- SAIB -

XLII Reunión Anual

Sociedad Argentina de Investigación en Bioquímica y Biología Molecular

42th Annual Meeting

Argentine Society for Biochemistry and Molecular Biology

November 12-15, 2006

Rosario, Santa Fe
República Argentina

ACKNOWLEDGMENTS

THE FOLLOWING INSTITUTIONS SUPPORTED THE ORGANIZATION OF THE XLII SAIB MEETING:

CONSEJO NACIONAL DE INVESTIGACIONES CIENTÍFICAS Y TÉCNICAS (CONICET)

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EUROPEAN MOLECULAR BIOLOGY ORGANIZATION (EMBO)

SECRETARÍA DE PRODUCCIÓN, PROMOCIÓN DE EMPLEO Y COMERCIO EXTERIOR DE LA MUNICIPALIDAD DE ROSARIO

SUBSECRETARÍA DE CIENCIA, TECNOLOGÍA E INNOVACIÓN DE LA PROVINCIA DE SANTA FE

FACULTAD DE CIENCIAS BIOQUÍMICAS Y FARMACÉUTICAS, UNIVERSIDAD NACIONAL DE ROSARIO

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ERRATA

SCIENTIFIC SECTIONS DELEGATES

-Cell Biology-
Dr. Claudia Nora Tomes
IHEM-CONICET, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo

-Lipids-
Dr. María del Carmen Fernández Tome
IQUIFIB, Facultad de Farmacia y Bioquímica, Universidad Nacional de Buenos Aires

-Plants-
Dr. Elena Graciela Orellano
IBR-CONICET, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario

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

Colegio Internacional Parque España
Centro Cultural Parque España
Bioceres S.A.
Fundación Josefina Prats

A Satellite Activity has been included in the program:

MONDAY, November 13, 2006

20:30-21:30 ***Act in honor of Drs. Juan José Cazzulo and Rubén Héctor Vallejos***
For their contribution to the development of the Rosario Biochemistry Research School
Fundación Josefina Prats, Córdoba 1995, Rosario

An additional Workshop has been included in the program:

WEDNESDAY, November 15, 2006

13:00-14:30 Room A
"Simposio Bioceres de Biotecnología Vegetal"

Carlos Pérez
Bioceres S.A., Rosario, Argentina
"Bioceres, del laboratorio al campo y del campo al laboratorio"

Raquel L. Chan
Facultad de Ciencias Bioquímicas y Farmacia, Universidad del Litoral, Santa Fe, Argentina
"Obtención y análisis de herramientas moleculares para el mejoramiento vegetal utilizando ingeniería genética"

The schedule of this poster has been changed as follows:

CB-P44- EFFECT OF THE ANTINEOPLASTIC AGENT TAMOXIFEN IN EXPERIMENTAL PORPHYRIA INDUCED BY HEXACHLOROBENZENE. Llambias, E.B.C.; Mazzetti, M.B.; Aldonatti, C.; San Martin de Viale, L.C. Departamento Química Biológica, Facultad Ciencias Exactas y Naturales, Universidad Buenos Aires.
From: Tuesday 14th; 17:00-19:00 hs, Poster Session
To: Monday 13th; 17:00-19:00 hs, Poster Session

The list of authors of these abstracts is as follows:

LI-P07- CHANGES IN RAT SPERMATOZOA LIPIDS AND FATTY ACIDS DURING EPIDIDYMAL MATURATION. **Zanetti SR; Monclus MA; Rensetti DE; Aveldaño MI, Fornés MW.** IHEM, CONICET-UNC, 5500 Mendoza e INIBIBB, CONICET-UNS, 8000 Bahía Blanca, Argentina.

LI-P27- EFFECTS OF CAPACITATION AND ACROSOME REACTION ON RAT SPERMATOZOA LIPIDS AND FATTY ACIDS. **Zanetti SR; Monclus MA; Rensetti DE**; **Aveldaño MI, Fornés MW.** IHEM, CONICET-UNC, 5500 Mendoza e INIBIBB, CONICET-UNS, 8000 Bahía Blanca, Argentina.

The following oral communication will be presented as a poster:

BT-C02- PLANT SPECIFIC INSERT (PSI) OF *St*APs: CLONING, EXPRESSION AND ANTIMICROBIAL ACTIVITY Muñoz, F.F.; Mendieta, J.R.; Pagano, M.R.; Daleo, G.R.; Guevara, M.G. Instituto de Investigaciones Biológicas, Universidad Nacional de Mar del Plata.
Poster presentation: BT-P34- Wednesday 15th; 17:00-19:00 hs.

PROGRAM

SUNDAY, November 12, 2006

14:00-18:00 Secretary of the Congress
REGISTRATION

18:00-18:15 Room A
OPENING CEREMONY

18:15-19:15 Room A
OPENING LECTURE
Ernesto Podestá
Departamento de Bioquímica, Universidad Nacional de Buenos Aires, Argentina
"Does the source of intramitochondrial arachidonic acid define the cell fate (proliferation or apoptosis)?"
Chair: Rodolfo Brenner (Universidad Nacional de la Plata, Argentina)

19:15-20:15 Room A
"**EMBO" LECTURE**
Randy Schekman
Department of Molecular and Cell Biology U. C. Berkeley, USA
"The morphogenesis of a transport vesicle: mechanism and disease implications"
Chair: Hugo J. Maccioni (Universidad Nacional de Córdoba, Argentina)

21:00 **COCKTAIL**

MONDAY, November 13, 2006

8:30-9:30 Room A
PLENARY LECTURE
Tom Jovin
Department Molecular Biology, Max Planck Institute Biophysical Chemistry Goettingen, Germany
"Surface tracking and intracellular trafficking of growth factor receptors of living cells using quantum dot ligands and new high-speed optical sectioning microscopy"
Chair: Francisco Barrantes (Universidad Nacional del Sur, Argentina)

9:30-10:00 **Coffee break**

10:00-12:30 **ORAL COMMUNICATIONS**
Room A
Microbiology (MI-C01/MI-C08)
Chairpersons: José Echenique (Universidad Nacional de Córdoba)
Teresita Lisa (Universidad Nacional de Río Cuarto)

10:00-10:15
MI-C01
IN VIVO LOCALIZATION OF THE *SALMONELLA ENTERICA* PHOP/PHOQ TWO-COMPONENT SYSTEM

Sciara, M.I.; Soncini, F.C.; García Véscovi, E.
IBR-CONICET, Fac. de Ciencias Bioquímicas y Farmacéuticas, UNR, Rosario, Argentina.

10:15-10:30
MI-C02
E. COLI MALIC ENZYMES: A NAD(P)-ME AND A MULTIMODULAR NADP-ME WITH DUAL ENZYMATIC ACTIVITY
Bologna, Federico P.; Andreo, Carlos S.; Drincovich, M.F.
CEFOBI-Fac. de Cs. Bioquímicas y Farmacéuticas, UNR, Rosario, Argentina.

10:30-10:45
MI-C03
A NEW DRUG TARGET: ACCASE6, AN ESSENTIAL ACETYL-COA CARBOXYLASE OF _MYCOBACTERIUM TUBERCULOSIS_
Kurth, D.; Gago, G.; Diacovich, L.; Gramajo, H.C.
Instituto de Biología Molecular y Celular de Rosario, UNR-CONICET, Rosario, Argentina.

10:45-11:00
MI-C04
ROLE OF RND EFFLUX SYSTEMS IN THE _BRUCELLA SUIS_ PHYSIOLOGY
Martín, F.A.; Posadas, D.M.; O'Callaghan, D.; Zorreguieta, A.
Fundación Instituto Leloir. FCEyN, UBA. INSERM U-431 Nimes, France.

11:00-11:15
MI-C05
CHARACTERIZATION OF THE CITRATE TRANSPORTER AND REGULATION OF CITRATE METABOLISM IN _E. FAECALIS_
Blancato, V.S.; Lolkema, J.S.; Magni, C.
University of Groningen, Holanda. UNR, IBR-CONICET, Rosario, Argentina.

11:15-11:30
MI-C06
REGULATION OF _PCHP_ GENE IN _PSEUDOMONAS AERUGINOSA_
Massimelli, M.J.; Choi, K.H.; Beassoni, P.R.; Domenech, C.E.; Schweizer, H.P.; Lisa, A.T.
Dpto. Biología Molecular, UNRC, Argentina; Dept. of Microbiology, CSU, CO, USA.

11:30-11:45
MI-C07
REGULATION OF THE _ESCHERICHIA COLI_ NADH DEHYDROGENASE-2 GENE EXPRESSION BY PHOSPHATE AND GLYCEROL
Schurig-Briccio, L.A.; Rintoul, M.R.; Baldoma, L.; Badia, J.; Rodríguez-Montelongo, L.; Rapisarda V.A.
Dpto. Bioq. Nutrición, INSIBIO y FBQF-UNT. Dpto. Bioq. y Biol. Molec., Univ. Barcelona.

11:45-12:00
MI-C08
SELECTIVE DETERMINANTS INVOLVED IN THE CueR-LIKE REGULATORS/ TARGET PROMOTERS INTERACTION
Pérez Audero, M.E.; Podoroska, B.M.; Checa, S.K.; Soncini, F.C.
IBR-CONICET, Facultad de Ciencias. Bioquímicas y Farmacéuticas, UNR, Rosario, Argentina.

10:00-12:30 **ORAL COMMUNICATIONS**

Room B

Cell Biology (CB-C01/CB-C10)

Chairpersons: Nicolás Koritschoner (Universidad Nacional de Córdoba)
José Luis Daniotti (Universidad Nacional de Córdoba)

10:00-10:15

CB-C01

P300 AND P53 COLOCALIZE IN INCLUSIONS RESEMBLING AGGRESOMES IN TUMOR-DERIVED MAMMARY MURINE CELLS

Curino, A.C.; Gonzalez-Pardo, V.; Gigola, G.; Gandini, N.A.; Perez, J.; Russo de Boland, A.; Boland, R; Facchinetti, M.M.

Dpto. de Biología, Bioquímica y Farmacia, UNS y INIBIBB, Bahía Blanca.

10:15-10:30

CB-C02

SCREENING OF THE MANNOSE RECEPTOR FAMILY PROTEIN uPARAP/Endo180 IN HEAD AND NECK TUMORS

Facchinetti, M.M.; Molinolo, A.A.; Rivadulla, M.G.; Maturi, H.V.; Gandini, N.A.; Bugge, T.H.; Curino, A.C.

