.
ROLE OF GPI-ANCHORED PROTEINS IN EPHA3-INDUCED RETINAL AXON GROWTH
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Nasal retinal ganglion cells connect to the caudal tectum and temporal ones contact the rostral tectum. Eph receptors and their ligands, the ephrins, are expressed in complementary gradients in both organs. EphrinAs located in caudal tectum repel EphA3-bearing temporal axons. Our results suggested that EphA3 in rostral tectum promote nasal axon growth to caudal tectum. As ephrinsA (GPI-anchored proteins) are expressed in optic fibers, we investigated if axonal GPI-anchored proteins mediate this effect.
Retinal explants from 6 days-old chick embryos were cultured on EphA3-Fc or Fc. They were treated with increasing doses of PI-PLC, an enzyme that sheds GPI-anchored proteins. EphA3-Fc-stimulated nasal explants increase their axonal length at low PI-PLC doses, but reduce it at high PI-PLC doses. Control explants increase axonal length at every PI-PLC dose. Axonal density of nasal and temporal explants shows the same pattern of response as nasal axon length.
The positive effect of PI-PLC treatment on control nasal axons suggests that endogenous ephrinAs could reduce axonal growth upon axonal EphA4 activation. The dual effect on EphA3-stimulated nasal axons also suggests that the lack of ephrinAs impairs EphA3-elicited axonal growth. It is possible that ephrinAs could act as receptors of EphA3. The weaker effect on temporal axons agrees with their lower expression of ephrinAs. This suggests that GPI-anchored proteins could participate in axonal guidance and ephrinAs could have a double role as both ligands and receptors.
This work was supported by grants from CONICET and UBA. Argentina.

DN-P9.
IS CYTOPLASMIC c-FOS REQUIRED FOR NEURONAL DIFERENTIATION?
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c-Fos participates, as a component of AP-1 family of transcription factors, in events related to cell growth and differentiation. A few years ago we found an additional activity for this protein: that is the capacity of c-Fos to activate the phospholipid synthesis machinery. This activation is a cytoplasmic phenomenon involving an association between c-Fos and the endoplasmic reticulum (ER) as was described in the cell lines NIH 3T3, PC12, cells. Herein, it was examined if this c-Fos activated phospholipid synthesis is also observed in primary cultures of pyramidal hippocampus cells. We found that c-Fos also associates to the ER and activates phospholipids synthesis. This phenomenon is relevant to growth since blocking of c-Fos/ER association with a c-Fos antibody also blocks phospholipid synthesis activation and neuronal differentiation. Subcellular location of c-Fos showed c-Fos/ER co-localization both in the cell soma and in growing axons, the latter mainly ascribed to branching zones. These results support the notion that c-Fos is required to sustain growth by activating the synthesis of phospholipids. At the present we are examining c-Fos depletion by an especific siRNA. Furthermore we are testing c-Fos subcelular localization in growing axons. Herein we postulate c-Fos as necessary step that activates the phospholipids synthesis machinery that sustain neuronal grow.

DN-P10.
EXPRESSION AND ACTIVITY OF MMP-2 IN THE PROCESS OF THE OPTIC LOBE LAMINATION
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Matrix metalloproteinases (MMPs) constitute a family of extracellular enzymes, which that participate in remodelling of pericellular environment through the cleavage of ECM proteins. MMP-2 and MMP-9 present gelatinolytic activity that is involved in tissue remodeling and development.
In this work we investigate the pattern of gelatinases activity in the developing chicken optic lobe (OL). The OL shows a cortical structure organized in alternating neuronal and fibrous laminae. OLs from different embryonic ages (E) from E6 to newly hatched chicks (NH) were analized.
We found MMP-2 but not MMP-9 activity in the developing OL. The zimographic activity pattern exhibits a significant increase between E12 and E14 and an abrupt decrease was observed from E16 until NH. The *in situ* activity was mainly detected in ventricular zone, in the neuronal layers and significant activity was detected in cells located around the radial glia. Topographic and temporal pattern of MMP-2 activity and expression suggests that it´s related to cell migration in the OL laminar organization
This work was supported by grants from UBACYT and CONICET. Argentina.

DN-P11.
ZEBRAFISH CELLULAR NUCLEIC ACID BINDING PROTEIN: EMBRYONIC EXPRESSION AND FUNCTION
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Cellular nucleic acid binding protein (CNBP) is single-stranded nucleic acid binding protein with nucleic acid chaperone activity. Despite its striking conservation among vertebrates, its biological function remains elusive. Here we analyze the zebrafish CNBP (*z*CNBP) developmental gene expression pattern and function.
*z*CNBP-mRNA distribution was homogeneous in embryos up to 24 hpf. Thereafter, it was specifically detected in eyes, tectum, myotomes and skeletal system. After 48 hpf, expression appeared also in liver, pectoral fins and retina. *z*CNBP gene is organised in five exons and four introns as all the vertebrates CNBP genes, being noticeable the absence of a predicted canonical promoter region. Reporter GFP gene was used to analyze fragments of the putative promoter region, identifying a specific control region within the first intron. Finally, *z*CNBP *in vivo* function was analyzed by transient modification of the *z*CNBP levels in embryos. Changes of maternal *z*CNBP levels caused malformations in the posterior trunk and tail development, while reduction of embryonic *z*CNBP induced abnormal formation of the midbrain/hindbrain border. Based on TUNEL and Br-dU assays, and *in situ* hybridization analysis of key tissue marker genes, we propose that these phenotypes may be related to cell proliferation/apoptosis unbalance.

DN-P12.
SEROTONINERGIC SYSTEM DEVELOPMENT IS VISUAL-ACTIVITY DEPENDENT IN CHICK RETINA
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Visual experience is a main issue in visual system maturation. This process includes a refinement of retinal synaptic circuitry mediated by a complex interplay between genetic and epigenetic factors.
There are increased evidences that light ordark rearing play a role in the morphological and functional maturation ofretinal circuits.We have previously described 5HT-amacrine cells of chick retina during pre and postnatal development. In addition, we have showed the darkness induced neuroplastic changes over those amacrine cells (Fosser *et al*., 2005). These changes could be necessary to decrease retinal threshold, and thus increase visual sensitivity. Besides red light rearing produces a lack of pruning of 5HT amacrine dendrites.
In this work we have extended our immunocytochemical studies on the serotoninergic system of chick retina through the analysis of the expression pattern of three serotoninergic receptors (5HTR1A, 2C and 3) during visual hypostimulation conditions.
Our preliminary data suggests that the expression pattern of serotoninergic receptors are conditioned by visual-activity. Besides, light affects not only the pruning of the serotonin amacrine-cells arborizations, but also spatial distribution of serotonin receptors.
(UBACyTO007).

DN-P13.
MOTOR ACTIVITY PRODUCES A NMDA RECEPTOR REDUCTION IN RAT STRIATUM DURING POSTNATAL DEVELOPMENT

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NMDA glutamate receptors have an established role in the regulation of motor behaviour by the basal ganglia. The present study was performed to investigate, through a circling training test (CT), the effects of motor activity on NMDA receptors in rat striatum. Recently we have shown that motor stimulation developed during cholinergic-dopaminergic striatal critical period (PN 30-37) does not alters the NMDA receptor number (Bmax of [3H] MK-801) or mRNA expression in adult animals (PN 75). If training took place, out of this period, during postnatal days 40-47 no change was observed. Due to the fact that glutamatergic fibers represent the primary cortical afference to striatum and having an activity maturation prior to cholinergic and dopaminergic fibers, in this new study, CT was performed during PN 20-27. Results show a permanent reduction of 33% in receptor number and 40% in mRNA levels of NR1, NR2A y NR2B subunits of such receptor. To synaptic level this effect correlates with PSD-95 protein reduction, which serves to cluster NMDA receptors at the postsynaptic membrane, and no modification of Synaptophysin (a presynaptic plasma membrane protein) in striatum of trained animals. These observations suggest an activity dependent synaptic adjustment prior to critical period of modulatory cholinergic-dopaminergic fibers evidencing the existence of multiple periods of activity dependent synaptic adjustment.

DN-P14.
DIFFERENTIAL EXPRESSION AND LOCALIZATION OF ANG II RECEPTORS DURING RAT HINDBRAIN DEVELOPMENT

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Angiotensin II (Ang II) exerts its physiological effects through binding to two receptor subtypes: AT_1 and AT_2 receptors. Ang II receptor expression is highly modulated during development. In rodents, cerebellum development takes places during 15-20 days after birth. Localization of Ang II receptors was studied by binding autoradiography in hindbrain of pups at different stages after birth: PND1 to PND60. Ang II receptor expression was also determined by semiquantitative RT-PCR. AT_1 Ang II receptor mRNA was lower at PND1 and PND8 while similar levels were observed for PND15, PND30 and PND60. By Autoradiography, several nuclei were observed in PND8 animals, most of them expressing AT_2 receptors: inferior colicullus (CIC), facial nucleus (7), supragenual nucleus (SGe) and inferior olive (IO). SGe nucleus was observed in PND8 up to PND60, while binding to the CIC was higher at PND15. At the age of PND60, the IO nucleus is present in the brainstem, expressing AT_2 receptors. At this age, most of the nuclei present in the brainstem do not express Ang II receptors. The present results suggest that Ang II receptor expression is developmentally regulated in cerebellum and brainstem with higher expression of AT_2 receptor in PND15 in concordance with the establishment of the olivo-cerebellar connection, a critical stage on hindbrain development.

DN-P15.
BUSPIRONE EFFECT ON PRENATAL ETHANOL-INDUCED DAMAGE TO FRONTAL CORTEX

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Prenatal ethanol exposure (PEE) alters the 5HT system development, reduces the number of 5HT neurons, decreases the 5HT reuptake sites and 5HT brain concentration. After the stimulation of its $5HT_{1A}$ receptors, astrocytes release S100b protein, a neurotrophic factor that promotes the stabilization of different cytoskeletal proteins such as MAP2 (usually used as a dendritic marker). Buspirone (B) is a partial agonist of $5HT_{1A}$ receptors. We studied MAP2 expression in frontal cortex when B was administered during PEE. Female Wistar rats were exposed to ethanol 6.6% (v/v) in drinking water for 6 weeks before breeding and during gestation; control group received water. Pregnant rats were divided into four treatment groups: CS (ctrol-saline), CB (ctrol-B), ES (EtOH-saline) and EB (EtOH-B). From E13 to E20 each group received a daily subcutaneous dose (4.5 mg/kg) of B or an equivalent volume of saline. EtOH was discontinued at birth. On P21, P35 and P60 pups' brain were processed by immunohistochemistry using primary antibodies to MAP2. Relative area of MAP2+ fibers were measured by computer-assisted image analysis. The results showed significant decreases in the dendritic arborization in frontal cortex in ES group compared with CS, CB or EB in all ages. Thus, B, prenatally administered to PEE rats, is capable to restore to control values the MAP2+ fibers in the frontal cortex.
Grant UBACyT M-072.

DN-P16.
HALOPERIDOL ADMINISTERED EXCLUSIVELY DURING THE STRIATAL CRITICAL PERIOD (POSTNATAL DAYS 30-37) ALTERS CIRCLING MOTOR BEHAVIOR IN RAT ADULTHOOD

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During rat striatum postnatal (PN) development exist a critical period (PN30-37) of activity-dependent plasticity. Circular motor activity developed in Circling Training (CT) during this period elicits a permanent drop of ~45% in dopaminergic D2 receptors expression. Here, we have studied long-lasting motor behavioral alterations elicited by the antipsychotic drug haloperidol (D2 antagonist) administered at clinically relevant doses before (PN 20-27), during (PN 30-37) or after (PN 40-47) striatal critical period. Male rats Sprague Dowley were treated between PN 20-27, PN 30-37 or PN 40-47 with haloperidol (i.p. 0,7 o 2,5 mg/kg/day) or physiological solution (control). Then, we have tested motor performance in CT during adulthood (PN 90). Rats treated with haloperidol during the critical period exhibits exacerbated motor response in CT in respect to control animals for both doses ($p<0,01$). In contrast, drug administered before or after critical period have not shown alterations in circling behavior. These results uncovers a particular susceptibility period to the known haloperidol effects during postnatal development with permanence in adulthood.

DN-P17.
FOLIC ACID DEFICIENCY AND RETINAL DEVELOPMENT
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The important role of folate on DNA metabolism (methylation and synthesis) and its relationship with the etiology of Neural Tube Defects, made us think in a possible link between Folic Acid deficiency and extrinsic/intrinsic signals acting during cellular chick retinal fate determination, because is one of the most highly conserved parts of the central nervous system. We used the antifolate Methotrexate® (MTX), injected intra-ovo at three concentrations at Embryonal day 0 (E0) and E4. The animals were sacrificed at E14, when the final retinal configuration is reached and the retinal sections were compared between groups. There were no significant differences between weights of total control animals (n=20): 11,82g ± 0,50 vs. vehicle injected animals (VIA, n=20): 12,02g ± 0,51. All the E0 $MTX^{10\text{-}5\ Mol}$ injected animals (n=20) did not develop. With $MTX^{10\text{-}6\ Mol}$ and $MTX^{10\text{-}7\ Mol}$ only grew up 6 of 20 and 7 of 20 respectively ($p<0.005$ compared with VIA). Only 14 of 20 and 17 of 20 injected animals at E4 developed with $MTX^{10\text{-}5\ mol}$ and $MTX^{10\text{-}6\ Mol}$ respectively, and their weights were statistically inferior to the VIA group. In conclusion, our study indicates that MTX treatment slow down the total embryo development at E0 by all doses administered and at E4 except for $MTX^{10\text{-}7\ Mol}$. The chick retina was grossly abnormal and with decreased neuronal numbers. There are less retinal ganglion cells and the inner and outer plexiform layers are not well structured in treated embryos.