INIBIBB y Dpto. Biología, Bioquímica. y Farmacia, UNS, Bahía Blanca.

10:30-10:45

CB-C03

CLONING AND FUNCTIONAL CHARACTERIZATION OF Dlg TUMOUR SUPPRESSOR PROMOTER

Cavatorta, A.L.; Giri, A.A.; Banks, L.; Gardiol, D.

Area Virología, IBR (CONICET), Fac. Cs. Bioquímicas, Rosario, Argentina. ICGEB-Trieste, Italy.

10:45-11:00

CB-C04

A NOVEL ONCOLYTIC ADENOVIRUS THERAPEUTICALLY EFFECTIVE IN MELANOMA TUMOR

Lopez M.V.; Viale, L.D.; Cafferata, E.G.; Maccio D.R.; Carbone C.; Gould, D.; Chernajovsky, Y.; Podhajcer, O.L.

Instituto Leloir-CONICET, CeNaGeM-ANLIS "CM", Facultad de Veterinaria, UNLP and University of London.

11:00-11:15

CB-C05

A NOVEL SPECIFIC ONCOLYTIC ADENOVIRUS DRIVEN BY HUMAN A33 PROMOTER FOR COLORECTAL CANCER THERAPY

Cafferata, E.G.A; Macció, D.R; López, M.V; Viale, D.L; Carbone, C; Podhajcer, O.L

Instituto Leloir-CONICET, CeNaGeM-ANLIS "Carlos Malbrán" Facultad de Veterinaria, UNLP.

11:15-11:30

CB-C06

CHARACTERIZATION OF A SPECIFIC SPARC PROMOTER SUITABLE FOR CANCER GENE THERAPY

Viale, D.L.; Lopez, M.V.; Cafferata, E.G.; Gould, D.; Chernajovsky; Y.; Podhajcer, O.L.

Instituto Leloir-CONICET, CeNaGeM-ANLIS "CM" and University of London.

11:30-11:45
CB-C07
CNBP BINDS PURINE-RICH ssDNA AND ITS ACTIVITY DEPENDS ON THE RGG BOX
Armas, P.; Nasif, S.; Agüero, T.; Aybar, M.; Calcaterra, N.B.
IBR-CONICET, FCByF, UNR. INSIBIO (CONICET-UNT). MNDB, ICM, Univ. Chile.

11:45-12:00
CB-C08
CNBP IS REQUIRED FOR CRANIOFACIAL SKELETON DEVELOPMENT IN ZEBRAFISH
Weiner, A.M.J.; Allende, M.L.; Becker, T.S.; Calcaterra, N.C.
IBR-CONICET, UNR, Argentina. MNDB, Univ. Chile, Chile. Sars Centre, Norway.

12:00-12:15
CB-C09
UNRAVELLING THE INTERPLAY BETWEEN CHROMATIN AND THE CELL CYCLE INHIBITOR p19INK4d
Sirkin, P.F.; Carcagno, A.F.; Ceruti, J.M.; Cánepa, E.T.
Laboratorio de Biología Molecular, Dpto. Química Biológica, FCEN, UBA, Buenos Aires, Argentina.

12:15-12:30
CB-C10
KLF6 BEHAVES AS AN ANTI-APOPTOTIC FACTOR IN MAMMALIAN CELLS
D'Astolfo, D.S.; Gherau, R.C.; Bocco, J.L.; Koritschoner, N.P.
Dpto. Bioquímica Clínica, CIBICI-CONICET, Fac. Cs. Químicas, Univ. Nacional de Córdoba, Argentina.

10:00-12:30 **ORAL COMMUNICATIONS**

Room C

Signal Transduction (ST-C01/ ST-C05) and Lipids (LI-C01/LI-C04)

Chairpersons: Teresa Machado (Universidad Nacional de Río Cuarto)
Cristina del Valle Paz (Universidad de Buenos Aires)

10:00-10:15
ST-C01
EGF RELEASES AA THROUGH ACS4 AND ACOT2 ACTIONS IN STEROIDOGENIC CELLS
Castilla, R.; Gadaleta, M.; Duarte, A.; Podestá, E.J.
Dep. of Biochemistry, School of Medicine, University of Buenos Aires, Argentina.

10:15-10:30
ST-C02
EXTRA-NUCLEAR LOCALIZATION OF α AND β ESTROGEN RECEPTORS IN C2C12 SKELETAL MUSCLE CELLS
Milanesi, L.; Russo de Boland, A.; Boland, R.
Laboratorio de Química Biológica, Universidad Nacional del Sur, Bahía Blanca, Argentina.

10:30-10:45
ST-C03
MULTIPLE KINASES ARE INVOLVED IN P19INK4D INDUCTION AND PHOSPHORYLATION IN RESPONSE TO GENOTOXICS
Ogara, M.F.; Marazita, M.C.; Scassa, M.E.; Cánepa, E.T.
Laboratorio de Biología Molecular, Dpto. Química Biológica, FCEN, UBA, Buenos Aires, Argentina.

10:45-11:00
ST-C04
DIFFERENTIAL INVOLVEMENT OF SAC INFLUX IN ATP MODULATION OF MAPKs IN OSTEOBLASTS AND BREAST CELLS
Scodelaro Bilbao P.; Katz S.; Santillán G.
Dpto. Biol., Bioq. y Fcia, Universidad Nacional del Sur, Bahía Blanca, Argentina.

11:00-11:15
ST-C05
BCL-XL EXPRESSION IS REGULATED BY EPIDERMAL GROWTH FACTOR (EGF) IN MAMMARY EPITELIAL CELLS (HC11)
Romorini, L.; Coso, O.A.; Pecci, A.
Dpto. Química Biológica, FCEN-UBA. Dpto. FBMC, FCEN-UBA. IFIByNE-CONICET.

11:15-11:30
LI-C01
MODULATION OF UNSATURATED FATTY ACID BIOSYNTHESIS BY INSULIN AND NUCLEAR TRANSCRIPTION FACTORS
Montanaro, M.A.; González, M.S.; Bernasconi, A.M.; Brenner, R.R.
Instituto de Investigaciones Bioquímicas de La Plata, Fac.Cs.Médicas, UNLP, La Plata, Argentina.

11:30-11:45
LI-C02
HORMONAL REGULATION OF ARACHIDONIC ACID RELEASE IN A SPECIFIC COMPARTMENT OF THE CELL
Castillo, A.F.; Cornejo Maciel, F.; Castilla, R.; Maloberti, P.; Paz, C.; Podestá, E.J.
Department of Biochemistry, School of Medicine, University of Buenos Aires, Argentina.

11:45-12:00
LI-C03
EFFECT OF MELATONIN AND STRUCTURAL ANALOGS ON LIPID PEROXIDATION OF TRIGLYCERIDES RICH IN ω-3 PUFAs
Fagali, N.S.; Catalá, A.
INIFTA, Facultad de Ciencias Exactas, CONICET, La Plata, Argentina.

12:00-12:15
LI-C04
ENHANCED CYTIDYLYLTRANSFERASE TRANSCRIPTION AND TRANSLATION BY NS398 IN HYPERTONIC MDCK CULTURES
Favale, N.O.; Lepera, L.G.; Gerardi, G.; Sterin-Speziale, N.; Fernández Tome, M.C.
Cátedra de Biología Celular, Fac. Farmacia y Bioquímica, UBA, IQUIFIB-CONICET.

12:30-15:00 **Lunch**

15:00-17:00 Room A

SYMPOSIUM

"Lipids"

Chairpersons: María del Carmen Fernández Tome (Universidad Nacional de Buenos Aires)
Marta Aveldaño (Universidad Nacional del Sur)

15:00-15:30 ***Horacio Garda***

Instituto de Investigaciones Bioquímicas, Universidad Nacional de La Plata, Argentina

"Structural and functional properties of a central domain of human apolipoprotein A-I"

15:30-16:00 ***José Luis Soulages***

Department of Biochemistry and Molecular Biology, Oklahoma State University, USA

"Lipolysis regulation in adipocytes"

16:00-16:30 ***Norma Sterin-Speziale***

IQUIFIB-CONICET, Universidad Nacional de Buenos Aires, Argentina

"Membrane lipids and tissue organization"

16:30-17:00 ***Lina M. Obeid***

Medical University of South Carolina, General Internal Medicine/Geriatrics, USA

"Bioactive lipids in inflammation and cancer"

17:00-19:00 **POSTERS**

Microbiology (MI-P01/MI-P50)
Structural Biology (SB-P01/SB-P21)
Cell Biology (CB-P01/CB-P40)
Enzymology (EN-P01/EN-P22)

19:00-20:15 Room A

"Homage to Dr. Luis Federico Leloir"

Chair: Armando Parodi (Fundación Instituto Leloir, Buenos Aires)

19:15-19:45 ***Fernando Goldbaum***

Fundación Instituto Leloir, Buenos Aires, Argentina

"Structural, functional and immunological studies of lumazine synthase, a new virulence factor of Brucella spp."

19:45-20:15 ***Andrea Gamarnik***

Fundación Instituto Leloir, Buenos Aires, Argentina

"Mechanism of dengue virus RNA recognition by the viral polymerase"

TUESDAY, November 14, 2006

8:30-9:30 Room A

PLENARY LECTURE

Yusuf Hannun

Department of Biochemistry and Molecular Biology
Medical University of South Carolina, Charleston, USA

"The extended family of neutral sphingomyelinase: role and regulation"

Chair: Norma Sterin-Speziale (Universidad de Buenos Aires)

9:30-10:00 **Coffee break**

10:00-12:30 **ORAL COMMUNICATIONS**

Room A

Microbiology (MI-C09/MI-C14) and Biotechnology (BT-C01/BT-C03)

Chairpersons: Mario Baigori (Universidad Nacional de Tucumán)
Claudia Studdert (Universidad de Mar del Plata)

10:00-10:15

MI-C09

TWO TRANSCRIPTIONAL REGULATORS COMPETE WITH IHF FOR THE BINDING TO THE *B. ABORTUS* VIRB PROMOTER

Sieira, R.; Comerci, D.J.; Ugalde, R.A.
IIB-INTECH, Universidad Nacional de General San Martín, Buenos Aires, Argentina.

10:15-10:30

MI-C10

THE ACID-INDUCED AUTOLYSIS IS CO-MEDIATED BY A QUORUM SENSING-INDEPENDENT PATHWAY IN *PNEUMOCOCCUS*

Piñas, G.; Albarracin Orio, A.; Cortes, P.; Echenique, J.
Dpto. Bioquímica Clínica, CIBICI-CONICET, Fac. de Cs. Químicas, UNC, Córdoba, Argentina.

10:30-10:45

MI-C11

A LOV-DOMAIN HISTIDINE KINASE IS INVOLVED IN *BRUCELLA ABORTUS* INFECTION

Paris, G.; Swartz, T.E.; Spera, J.M.; Comerci, D.; Bogomolni, R.; Goldbaum, F.A.
Instituto Leloir, Buenos Aires. Dept of Chem & Biochem, UCSC, USA. IIB-UNSAM CONICET, San Martín.

10:45-11:00

MI-C12

THE ERROR-PRONE DNA POLYMERASE IV IS INVOLVED IN *PSEUDOMONAS AERUGINOSA* MUCOID CONVERSION

Moyano, A.J.; Luján, A.M.; Argaraña, C.E.; Smania, A.M.
CIQUIBIC-CONICET, Facultad de Ciencias Químicas, UNC, Córdoba, Argentina.

11:00-11:15

MI-C13

ISOLATION OF BIOEMULSIFIER-PRODUCING SPORE-FORMING MICROORGANISMS FROM PETROLEUM CONTAMINATED SITES

Costas L; Pera LM; Rodriguez E; Baigori M.
PROIMI-CONICET, Tucumán.

11:15-11:30
MI-C14
STUDY OF PARASPORAL INCLUSIONS BY NUMERICAL ANALYSIS OF THEIR SDS-PAGE PROTEINS PROFILES
Alvarez A; Pera LM; Virla E; Baigori M.
PROIMI-CONICET, Tucumán.

11:30-11:45
BT-C01
TETRADECYLTRIMETHYLAMMONIUM DEGRADATION BY *P. PUTIDA* IS MORE EFFICIENT IN PRESENCE OF ALUMINIUM
Liffourrena, A.S.; Salvano, M.A.; Domenech, C.E.; Lucchesi, G.I
Dpto. Biología Molecular, FCEFQyN, UNRC, Argentina.

11:45-12:00
BT-C02
PLANT SPECIFIC INSERT (PSI) OF *St*APs: CLONING, EXPRESSION AND ANTIMICROBIAL ACTIVITY
Muñoz, F.F.; Mendieta, J.R.; Pagano, M.R.; Daleo, G.R.; Guevara, M.G.
Instituto de Investigaciones Biológicas, Universidad Nacional de Mar del Plata, Argentina.

12:00-12:15
BT-C03
MICROARRAY TRANSCRIPTIONAL ANALYSIS IN SPARC GAIN OF FUNCTION CONDITION USING *D. MELANOGASTER* EMBRYOS
Prada, F.; Wappner, P.; Podhajcer, O.
Fundación Instituto Leloir, Buenos Aires, Argentina.

10:00-12:30 **ORAL COMMUNICATIONS**

Room B

Cell Biology (CB-C11/CB-C19)

Chairpersons: Luis A. Quesada Allué (Fundación Instituto Leloir)
Cecilia Álvarez (Universidad Nacional de Córdoba)

10:00-10:15
CB-C11
TWO CRM1-DEPENDENT NUCLEAR EXPORT SIGNALS ARE INVOLVED IN THE REGULATION OF HIF1/SIMA
Romero, N.M.; Irisarri, M.; Cahuerff, A.; Wappner, P.
Fundación Instituto Leloir, Buenos Aires, Argentina.

10:15-10:30
CB-C12
RHO FAMILY PROTEINS ARE INVOLVED IN THE FORMATION OF *COXIELLA BURNETII REPLICATIVE COMPARTMENT*
Aguilera, M.; Rosales, E.; Salinas, R.; Berón, W.
IHEM-CONICET, Facultad Ciencias Médicas, Univ. Nac. Cuyo, Mendoza, Argentina.

10:30-10:45
CB-C13
RAB3A TRIGGERS CORTICAL GRANULE EXOCYTOSIS IN MOUSE EGGS
Michaut, M.A.; López, C.; Carabajal, M.; Mayorga, L.S.
Lab. Biología Celular y Molecular, IHEM-CONICET, Univ. Nacional Cuyo, Mendoza, Argentina.

10:45-11:00
CB-C14
PREFERENTIAL TRANSFER OF THE COMPLETE GLYCAN IS DETERMINED BY THE OLIGOSACCHARYLTRANSFERASE COMPLEX
Castro, O.A.; Movsichoff, F.; Parodi, A.J.
Fundación Instituto Leloir, Buenos Aires, Argentina.

11:00-11:15
CB-C15
IDENTIFICATION OF ER UDP-Glc TRANSPORTERS IN YEAST: THE SEARCH CONTINUES
D´Alessio, C.; Bredeston, L.; Parodi, A.J.
Fundación Instituto Leloir- IIBBA, CONICET, Buenos Aires, Argentina.

11:15-11:30
CB-C16
PHYSICAL AND FUNCTIONAL INTERACTION BETWEEN GalT2 AND CALSENILIN/CALP
Quintero, C; Valdez Taubas, J; Ferrari, M; Haedo, S.; Maccioni, H.
Dpto. Química Biológica, CIQUIBIC-CONICET, Facultad de Ciencias Químicas, Univ. Nacional de Córdoba, Argentina.

11:30-11:45
CB-C17
PHOTOACTIVABLE MOLECULES GENERATE TISSULAR DAMAGE BY ROS ON IMMATURE STAGES OF DIPTERANS
Massaldi, A.; Pujol Lereis, L.; Filiberti, A.; Radrizzani, M.; Rabossi, A.; Quesada Allué. L.
FCEyN-UBA, Fund. Inst. Leloir; Dpto Química Biol. UNC,CIQUIBIC-CONICET.

11:45-12:00
CB-C18
NOVEL BIOLOGICAL ACTIVITIES OF BENZNIDAZOLE: EFFECT ON THE ACTIVATION AND GROWTH OF RAW 264.7 CELLS
Pascutti, M.F.; Manarin, R.; Ruffino, J.P.; Revelli, S.; Serra, E.
IBR-CONICET, Facultad Cs. Bioq. y Farm; Instituto de Inmunología, Facultad Cs. Médicas, UNR.

12:00-12:15
CB-C19
MITOCHONDRIAL ATP SYNTHESIS AND ITS ROLE IN ARACHIDONIC ACID RELEASE AND METABOLISM IN THIS ORGANELLE
Duarte, A.; Castillo, A.F.; Castilla, R.; Maloberti, P.; Paz, C.; Podesta, E.J.; Cornejo Maciel, F.
Department of Biochemistry, School of Medicine, University of Buenos Aires.

10:00-12:30 **ORAL COMMUNICATIONS**

Room C

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

Chairpersons: Raquel Chan (Universidad Nacional del Litoral)
Ana María Laxalt (Universidad Nacional de Mar del Plata)

10:00-10:15

PL-C01

ATP MODULATES PEROXIDASE ACTIVITY AND ELICITS AUTOPHOSPHORYLATION OF RAPESEED 2-CYS PEROXIREDOXIN

Aran, M.; Caporaletti, D.; Senn, A.; Etchegoyen, J.I.; Tellez de Iñon, M.T.; Wolosiuk, R.A.
Instituto Leloir, Buenos Aires and INGEBI, Buenos Aires, Argentina.

10:15-10:30

PL-C02

NADP-MALIC ENZYME FAMILY FROM *A. THALIANA*: FUNCTIONAL CHARACTERIZATION OF T-DNA INSERTION MUTANTS

Gerrard Wheeler, M.; Zanor, M.I.; Drincovich, M.F.; Andreo, C.S.; Maurino, V.
CEFOBI-UNR, Rosario, Argentina and Botanisches Institut, Universität zu Köln, Cologne, Germany.

10:30-10:45

PL-C03

DOMAINS IMPLICATED IN TETRAMERIZATION AND MALATE INHIBITION OF MAIZE NADP-MALIC ENZYME

Detarsio, E.; Alvarez, C.E.; Saigo, M.; Andreo, C.S.; Drincovich, M.F.
CEFOBI-Facultad Cs Bioquímicas y Farmacéuticas (UNR), Rosario. Argentina.

10:45-11:00

PL-C04

HIGH LEVEL OF NADP-MALIC ENZYME IN PLASTIDS REDUCES PROLONGED DARKNESS TOLERANCE IN *A. THALIANA*

M. Saigo; H. Fahnenstich; M. Niessen; M.F. Drincovich; C. Andreo; U-I. Flügge; V. Maurino.
CEFOBI-UNR, Rosario, Argentina and Botanisches Institut, Universität zu Köln, Cologne, Germany.

11:00-11:15

PL-C05

THE BIOGENESIS OF THE PHOTOSYNTHETIC APPARATUS IS REPPRESED BY THE TRANSCRIPTION FACTOR HAHB-4

Manavella, P.A.; Dezar, C.A.; Ariel, F.D.; Drincovich, M.F.; Chan, R.L.
Cátedra de Biología Celular y Molecular, FBCB (UNL) Santa Fe, Argentina.

11:15-11:30

PL-C06

MicroRNA EXPRESSION IN MAIZE AND WHEAT

Berdat, C.; Margarit, E.; Reggiardo, M.; Vallejos, R.H
CEFOBI-Facultad Cs Bioquímicas y Farmacéuticas (UNR). Rosario. Argentina.

11:30-11:45

PL-C07

TOBACCO TRANSCRIPTOME ANALYSIS IN RESPONSE TO *XANTHOMONAS AXONOPODIS* PV. *CITRI*
Daurelio, L.D.; Petrocelli, S.; Ottado, J.; Orellano, E.G.
IBR, CONICET, Facultad Ciencias Bioquímicas Farmacéuticas, UNR, Rosario, Argentina.

11:45-12:00
PL-C08
IDENTIFICATION OF PROTEIN-PROTEIN INTERACTIONS BETWEEN *XANTHOMONAS AXONOPODIS* PV. *CITRI* AND ORANGE
Dunger, G.; Pereda Rosa, M.C.; Farah, C.S.; Orellano, E.G.; Ottado, J.
IBR-CONICET, Fac. Cs. Bioq. UNR, Rosario, Argentina; Dpto. Bioq., IQ, USP, São Paulo, Brasil.