NR-P1.
ACTIVATION OF ALPHA-7 NICOTINIC RECEPTORS BY ANTHELMINTIC AGENTS
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Nicotinic acetylcholine receptors (AChRs) play key roles in chemical synapses. AChRs mediate neuromuscular transmission in nematodes and they are targets for antiparasitic drugs. The anthelmintic agents levamisole and pyrantel are potent agonists of nematode muscle AChRs but weak agonists of mammalian muscle AChRs. To understand the structural basis of the differential activation of anthelmintics among AChR subtypes, we studied the activation of α7 AChRs by these agents at the single-channel and macroscopic-current level. We used the high conductance form of the α7-$5HT_{3A}$ receptor, which is a good model for pharmacological studies of α7. Macroscopic and unitary current recordings show that at α7 levamisole is a weak agonist, whereas pyrantel is more potent than ACh ($EC50_{ACh}$ = 200 µM, $EC50_{pyr}$ = 45 µM). To identify determinants of this differential activation, we replaced residues of the complementary face of the binding site by the equivalent in muscle e subunit and evaluated the kinetic changes. The mutation Q57G does not affect the activation by ACh. However, it increases the EC50 and decreases the maximal response elicited by pyrantel. Single channels can be detected at 10-fold higher concentrations and the duration of bursts of openings significantly decreases with respect to the control chimera. The decreased sensitivity of Q57G-α7 to pyrantel agrees with its weak action at muscle AChRs, indicating that this residue may be involved in the differential activation of AChR subtypes by pyrantel.

NR-P2.
NEURONAL α7 NICOTINIC RECEPTORS: ROLE IN THE ACTIVATION OF HUMAN LYMPHOCYTES
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The neuronal nicotinic receptor α7 has been shown to be present in human lymphocytes. However, little is known about its functional role. In the present study we investigated the contribution of α7 to lymphocyte activation. We determined by RT-PCR the presence of α7 in lymphocytes after phytohemagglutinin (PHA) activation. α7 mRNA was detected in lymphocytes incubated for 48 hrs in the presence of PHA or PHA + bungarotoxin (BTX), an α7 antagonist. The modulation of lymphocyte activation by α7 was evaluated by measuring cell proliferation using ^{3}H-thymidine incorporation. Our results reveal that the presence of BTX during activation by PHA leads to a 2-fold increase in cell proliferation. In addition, BTX alone was unable to induce cell proliferation. Secretion of acetylcholine (ACh) and activation of muscarinic receptors (mAChRs) have been suggested to be involved in lymphocyte activation. We propose that the increment in the proliferation promoted by PHA+ BTX is due to an increase in the synthesis of ACh. To test this hypothesis, we performed RT-PCR to detect choline acetyltransferase (CHAT) mRNA after cell incubation with either PHA or BTX. The increase in CHAT, which catalizes ACh synthesis, is an indirect measurement for the increase of secreted ACh. We show that PHA and BTX induce CHAT gene expression. These results suggest that the blockade of α7 by BTX modulates ACh synthesis, and this event could affect lymphocyte activation through mAChRs.

NR-P3.
LEUKOCYTE ANTIGEN-RELATED PROTEIN TYROSINE PHOSPHATASE RECEPTOR: A SMALL ECTODOMAIN ISOFORM FUNCTIONS AS A HOMOPHILIC LIGAND AND PROMOTES NEURITE OUTGROWTH
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The identities of ligands interacting with protein tyrosine phosphatase (PTP) receptors to regulate neurite outgrowth remain mainly unknown. Analysis of cDNA and genomic clones encoding the rat leukocyte common antigen-related (LAR) PTP receptor predicted a small, 11 kDa ectodomain isoform, designated LARFN5C, containing a novel N terminal followed by a C-terminal segment of the LAR fifth fibronectin type III domain. RT-PCR and Northern blot analysis confirmed the presence of LARFN5C transcripts in brain. Transfection of COS cells with LARFN5C-Fcc DNA resulted in expression of the predicted protein, and Western blot analysis verified expression of 11 kDa LARFN5C protein *in vivo* and its developmental regulation. Beads coated with rLARFN5C demonstrated aggregation consistent with homophilic binding, and pull-down and immunoprecipitation assays demonstrated that rLARFN5C associates with the LAR receptor. rLARFN5C binding to COS cells was dependent on LAR expression, and rLARFN5C binding to LAR_/_hippocampal neurons was fivefold greater than that found by using LAR-deficient (-/-) neurons. Substratum-bound rLARFN5C had potent neuritepromoting effects on LAR-/- neurons, with a fivefold loss in potency with the use of LAR-/- neurons. rLARFN5C in solution at low nanomolar concentrations inhibited neurite outgrowth induced by substratum-bound rLARFN5C, consistent with receptor-based function. These studies suggest that a small ectodomain isoform of a PTP receptor can function as a ligand for the same receptor to promote neurite outgrowth.

NR-P4.
DIFFERENTIAL EFFECTS OF CERAMIDE ON NICOTINIC RECEPTOR DISTRIBUTION IN CHO CELLS
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With the aim to analyze the mechanism of modulation exerted by sphingolipids on AChR distribution and traffic in CHO cells, we studied the effects of long- and short-chain ceramides (Cer) on plasma membrane and intracellular AChR by ligand binding and fluorescence microscopy. We also compared the effects of natural (bovine brain) Cer and sphingosine. After 24 h treatment with a low (5 μM) concentration of C6-cer or natural Cer, increases of up to 50% were observed in the surface fluorescence of cells labeled with Alexa^{488}α-bungarotoxin, whereas intracellular label decreased. This effect was specific for the AChR, because the surface expression of VSVG-GFP was not modified. Treatment of CHO cells with 12.5 μM Cer did not alter this profile. Unexpectedly, higher Cer concentrations (25-37.5 μM) had the opposite effect, raising the levels of cell-surface AChR. These effects were not observed with a related lipid, sphingosine. Endogenous generation of Cer by sphingomyelinase decreased cell-surface AChR. The results indicate that ceramides modulate AChR distribution at the surface and intracellular compartments.

NR-P5.
NICOTINIC RECEPTORS AT THE NEUROMUSCULAR ENPLATE OF *Caenorhabditis elegans*
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Nicotinic acetylcholine receptors (AChRs) are pentameric ligand-gated ion channels that mediate synaptic transmission in both vertebrates and invertebrates. Nematode AChRs are of interest because they are targets for anthelmintic drugs as levamisole. The adult *C.elegans* neuromuscular junction contains two distinct nAChRs: the levamisole-sensitive receptor (LSR), and the levamisole-insensitive receptor. The subunit composition and activation kinetics of these AChRs remain unknown. By setting up a primary culture method that allows differentiation of *C. elegans* embryonic cells larva 1 (L1) muscle cells *in vitro* we described for the first time single-channel currents from AChRs of this nematode. Our results show that channels can be activated by both ACh and levamisole. Amplitude histograms of AChRs activated by ACh or levamisole at a membrane potential of -100 mV can be fitted by a main component of about 3.7 pA. The mean open time values are very similar for ACh- and levamisole-activated channels. These results suggest that a single AChR population is present at the L1 stage. Furthermore, single-channel recordings from levamisole-resistant mutants lacking the UNC-38 subunit, which has been shown to form the LSR, are similar to those obtained from wild-type nematodes. Our observations suggest that nematode AChRs have different developmental expression patterns and provide new insights into the relationship between antiparasitic drug sensitivity and developmental stage.

NR-P6.
INTERACTIONS BETWEEN HIGHLY HYDROPHOBIC MOLECULES AND THE ACETYLCHOLINE RECEPTOR IN NATIVE SYSTEM
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A group of highly hydrophobic molecules, steroids and free fatty acids (FFA), act as non-competitive inhibitors of the nicotinic acetylcholine receptor (AChR). The details of these interactions are still unknown. In this work, we used the fluorescence resonance energy transfer (FRET) between the intrinsic AChR fluorescence and the probe Laurdan to study effects of FFA and steroids on AChR-rich membranes from *T. californica*. Structurally different steroids and FFAs produced similar decreases in FRET efficiency (*E*), and competitive studies between them suggested the occurrence of equivalent sites for both types of molecules at the lipid-protein interface. Endogenous production of FFAs by controlled digestion of membrane phospholipids with phospholipase A2 produced similar decreases of *E*. Controlled hydrolysis of *T. californica* membranes with proteinase K suggests that the interaction sites for steroids and FFA on the AChR are located in the transmembrane regions. Experiments using desensitized AChR (1mM Carb) showed different diminutions of *E*, suggesting that the sites for hydrophobic molecules are sensitive to AChR conformation, and in particular to rearrangement of the transmembrane portions, making some of these lipid-protein interface sites (i.e. non-annular sites) no further accessible to exogenous molecules.

NR-P7.
CONFORMATIONAL REARRANGEMENT OF THE NICOTINIC ACETYLCHOLINE RECEPTOR INDUCED BY ITS LIPID ENVIRONMENT
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Perturbations of the nicotinic acetylcholine receptor (AChR) native lipid environment alter its function, pointing to the lipid-protein interface as the locus where "lipid signals" are gated to unleash the subsequent ion pore opening and closure, probably initiated by changes in the topology of the outer ring TM segments and ultimately leading to a conformational change of the whole AChR. The possible AChR conformational changes induced by perturbations of its native lipid microenvironment in *T. californica* AChR-rich membranes were studied with fluorescence spectroscopy using the probe crystal violet (CrV). This probe behaves as an AChR ion blocker, displaying higher affinity for the desensitized conformation (D, in the presence of agonist) than for the resting conformation (R, absence of agonist) of the AChR. We took advantage of these AChR-CrV differences in affinity to monitor AChR conformational states. K_D values for AChR-CrV interaction measured in the absence of agonist decreased both in cholesterol-depleted or cholesterol-enriched membranes, suggesting the occurrence of AChR intermediate conformational state(s). Addition of highly hydrophobic molecules that act as AChR non-competitive inhibitors (free fatty acids and steroids) to native membranes in the absence of agonist decreased the K_D values to levels obtained in the presence of agonist. The results indicate a direct correlation between lipid environment of the AChR and its conformational state.
Supported by grants from UNS, FONCYT and TWAS.

NR-P8.

EXPRESSION OF NMDA RECEPTOR SUBUNITS IS DIFFERENTIALLY MODIFIED BY ADMINISTRATION OF ENDOBAIN E, AN ENDOGENOUS Na^+, K^+-ATPase INHIBITOR

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Previous studies showed that endobain E, an endogenous Na^+, K^+-ATPasa inhibitor, decreases dizocilpine binding to glutamatergic ionotropic NMDA receptor. In the search of interplay between NMDA receptor and Na^+, K^+-ATPasa, herein we analysed the expression of NMDA subunits after endobain E administration. Endobain E or saline (control) solutions were administered i.c.v. and 3 days later animals were decapitated, cerebral cortex and hippocampus removed and tissues were subjected to differential centrifugation to isolate crude membranes fractions. Western blot analysis by 7.5% SDS-PAGE were performed. Nitrocellulose membranes were blocked for 1 hour, then incubated with the antibodies against subunists NR1, NR2A, NR2B and of NMDA receptor. The bands were visualised by quimiluminscence. After endobain E administration, expression of NR1, the most abundant subunit, highly increased in cerebral cortex and hippocampus. NR2A expression resulted enhanced or diminished in cerebral cortex and hippocampus, respectively. NR2B, the less abundant subunit, failed to change by the treatment. Results indicate that endobain E produced differential modifications in the expression of NMDA receptor subunits and support the hypothesis of a relationship between this receptor and Na^+, K^+-ATPasa.

NR-P9.

SYSTEMIC INJECTION OF BOTH EPINEPHRINE OR INSULIN IN CHICKS INCREASED, BY DIFFERENT MECHANISMS, THE GABAA RECEPTOR DENSITY IN THE SYNAPTOSOMAL MEMBRANE SURFACE

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The [^{3}H]-flunitrazepam receptor density measured ex vivo in the synaptosomal membranes surface at 4ºC was recruited by acute stress of chicks possibly through the phosphorylation of the $GABA_A$ receptors and/or their associated proteins. A systemic injection of epinephrine at 0.25 mg and 0.5 mg/kg of body weight increased the $GABA_A$ receptor expression in synaptosomes in a stress dependent way and in addition to the increase induced by stress. No changes were observed with propranolol. By other hand insulin at different doses increased the receptor density surface in non-stressed but not in the stressed chicks suggesting that insulin induced the recruitment of receptors, which is a different mechanism than occurs by the epinephrine. The results suggest that both epinephrine and insulin increased the $GABA_A$ receptors by a recruitment of receptors depending of the previous phosphorylation of the $GABA_A$ receptors and/or their associated proteins.