12:00-12:15
PL-C09
HEME OXYGENASE UP-REGULATION IN UV-B IRRADIATED SOYBEAN PLANTS INVOLVES REACTIVE OXYGEN SPECIES
Yannarelli, G.G.; Noriega G.O.; Santa-Cruz, D.; Batlle, A.; Tomaro, M.L.
Dpto. de Química Biológica, Fac. Farmacia y Bioquímica, UBA. CIPYP-CONICET, Buenos Aires.

12:30-15:00 **Lunch**

13:40-14:30 **Technical Worshop** (Room B)
"New Technologies in Pipetting Instruments"
Lic. Ethel Coscarello, Gilson Product Specialist, Bio Esanco S.A.

15:00-17:00 Room A
SYMPOSIUM
"Cell Biology"
Chairperson: Claudia Tomes (Universidad Nacional de Cuyo)
Carlos Arregui (Universidad de San Martín)

15:00-15:30 ***Lisette Leyton***
ICBM-Facultad de Medicina, Universidad de Chile, Santiago, Chile
"Thy-1/αVβ3 integrin interaction activates bidirectional signaling in both neurons and astrocytes"

15:30-16:00 ***Silvia Moreno***
Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina
"Protein kinase A activation in lower eukaryotic model systems"

16:00-16:30 ***Mario D. Galigniana***
Fundación Instituto Leloir, Argentina
"Role of high MW immunophilins in the subcellular localization of signaling proteins"

16:30-17:00 ***Marisa Colombo***
Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Argentina
"Microorganisms and Autophagy: the autophagic response, a pathogen driven process"

15:00-17:00 Room B

SYMPOSIUM

"Microbiology"

Chairperson: Hugo Gramajo (Universidad Nacional de Rosario)
José Luis Bocco (Universidad Nacional de Córdoba)

15:00-15:30 ***Mary Fillon-Jackson***

Unité de Génétique Mycobactérienne, Pasteur Institute, Paris, Francia

"The polyketide-derived lipids of *Mycobacterium tuberculosis*"

15:30-16:00 ***Keith Chater***

Department of Molecular Microbiology, John Innes Center, Norwich, United Kingdom

"Influence of a tRNA on the extracellular biology of *Streptomyces*"

16:00-16:30 ***Gustavo E. Schujman***

IBR/CONICET, Universidad Nacional de Rosario, Argentina

"Regulation of lipid biosynthesis in Gram positive bacteria"

16:30-17:00 ***Hugo Gramajo***

IBR/CONICET, Universidad Nacional de Rosario, Argentina

"Acyl-CoA carboxylases in Mycobacteria: finding new targets for drug development"

17:00-19:00 **POSTERS**

Microbiology (MI-P51/ MI-P93)
Cell Biology (CB-P41/ CB-P85)
Plant Biochemistry and Molecular Biology (PL-P01/ PL-P46)

19:00-20:00 Room A

PLENARY LECTURE

Carlos Gómez Moreno

Department of Biochemistry Molecular and Cellular Biology, Faculty of Sciences, University of Zaragoza, Spain

"Molecular mechanism of protein-protein recognition"

Chair: Néstor Carrillo (Universidad Nacional de Rosario)

20:30 **SAIB General Assembly**

WEDNESDAY, November 15, 2006

8:30-9:30 Room A

PLENARY LECTURE

Wolfgang Junge

Division of Biophysics, University of Osnabrück, Germany

"Nanomechanics of rotary ATP synthase"

Chair: Oscar Roveri (Universidad Nacional de Rosario)

9:30-10:00 **Coffee break**

10:00-12:30 **ORAL COMMUNICATIONS**

Room A

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

Chairpersons: Luis Ielpi (Fundación Instituto Leloir)
Alberto Iglesias (Universidad Nacional del Litoral)

10:00-10:15
SB-C01
CRYSTAL STRUCTURE OF PIMA, AN ESSENTIAL MANNOSYLTRANSFERASE FROM *MYCOBACTERIUM SMEGMATIS*
Guerin, M.E.; Buschiazzo, A.; Schaeffer, F.; Kordulakova, J.; Giganti, D.; Svetlikova, Z.; Mikusova, K.; Gicquel, B.; Jackson, M.; Alzari, P.M.
Institute Pasteur, Paris, France. Comenius University, Fac Nat Sci, Bratislava, Slovakia.

10:15-10:30
SB-C02
CRYSTALLOGRAPHIC AND ENZYMATIC STUDIES ON LUMAZINE SYNTHASES RIBH1 AND RIBH2 FROM *Brucella spp.*
Klinke, S.; Zylberman, V.; Bonomi, H.; Guimarães, B.G.; Braden, B.C.; Goldbaum, F.A.
Fundación Instituto Leloir, Bs. As. LNLS, Campinas, Brazil. Bowie State Univ., Bowie, MD, USA.

10:30-10:45
SB-C03
CRYSTAL STRUCTURE OF GUMK, A BETA-GLUCURONOSYLTRANSFERASE INVOLVED IN XANTHAN BIOSYNTHESIS
Barreras, M.; Kampel, M. A.; Ielpi, L.
Fundación Instituto Leloir, Buenos Aires. Argentina.

10:45-11:00
SB-C04
CRITICAL ACTIVE-SITE RESIDUES IN *PSEUDOMONAS AERUGINOSA* PHOSPHORYLCHOLINE PHOSPHATASE
Beassoni, P.R.; Otero, L.H.; Massimelli, M.J.; Lisa, A.T.; Domenech, C.E.
Dpto. Biología Molecular, UNRC, Córdoba, Argentina.

11:00-11:15
EN-C01
INHIBITION OF THE MITOCHONDRIAL ATP SYNTHESIS BY POLYGODIAL AND OTHER DRIMANE ANALOGUES
Castelli, M.V.; Fábregas, J.I.; Zacchino, S.A.; Roveri, O.A.
Areas Biofísica y Farmacognosia, Fac. Cs. Bioquímicas y Farmacéuticas, UN Rosario, Argentina.

11:15-11:30
EN-C02
TRANSESTERIFICATION ACTIVITY OF A MYCELIUM-BOUND LIPASE FROM *Aspergillus niger* MYA 135
Romero C.M.; Pera L.M.; Baigorí M.
PROIMI-CONICET, Tucumán.

11:30-11:45
EN-C03
STRUCTURE-FUNCTION RELATIONSHIP OF UROPORPHYRINOGEN DECARBOXYLASE (UROD)
Graziano, M.; Romero, D.M.; Ríos de Molina, M.C.
Dpto. Química Biológica, FCEyN, UBA. CONICET.

10:00-12:30 **ORAL COMMUNICATIONS**

Room B

Lipids (LI-C05/LI-C10) and Neurosciences (NS-C01/NS-C02)

Chairpersons: María Gonzalez Baro (Universidad Nacional de La Plata)
Claudia Banchio (Universidad Nacional de Rosario)

10:00-10:15
LI-C05
SP1 BINDS ELEMENTS REGULATED BY RB AND REGULATES CTP:PHOSPHOCHOLINE CYTIDYLYLTRANSFERASE EXPRESSION
Banchio, C.; Vance, D.
IBR CONICET, Universidad Nacional de Rosario, Rosario, Argentina.

10:15-10:30
LI-C06
BIOSYNTHESIS OF PHOSPHATIDYLCHOLINE DURING NEURONAL DIFFERENTIATION
Marcucci, H.J.; Gilardoni, P.; Banchio, C.
IBR CONICET, Universidad Nacional de Rosario, Rosario, Argentina.

10:30-10:45
LI-C07
PHOSPHOLIPIDS INVOLVED IN THE SIGNAL TRANSDUCTION CASCADE LEADING TO ACROSOMAL EXOCYTOSIS
Belmonte, S.A.; Suhaiman, L.; López, C.I.; Pelletán, L.; Mayorga, L.S.
Lab. Biología Celular y Molecular, Inst. Histología y Embriología, Facultad Cs. Médicas, UN Cuyo, Mendoza, Argentina.

10:45-11:00
LI-C08
UNEVEN DISTRIBUTION OF LIPIDS AND FATTY ACIDS BETWEEN HEAD AND TAIL IN RAT SPERMATOZOA
Oresti, G.M.; Ayuza Aresti, P.L.; Furland, N.E.; Aveldaño, M.I.
INIBIBB, CONICET-UNS, Bahía Blanca, Argentina.

11:00-11:15
LI-C09
2-HYDROXY FATTY ACIDS WITH VERY LONG CHAINS IN SPHINGOLIPIDS FROM RAT TESTES AND SPERMATOZOA
Zanetti, S.R.; Aveldaño, M.I.
INIBIBB, CONICET-UNS, Bahía Blanca, Argentina.

11:15-11:30
LI-C10
INHIBITION OF SK1 STIMULATES SPHINGOLIPIDS DE NOVO SYNTHESIS, ENHANCING CERAMIDE ACCUMULATION
Leocata Nieto, F.A.; Pescio, L.; Salama, F.; Sterin-Speziale, N.
Cátedra de Biología Celular. Fac. Farmacia y Bioquímica, UBA. IQUIFIB-CONICET.

11:30-11:45
NS-C01
MOLECULAR DYNAMICS OF ACETYLCHOLINE RECEPTOR ION CHANNEL BLOCK BY THE NEUROLEPTIC CHLORPROMAZINE
Y. Xu; J. Shen; X. Luo; W. Zhu; K. Chen; H. Jiang; F. J. Barrantes.
UNESCO Chair of Biophysics & Molecular Neurobiology / INIBIBB. Bahía Blanca, Argentina.

11:45-12:00
NS-C02
PRESENCE OF A GLOBIN mRNA AND MIGRATION OF BONE MARROW CELLS AFTER SCIATIC NERVE INJURY
Setton-Avruj, C.P; Musolino, P.L.; Salis, C.; Alló, M.; Bizzozero, O.; Villar, M.J.; Soto, E.F; Pasquini, J.M.
Dept. Quím. Biol., FFyB, IQUIFIB, UBA-CONICET. Fac. Ciencias Médicas, Universidad Austral.

12:30-15:00 **Lunch**

15:00-17:00 Room A

SYMPOSIUM

"Plant Biochemistry and Molecular Biology"

Chaipersons: Elena Orellano (Universidad Nacional de Rosario)
Jorge Muschietti (INGEBI-Universidad de Buenos Aires)

15:00-15:30 ***Marcelo Yanovsky***
Facultad de Agronomía, Universidad de Buenos Aires, Argentina
"Light signaling pathways mediating shade avoidance responses in *Arabidopsis*"

15:30-16:00 ***Xavier Jordana***
Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile Santiago, Chile
"Mitochondrial complex II is essential for gametophyte development in *Arabidopsis thaliana"*

16:00-16:30 ***Ariel Goldraij***
Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina
"New insights on pollen rejection in self-incompatible *Nicotiana*"

16:30-17:00 ***Daniel H. González***
Facultad Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina
"Transcriptional coordination of nuclear genes encoding plant mitochondrial respiratory chain components"

15:00-17:00 Room B

SYMPOSIUM

“Structural Biology”

Chairpersons: Fernando Goldbaum (Fundación Instituto Leloir)
Eduardo Ceccarelli (Universidad Nacional de Rosario)

15:00-15:25 ***Gonzalo de Prat Gay***

Fundación Instituto Leloir, Buenos Aires, Argentina

“Oligomerization, localization and function of the E6 and E7 oncoproteins from human papillomavirus”

15:25-15:50 ***Alejandro Buschiazzo***

Institut Pasteur France and Intituto Pasteur Montevideo

"Crystal structure, catalytic mechanism, and mitogenic properties of *Trypanosoma cruzi* proline racemase"

15:50-16:15 ***Ana Cauerhff***

Fundación Instituto Leloir, Buenos Aires, Argentina

"Structural mechanism for affinity maturation of an anti-lysozyme antibody"

16:15-16:40 ***Mario Ermácora***

Universidad Nacional de Quilmes, Buenos Aires, Argentina

"Mapping the distribution of conformational information throughout a protein sequence"

16:40-17:05 ***Roberto Bogomolni***

Department of Chemistry, University of California at Santa Cruz, USA

"Structural biology and biochemistry of plant and bacterial phototropin LOV domains"

17:00-19:00 **POSTERS**

Plant Biochemistry and Molecular Biology (PL-P47/PL-P76)
Signal Transduction (ST-P01/ST-P30)
Neuroscience (NS-P01/NS-P07)
Biotechnology (BT-P01/BT-P33)
Lipids (LI-P01/LI-P31)

19:00-20:00 Room A

“Alberto Sols” LECTURE

Joaquim Ros

Departamento Ciencias Médicas Básicas, Universidad de Lérida, España

"Protein oxidative damage in yeast"

Chair: Néstor Cortez (Universidad Nacional de Rosario)

21:00 **Closing Dinner**

SYMPOSIA

S1- S23

S-01

STRUCTURAL AND FUNCTIONAL PROPERTIES OF A CENTRAL DOMAIN OF HUMAN APOLIPOPROTEIN A-I

Garda, H. A.
Instituto de Investigaciones Bioquímicas de La Plata (CONICET-UNLP) Calles 60 y 120. 1900-La Plata
E-mail: hgarda@atlas.med.unlp.edu.ar

Apolipoprotein A-I (apoAI) plays a key role in several steps of reverse cholesterol transport, a process of antiatherogenic relevance that removes cholesterol excess of peripheral tissues. ApoAI is constituted almost exclusively by amphipathic α-helices and undergoes large conformational changes to exchange among lipid-free and different lipid-bound states during its functional cycle. Identification of domains involved in the different apoAI functions, and knowing the characteristics that make them critical for its activity, would provide valuable information on the mechanism of cellular lipid efflux mediated by this protein. I will focus here on a central pair of α-helices having a particular charge distribution in their polar face. The behavior of a synthetic peptide suggests that these helices constitute a functional and structural domain with relative independence from the rest of apoAI molecule. This domain inserts preferentially into cholesterol-containing membranes where it facilitates cholesterol desorption. Recent studies revealed that the central domain is also responsible for triggering mobilization of intracellular cholesterol depots toward the cell membrane. For its activity, a specific sequence is not required, but the charge distribution of class "Y" amphipathic α-helices and an adequate orientation of the hydrophobic and hydrophilic helix faces would be necessary.

S-02

LIPOLYSIS REGULATION IN ADIPOCYTES

Soulages, José Luis
Department of Biochemistry and Molecular Biology; Noble Research Center, Oklahoma State University, Stillwater, OK 74078. E mail: jose@biochem.okstate.edu

The development and pathology of non insulin dependent diabetes mellitus, NIDDM, are associated to a high concentration of plasma free fatty acids (FFA). The thiazolidinediones (TZDs) rosiglitazone (RZG) and pioglitazone are currently used to treat patients with NIDDM. Treatment with TZDs results in lower plasma levels of glucose and free fatty acids. TZDs are potent ligands of the transcriptional factor peroxisome-proliferator activated receptor-gamma (PPAR-γ). However, the mechanism by which TZDs improves the metabolism of glucose and lipids is not clearly understood. Two models have been proposed to explain the RGZ dependent reduction in plasma FFA. It has been hypothesized that the increase in glycerol kinase expression observed in RGZ treated adipocytes could induce an overall reduction in plasma FFA by increasing the rate of FA esterification in adipose tissue. It has also been suggested that the induction of the expression of PEPCK by RGZ could enhance glyceroneogenesis and thus FA-re-esterification in adipose tissue. The present study was undertaken to investigate possible effects of RGZ on the utilization of glucose for the synthesis of triglycerides in 3T3 L1 adipocytes. The utilization of glucose carbons for the synthesis of lactate, glyceride-glycerol and fatty acids was studied under different metabolic conditions: low lipolysis (insulin), high lipolysis (isoproterenol), and moderate lipolysis. The results obtained indicate that RGZ treatment enhances the rate of glycolysis to fuel the increase in the rate of the TG cycle (hydrolysis/re-synthesis).

S- 03
MEMBRANE LIPIDS AND TISSUE ORGANIZATION

Sterin-Speziale, Norma
Biología Celular. FFyB, UBA. IQUIFIB-CONICET. Buenos Aires, ARGENTINA.
E-mail: speziale@ffyb.uba.ar

The adhesion of cells to the extra cellular matrix (ECM) is essential for the maintenance of the tissue integrity. Focal adhesions (FA), the most stable points of cell tethering to the ECM occur by the interaction of its cytoplasmic domain integrin with the FA protein talin which, in turn, bounds vinculin. The assembly of talin and vinculin into FA depend on their activation by binding to phosphatidylinositol-4,5-biphosphate $PI(4,5)P_2$. Plasma membrane $PI(4,5)P_2$ stabilizes integrant binding of talin and promotes the recruitment of vinculin to the membrane, initially via an interaction between the negatively charged phospholipid head group of the phospholipid membrane. Earlier "in vitro" experiments demonstrated that the insertion of either vinculin or talin into lipid bilayer depends on its phospholipid composition. While the formation and modulation of FA complexes have been intensively studied, the role of the membrane lipid environment in cellular systems is studied less often. We studied the effect of changes in membrane lipid composition on the preservation of FA plaques and demonstrated that the membrane lipid composition play a central role for the preservation of cell-matrix adhesion structures. This study may constitute an explanation for the deleterious effect exerted by membrane affecting agents of pharmacological use on tissue organization.

S-04
BIOACTIVE SPHINGOLIPIDS IN CANCER AND INFLAMMATION

Obeid, Lina M.
Medical University of South Carolina, Dep. Medicine, Biochemistry, Molecular Biology, Charleston.

Our laboratory focuses on the study of bioactive sphingolipids and their role in cell growth regulation. Specifically ceramide has been shown to mediate cell growth arrest, apoptosis, and senescence, whereas sphingosine-1-phosphate mediates cell proliferation and angiogenesis. Ceramidases breakdown ceramide to sphingosine, which is in turn phosphorylated to sphingosine 1 phosphatase (S1P). It is, therefore, evident that regulation of these enzymes has a critical role in the balance of cellular levels of ceramide and S1P. Moreover, we have recently implicated sphingosine kinase and S1P in regulation of the cyclooxygenase-2 (COX-2) pathway of inflammation. Studies on the regulation of ceramidases and sphingosine kinase are underway in our laboratory and indicate that both these enzymes are activated by proinflammatory cytokines. Our recent data implicate the SK/S1P/COX2 pathway in colon cancer. Moreover a differential role for SK/S1P/COX2 pathway is emerging in the innate immune response by lipopolysaccharide (LPS) as compared to the response to tumor necrosis factor (TNF). These studies may have implications to understanding the regulation of different inflammatory and cancer processes.

S-05

THY-1/αVβ3 INTEGRIN INTERACTION ACTIVATES BIDIRECTIONAL SIGNALING IN BOTH NEURONS AND ASTROCYTES

Leyton, Lisette
Center for Molecular Studies of the Cell, Fac. de Medicina, Univ. de Chile. Santiago, Chile. E-mail: lleyton@med.uchile.cl

Upon brain injury, astrocytes enlarge, divide and migrate to form a glial scar which creates a non-permissive environment for neuronal regeneration in a process known as astrogliosis. Inhibitory mechanisms of neuronal regeneration occurring in the adult central nervous system (CNS) include the redundant presence of molecules that prevent axonal regrowth. We have focussed our interest on Thy-1, an abundant mammalian-neuronal glycoprotein that has been implicated in inhibiting neurite outgrowth. Although this role for Thy-1 was suggested a few decades ago, its function remained obscure until we identified αVβ3 integrin as Thy-1 receptor/ligand molecule on astrocytes. We also showed that Thy-1/αVβ3 integrin interaction triggers focal adhesion and stress fiber formation in astrocytes, thereby promoting cell attachment via integrin clustering and activation of the Rho GTPase and its effector, RhoA/p160ROCK. Moreover, we obtained evidence indicating that sustained Thy-1 stimulation induces migration but not proliferation of astrocytes. Morphological changes and subsequent migration observed in Thy-1-treated astrocytes in vitro may be related to events triggered in vivo during astrogliosis. Additionally, using the CAD cell line from mouse CNS, we explored the effect of αVβ3 on neuronal differentiation induced by serum deprivation. Our results indicate that Thy-1/αVβ3 integrin interaction causes the inhibition of neurite outgrowth as assessed by morphological and elecrophysiological parameters. These results implicate Thy-1/αVβ3 integrin in bidirectional signaling between neurons and astrocytes.