NR-P10.

PRESTIN, A KEY PLAYER IN OUTER HAIR CELL ELECTROMOTILITY, AND THE ?10 NACHR SUBUNIT SHOW POSITIVE SELECTION SIGNATURES IN THE MAMMALIAN LINEAGE

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The presence of outer hair cells (OHC) only in the mammalian cochlea and of OHCs active mechanisms, makes the mammalian cochlea a unique hearing device which has the capability of detecting a wide range of frequencies including the highest (more than 100 kHz) in the animal world. Several lines of evidence suggest that the motor protein prestin and α9α10-containing nicotinic receptors (nAChRs) are key players in the function or regulation of OHC active mechanisms. In order to understand how this system evolved only in the lineage leading to mammals we performed an evolutionary molecular analysis of these proteins. Prestin underwent some dramatic changes, especially in the sulphate transporter and antisigma-factor antagonist domain, after the split between mammals and birds. In addition, in placental mammals this gene is under strong purifying selection, suggesting that its function is highly important for these organisms' fitness and adaptation. On the other hand, the α10 nAChR subunit (but not α9) shows signatures of positive selection along the lineage conducting to mammals, suggesting a possible correlation between unique OHC characteristics and the evolution of new functions for this receptor subunit. Thus, we describe at the molecular level signatures of adaptive evolution of two OHC proteins in the lineage leading to mammals reflecting the importance of these proteins in OHC somatic electromotility.

NR-P11.

DIFFERENT SITES OF INTERACTION OF QUINOLINE DERIVATIVES WITH α9α10 nAChRs

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In this study, we report the effects of the quinoline derivatives quinine (Qn), its optical isomer quinidine (Qd), and chloroquine (Cl) on α9α10 nicotinic acetylcholine receptors (nAChRs). The compounds blocked acetylcholine (ACh)-evoked responses in α9α10-injected *Xenopus laevis* oocytes in a concentration-dependent manner, with a rank order of potency of Cl (IC_{50}=0.39μM) > Qn (IC_{50}=0.97μM) ~ Qd (IC_{50} =1.37μM). Moreover, Cl blocked ACh-evoked responses on rat cochlear inner hair cells with an IC_{50} value of 0.13μM. Block by Cl was purely competitive, whereas Qn inhibited ACh currents in a mixed competitive/noncompetitive manner. The competitive nature of the blockage produced by the three compounds was confirmed by equilibrium binding experiments using [H^3]methyllycaconitine. Block by Qn was found to be only slightly voltage-dependent, thus precluding open-channel block as the main mechanism of interaction of quinine with α9α10 nAChRs. The present results add to the pharmacological characterization of α9α10-containing nicotinic receptors and indicate that the efferent olivocochlear system that innervates the cochlear hair cells is a target of these ototoxic antimalarial compounds.

NR-P12.
AMPHETAMINE INDUCED RELEASE OF DOPAMINE IN PREFRONTAL CORTEX OF PRENATALLY STRESSED RATS
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Several authors have demonstrated the relationship between prenatal environment alterations and the etiology of brain disorders. We have previously shown that prenatal stress (PS) increases D2-type dopamine (DA) receptor levels in medial prefrontal cortex (MPC) and others authors have demonstrated an amphetamine vulnerability in male rat adult offspring. We analyzed DA MPC levels by microdialysis studies in freely-moving PS male offspring following amphetamine s.c. administration. Regarding the importance of pubertal stage in DA neurotransmission regulation, we performed this study in prepubertal (4 weeks) and adult (8 weeks) PS male offsprings. Basal DA levels were not significantly changed between control and PS prepubertal and adult offspring. DA MPC levels found in response to amphetamine challenge were lower in prepubertal prenatal stressed offspring comparing to control. These data might suggest that the reduced effect of amphetamine in MPC of prepubertal PS offsprings might be related to the elevated levels of dopamine receptor observed in the same region in the adult PS offsprings.

NR-P13.
PRESSOR EFFECT OF NORADRENALINE INJECTION INTO THE SUPERIOR COLLICULUS OF THE RAT BRAIN
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INTRODUCTION AND GOALS: The superior colliculus (SC) is involved in central blood pressure control. Chemical stimulation of SC evoked either pressor (excitatory amino acids and Angiotensin II) or depressor responses (endotelin-1). Although noradrenergic nerve terminals have been shown to be present in the SC, there are no reports on the effects of local injections of noradrenaline (NA) into this area. Taking that into consideration we studied the cardiovascular effects of the injection of NA into SC of unanesthetized rats. METHODS: Male Wistar rats were used (220-270g). Guide cannulas were implanted into the SC commissure. A catheter was introduced into the right femoral artery for blood pressure recording. RESULTS: NA injections (15nmol/50nL) evoked blood pressure increase (ΔMAP= +41.0 ± 4.5 mmHg, n= 6) and heart rate decrease (ΔHR= -42.5 ± 5.8 mmHg, n=6) in unanesthetized rats. The pressor response to NA was blocked by intravenous pretreatment with mecamylamine. CONCLUSION: The results suggest the existence of a sympathetic pressor noradrenergic mechanism in the SC of the rat.
FINANCIAL SUPPORT: G.G. Pelosi (fellowship from FAPESP - 02/14149-7).

NR-P14.
ABSENCE OF ß-ENDORPHIN CAUSES AN INCREASE IN SYSTOLIC BLOOD PRESSURE AND SYMPATHETIC ACTIVITY DURING SODIUM OVERLOAD
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The objective of this study was to test the hypothesis that low levels of ß Endorphins are involved in the development of salt sensitivity hypertension probably due to an enhance in sympathetic activity.
ß-Endorphin KO ($ßend^{-/-}$), HT ($ßend^{+/-}$) and WT ($ßend^{+/+}$) mice were submitted for two weeks with either low- or high-sodium diet (HSD) and systolic blood pressure (SBP), urinary catecholamines and brain pattern of Fos immunoreactivity (Fos-ir) were evaluated in each group.
HSD caused a significant increase in SBP in $ßend^{-/-}$ mice ($P < 0.01$) but non-changes were observed in $ßend^{+/+}$ and $ßend^{+/-}$ mice when kept on the same experimental conditions. With regard to brain activity, $ßend^{-/-}$ mice maintained on a HSD showed a significant increase in Fos-ir neurons in the median preoptic nucleus ($p< 0.01$) when compared to $ßend^{+/-}$ and $ßend^{+/+}$ animals. Additionally, $ßend^{-/-}$ mice had higher levels of urinary epinephrine excretion ($p < 0.05$) when placed on a HSD in comparison to $ßend^{+/+}$ and $ßend^{+/-}$ animals. Thus, these results suggest that the ß-endorphinergic system may participate in blood pressure regulation during sodium overload probably inhibiting sympathethic activity.
Supported by ANPCyT and CONICET.

NR-P15.
MODIFICATIONS IN THE EXPRESSION OF S100ß AND SEROTONIN IN TRIGEMINAL SUBNUCLEUS AFTER PULPAR MOLAR INJURY
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Orofacial pain is modulated in trigeminal subnucleus before it reaches higher centers of the sensitive pathways. In a previous work we have studied the expression of the astrocytes protein S100 and S100ß in a region of the caudal spinal trigeminal nucleous. In this work we analyze some modifications of serotoninergic fibers in the trigeminal caudal and intermediate spinal nucleus, as also the astroglial protein S100ß along these nucleous.
Adult Wistar rats were used, and the first left inferior molar pulpar chamber was unroofed and the pulp exposed. This procedure produces an inflammatory response accompanied with pain and animals showed a behaviour which may be equivalent to human pain. Glial and neuronal modifications, at different levels of the trigeminal caudal subnucleous, were done at 2 hours, 7 and 35 days post pulpar injury.
Our results show that there is an astroglial reaction based on an increase in S100ß positive astrocytes and that these modifications are evident in the caudal subnucleus. On the other hand, serotoninergic fibers, widespread throughout the spinal trigeminal nucleous, show an increment in its extension and in the number of varicosities. These changes were significative in the superficial zones (laminae I/II) of the injured side of caudal subnucleus.
(UBACyTO007).

NR-P16.
KETAMINE: DIFFERENTIAL ACTIVATION OF MESENCEPHALIC RAPHE NUCLEI
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Drug challenges induce transient neuronal expression of the cFos protein. Ketamine (KET) produces analgesia and psychoto-mimetic effects. We studied the midbrain neuronal activation under KET administration as showed by cFos inmuno-fluorescence (-if). Male Wistar rats (250-300g) received KET 100mg.kg^{-1} ip (n=6) or saline (n=6). Brain sections were processed by double-if detection of cFos + TPH or TH. Sections were photographed and analysed. The rostral portion of the linear nucleus (rLN) showed an intense KET-induced cFos-if; all neurons were negative for TPH/TH. Activation of the ventral tegmental area (VTA) (21,15% of 520 neurons analysed) was restricted to large cells, while small ones did not show cFos-if. Only a minimal amount of TPH-if neurons showed a concomitant cFos-if: in midbrain dorsal raphe nucleus (7,40%, n=324), caudal LN (8,23% n= 265) and medial lemniscus nucleus (8,10% n=278). Raphe nuclei showed many cFos-if TPH/TH negative cells. The rLN exhibited a strong activation, and lacked TH-/TPH-if neurons. The VTA and the 5-HT nucleus showed activation mainly of large neurons, suggesting that KET actions may be mediated by projection neurons. Further studies are needed to identify the chemical identity of KET-activated raphe TPH/TH negative neurons. Previous studies have demonstrated that anti-5-HT drugs suppresses KET analgesia while those inhibiting cathecolaminergic neurotransmission, like α_2-agonists, diminished psychotomimetic effects suggesting, in concordance with the present work, that the midbrain raphe nuclei may be an important target of KET action.
Grant UBACyT M-072.

NR-P17.
NEUROENDOCRINE REGULATION OF SODIUM INTAKE INDUCED BY PERITONEAL DIALYSIS
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The goal of the present work was to study the neuroendocrine response before and after salt-induced ingestion stimulated by peritoneal dialysis (PD).
Adult male Albino rats were sodium depleted by peritoneal dialysis. Twenty-four hours after PD or PD sham, the groups were designed as control (PD sham) with no access to water and 2% NaCl (C-NA), PD with no access to water and 2% NaCl (PD-NA), control (PD sham) with access to water and 2% NaCl (C-A), and PD with access to water and 2% NaCl (PD-A).The animals were decapitated and bled before and after test. The trunk blood was collected and plasma levels of ANP and OT were measured by radioimmunoassay as previously described by Godino et al. (2005).
Compared with the others groups PD-A showed an increase in the plasma levels of ANP and OT ($p<0.05$). Moreover, the plasma level of ANP in the PD-A group had a higher significant increase compared to PD-NA ($p<0.001$).
Our data indicate that plasma OT and ANP are released during the satiety process of sodium appetite. ANP also participate during the sodium appetitive phase. This evidence suggest that sodium body status modulates the OT and ANP release to blood circulation.
Supported by CONICET and CNPq.

NR-P18.
ANGIOTENSIN-(1-7) ENHANCES PHOSPHOINOSITIDE HYDROLYSIS IN NEONATAL RAT BRAIN
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Ang-(1-7) is an endogenous peptide hormone of the renin-angiotensin system which exerts diverse biological actions some of them involving noradrenergic system. Since the ability of norepinephrine to modify phosphoinositide (PI) turnover has been previously described, the purpose of this study was to evaluate potential effect of Ang-(1-7) on such signalling system. Neonatal rat brain prisms were incubated with [^{3}H]*myo*inositol in Krebs-Henseleit buffer during 60 min. Then, drugs were added and incubation continued for 30 min. Reaction was stopped with cloroform/methanol and [^{3}H]inositol-phosphates (IPs) accumulation was quantified. It was observed an enhancement of PI hydrolysis, which ranged from 30% to 60% in the presence of 0.01 nM to 100 nM Ang-(1-7). Since biological actions of Ang-(1-7) are blocked with [D-Ala7]Ang-(1-7), experiments with peptide were also carried out. It was observed that with 0.1 nM Ang-(1-7) IPs accumulation attained 161 ± 26.5% (n = 8), a value which was even higher (213 ± 23.5%, n = 5; $p< 0.01$) in the presence of [D-Ala7]Ang-(1-7). This antagonist alone was likewise able to increase PI turnover (153 ± 5.1%, n =3). Results indicate that Ang-(1-7) is able to modify cell signalling system mediated by PI hydrolysis, an effect most likely independent of Ang-(1-7) receptor which is blocked by [D-Ala7]Ang-(1-7).