S-06

PROTEIN KINASE A ACTIVATION IN LOWER EUKARYOTIC MODEL SYSTEMS

Moreno, Silvia
Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, UBA. E-mail: smoreno@qb.fcen.uba.ar

Our main interest is to understand the mechanism of activation of the cAMP-dependent protein kinase within the cell, contributing to this field with three lower eukaryotic models: the fungus Mucor (*M. rouxii* and *M. racemosus*) and *Saccharomyces cerevisiae*. In Mucor, signal transduction via cAMP exerts a clear physiological effect upon morphology. Biochemical, genetical and physiological approaches have been undertaken with this model. *S. cerevisiae* has the advantages of its complete genome sequence as well as the tools available both for genetic and biochemical experiments. The protein kinases A (PKA) from both models have been characterized in vitro, and protein substrates have been identified or proposed. The affinities between R and catalytic (C) subunits in both models is completely different as judged by biochemical and genetic parameters, being the affinity between R and C in Mucor PKA much higher. The reasons for the differences in affinities has been approached biochemically, using whole proteins or peptides, genetically and bioinformatically. Activation of yeast PKA has been measured *in situ* during a physiological increase in cAMP levels; our results show that during this endogenous activation the C subunit (Tpk1) is phosphorylated with a change in its kinetic parameters, and suggest that only locally produced cAMP can activate the holoenzyme in its endogenous location.

S-07

ROLE OF HIGH MW IMMUNOPHILINS IN THE SUBCELLULAR LOCALIZATION OF SIGNALLING PROTEINS

Galigniana, M.D.
Fundación Instituto Leloir & Departamento de Química Biológica, Facultad de Ciencias Exactas, UBA. E-mail: mgali@leloir.org.ar

Most soluble proteins are not confined to the cytoplasm or the nucleus in a static manner. They are able of shuttling dynamically through the nuclear pore, even when the number of molecules in a given compartment is overwhelmingly larger than the number located in the other compartment. Protein mistargeting has dire cellular consequences and leads to a large variety of pathologies. More than 300 diseases have been related to date with failures in the transport or mislocalization of proteins. As such, a major unsolved problem in Cell Biology that pertains to all signalling pathways relates to how these factors move to their sites of action. Corticosteroid receptors are superb tools for studying this conundrum. They are primarily cytoplasmic in the absence of ligand and rapidly move towards the nucleus upon hormone binding. A classical model proposed 25 years ago posited the notion that the hsp90-based heterocomplex associated to the steroid receptor must dissociate to allow its nuclear translocation, simple diffusion being the driving force. Although heuristic, this model has no experimental support. We provide direct evidence that the hsp90-high MW immunophilin complex bound to steroid receptors is not dissociated at early times upon ligand binding. Actually, it is required for the active retrograde movement of the receptor, a property also shared by other soluble factors such as p53.

S-08

MICROORGANISMS AND AUTOPHAGY: THE AUTOPHAGIC RESPONSE, A PATHOGEN DRIVEN PROCESS

Romano, P.(1); Saka, A.(2); Gutierrez, M.(1); Zoppino, M.(1); Bocco, J. L.(2); Colombo, M. I.(1)
1IHEM-CONICET, Fac. de Cs Méd., U. N. Cuyo Mendoza.
2CIBICI-CONICET, Fac. de Cs Qcas, UNC, Córdoba.
E-mail: mcolombo@fcm.uncu.edu.ar

Coxiella burnetii is an intracellular obligate parasite that resides and replicates in large vacuoles with autophagolysosomal characteristics. We have examined, at earlier stages of infection, the distribution and roles of the small GTPases Rab5 and Rab7 on *C. burnetii* trafficking. Coxiella phagosomes (CPh) acquire these two Rab proteins sequentially during infection and overexpression of Rab5 and Rab7 dominant negative mutants inhibited vacuole formation. Interestingly, CPh colocalized with the autophagy protein LC3 as early as 5 min after infection and this recruitment was dependent on the microorganism protein synthesis. Our results indicate that *C. burnetii* actively interacts with autophagosomes at early times after infection. Though interactions of whole pathogens with the autophagy pathway have been described, specific bacterial components modulating autophagy have not been identified so far. We have evidences of a link of autophagy with extracellular pathogens, like *Vibrio cholerae*, through a secreted molecule termed *V. cholerae* cytolysin (VCC).VCC-induced vacuoles colocalized with LC3, indicating the interaction with autophagic vesicles. In cells knock out for the autophagy protein Atg5, VCC failed to induce vacuolization and cell survival was dramatically impaired, indicating that autophagy also acts as a cellular defense pathway against a secreted bacterial toxin.

S-09

THE POLYKETIDE-DERIVED LIPIDS OF *MYCOBACTERIUM TUBERCULOSIS*

Jackson, M.; Rousseau, C; Stadthagen, G; Bordat, Y.; Gicquel, B.
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Mycobacterium tuberculosis, the causative agent of tuberculosis in humans, remains a major cause of mortality and morbidity worldwide. Although much remains to be done in identifying the molecular determinants of the pathogenicity of this bacterium, there is increasing evidence that the complex envelope of the bacterium plays a major role in pathogenesis. Among the unique features of the envelope of pathogenic mycobacteria are the complex lipids esterified with up to five multiple methyl-branched long-chain fatty acids. In *M. tuberculosis*, these lipids include the phthiocerol dimycocerosates (PDIM) and the closely related phenolic glycolipids (PGL), the trehalose ester families that include sulfatides (SL), diacyltrehaloses (DAT), triacyltrehaloses (TAT) and polyacyltrehaloses (PAT), and the mannosyl-β-1-phosphomycoketides. Using various genetic approaches, we have isolated a number of *M. tuberculosis* mutants deficient in the production of these lipids. *In vitro* and *in vivo* studies performed on these mutants allowed us to gain significant insights into the biosynthesis, regulation, transport across the different layers of the cell envelope and biological functions of these lipids.

S-10

EFFECTS OF DELETION OF A tRNA GENE ON THE SPECIALISED BIOLOGY OF *STREPTOMYCES COELICOLOR*

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Streptomycetes grow as mycelial colonies that develop overtly differentiated layers ("tissues"). The biomass-accumulating vegetative mycelium, in comparatively close contact with the food source (usually soil), supports the growth of aerial braches that may serve as exploratory organs or as sporophores. The region between the active vegetative mycelium and the sporulating aerial mycelium shows much mycelial death, presumably associated with the reuse of the biomass for aerial growth. It also seems to be the main focal point for the production of antibiotics, since [at least in the model strain *Streptomyces coelicolor* A3(2)] two coloured antibiotics are visible only in this zone. Among *S. coelicolor* mutants affecting aspects of colony development, one type, defective in bldA, has particularly pleiotropic defects in aerial growth and antibiotic production. The bldA gene product is the tRNA for the UUA codon, which is the rarest codon in GC-rich Streptomyces genomes. Deletion of bldA does not diminish growth, but it does virtually eliminate the expression of most TTA-containing genes tested. A combined proteomic and microarray analysis has revealed that bldA deletion affects important aspects of both intra- and extracellular physiology. Only a few of the TTA-containing genes of *S. coelicolor* are conserved among different streptomycetes.

S-11
REGULATION OF LIPID BIOSYNTHESIS IN GRAM POSITIVE BACTERIA

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Fatty acids and their derivatives play essential roles in all living organisms as components of membranes and energy sources. Biosynthesis of these compounds is carried out by a single multifunctional polypeptide (type I system) in higher eukaryotes and by dissociable enzymes (type II system) in bacterial cells, plant chloroplasts and malaria parasites. Yet, despite the complexity of these biosynthetic pathways, biological membranes maintain stable compositions that are characteristic for each cellular type. Nevertheless, the precise homeostatic mechanisms maintaining the concentration of lipids at particular levels are largely unknown. Here we report a novel role for malonyl-CoA, a universal intermediate in fatty acid biosynthesis, as a negative effector of the global transcriptional repressor FapR that regulates the expression of several genes encoding fatty acid and phospholipid biosynthetic enzymes in *Bacillus subtilis*. Crystallographic and binding studies demonstrate that malonyl-CoA specifically binds to the thioesterase-like effector domain of FapR, inducing a conformational rearrangement that dissociates the FapR-DNA complex or prevents its formation. Furthermore, mutations disrupting the FapR-malonyl-CoA interaction result in a lethal phenotype, suggesting that this homeostatic signaling pathway is a target for novel chemotherapeutic agents against Gram-positive pathogens.

S-12
ACYL-CoA CARBOXYLASES IN MYCOBACTERIA: FINDING NEW TARGETS FOR DRUG DEVELOPMENT

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The most relevant lipids present in *Mycobacterium* cell envelope are the mycolic acids, long-chain α-alkyl, β-hydroxy fatty acids, and the characteristic methyl-branched long-chain acids. These unusual fatty acids are essential for the survival, virulence and antibiotic resistance of *M. tuberculosis*. Acyl-CoA carboxylases (ACCases) commit acyl-CoAs to the biosynthesis of these unique fatty acids. Unlike other organisms, *M. tuberculosis* contains six ACCase carboxyltransferase (CT) domains, whose specific roles in the pathogen are not well defined. The biochemical characterization of ACCase5 of *M. tuberculosis*, led us to propose this complex as the propionyl-CoA carboxylase enzyme that produces methylmalonyl-CoA for the biosynthesis of the multimethyl-branched fatty acids. ACCase6, instead, appears to be the acetyl-CoA carboxylase complex that provides malonyl-CoA for the biosynthesis of mycolic acids. Crystal structures of the CT components, AccD5 and AccD6, of these two enzyme complexes showed a highly conserved active site and supported the biochemical roles of these enzymes. *In silico* screening of NCI database, resulted in the identification of inhibitors of the two CTs, that were also capable of inhibiting growth of *M. smegmatis* and *M. bovis* BCG. Our functional and structural studies provide a new structure-based drug design target for tuberculosis therapeutic development.

S-13

OLIGOMERIZATION OF PAPILLOMAVIRUS ONCOPROTEINS: UNEXPECTED FUNCTION AND CELLULAR LOCALIZATION

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Human papillomavirus E6 and E7 oncoproteins transform ephithelial cells in various virus linked neoplasias, where the most relevant is cervical cancer in women. HPV E6 and E7 have also been instrumental in elucidating fundamental aspects of p53 and retinoblastoma tumour suppressor cell cycle control, with counterpart activities in various DNA tumour viruses. E7 is a ca100 amino acid acidic protein and E6 is a 150 amino acid basic protein, both with cystein mediated zinc binding motifs but with no biochemical function other than binding reported for them. Given their small size, it is difficult to explain the over 50 protein targets that have been reported for the proteins. Both proteins readily form large soluble oligomers, indicating that the monomeric or dimeric forms are not the only possible conformations. E7 is an extended dimer, 50 nm spherical oligomers formed upon removal of zinc are regular and we found that they have chaperone holdase activity, which may explain its apparent target promiscuity. Recombinant high risk E6 oncoproteins refold into oligomers of ~1.2 MDa molecular weight. Contrary to the monomeric forms, the E6 oligomers are deficient in promoting p53 degradation in vitro. We detect and localize the endogenous oncoproteins in HPV transformed cell lines for the first time and describe a differential localization for each conformer.

S-14

CRYSTAL STRUCTURE AND CATALYTIC MECHANISM OF THE PROLINE RACEMASE, A B-CELL MITOGEN FROM TRYPANOSOMA

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Amino acid racemases catalyze the stereoinversion of the chiral Ca to produce the D-enantiomers that participate in biological processes, such as cell wall construction in prokaryotes. Within this large protein family, bacterial proline racemases have been extensively studied as a model of enzymes acting with a pyridoxal-phosphate-independent mechanism. Here we report the crystal structure of the proline racemase from the human parasite Trypanosoma cruzi (TcPRACA), a secreted enzyme that triggers host B cell polyclonal activation, which prevents specific humoral immune responses and is crucial for parasite evasion and fate. The enzyme is a homodimer, with each monomer folded in two symmetric a/b subunits separated by a deep crevice. The structure of TcPRACA in complex with a transition-state analog, pyrrole-2-carboxylic acid, reveals the presence of one reaction center per monomer, with two Cys residues optimally located to perform acid/base c talysis through a carbanion stabilization mechanism. Mutation of the catalytic Cys residues abolishes the enzymatic activity but preserves the mitogenic properties of the protein. In contrast, inhibitor binding promotes the closure of the interdomain crevice and completely abrogates B cell proliferation, suggesting that the mitogenic properties of TcPRACA depend on the exposure of transient epitopes in the ligand-free enzyme.

S-15

STABILITY AND PLASTICITY PLAY IMPORTANT ROLES IN THE AFFINITY MATURATION OF ANTI-PROTEIN ANTIBODIES

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The biotechnological and industrial applications of proteins are usually limited by deficient protein stability. The objective of this work is to study the structural basis of the affinity maturation of two anti-lysozyme (HEL) antibodies, in relationship with the stability of their variable domains. These two mAbs, F10.6.6 and D44.1, are derived from the same germlines genes, and recognize the same epitope on HEL surface, but F10.6.6 has ~1000 higher affinity towards HEL than D44.1.To study the contribution of each variable region of both mAbs in binding kinetics, thermodynamics and stability, chain shuffling experiments were performed. It was noticeable that VH domain drives the association rates to HEL while VL domain modulates the dissociation rate and binding enthalpy. Crystallographic analysis of the free and bound form of both antibodies shows that F10.6.6 losses more contacts in VH-VL interaction than D44.1 upon binding to HEL. Chemical and thermal stability was studied by circular dichroism and intrinsic fluorescence measurements. Fv F10.6.6 has an increased thermal and chemical stability compared with Fv D44.1, showing a cooperative unfolding transition. As a conclusion, an improvement of the stability of the variable domain that increases the plasticity of the VH-VL interaction results in the improvement of the binding properties of the antibody towards the antigen.

S-16

LONG RANGE INTERACTIONS ARE NOT ESSENTIAL TO THE 3D STRUCTURE OF A TOPOLOGICALLY COMPLEX PROTEIN

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Even though sequence homology classifies proteins into structural classes, it failed so far to identify signatures characteristic of each kind of protein fold.
We devised an experimental procedure for the identification of sequence segments that may carry essential conformational information. The procedure involves terminal truncation of circularly permuted proteins and sliding a deletion window along the chain. The resulting abridged variants reveal the structural consequences of lacking specific parts of the sequence. Twenty five circularly permuted and truncated variants of ESBL, a class A beta lactamase, were prepared. In these variants more than 90% of the sequence and 92% of the non local atom atom contacts were removed. The results indicate that the deletions affects the yield of folding but do not impede the chain to find its native conformation. In other words, all the preparations of truncated ESBL variants contain, to greater or lesser extent, molecules with the essential features of the ESBL native fold. Systematic permutation and truncation is a stringent test for almost every aspect of folding theories, and the results challenge established ideas on the cooperative nature of the protein structure based in long range interactions. Arguments will be presented favoring a folding model based in local information and a modular character of protein structure.

S-17

LOV DOMAIN PHOTORECEPTORS IN PLANTS AND BACTERIA

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The flavin-binding LOV domain (member of the PAS domain super family) functions as a light-sensory module in plant, algae, and fungal blue-light receptors. LOV domains are photo-activated by a unique mechanism that involves formation of a cysteinyl adduct (a C-S bond between the sulfur of a cysteine and a carbon of the flavin). Phototropin (the plant blue-light receptor that mediates phototropism, chloroplast relocation and stomatal opening) has two N-terminus LOV domains that activate a C-terminal serine/threonine kinase. Many bacteria, including *B. melitenesis*, *E. litoralis*, and *P. syringae*, contain genes that code for proteins in which a LOV domain is coupled to a histidine kinase. Sensor histidine kinases are essential in environmental sensing by bacterial two-component systems, which are generally involved in gene transcription regulation. We have cloned, expressed in E. coli and affinity purified four LOV-domain-histidine kinase proteins (LOV-HKs). All four LOV-HKs bind a flavin chromophore (provided by the *E. coli* host) and undergo light-induced absorption changes typical of LOV-domain receptor modules. We have demonstrated that all four proteins act as light-activated histidine kinases. Although this strongly suggests that these proteins all function as light receptors and belong to a new family of photoreceptors, the light responses these proteins mediate are unknown.

S-18

LIGHT SIGNALING PATHWAYS MEDIATING SHADE AVOIDANCE RESPONSES IN ARABIDOPSIS

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Light signals caused by the presence of neighbors accelerate stem growth, flowering, and induce a more erect position of the leaves, a developmental strategy known as shade-avoidance syndrome. The perception of neighbors is mediated by the phytochrome family of photoreceptors, but the molecular mechanisms connecting these photoreceptors to the physiological responses remain largely unknown. The work in our laboratory combines molecular-genetic and functional genomic approaches to improve our understanding of how light modulates plant development. We have isolated several mutants defective in light signaling and we are using microarray studies to understand the molecular basis of their developmental defects. For example, the novel csa1mutant shows a shade avoidance phenotype in the absence of shade and this phenotype is caused by a dominant negative mutation in a TIR-NBS-LRR gene. TIR-domain proteins had been implicated in defense responses in plants. Our results indicate that responses to pathogens and neighbors share `core-signaling components in Arabidopsis. Using Affymetrix microarrays we have detected that the levels of PIF3, a bHLH protein that acts as a negative regulator of phytochrome signaling, are increased 15 times in the csa1 mutant compared to wild-type plants, suggesting that PIF3 may act as the molecular link between phytochrome and defense signaling pathways.

S-19

MITOCHONDRIAL COMPLEX II IS ESSENTIAL FOR GAMETOPHYTE DEVELOPMENT IN *Arabidopsis*

thaliana

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Complex II subunits are all nuclear-encoded in Arabidopsis. Two nuclear genes, *SDH1-1* and *SDH1-2*, encode the flavoprotein subunit, and three nuclear genes, *SDH2-1*, *SDH2-2* and *SDH2-3*, encode the iron-sulfur subunit. To find clues about their role in complex II biogenesis, we have undertaken the analysis of their expression and the characterization of insertional mutants. Our results show that only one of the two *SDH1* genes is expressed at a significant level, and that its inactivation leads to pollen abortion and alterations in embryo sac development. Therefore, complex II is essential for gametophyte development, highlighting the importance of mitochondrial metabolism in the gametophytes. *SDH2-1* and *SDH2-2* likely arose via a relatively recent duplication event, are expressed in all organs from adult plants and are redundant. In contrast, *SDH2-3* is only expressed during seed development. *SDH2-3* transcripts appear during seed maturation, are abundant in dry seeds, and markedly decline during germination. Promoter elements important for transcriptional activation have been identified, and the role of ABI3 transcription factor has been established. In contrast to *SDH2-3* transcripts, *SDH2-1* and *SDH2-2* mRNAs are low in dry seeds and increased during germination and post-germinative growth, suggesting the replacement of the SDH2-3 polypeptide by SDH2-1 or SDH2-2 in mitochondrial complex II.

S-20

COMPARTMENTALIZATION OF S-RNASE IN SELF-INCOMPATIBLE *NICOTIANA*

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Flowering plants have developed a variety of strategies to control fertilization. Self-incompatibility is a mechanism that allows plants to distinguish and reject their own or genetically similar pollen. Thus, self-incompatible plants avoid inbreeding and promote genetical diversity. In Solanaceae and other families, pollen recognition occurs in the upper part of the style and involves a ribonuclease (S-RNAse) secreted by the transmitting tissue to the extracellular matrix and taken up by pollen tubes. Inside pollen tubes, S-RNases interact with the SLF protein, the recognition factor of male side. The consequence of this interaction is the rejection (incompatible reaction) or acceptance (compatible reaction) of pollen by the pistil. Pollen rRNA is selectively degraded in incompatible reactions suggesting that S-RNase has a cytotoxic effect during pollen rejection. Using immunocytochemistry and confocal microscopy, S-RNase was shown to be compartmentalized in the vacuole of pollen tubes. In compatible pollinations, the vacuole appears to be intact while in incompatible pollinations, vacuole is disrupted when pollen tubes are rejected, releasing S-RNase to the cytoplasm. This result suggests that compartmentalization plays a key role in the process of selecting the successful pollen.