NR-P19.
BRAIN FOS IMMUNOREACTIVITY PATTERN AFTER FLUID REESTABLISHMENT IN WATER DEPRIVED RATS
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Adult male rats deprived of water for 36 h were allowed to rehydrate either, by ingesting only water for 90 minutes (WD/WR group) or water and 0.9% NaCl for 120 minutes (WD/WSR group).
Compared with control sham deprivated group the number of Fos immunoreactive cells (Fos-ir), increased in the subfornical organ (SFO), median preoptic nucleus (MnPO), ventral, medial and posterior subdivisions of the paraventricular nucleus (PaV, PaMP, and PaPo, respectively) and in the lateral parabrachial nucleus (LPBN), in both WD/WR and WD/WSR groups.
After rehydration with water and isotonic sodium solution we observed a decreased number of Fos-ir neurons in the SFO, MnPO, PaV, PaMP, PaPo and the LPBN, compared to animals rehydrated only with water.
Our results indicate changes in brain Fos-ir neuronal pattern after water or water and sodium reestablishment. These evidences identify the brain neuronal cluster involved in the different phases of hydro-electrolyte restore.
Supported by ANPCyT and CnPQ.

NR-P20.

REGULATION OF EXTRACELLULAR ATP IN THE ZEBRAFISH RETINA: ECTONUCLEOTIDASES LOCALIZATION AND ACTIVITY

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ATP is released by neurons and acts as a neurotransmitter and neuromodulator via purinergic receptors. Extracellular ATP concentrations are precisely regulated by ecto-nucleoside-triphosphate diphosphohydrolases (ENTPDases). Resulting AMP is further metabolized extracellularly to adenosine, a potent inhibitory neuromodulator. We showed that two major subtypes of ectonucleotidases: ENTPDase 1 and 2 are expressed in the zebrafish retina both by western blot and immunohistochemistry on 10 µm tissue sections. Both enzymes are heterogeneously distributed among retinal layers, principally expressed in the inner half of the retina but their expression pattern overlaps only partially. ENTPDases 1 and 2 activities can be distinguished one from each other by autoradiography using radioactive nucleotides as substrates. To date, we demonstrated extracellular ATPase activity on zebrafish retinal sections by this methodology. We are currently comparing whether the site of ATP hydrolysis matches the immunoreactivity patterns for both enzymes. In conclusion, the retina of zebrafish contains two different ENTPDases with ATPase activity. Interestingly, these enzymes show a specific distribution on cell subtypes and synaptic retinal layers. Since ENTPDase 1 produces AMP in a much higher rate than ENTPDase 2, our results suggest a localized regulation of extracellular adenosine levels.

NR-P21.

COMPENSATION OF P/Q-TYPE CALCIUM CHANNELS ABLATION AT MICE LATERAL SUPERIOR OLIVE INHIBITORY SYNAPSES

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Ataxic transgenic mice in which α_{1A} subunit of the P/Q-type calcium channels are ablated, knock-out (KO), provide a powerful tool with which to test compensatory mechanisms at synapses. We used whole cell voltage clamp recordings in mice auditory brainstem slices to study calcium channels involved in glycinergic transmission from the medial nucleus of the trapezoid body (MNTB) to the lateral superior olive (LSO). Evoked inhibitory postsynaptic currents (IPSCs) were recorded in wild type and KO and specific toxins were applied to investigate the contribution of the different channels to transmitter release. In wild type, glycinergic release was mediated with similar efficacy by both P/Q-type and N-type calcium channels and a smaller but significant contribution of L-type calcium channels. KO inhibitory synapses strength was preserved by both N and L-type calcium channels which functionally compensate for the absence of the P/Q subtype. When N-type calcium channel blocker, ω-conotoxin GVIA, was applied first, it completely abolished the IPSC while in the same conditions L-type calcium channel blocker, nitrendipine, blocked IPSCs by only 50 %. This indicates that both calcium channels contribute to the release of a single vesicle but N-type calcium currents predominate. This may increase the susceptibility to presynaptic inhibition by G protein mediated signaling.

NR-P22.

ABLATION OF P/Q-TYPE CALCIUM CHANNELS AT THE MICE LATERAL SUPERIOR OLIVE INHIBITORY SYNAPSES

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We used whole cell voltage clamp recordings in mice auditory brainstem slices to study calcium channels involved in glycinergic transmission from the medial nucleus of the trapezoid body (MNTB) to the lateral superior olive (LSO). Evoked inhibitory postsynaptic currents (IPSCs) were recorded in wild type and transgenic mice, knock-out (KO), in which α_{1A} channels are ablated and specific toxins were applied to investigate the contribution of the different channels to the transmitter release.
Our results show that, in wild type, glycinergic release was mediated with similar efficacies by both P/Q-type and N-type calcium channels but with a significant contribution from L-type calcium channels, whereas in KO we found that both N and L-type calcium channels functionally compensate for the absence of the P/Q subtype but in different ways. When N-type calcium channel blocker, ω-conotoxin GVIA, was applied first it completely abolished the IPSC. In contrast, when it was applied after the L-type calcium channel blocker, nitrendipine, it blocked only 50%. This indicates that both calcium channels contribute to the release of a single vesicle but N-type calcium currents are predominate.

NP-P23.

Ca^{2+} INFLUX THROUGH L-TYPE CHANNELS MODULATES VESICLE RECYCLING AT MOUSE MOTOR NERVE TERMINALS

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Electrophysiological and immunocytochemical studies have shown that different types of calcium channels are expressed at adult motor nerve terminals, but their physiological role is not yet completely understood. We have studied synaptic vesicle recycling using FM dyes. In agreement with previous electrophysiological studies, destaining (exocytosis) of FM loaded nerve terminals was strongly inhibited by blockers of the P/Q type channels, but was not affected by N- or R- type channel blockers. In contrast, L-type channel blocker nitrendipine alters destaining kinetics at stimulation frequencies over 20Hz. Endocytosis was studied by measuring the uptake of FM after a period of strong exocytosis and its time course was estimated by adding dye at different times after tetanus. Uptake of FM decayed to zero a few minutes after the tetanus. Preincubation with nitrendipine increases and prolongs the after-tetanus uptake, suggesting an important role of L-type channels in endocytosis and vesicle recycling processes.

NR-P24.
TRANSMITTER RELEASE AND ITS REGULATION BY PRESYNAPTIC RECEPTORS AT THE CALIX OF HELD SYNAPSES OF KNOCK OUT MICE LACKING P/Q TYPE CALCIUM CHANNELS

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Calcium channels of the P/Q subtype mediate transmitter release at various central synapses. Transgenic mice in which a1A channels are ablated provide a powerful tool to study compensatory mechanisms and presynaptic regulation associated with expression of P/Q channels. We use the technique of whole cell patch clamp to measure presynaptic Ca^{2+} currents (Ica) from the calyx of Held nerve terminal and excitatory postsynaptic currents (EPSCs) at the soma of the neurons in the medial nucleus of the trapezoid body (MNTB). We demonstrate that neurotransmitter release at KO mice (where transmission is mediated by N-type Ca^{2+} channels) fail to undergo pair pulse facilitation due to the absence of pair pulse facilitation in the presynaptic Ica. In addition, the relation between calcium current and induced EPSC is altered, indicating high calcium cooperativity at synapses from KO mice. This relation can be approximated by $EPSC=Ica^n$ with $n=2.62\pm0.21$ for WT and 3.44 ± 0.22 for KO. We also study the modulatory role of different type of presynaptic receptors in transmitter release. Activation of presynaptic $GABA_B$ receptors generates a more potent inhibition of Ica in KO mice (through N-type Ca channels) than in WT mice (through P/Q-type Ca channels). Consequently, glutamate release and EPSCs shows a higher sensitivity to the selective $GABA_B$ agonist baclofen, which causes a potent inhibition of synaptic transmission in a dose dependent manner.

NP-P1.
DECREASED LEVEL OF NITRIC OXIDE PRODUCTION AND RESISTANCE TO EAE IN AGED RATS

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The primary effector mechanisms of the immune system leading to tissue destruction and clinical signs in EAE remain unclear. Macrophages invading the CNS and its inflammatory mediators like NO are suggested to be involved. Aged rats are resistant to the induction of the classic EAE paralysis signs when compared with the highly susceptible young rats. To test the contribution of NO on EAE pathogenesis, aged and young Wistar rats were immunized with myelin antigens in CFA and analyzed with respect to the capacity of macrophages to invade CNS and to produce NO. Demyelination degree and immunological response were also evaluated. Histological analysis of spinal cord and cerebellum showed no differences in inflammation or extent of demyelination between both age groups. The ratio between proinflammatory ($CD4^+IFN\text{-}\gamma^+$) and anti-inflammatory ($CD4^+IL\text{-}4^+$) T-cells was lower in aged animals as assessed by flow cytometry analysis of intracellular cytokines. Enriched macrophage populations obtained by plate cell adhesion after peritoneal lavage were used to quantify NO production. Determination by Griess reaction of NO amounts showed that during EAE macrophages from aged rats are less capable to produce NO than those from young ones. It can be concluded that NO production during EAE development can play an essential role in the pathogenesis of active EAE.

NP-P2.
CHANGES IN SYNAPSIN I PHOSPHORYLATION DURING THE ACUTE STAGE OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE)

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Significant morphological and functional alterations of synapses have been described in EAE. Also, it has been shown that antibodies and T cells against the encephalitogenic myelin basic protein (MBP) immunocrossreacts with Synapsin I (Syn), a neuron-specific protein located mainly in the presynaptic terminals. Syn phosphorylation controls its association to synaptic vesicles and actin filaments, playing an important role in the regulation of neurotransmitter release. In this work we study the state of phosphorylation of Syn during the development of EAE induced in Wistar rats. The synaptosomal fractions (P2) were purified from brain, cerebellum and spinal cord of EAE and control animals, and subsequent quantification of total Syn and phosphorylated Syn at Site 1 (phosphoSer9) was done by immunoblot analysis. We found that in the acute and recovery stages of EAE the amount of total Syn was similar in all the tissue samples. However, phosphorylation of Syn was significantly diminished (~40%) in the lumbosacral spinal cords in the acute period but it was restored to normal values after the recovery period. These observations suggest that an alteration on the mechanism of neurotransmitter release could be involved in the development of the clinical signs of EAE.

NR-P3.
DIMINUTION OF IONOMYCIN-TRIGGERED GLUTAMATE RELEASE FROM SYNAPTOSOMES ISOLATED FROM RATS WITH EAE

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Recent evidence indicates that, besides demyelination, axonal and neuronal degeneration also occurs in both multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE), an animal model for MS. The mechanisms underlying neuronal dysfunction in MS and EAE remain elusive. To address this issue, we have analyzed the neurotransmitter release from cerebrocortical synaptosomes from EAE and control animals. The effect on evoked release of glutamate stimulated by KCl or ionomycin was monitored using an enzyme-linked fluorometric assay. The results showed a diminished ionomycin-triggered glutamate release from EAE synaptosomes compared with both that from control synaptosomes and the K+-evoked glutamate release from the same EAE synaptosomes. This inhibited synaptosomal activity was restored by increasing extracellular Ca+2 levels. The observed alteration in neurotransmitter release could be due to a possible variation of the synaptosome membranes isolated from animals with EAE, which as well could affect the normal operation of the cerebrocortical nerve terminals.

NP-P4.
DEVASCULARIZING LESIONS OF BRAIN MOTOR CORTEX FOR THE STUDY OF NEUROPROTECTION
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Most models of ischemic damage in rodent brain require occlusion of one of the major cerebral vessels. However, human brain disabilities often represent the sum of minor ischemical lesions. To analyze the recovery of such kind of lesions in mice, we have developed a model of motor disability produced by selective devascularization of sensory-motor cortex. Here, we demonstrate the recovery pattern of these lesions and show that it can be significantly improved by treatment with Granulocyte Colony Stimulation Factor (G-CSF).
C57Bl/6J mice were used. Motor function was evaluated with the Beam Walking Test 3 days before and 16 days after surgery. After devascularization, animals were separated in two groups receiving 50 mg/kg/day G-CSF (Filgrastim. Biosidus) or saline during 10 days after surgery.
Analysis of behavioural data indicated that mice receiving G-CSF had a better motor performance since the beginning of treatment. Those mice showed complete recovery 10 days after surgery, whereas control mice still showed motor dysfunction after 16 days. Devascularizing cortical lesions appear like a suitable model for the study of neuroprotective therapies and seem to be sensitive to G-CSF effects as shown in other studies.

NP-P5.
NEURONAL ALTERATIONS DUE TO FOCAL CHEMICAL HYPOXIA
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Hypoxia is one of the pathological factors inducing neuronal injury. Cobalt chloride ($CoCl_2$) is a synaptic blocker and activator of the hypoxia-inducible factor-1. Adult male Wistar rats were anaesthesized, placed in a stereotaxic device and intracerebrally injected with 2 µl of 50 mM $CoCl_2$ in the right hemisphere and with 2 µl of saline solution (SS) in left hemisphere. The site of injection was layer 2-3 of fronto-parietal cortex (Bregma -1.30 mm). One group of animals was fixed 6 hours after the surgery and other group 5 days later. The lesion area was studied by electron microscopy and neuronal death was determined by Hoescht 33342 and TUNEL techniques. Larger intercellular spaces and a disruption of neuronal and glial morphology with extended processes forming an irregular network were observed. In the penumbra area abundant images of nuclear cleavage, cytoplasmic multivesicular contents and altered mitochondria ultrastructure (abnormal crests with swelling images). There were abnormal presynaptic structures as well as lamellar images of membranous processes included in the matrix between neurons and glial cells. Outside the penumbra limits and in the SS injection zone, the brain tissue presented the typical morphology. Abundant TUNEL+ nucleus and with various lobes (stained with Hoescht 33342) indicated cellular death 6 hs after the treatment but not at 5 days. Our results suggest that $CoCl_2$ is able to induce local neuronal and glial alterations in the $CoCl_2$-exposed brain tissue that persist 5 days after the lesion. The localized damage makes this model useful to induce a very focal brain injury.
Grant UBACyT M-072.

NP-P6.
NEUROFIBRILLARY TANGLES AND Al^{3+} ACCUMULATION IN RAJIDAE ELECTRIC ORGAN: AN X-RAY MICROANALYSIS
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An association between the presence of Al^{3+}, Si^{2+} and Alzheimer's disease (AD) has been reported. At the evidence of the accumulation of oxygen, Al^{3+} and Si^{2+} in Rajidae electrocyte by X-ray microanalysis, it was decided to explorer the presence of neurofibrillary tangles (NFT´s). *Psammobatis extenta* is an electrical fish from Rajidae family. According to their semicircle electrocyte shape, they are the less derived of this family. Electric organ cryostat sections of 8 µm of *P. extenta*, were performed along its antero-posterior axis; sections were treated by a modified Bielschowski method. It was observed this lesions, by a light microscope, distributed in the electrocyte cytoplasm. NFT´s have a circular shape and are around of 2-3 µm. In accompanied muscle cells, NFT´s are absent. This observation support the idea of an important role of Al^{3+} accumulation and oxidative stress in NFT´s development. In AD and in Rajidae electrocyte, cholinergic innervation are affected. *P. extenta* electrocyte can be use as an animal model for AD.
*Supported by grants to MPF, from the SGC y T, UNS, Argentina. [2]*IBRO Fellowship/short-stays 2005. Present address: CIBN, Universidad Nacional Mayor de San Marcos, Perú.*

NP-P7.
THE FORMATION OF A STABLE COMPLEX BETWEEN Aβ AND INSULIN-DEGRADING ENZYME: A POSSIBLE *TRANS*-ACTING PATHWAY OF AMYLOID SELF-PROPAGATION IN THE BRAIN
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The accumulation of aggregated amyloid β of 40-42 residues in the brain is a major pathogenic mechanism in several neurodegenerative diseases. Insulin degrading enzyme (IDE) is a zinc-metalloendopeptidase that removes brain soluble Aβ under physiologic conditions. In the course of our study of Aβ-IDE interaction, we found that upon incubation *in vitro*, in presence or absence of specific inhibitor for IDE, Aβ was capable of forming a complex with the enzyme that resisted boiling in 4% SDS-0.1M DTT. Moreover, this component was not dissociated in 8 M urea or 70% formic acid, underscoring its high stability. We further mapped the interaction to the region 16-28 of Aβ, by using an N-terminal fluorescein-labeled Aβ16-28. We found that complex formation was a very slow process (*t1/2*~ 45 min) and it was specifically out competed with insulin, another substrate of IDE. These findings suggest that although the formation of stable Aβ-IDE complexes may not be dependent on the catalytic mechanism, it requires the interaction of the central region of Aβ with the substrate binding site of IDE, or at least a part of it. The full characterization of a non-productive pathway in Aβ-IDE interaction and its detection *in vivo* may provide novel insights about the self-propagation of the amyloidogenic process in the brain.
(Alzheimer's Association IIRG035312, PICT2002-10599).

NP-P8.

INSULIN-DEGRADING ENZYME AS A DOWNSTREAM TARGET OF STRESS AND GROWTH FACTORS SIGNALING CASCADES: IMPLICATIONS FOR ALZHEIMER'S DISEASE INTERVENTION

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Insulin-Degrading Enzyme (IDE) is one of the proteins that has been demonstrated to play a key role in degrading amyloid β (Aβ) *in vitro* and *in vivo*, raising the possibility of upregulating IDE as an approach to reduce Aβ. Little is known, however, about the cellular and molecular regulation of IDE protein. The aim of this work was to evaluate the role of stress and growth factors (GF) cell signaling pathways on IDE protein and mRNA expression levels. In this study, primary astrocytes from new-born rats, human THP-1 monocytic cells and N2a mouse neuroblastoma cells (wild type and over-expressing Aβ) were used as cellular models in providing mechanisms by which IDE may be regulated in the brain. Cell-cultures were subjected to stressor agents such as Aβ exposure at 1 μM concentration (sub-optimal for neurotoxicity), 1 mM H_2O_2 treatment, serum deprivation and *in vitro* replicative aging according to standard protocols. Immunofluorescence confocal microscopy was used to investigate sub-cellular IDE expression. IDE transcripts levels were monitored by RT-PCR. The findings in this study tentatively suggest that GF and stress signaling may strongly induce IDE expression in the brain. We propose that IDE may be modulated as a novel therapeutic intervention in Alzheimer`s disease.
(PICT2002 10599, IIRG 03-5312).

NP-P9.

PHOSPHORYLATION OF COFILIN BY LIMK1 MEDIATES AMYLOID β-INDUCED DEGENERATION IN ALZHEIMER´S DISEASE

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Deposition of Amyloid β fibrils (fAβ) plays a critical role in Alzheimer´s disease (AD). FAβ-induced dystrophy requires the activation of focal adhesion proteins and the formation of aberrant focal adhesion structures. Focal adhesions are actin-based structures that provide estructural link between the extracellular matrix and the cytoskeleton. To gainfurther insight in the molecular mechanism of neuronal degeneration in AD, here we explored the involvement of LIM kinase 1 (LIMK1) and cofilin (Cofilin) in Aβ-induced dystrophy. Cofilin is an actin binding protein that plays a central role in actin filament dynamics, and LIMK1 is the kinase that phosphorylates and thereby inhibits Cofilin. Our data indicate that treatment of hippocampal neurons with fAβ increases the level of phospho-Cofilin and phospho-LIMK1, accompanied by a dramatic remodeling of actin filaments and neuritic dystrophy. A synthetic peptide, S3 peptide, which acts as a specific competitor for Cofilin phosphorylation by LIMK1, inhibited fAβ-induced Cofilin phosphorylation, preventing dystrophy, indicating the involvement of LIMK1 in Aβ-induced degeneration *in vitro*. Immunofluorescence analysis of APP23 and 3XTg-AD transgenic mice, and AD brain showed high level of P-LIMK1 in pathologically affected areas and in neurons depicting early signs of AD-pathology. Thus, LIMK1 activation may play a key role in the pathologic neuroplastic remodeling of neurites in AD.

NP-P10.

Si^{2+} AND CHALCEDONY PRECIPITATION DURING OXIDATIVE STRESS IN RAJIDAE ELECTROCYTE: A MINERALOGICAL STUDY

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At the evidence of the accumulation of Oxygen, Al^{3+} and Si^{2+} in Rajidae electrocyte by X-ray microanalysis it was decided to explorer the presence of Si minerals by a transmitted light petrographic microscope. Fish from San Antonio Oeste, Río Negro Province, were used because electrocyte Si^{2+} peaks are more important than the same peaks present in fish from Bahía Blanca Estuary. Electric organ cryostat sections of 8 μm of *Psammobatis extenta*, were performed along its antero-posterior axis, fixed for 15 min at 4°C in 3.7% formaldehyde in 0.1 M PBS, pH 7.2, then washed with PBS and mounted with PBS/glicerol (1:1). It was observed chalcedony distributed in the electrocyte cytoplasm. Chalcedony is a typical mineral present in fossilization process in different organic matter from a wide geological record. Relationship between pH and the solubilities of calcite, quartz, and amorphous silica is well known. High hydrogen ion concentration cause precipitation of SiO_2. This observation support the idea of an important role of SiO_2 accumulation and pH, in electrocyte fossil formation. In Alzheimer's disease (AD) and in Rajidae electrocyte, cholinergic innervation are affected.
Supported by grants to MPF, from the SGC y T, UNS, Argentina.

NP-P11.

INSULIN DEGRADING ENZYME: THE SECRETORY PATHWAY IMPLICATED IN THE CLEARANCE OF AMYLOID β

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Newly generated soluble amyloid β (Aβ) peptide is rapidly cleared from the brain and this process may be defective in Alzheimer`s disease. Insulin degrading enzyme (IDE) is a major brain protease capable of degrading Aβ but little is known about the sub-cellular compartment where IDE-Aβ interaction occurs. It has been recently proposed that Aβ generation and oligomerization takes place in cholesterol and glycosphingolipids-rich membrane regions defined as *lipid-rafts*. We hypothesize that membrane associated and secreted IDE isoforms are critical in the process that sustains the levels of soluble brain Aβ peptide within a physiological range. Aims: 1- Characterization of membrane-associated IDE. 2- Identification of the mechanisms involved in IDE secretion. Methods: Detergent resistant microdomains (DRMs) were isolated from rat brain and N2a cells by Triton X-100 treatment and sucrose gradient flotation. To visualize IDE in lipid rafts of living cells immunofluorescence was made using Alexa fluor 594-cholera toxin subunit B and 1C1 anti-IDE monoclonal antibody. IDE/lipid rafts-cholesterol association was study by Methyl β-cyclodextrin (MβCD) treatment followed by DRMs isolation. IDE release was tested by pulse chase and immunoprecipitation in the presence of Monensin, Brefeldin A, MβCD and low temperature. IDE post-translational (PT) modification were assessed by metabolic labeling using ^{35}S-methionine, ^{3}H-etanolamine and ^{3}H-palmitic acid. Exosomes were purified from culture media of N2a cells by sequential ultra-centrifugation. Characterization of the pellets was performed by western blot using BC2 (anti-IDE polyclonal antibody), anti-Bip, and anti-flotillin. An aliquot was fixed with 4% paraformaldehide, negatively stained with uranyl acetate and visualized under electron microscopy. Results: 1-A pool of membrane IDE resists alkaline treatment and is active in DRMs. 2- IDE lacks GPI and palmitoylation PT modifications 3- Cholesterol depletion disrupts IDE-DRMs association, 4- IDE is associated with lipid rafts in living cell, 5- IDE release is not mediated by the ER-Golgi pathway and exosomes may be involved in this process. Conclusions: our experimental evidences indicate that specific pools of IDE may associate with lipid raft or translocate from the cytosol to extracellular compartment to allow IDE-mediated proteolysis of soluble Aβ.

NP-P12.
INSULIN DEGRADING ENZYME OVER-EXPRESSION IS ASSOCIATED WITH AMYLOID β PLAQUE DEVELOPMENT
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Insulin degrading enzyme (IDE) is implicated in amyloid β (Aβ) degradation. Previously we reported the presence of IDE around the amyloid plaques from old transgenic mice Tg2576 (Tg) overexpressing a mutated form of human Aβ precursor protein used as animal model of Alzheimer`s disease (AD). The aim was to evaluate if IDE distribution, its expression and Aβ accumulation are time-course related events. Cryostat coronal brain sections of 11, 15, 17 and 23 months of age (m) Tg and non-transgenic (NTg) mice were subjected to semi-quantitative immunohistochemistry (ImagePro software) (n=4/group) to evaluate Aβ (S40 antibody), IDE (1C1 antibody), astrocytes (anti-GFAP) and microglia (tomato lectin, lycopersicon esculentum) reactivity. Tg mice beyond 11 m presented Aβ deposits in hippocampus, cerebral cortex, amygdala and corpus callosum, with a sharp age-dependent increase in the number of the deposits between 17 and 23 m. Aβ deposition showed reactive gliosis, with increasing numbers of astrocytes and microglial cells surrounding the plaques. IDE was observed at similar levels in the cytoplasm of neurons in Tg and NTg, at all evaluated ages. In addition, a strong IDE immunoreactivity, that co-localized with activated glial cells, was detected surrounding 14% of Aβ deposits in 15 m Tg and 100 % of plaques in 17 and 23 m Tg. IDE plaques mean diameter ranged form 30 to 100 μm, with no size variations among different ages. These results suggest that Aβ plaque-deposition is synchronous with glial IDE overexpression and may limit plaque growth in this model of AD.
(IIRG 03-5312, PICT 05-10599).

NP-P13.
PROLYL OLIGOPEPTIDASE INACTIVATES BRADYKININ IN BRAIN OF RATS SUBMITTED TO AN EXPERIMENTAL MODEL OF ALZHEIMER'S DISEASE
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The nervous system in mammals contains a large number of peptides and proteases that play a role in neurological pathologies such as the Alzheimer's disease (AD). The presence and relevance of kallikrein-kinin system components has been suggested to contribute to AD pathology. In this work, we have studied the bradykinin (BK) releasing and its inactivation in a murine model of AD based on the chronic cerebroventricular infusion of β-amyloid peptide (βA). The neurodegeneration was confirmed by hystological analysis of the brain samples. In cerebrospinal fluid of animals infused with βA BK concentration, determined by radioimmunoassay was increased. However, in the brain of β-amyloid group, we only detected the peptide Arg-Pro-Pro, purified by reversed phase chromatography, and characterized by liquid chromatography-electrospray ionization mass spectrometry. This particular cleavage of bradykinin at Pro residue indicated the participation of a prolyl oligopeptidase as an important brain enzyme in kinin metabolism.
(CNPq, FAPESP, FADA/UNIFESP).

NP-P14.
EFFECT OF REPETITIVE SEIZURES ON NR2B SUBUNIT OF NMDAR IN RAT HIPPOCAMPUS
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In previous work in our laboratory, we have reported modifications of specific ligand binding to rat brain NMDA receptors, after the administration of the convulsant drug 3-mercaptopropionic acid (MP). NMDAR is an heteromeric complexes containing NR1 and different NR2 subunits which determine distinct functional properties.
The objective of this work is to study the effect of MP-induced seizure on NR2B.
Methods: Wistar rats (250-300g) were injected with MP 45mg/kg i.p. during 4 and 7 days. Control rats were injected with saline. One day after the last injection, brain were processed for immunohystochemical assays using antiNR2B and the PAP technique.
Results: The studies on control rat hippocampal slices showed immunostaining in CA2 and CA3 and in hillus. In MP4 significant decrease with the control was observed, with almost undetectable immunoreactivity, a light negligible mark around pyramidal cells and a few cells in lacunosum molecular layer were stained and a similar declined mark in MP 7.
Conclusions: These results suggest that NR2B may play a role in modulating convulsive activity in MP-induce seizures.

NP-P15.
NIMODIPINE AVOIDS RAT DEATH DUE TO STATUS EPILEPTICUS IN A REFRACTORY EPILEPTIC MODEL TO PHENYTOIN
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In previous work in our laboratory we have observed CNS P-gp 170 overexpression in our experimental epilepsy model.The purpose of this work was to study the effect of Nimodipine (ND) and phenytoin (PHT) administration in this model of refractory Epilepsy. Methods: Lots of Wistar rats were divided in 5 groups. Three groups received a single dose of MP (45mg/kg, i.p.) daily injected during 13 days (MP13). During the same period, the fourth group, was daily treated with PHT (50 mg/kg), 30 minutes before MP injection (PHT+MP). The last treatment day, a group of MP13 was treated with PHT 30 minutes before MP, and other with Nimodipine (2 mg/kg), 30 minute previous to PHT and 60 minute before MP. As control the fifth group was treated with saline. Results: MP induced seizures from the first day. PHT protected from MP-induced seizures until the third day in the fourth group, however protective effect was completely lost at day 7. MP13 rats developed status epilepticus (SE) and die 100% of them, while rats with PHT+MP reduced to 50% of death. Nimodipine administration previous PHT protect rats from SE, they have light convulsive episodes, and 100% of them remained alive. Conclusions: Our results showed that the ND + PHT protect rats from SE death and can revert the refractory epilepsy state.

NP-P16.
PARTICIPATION OF 5-HT$_{1A}$ RECEPTORS IN THE CONTROL OF EPILEPTIC ACTIVITY IN RATS
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The present study evaluated the participation of 5-HT$_{1A}$ receptors in the control of the epileptic activity by using the agonists 8-hydroxy-2-di-n-(propylamino)tetralin (8-OH-DPAT, 0.01, 0.1, 0.3 and 1 mg/kg, s.c.) and Indorenate (1, 3, 10 mg/Kg, i.p.) in three different types of convulsive seizure in rats: primary generalized clonic-tonic seizures produced by pentylenetetrazol (PTZ, 60 mg/kg, i.p.); complex partial seizures with secondary generalization (kindled seizures) induced by electrical amygdala kindling (daily 1 s train of 0.1 ms pulses at 60 Hz and 400 μA); *Status Epilepticus* (SE) of limbic seizures caused by kainic acid (KA, 10 mg/kg, i.p.). To corroborate the participation of the 5-HT$_{1A}$ receptors it was employed the antagonist WAY100635 (1 mg/kg, s.c.). Regarding to the PTZ-produced convulsions, 8-OH-DPAT did not modify the latency to the convulsions induced by PTZ but diminished in a dose-dependent manner the mortality rate, and at 1 mg/kg enhanced twice the number of clonic seizures that suffered the rats. Indorenate at 10 mg/Kg augmented the latency to the first myoclonic convulsion and to the tonic extension induced by PTZ. Indorenate produced a dose dependent diminution in the percentage of rats that presented tonic seizure and death. Concerning to the kindled seizures, 8-OH-DPAT and Indorenate did not modify their expression. However, Indorenate but not 8-OH-DPAT decreased the seizure index (SI) during the postictal depression. About the KA-induced SE, 8-OH-DPAT produced a dose-dependent augment in the latency and decreased the incidence of the wet dog shakes (WDS), the generalized seizures (GS) and the SE. 8-OH-DPAT modified the incidence of SE depending of the doses: at 0.01 mg/kg this drug increased the incidence of SE, whereas at 0.1 and 1 mg/Kg decreased this parameter. Indorenate increased the latency to the WDS, the GS and SE and diminished the incidence of the GS, but this drug did not modify the percentage of animals that suffered SE. The changes induced by 8-OH-DPAT and Indorenate on the convulsions were partially or fully blocked by WAY100635.

NP-P17.
TIME COURSE OF CEREBELLAR CATALASE LEVELS AFTER NEONATAL IONIZING RADIATION
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Reactive oxygen species are physiologically generated as a consequence of aerobic respiration, but this generation is increased in response to external stimuli, including ionizing radiation.
The central nervous system (CNS) is vulnerable to oxidative stress due to its high oxygen consumption rate, its high level of polyunsaturated fatty acids and low levels of antioxidant defences. An important compound of this defence system is the antioxidant enzyme catalase, an heme protein that removes hydrogen peroxide from the cell by catalyzing its conversion to water.
The aim of the present work was to study if catalase is susceptible to oxidative stress generated by ionizing radiation on the cerebellum. Neonatal rats were irradiated with 5 Gy of X rays and the levels of catalase were measured at 15, 30 and 60 days of age.
Results show that there is a decrease in the activity of catalase in irradiated cerebellum at 15 (% respect the control, 65.6 ± 14.8), 30 (51.35 ± 5.8%), and 60 days (9.3 ± 0.34%).
Catalase activity at 15 and 30 days has shown to be positively correlated with the radiation-induced decrease in tissue's weight, while at 60 days there is an extra decrease. It would be suggested that, at long term, radiation exposure might induce, in addition to cerebellar atrophy, the oxidation of the radiosensitive heme group of the enzyme, leading to its inactivation. In conclusion, the antioxidant enzyme catalase has shown to be especially sensitive to ionizing radiation.

NP-P18.
PRESENCE OF GROWTH PROMOTING AND INHIBITING CELL ADHESION MOLECULES IN ADULT RAT SENSORY NEURONS AFTER AXOTOMY
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Peripheral nerve axotomy alters protein expression in DRG neurons to promote axon regrowth. In the PNS, this growth is often successful, while it usually fails in the dorsal root entry zone (DREZ), where the axons first encounter the CNS environment. It is known that DREZ astrocytes form at least part of the barrier to regrowth, although the molecules responsible for the inhibition are so far unidentified. METHODS: Unilateral sciatic nerve axotomies were performed on adult rats, and protein expression and distribution were determined by Western blot and immunohistochemistry in the corresponding nerves, ganglia (L4/5) and roots. RESULTS: We found high levels of expression of the Ig-CAM family members thy-1 and L1 in the DRG and central and peripheral processes, both in control and axotomized samples. As both these molecules are known to bind to $\alpha_v\beta_3$-integrin, we probed for it's expression in the dorsal roots, and found both subunits to be present regardless of axotomy. Several signal transduction proteins implicated in axon growth (GAP-43, src-kinases) were induced and transported centrally and peripherally, while others (caveolin, cdc42) were not induced. CONCLUSIONS: Neuronal thy-1 is postulated to inhibit axon growth. We show that it is present in centrally-projecting axons of DRG neurons and that it's cognate receptor is present in the dorsal roots where regeneration fails. We suggest that this interaction may underlie axonal inhibition in the DREZ.

NP-P19.
BONE MARROW STROMAL CELLS INJECTED INTO LUMBAR DRG INDUCE CHANGES IN THERMAL AND MECHANICAL SENSITIVITIES AFTER SCIATIC NERVE CONSTRICTION
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Single ligature nerve constriction (SLNC) and Bennett neuropathic pain models have been used for the study of pain riggered by peripheral nerve injury. Bone marrow stromal cells (MSCs) have been involved in migration and fuctional recovery after traumatic injured rat brain and spinal cord. In this study we have analyzed changes in thermal and mechanical pain behavior in animals following SLNC of the SN and also after SLNC and MSCs injection into the L4 DRG. MSCs were isolated by their adherence to plastic, cultured until they reached confluence, labeled with Hoechst 33258 and harvested using trypsin-EDTA. Four groups of SLNC animals were prepared. The 1st group was injected with 3 x 10^6 MSCs into the L4 DRG. The 2nd group was injected with non adherent mononuclear cell fraction (MCF). The 3rd group was only constricted and the 4th was the saline sham. Animals were tested for mechanical and thermal withdrawal thresholds before surgery and after 1, 3, 7 days using Von frey and Choi tests. The results showed a significant decrease in allodynia generation after SLNC by MSCs injection. Animals treated with MCF cells or saline showed no changes in pain response. These results suggest a protective function of MSCs on pain generation.

NP-P20.
UNDERSTANDING THE INTERRELATION AMONG NEURON AND SCHWANN CELLS IN PERIPHERAL NERVOUS SYSTEM IN CHARCOT MARIE TOOTH DISEASE

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Charcot Marie Tooth (CMT) is a syndrome including different inherited peripheral neuropathies It is the most frequent peripheral degenerative disease (1/2500). Mutations causing CMT have been identified in several different chromosomes. They may be either dominant or recessive. Besides, these mutations can either affect genes specifically expressed in neurons or Schwann cells, causing a primarly Axonal (CMT2) or a primarly Demyelinating (CMT1) type respectively. However, the CMT1 type also shows an altered axonal cytoskeleton, interrupted axonal transport and decreased Nerve Conduction Velocities (NCV). In contrast, NCV is normal in CMT2 patients, that shows axonal damage and altered myelin sheath. The relevance of axon-glial relationship and communication became evident in these pathologies, especially those related to structural and physiological maintenance. Furthermore, local protein synthesis in axons is a clue issue for understanding this mechanisms. Its relevance could be highlighted if we consider that axonal protein synthesis machinery could be provided by Schwann cells. CMT patients sural nerves were immunostained to detect transport and synthesis machinery proteins distribution.
Schmidt-Lantermann clefts, vesicles and axonal sprouting immunostaining were especially analyzed, regarding their possible involvement in the above mentioned comunication process. We found ribosome staining and Yoyo-1 staining in inner and outer perimyelinic domain, in Schmidt-Lantermann cleft, as well as in the point of origin of axonal sprouts.

NP-P21.
HIPPOCAMPAL MITOCHONDRIAL DYSFUNCTION IN EXPERIMENTAL PORTAL HYPERTENSION

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Portal hypertension is a major complication of human cirrhosis that frequently leads to hepatic encephalopathy. In this syndrome, hyperammonemia has been observed, in association with mitochondrial damage, energy impairment, and increased free radical formation. In our study, portal hypertension was produced by performing a calibrated stenosis of the portal vein. Mitochondrial function and morphology as well as of mtNOS activity and expression were determined. Succinate-dependent respiratory rates decreased by 29% in controlled state 4 and by 42% in active state 3, and respiratory control diminished by 20% in hippocampal mitochondria from portal hypertensive rats. The activity and expression of mtNOS were significantly decreased in hippocampus from portal hypertensive rats. Ultrastructural damage consisting of swollen mitochondria, loss of cristae and rupture of outer and inner membrane, was observed in the mitochondria of hippocampal astrocytes and endothelial cells of the blood-brain barrier. The moderate increase in plasma ammonia that followed portal hypertension was the potential primary cause of the observed alterations.

NP-P22.
STUDY OF Cu/Zn SOD AND ASOCIATED PROTEINS IN A NEURODEGENERATIVE PROCESS

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Cu/Zn superoxide dismutase (SOD) is found aggregated in amyotrophic lateral sclerosis (ALS). Transgenic rats expressing human mutant SOD (hSODG93A) develop ALS symptoms, including protein aggregation and motoneurons death. As an approach to understand *in vivo* protein folding and aggregation mechanisms, we developed a method to extract hSOD by affinity chromatography using policlonal antibodies which specifically react against human SOD. This antibodies were purified from rabbit serum and immobilized on CNBr-Sepharose in order to fractionate protein extracts by affinity chromatography. Liver and brain proteins from G93A transgenic rat retained in this sepharose were analyzed by SDS-PAGE and western blot. Our results show: 1) the system captures hSODG93A but does not retain rat SOD; 2) co-purification of hSODG93A and SOD from rat only occurs in liver protein extracts, this was not observed in brain extracts; 3) other proteins which co-purify with hSODG93A can be isolated by this method and identified by mass spectrometry. In conclusion, we developed a method to partially purify hSOD that can contribute to the identification of proteins associated to hSOD in different tissues in neurodegenerative processes.

NP-P23.
PRION PROTEIN AND METAL-IONS QUANTIFICATION IN CEREBROSPINAL FLUID OF CREUTZFELDT- JAKOB PATIENTS AND CONTROLS

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Creuztfeldt Jakob (CJ) is a disease with a difficult diagnosis, it is produced by a conformational change in prion protein causing cerebral spongiosis. In an effort to improve diagnosis we compared quantities of iron, copper and prion protein in cerebrospinal fluid (CSF) of patients and controls. Since prion protein binds copper we expected a relationship between these parameters in both groups. We studied the CSF from patients diagnosed with sporadic and familiar CJ and compared with patients that were initially diagnosed as CJ, but during the development of the disease, this pathology was rejected. By immunological methods we quantified the prion using a standard protein, and metals by atomic absorption spectrometry. Total proteins wee higher in most of the familiar CJ respect to controls and sporadic CJ. No statistically significant differences were found either in metal content or in prion protein concentration. In conclusion: the parameters measured are inadequate to improve CJ diagnosis.
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NP-P24.
NEUROFILAMENT-LIGHT AND NITRATED PROTEINS IN CSF OF HTLV-I INFECTED PATIENTS

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HTLV-I is the etiologic agent of tropical spastic paraparesis (TSP/HAM). It is a pathology that involves the CNS, characterized by a slowly progressive axomyelinic degeneration of the corticospinal tracts. Neurofilament-light (NF-L), one of the components of axonal cytoskeleton, has been reported to be elevated in cerebrospinal fluid (CSF) of patients with inflammatory neurological disease (IND), where also the oxidative stress plays an important role. There are controversies in considering TSP/HAM as of inflammatory or degenerative type. The aims of this work were to compare by immunological methods in CSF in controls, TSP/HAM and IND groups: a) levels of NF-L and nitrated NF-L and b) levels of general nitrated proteins. The results indicated that NF-L is significantly elevated only in IND group, being equally nitrated in the three groups. The total nitrated proteins are increased in IND groups compared to controls and TSP/HAM patients. These results suggest the lack of a considerable oxidative damage in TSP/HAM.
Financed by grant: Fondecyt 105-0784.

NP-P25.
A MOUSE SPINAL CORD CELL LINE ¿A MODEL TO STUDY HTLV-I -INDUCED NEURONAL DISEASE?

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Tropical spastic paraparesis is a myelopathy with a slowly progressive damage to the longest axons of the corticospinal tracts, associated to HTLV-I infection (TSP/HAM). How the HTLV-1 retrovirus causes this disease in the CNS it is unknown. The axonal damage in response to secreted viral proteins may impair axonal transport. Therefore, the goal of this work is to determine the effect of viral proteins secreted from MT2 cell (infected with HTLV-I) on a mouse neuronal cell line (M4b cells) following neurite extensions, and protein phosphorylation. Coculture and indirect culture through a semipermeable membrane (cell culture inserts) allowing the release of viral proteins were performed. In short culture period some neurite reduction in the population cell was observed. No changes in M4b viability was observed followed by calcein AM incorporation. An impairment of normal axonal cytoskeletal organization could lead to axonal transport damage, where alteration of normal neurofilament and microtubule phosphorylation can be involved.
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NP-P26.
AFFINITY PURIFICATION OF CSF TAU FOR STUDYING ABNORMAL PHOSPHORYLATION

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Tropical spastic paraparesis or human T-cell lymphotropic virus associated myelopathy (TSP/HAM), is a neurological manisfestation with a distal alteration of corticospinal tracts. The goal of the present work was to explore the possibility of abnormal phosphorylation sites in tau that could be related with alteration of the microtubule assembly. Affinity purification of CSF tau from TSP/HAM patients and control subjects, using monoclonal antibodies against tau (clon-5), showed after immunowestern blot a single band with Mr 55 kDa. The blots were sequentially stripped and reblotted with antibodies to phosphoserine and phosphotreonine, visualizing in both cases a positive reaction of the 55 kDa-band. Studies using 9 phosphorylation dependent tau antibodies showed some differences in the imunoreactivity towards these antibodies. This information will be useful to determine which protein kinases have the potential to phosphorylate tau. Abnormal tau phosphorylation could be associated with TSP/HAM axonal pathology.
Financed by grant: Fondecyt 105-0784

NP-P27.
HISTOLOGY AND PHISIOLOGY OF THE AUDITORY PATHWAY IN DEAFENED ANIMALS

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TX-P1.
THALLIUM INDUCES APOPTOSIS IN RAT PHEOCHROMOCYTOMA (PC12) CELLS

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Thallium (Tl) is a non-essential heavy metal, with two oxidation states (Tl^+ and Tl^{3+}). Tl^+ causes mitochondrial swelling, neurodegeneration, and an increase in lipid oxidation products content. Working with PC12 cells, we investigated the cytotoxic effects of Tl^+ and Tl^{3+} (10-250 µM) (1-72 hs). The number of viable cells, assesed by the incorporation of the probes calcein AM and ethidium homodimer and the reduction of MTT, decreased in Tl-containing samples in a concentration-and time-dependent manner. GSH content decreased at 24 hours of incubation in the presence of the metal as evaluated by the incorporation of the probe mBCI. Mitochondrial membrane potential decreased, as evaluated by the uptake of the fluorescent probe rhodamine 123, effect that was accompanied by an increase in H_2O_2 content, measured by the oxidation of the probe dihydrorhodamine 123. An increase in the number of apoptotic cells was found when the nuclear morphology was assesed using the probes acridine orange and ethidium bromide. Western blot analysis showed a higher PARP cleavage. In summary, Tl causes a decrease in GSH, alters mitochondrial functionality increasing H_2O_2 content, molecule that could trigger cell apoptosis.

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TX-P2.
LAMOTRIGINE PROTECTS CHO-K1/A5 CELLS FROM CELL DEATH INDUCED BY SERUM DEPRIVATION AND LITHIUM

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The nicotinic acetylcholine receptor (AChR) is a membrane protein present in the postsynaptic regions of muscle cells and pre-, peri- and postsynaptic regions in nervous cells. Lamotrigine (LTG) blocks in a dose-dependent way the muscle AChR channel in CHO-K1/A5 cells through a mechanism that is compatible with that of open channel blockers. We evaluated if the blockage seen with LTG on the muscle AChR affected cell viability, as it was previously seen with Carbamazepine, another antiepileptic drug that also acts as an open channel blocker. We studied the neuroprotective action of lithium (Li) and LTG in cultured CHO-K1/A5 cells. Li is best known for its neuroprotective action against various insults. In the concentrations tested (50-200 µM) LTG did not modify cell viability as judged by the MTT assay. We then induced cell death by removing serum from the culture for 24 h. Cell survival was reduced significantly (25%). Co-incubation with 50 µM LTG protected cells from death against serum deprivation. However 10 mM Li did not prevent cell death. Furthermore, 10 mM Li enhanced cell death by 40% in the absence of serum. We established a TD_{50} = 25 mM for Li in the presence of serum in the medium. Thus, the antiepileptic drug LTG has a robust protective effect on CHO-K1/A5 cell death induced by serum deprivation and Li in the culture medium. These data provide new avenues to study the cellular and molecular mechanisms of anticonvulsive drug action.

TX-P3.
MELATONIN DECREASE THE OXIDATIVE STRESS PRODUCED BY 2,4-DICHLOROPHENOXYACETIC ACID

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2,4-dichlorophenoxyacetic acid (2,4-D) is a herbicide widely used in the world. We demonstrated that 2,4-D to increase the oxidative stress and induction of death in rat cerebellar granule cell (CGC) cultures. Recently it found that melatonin would be a true radical scavenger since it protect against the toxic action of kainic acid.
The aim of the present work was to determinate in rat CGC cultures, exposed to 2,4-D (1mM) and/or Melatonin (0.1 and 0.5 mM) for 48 hs, cell viability for MTT method, reactive oxygen species (ROS) using a fluorescent compound (DCF), production of NO by Griess's method, catalase (CAT) activity by method of Beers and Sizer, both Mn- and Cu, Zn-superoxide dismutase (SOD) activity by the method of Fridovich, glutathione peroxidase (GPx) activity by method of Rice-Evens and level of reduced glutathione (GSH) by Thomas's method.
We observed that the treatment with both drugs presented higher viability (87±2), CAT activity (12.1±0.8) and GSH levels (2.3±0.2) than the 2,4-D alone (46±4, 6.5±0.8 and 1.13±0.03 respectively). The ROS levels (5.0±0.5) were lower when we compared to the same groups (3.8± 0.5)
In conclusion, melatonin would be important as mechanism of protection against the toxicity of this herbicide

TX-P4.
MELATONIN PREVENTS *IN VIVO* CADMIUM-INDUCED OXIDATIVE STRESS

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Cd is a potent endocrine disruptor that induces oxidative stress in the anterior pituitary (AP). Melatonin (Mel) can function as an antioxidant Here we used Mel to reduce Cd-induced oxidative stress in the gland. Adult male rats were exposed for 1 month to 5 ppm Cd in the drinking water with or without 30 microg/ml of Mel. Mel prevented Cd-induced oxidative stress as measured by lipid peroxydation (TBARS, % of control, Cd: 134 ± 4*, Mel: 93 ± 7, Cd+Mel: 92 ± 9Δ, *p<0.05 vs. control, Δp<0.05 vs. Cd, n=8) and HO-1 mRNA expression (rt-RT-PCR, relative expression, control: 1.1 ± 0.2, Cd: 4.8 ± 0.5***, Mel: 2.0 ± 0.3, Cd+Mel: 2.6 ± 0.1*ΔΔ *p<0.05 **p<0.001 vs. control, ΔΔp<0.01 vs. Cd, n=4). Mel also reduced the Cd-induced expression of NOS2 and NOS1 mRNA (NOS2: control 1.1 ± 0.2, Cd: 3.1 ± 0.4**, Mel: 0.12 ± 0.11**, Cd+Mel: 0.13 ± 0.12ΔΔΔ. NOS1: control: 1.0 ± 0.04, Cd: 1.7 ± 0.2**, Mel: 0.42 ± 0.04*, Cd+Mel: 0.94 ± 0.18ΔΔ, *p<0.05 **p<0.001 vs. control, ΔΔp<0.01, ΔΔΔp<0.001 vs. Cd, n=4). CONCLUSION: Mel is able to reduce Cd-induced oxidative stress in the AP this effect could be due, at least in part, by inhibiting de expression of pro-inflammatory NOS2.

TX-P5.

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) ON HYPOTALAMIC DOPAMINERGIC NEURONS OF ADULT RATS: A HISTOLOGICAL STUDY

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2,4-D is an herbicide used worldwide. Dopaminergic system alteration in rats exposed to 2,4-D through lactancy has been reported in previous histological and biochemical studies from our laboratory. In substantia nigra and ventral tegmental area a decreased neurons´ tyrosine hydroxylase (TH) immunostaining in 2,4-D-exposed pups was observed. The aim of this work was to perform an immunohistochemical quantitative study of the hypothalamic dopaminergic neurons on adult rats exposed to 2,4-D. Wistar rats were made pregnant and exposed to 2,4-D (50 mg/kg/day, through diet) from day 16th of gestation to weaning. After weaning, pups were divided in two experimental subgroups: $\mathbf{T_1}$: fed with untreated diet until sacrifice (postnatal day 90, PND_{90}). $\mathbf{T_2}$: treated until PND_{90}. Control animals received standard diet. Serial coronal sections -from plates 18 to 35 of the Paxinos and Watson atlas- were immunostained according to Sternberger's PAP technique using a monoclonal anti-TH primary antibody. Data showed a decrease in the number of dopaminergic neurons, in both peri- and paraventricular hypothalamic nuclei, in T_1 rats. 2,4-D exposure during pregnancy and lactation produced permanent neuronal decrease in both hypothalamic dopaminergic nuclei. However, with the chronical 2,4-D exposure an adaptable response was observed.

TX-P6.

NEURODEGENERATIVES STUDIES IN RATS EXPOSED TO 2,4 DICHLOROPHENOXYACETIC ACID (2,4-D)

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Chlorophenoxy herbicides are widely used for the control of broad-leaved. We previously reported a CNS myelination deficit, an increase in size and density of serotonin immunoreactive neurons as well as in fiber legth and an important astrogliosis reaction. The purpose of this study was to determine the neurotoxicity produced in male rats exposed to 2,4-D using immunocytochemical expression of c-*f*os, the amino-cupric-silver technique to evaluate neuronal degeneration, and odor preference test (OPT) to evaluate behavioral alterations produced by the herbicide. We observed isolated neuronal death in the piriforme (Pir) and lateral enthorrinal cortex (LEnt), the posteromedial cortical amygdaloid nucleus (PMCo) and an increase of *c-fos* expression in rats treated with 50 and 100 mg/kg of 2,4-D in Pir and LEnt ($p<0.001$), PMCo ($p<0.05$). Also we observed increase in *c-fos* expression in the following areas: accesory olfactory bulb ($p<0.05$), bed nucleus of the stria terminalis ($p<0.05$), medial and central amygdaloid nucleus ($p<0.05$). In the OPT we observed decrease of time permanence in the chamber with the olfactory cue derived from the urine of a proestrous female in both treatments ($p<0.01$ and $p<0.05$ for 50 and 100 mg/kg 2,4-D). These results indicate that acute and cronic doses of 2,4 D have neurotoxic effects in some areas of the CNS. The mechanisms have not been elucidated and remain to be studied.

TX-P7.

SEXUAL DIMORPHIC EFFECTS IN THE NEUROTOXICITY INDUCED BY MK-801 IN THE RAT RETROSPLENIAL CORTEX

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NMDA antagonists, like MK-801, are of potential therapeutic benefit for several conditions. However, their ability to produce neurotoxicity and psychosis has hampered their clinical use. A better understanding of these side effects and the mechanism underlying them could result in improving our understanding of psychotic illnesses. To study the mechanism and the extent of the effects produced by this drug we used acute moderate to high doses (5-10 mg/kgr) in male and female rats, and compared different protocols for detecting degeneration, such as the Amino Cupric Silver Technique (A-CuAg), Fluoro Jade B and GFAP imunohistochemistry. To further corroborate the sensitivity of these techniques, controls for degeneration with other animal models such as intra-srtiatal injections of quinolinic acid were analysed. Comparing the results obtained by the A-CuAg with the other protocols, it is concluded that A-CuAg was the most sensitive protocol for the detection and accurate appraisal of the neuronal degeneration produced. Thereby, we analysed both divisions of the granular (RSG) and agranular (RSA) Restrosplenial Cortex. The somatodendritic degeneration (SD) induced by MK-801 in the female rat was almost completely restricted to layer 4 and 5 of the RSG, instead in the male, besides showing much less cell damage ($p < 0.05$), SD invaded layer 5 of the immediately neighbouring RSA. Since RSA and RSG have different afferent and efferent connections, it is probable that the sexually dimorphic neuronal toxicity induced by MK-801 is reflected in mechanisms involving sensorial areas which may lead in turn to behavioural differences described in the literature.

TX-P8.

NEURONAL ACTIVATION (C-FOS, FOSB) AND DEATH IN THE BED NUCLEUS OF THE ACCESSORY OLFACTORY TRACT AFTER KAINIC ACID ADMINISTRATION

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The Medial Extended Amygdala (MExA) modulates the pheromonal perception, influencing the emotional and social behaviour, in a circuit integrated by the Bed Nucleus of the Accessory Olfactory Tract (BAOT), Medial Nucleus and Posteromedial Cortical Nucleus of the Amygdala and Bed Nucleus of the Stria Terminalis. Here the BAOT was studied by immunohistochemistry (ICC) (fos, FosB) for neural stress and neuronal death in rats after Kainic Acid (KA) administration.
Sixteen adult Wistar male rats were used. Eight animals received a single IP injection of KA (8 mg/kg), and eight (controls) were injected with saline. After 24 and 72 hs survival, animals were fixed, the brains were sectioned serially and stained for fos and fosB and for neuronal death with the Amino-Cu-Ag technique. Sections were observed in an Axioplan microscope. Nuclei and neurons were counted by a Scion Program. Data were analysed using one way ANOVA followed by the Fisher post hoc test.
Conclusions: 1) numerous ICC nuclei to fos and fosB were found in BAOT after 24 and 72 hs of KA, being different between them and from controls animals ($p<0.05$). 2) Fos decrease and fosB increase in the same period. 3) Neuronal death paralleled fos B reaction. Those findings show that KA produced deleterious changes and neuronal loss in BAOT, demonstrating the vulnerability of its neurons to the effects of KA.

TX-P9.

SEX DIFFERENCES IN THE EFFECT OF DIHYDROTESTOSTERONE (DHT) IN THE MEDIAL EXTENDED AMYGDALA (MExA) USING THE KAINIC ACID MODEL OF EPILEPSY

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Previously we shown in rats that MExA is damaged by Kainic Acid (KA). Both male and female gonadectomized (GX) were more sensitive to KA than normal animals, being GX males more vulnerable than GX females. Here we compared the effect of DHT in the Medial Nucleus of the Extended Amygdala in those groups GX female and male rats after KA administration.
Twenty-four adult Wistar female and male rats were used. Eight female were ovariectomized (OVX) and eight male were castrated (CD). Four female and male were not gonadectomized. Twenty-one days after surgery, four female rats received a single injection of DHT (OVX+DHT) and four males a single injection of T (CD+T). Three days after, all animals received a single injection of KA (8 mg/kg). Control animals were injected with saline. Twenty-four hours after the KA injections the brains were fixed, sectioned and stained for neuronal death with Amino-Cu-Ag technique. Neurons were counted using a Scion Program. Data were analysed with one way ANOVA followed by the Fisher post hoc test.
Conclusions: GX increased neuronal loss in females and males. DTH did not significantly reduce neural injury induced by KA. In both cases, males presented a higher amount of death neurons ($p<0.05$) This suggests higher vulnerability to KA of the MExA neurons in males, being the survival mechanisms other than T.

TX-P10.

THE NEUROTOXIC EFFECT OF CUPRIZONE ON OLIGODENDROCYTES REQUIRES THE PRESENCE OF TNFα AND IFNγ

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Cuprizone(CPZ) intoxication model has been widely used to induce demyelination. To explain the unknown mechanism of demyelination caused by CPZ, primary oligodendroglial cell (OLGc) cultures were treated with a wide range of CPZ concentrations, for 0/7 days and in presence or absence of conditioned medium from astrocytes previously treated with CPZ. Cell viability, evaluated by the MTT assay doesn't showed changes. The effect of CPZ on OLGc cell cycle and differentiation was also evaluated and no variations were detected. The inflammatory process plays a key role in the pathogenesis of degenerative disorders such as Multiple Sclerosis so we tested the effect of cytokines such as IFNγ and TNFα and none of them affected cell viability. However, in presence of cytokines and CPZ, we observed an important diminish in cell viability. Activities of mitochondrial complexes I/I-III evaluated on mitochondrias from glial cell cultures treated with CPZ (72h) show a decrease relative to controls. Production of free radicals evaluated using DCDCDHF, in cells treated with TNFα, CPZ or both, is significantly increased. Results suggest that CPZ demyelination is not a direct effect of the drug on OLGcs. Myelin loss and OLGc death seems to be mediated by molecules such as TNFα and IFNγ secreted by recruited microglia/macrophages and could be also consequence of a mitochondrial complexes activity diminish, with a loss in energy production and an increase in the production of free radicals.

TX-P11.

GLYCOLYTIC ENZYMES IN AN ACUTE PORPHYRIA MODEL

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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 behaviour of key enzymes of glycolysis such as hexokinase (HK), glucokinase (GK) and pyruvate kinase (PK) in an acute porphyria model induced by the administration of both porphyrinogenic drugs [3 variable doses of 2-allyl-2-isopropylacetamide (AIA) and a fixed dose of 3,5-diethoxycarbonyl 1,4-dihydrocollidine (DDC)]. The enzymatic activities of GK+HK were measured at glucose concentration of 200mM, whereas that of HK was measured at 1 mM glucose. The activity of PK was evaluated using lactic dehydrogenase as coupled enzyme and measuring the decrease of NADH. The results obtained make evident a diminution of GK depending on the dose of AIA. This decrease being of 70% respect to the control for the higher dose of AIA assayed. PK also showed a decrease dose dependent that reached 40% at the same condition. These results suggest that in this intoxication the enzymes of glycolysis respond in a such way as not to reduce even more the availability of glucose already diminished by the gluconeogenic blockade.

TX-P12.

ANTIMICROBIAL ACTIVITY OF *Bothrops alternatus* VENOM FROM THE NORTH EAST OF ARGENTINA

Bustillo S[1], Merino L[1], Leiva LC[1], Acosta OC[1], Bal de Kier Joffé E[2], Gorodner JO[1].
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Envenomation is a process associated with a low incidence of bacterial infection. This feature could indicate the presence of antibacterial molecules in the snake venoms. This secretion contains a large number of biologically active proteins and peptides that are usually similar in structure but not identical to that of prey physiological systems. The antibacterial activity of *Bothrops alternatus* venom, specie responsible for most of snakebites in North-eastern Argentina, was investigated against ATCC bacterial strains. Inhibitory halos values and reduction of colony-forming unites (CFU) by dilution plate technique were determined. Bactericidal activity was in the following decrescent order: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomona aeuruginosa* (ATCC 27853), and *Enterococcus faecalis*. (ATCC 29212). Results, lead to the conclusion that *B. alternatus* venom display bactericidal activity upon Gram positive and negative strains, but different mechanisms are involucrate. The aim of this work is to review the basis of antimicrobial mechanisms revealing snake venom as a feasible source for searching an antibiotic prototype.

TX-PI3.
CHARACTERIZATION OF CHOLINESTERASES IN TWO FRESHWATER INVERTEBRATES

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Cholinesterase (ChE) activity is widely used as biomarker for neurotoxic pesticides. However, prior to its use in a species is necessary to characterize the type of enzyme(s) present. The aim of this work was to characterize the ChE present in two freshwater invertebrates. Measurements of ChE activity from the whole body soft tissue of *Biomphalaria glabrata* and *Lumbriculus variegatus* were performed using two substrates: acetylthiocholine (AsCh) and butyrylthiocholine (BsCh) according to the method of Ellman. When using AsCh, *B. glabrata* presented almost all the activity in the 11,000 x g supernatant fraction (0.046 ± 0.006 µmol min^{-1} mg $protein^{-1}$). *L. variegatus* presented values approx. 6 and 20 times higher of ChE activity than *B. glabrata* either in the supernatant or in the pellet, respectively. When using BsCh, significant values of enzyme activity were only detected in *L. variegatus* (72% of the total ChE). Iso-OMPA produced an inhibition of 72 % of ChE in *L. variegatus*, but only a slight decrease in *B. glabrata*. Increased concentrations of AsCh induced a progressive decrease in the ChE activity in both organisms (80% of inhibition at 34 mM of substrate). In *L. variegatus* inhibition by an excess of BsCh was also observed. ChE activity from *L. variegatus* was more sensitive to eserine inhibition than that from *B. glabrata*. According to our results both invertebrates present marked differences in ChE activity, type of enzymes and subcellular location. In addition *L. variegatu*s was more sensitive to *in vivo* ChE inhibition induced by the organophosphate azinphos-methyl than *B. glabrata*.

TX-P14.
HISTOLOGICAL EXAMINATION OF HEPATIC INJURY DUE TO HEXACHLOROBENZENE (HCB) ON AVIAN EMBRYOS

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Histological examination of chick livers were performed after injection of 20 mg HCB/egg in the yolk and "in ovo" incubation by 36 and 60 hs. Samples were stained with hematoxilin-eosin and Masson's trichrome. Non observable changes occurred at 36 hs. Hepatic injury registered at 60 hs. of treatment consisted of parenchymal damage observed as partial tissue liver necrosis. This observation was outlighted by the presence of enlarged and foamy hepatocytes without nucleus, colapsed sinusoides and the absence of bile pigments in bile ducts and canaliculus. Lost of parenchymal integrity, damaged or destroyed hepatocytes, bile ducts and sinusoids, would diminished blood flow efficiency. This would impair cellular regeneration leading to embryo death by hepatic dysfunction. Changes of macroscopic pattern and density of lipids, revealed by cytoplasmic vacuolization, would promote changes in membrane fluidicity modifying the essential substance transport. All these effects decrease bile pigments conjugation and excretion, and taking together with the less amount of heam, would explain bile pigment missing in the bile ducts and canaliculus. These results compile sufficient evidence to support that HCB plays an important role in the hepatic injury. A precancerous patology remains to be elucidated.

TX-P15.
MOLECULAR AND ENZYMATIC RESPONSES TO ORGANOPHOSPHATES IN *Bufo arenarum* EMBRYOS

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Amphibians are particularly sensitive to environmental pollutants during embryonic development. We previously showed the adverse effects of sublethal doses of malathion (Mtn) and other organophosphates (OP) causing oxidative stress in *B.arenarum* embryo. We analyze here the effects of a low exposure to the OP chlorpyrifos (Cpf) (2 mg/L, 1/7 LC50) and Mtn (20 mg/L, ½ LC50) in early embryogenesis (48 h). Antioxidant enzyme activities were measured kinetically, AP-1 TRE-binding was determined by EMSA, and nuclear protein phosphorylation was performed with ^{32}P-ATP and analyzed by SDS-PAGE and autoradiography.
Cpf tended to lower catalase and GSH-S-transferase and increase GSH-reductase activities, while Mtn induced the 3 enzymes (25, 100 and 50% respectively). In turn, both OP clearly reduced AP-1 binding, and increased protein phosphorylation (29 KDa; 43 KDa). Western blot analyses of (p-)c-Jun and c-Fos gave yet no conclusive results. Enzyme responses are more evident in sublethal Mtn than in low Cpf exposures. However, molecular events regarding AP-1 and phosphorylation, point out that Cpf is affecting early development in *B. arenarum*. AP-1 downregulation may be reflecting other mechanisms shared by c-Jun, such as Antioxidant Response Element binding.

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