S-21

TRANSCRIPTIONAL COORDINATION OF GENES ENCODING PLANT MITOCHONDRIAL RESPIRATORY CHAIN COMPONENTS

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Biogenesis of the mitochondrial respiratory chain requires the expression of a vast set of genes from two different genomes. It is generally assumed that the expression of most genes that encode respiratory components must be somehow coordinated. We are studying the expression of Arabidopsis genes encoding proteins involved in respiration to determine the extent, conditions and mechanisms of this coordination in plants. Analysis of expression data from available microarray experiments indicates that most nuclear genes encoding respiratory chain components show similar expression characteristics and responses. Studies on the promoter of one of the Arabidopsis cytochrome c genes using deletions and mutagenesis indicated that DNA elements known as site II, that bind transcription factors of the TCP family, are essential for expression. Similar motifs are present in most promoters of nuclear genes encoding respiratory components. Mutagenesis of site II elements in several of these promoters revealed their importance for expression. Cluster analysis of microarray experiments allowed the identification of other genes that show similar expression characteristics. Genes encoding mitochondrial components and promoters with site II elements are enriched in this group of genes, reinforcing the notion of coordination of gene expression through these elements during mitochondrial biogenesis.

S-22

STRUCTURAL, FUNCTIONAL AND IMMUNOLOGICAL STUDIES OF A NEW VIRULENCE FACTOR OF Brucella spp.

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The penultimate step in the biosynthesis of riboflavin (vitamin B2) is catalyzed by lumazine synthase. Pathogenic Brucella species adapted to an intracellular lifestyle comprise two genes (designated ribH1 and ribH2) with similarity to lumazine synthase genes, which are located on different chromosomes. The ribH2 gene specifies a lumazine synthase with an unusual decamer structure and is an immunodominant antigen able to generate strong humoral as well as cellular immunity against B. abortus in mice. The ribH1 gene is located inside a small riboflavin operon, and appears to be the functional LS, whereas the decameric RibH2 is a virulence factor presumably acting as oxidative stress response factor. The latter observation prompted us to investigate further on the structural and enzymologic properties of RibH2, explaining at the structural level the low catalytic activity of this enzyme. Sequence comparison of lumazine synthases from bacteria, plants and fungi suggests a family of proteins comprising bona fide lumazine synthase, archaeal riboflavin synthase and the RibH2 proteins of Brucellae and Rhizobium which may have additional functions besides their documented lumazine synthase activity. This analysis shows that the lumazine synthase fold contains structural plasticity, conserving a pyrimidine binding site but adapting for several different functions.

S-23

MECHANISM OF DENGUE VIRUS RNA RECOGNITION BY THE VIRAL POLYMERASE

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RNA replication of plus-strand RNA viruses is mediated by a viral encoded RNA dependent RNA polymerase (RdRp). This enzyme, together with other cellular and viral components, ensures an efficient and specific amplification of the viral genome in the cytoplasm of the infected cell. In this process, the viral genome is first copied into a complementary minus strand, which in turn is used as template for plus strand amplification. RNA elements that regulate the level, polarity, and timing of RNA synthesis are present within both the minus and plus strand viral RNAs. Here, we used dengue virus (DV) as a model to search for RNA signals responsible for specific RdRp activity. We identified an RNA element present at the 5'UTR of the viral genome that functions as the core promoter for template discrimination and polymerase activity both in vivo and in vitro. Furthermore, using RNA binding assays, we found that the viral RdRp is an RNA binding protein that interacts with the promoter element with high affinity (K_d 12 nM). Interestingly, using atomic force microscopy, we were able to visualize individual RNA molecules interacting with the viral polymerase. Based on our findings, we propose a novel mechanism for minus strand RNA synthesis of a flavivirus, in which the viral polymerase binds the 5' end of the genome and reaches the site of initiation at the 3' end via long-range RNA-RNA interactions.

ORAL COMMUNICATIONS

Biotechnology (BT-C1/BT-C3)
Cell Biology (CB-C1/CB-C19)
Enzymology (EN-C1/EN-C3)
Lipids (LI-C1/LI-C10)
Microbiology (MI-C1/MI-C14)
Neurosciences (NS-C1/NS-C2)
Plant Biochemistry and Molecular Biology (PL-C1/PL-C9)
Structural Biology (SB-C1/ST-C4)
Signal Transduction (ST-C1/ST-C5)

BT-C01

TETRADECYLTRIMETHYLAMMONIUM DEGRADATION BY *P. putida* IS MORE EFFICIENT IN PRESENCE OF ALUMINIUM

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Pseudomonas putida can use tetradecyltrimethylammonium (TDTMA) as sole C and N source. The trimethylamine (TMA) produced in the first degradation step by action of monooxygenase activity, was used as N source and also accumulated inside the cell, affecting the bacterial growth. Here, we report that Al^{3+} acted as a Lewis' acid playing a role in the control of TMA intracellular through the reaction Al^{3+} + 2 $:N(CH3)_3$ —› Al3+:$[N(CH3)_3]_2$, allowing the bacterial growth until total consumption of TDTMA. The intracellular TMA-Al^{3+} complex was determined quantitatively by the relationship between TMA intracellular (detected by GC-MS), total Al^{3+} incorporated (calculated by disappearance of culture media) and free-Al^{3+} intracellular (detected by fluorescence of Al^{3+}-morin complex). Cells were growth in basal media with 50 mg l^{-1} TDTMA up stationary phase and Al^{3+} was added. In the presence of 0.1 mM Al^{3+}, the free-TMA intracellular concentration decreased to zero with total consumption of TDTMA. With 0.005 mM Al^{3+} the low level of intracellular Al^{3+} was not sufficient to sequester the TMA and neither bacterial growth and TDTMA consumption was observed. In this case, the level of intracellular free-TMA was 1.30 10^{-9} nmol $cell^{-1}$, sufficient for inhibit the monooxygenase activity. Thus, it is evident that the presence of Al^{3+} is a strategy for a more efficient utilization of TDTMA by *P. putida*.

BT-C02

PLANT SPECIFIC INSERT (PSI) OF *St*APs: CLONING, EXPRESSION AND ANTIMICROBIAL ACTIVITY

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Precursors of Plant Aspartic Proteases have, in their amino acid sequence, a domain named "Plant Specific Insert" (PSI). This domain has structural homology with saposin-like proteins, a family of proteins with capacity to destabilize microbial plasma membranes. We have previously reported the presence of PSI in mature monomeric Potato Aspartic Proteases (*St*APs), which have the capacity to permeabilize plasma membrane pathogens. In order to study the possible role of PSI in the antimicrobial activity of *St*APs, the aim of this work was to clone the PSI domain, to express it in a heterologue system and to analyse its antifungal activity. Viability assays demonstrated that recombinant PSI (*St*AP-PSIr) is able to kill spores of *F. solani* in a dose-dependent manner. Besides, localization experiments with FITC-labelled *St*AP-PSIr showed a direct interaction with the surface of spores and hyphae of *F. solani*. Moreover, incubation of spores and hyphae with *St*AP-PSIr resulted in membrane permeabilization as shown by the uptake of the probe SYTOX Green. In conclusion, we were able to obtain the recombinant PSI and assessed its antifungal activity towards *F. solani*. Therefore, these results could suggest that this domain plays an essential role in the antimicrobial activity previously reported for *St*APs.

BT-C03
MICROARRAY TRANSCRIPTIONAL ANALYSIS IN SPARC GAIN OF FUNCTION CONDITION USING *D. melanogaster* EMBRYOS

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The extracellular matrix (ECM) is an intricate arrangement of glycoproteins and proteoglycans that act not only as a physical scaffold for the attachment and organization of cellular structures, but also as a mediator of intracellular signaling through cell surface receptors that recognize them. Secreted protein acidic and rich in cysteine (SPARC) is a 42 kDa ECM component which regulates cell-shape, adhesion, proliferation, migration and differentiation. Although the biology of mammalian SPARC has been studied extensively, no receptors have been identified so far. For this reason we tried to elucidate the identity of SPARC signaling pathway using the D melanogaster model. We have previously reported that the SPARC gain of function in D melanogaster embryos was associated with phenotypes likely related to migration and adhesion failures. Here, full transcriptome microarrays have been performed using whole embryos (stage 14-15) overexpressing SPARC in ectoderm (69B driver) or in an ubiquitous manner (heat-shock driver). Labelled RNAs were hybridized to Affymetrix chips and analyzed using The R Project for Statistical Computing. The data technical validation is been performed by real time PCR and candidate genes will be verified by biological and genetic analysis, paving the route to new experiments.

CB-C01
P300 AND P53 COLOCALIZE IN INCLUSIONS RESEMBLING AGGRESOMES IN TUMOR-DERIVED MAMMARY MURINE CELLS

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The cellular coactivator p300 participates in the regulation of a wide range of biological processes, such as cell proliferation, differentiation, senescence and apoptosis. Recently, p300 has also been implicated in the regulation of p53. In an effort to better define the molecular mechanisms through which p300 contributes to the stability/degradation of p53, we studied the effect of different p300 mutants on p53 expression and localization and found that a core region of p300 encompassing amino acids 1514-1922 was able to bring p53 into defined cellular inclusions, likely resembling aggresomes. Immuno-fluorescence studies show that over expression of p300 (1514-1922) “mimics” treatment with a proteasome inhibitor in that it triggers the formation of inclusions where p53 accumulates. Further, an association of p300 with the 20S proteasomal subunit was observed. In primary cultures of mouse mammary tumors, but not in normal mammary tissue, the formation of these inclusions that stained for p53, p300 and ubiquitin was observed. The expression of these proteins, in tumor and normal tissue, was corroborated by immunohistochemistry. In addition, the physical association of p300 and p53 was studied. This preliminary evidence suggests that p300 recruits mutant cytoplasmic p53 to the aggresomes whereas it does not have the same effect with wild type p53. This is a novel activity that could be important for the role of this protein in oncogenesis.

CB-C02
SCREENING OF THE MANNOSE RECEPTOR FAMILY PROTEIN uPARAP/Endo180 IN HEAD AND NECK TUMORS

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We have recently demonstrated that genetic ablation of uPARAP/Endo180 impair collagen turnover that is critical to mouse mammary tumor expansion, as evidenced by the abrogation of cellular collagen uptake, tumor fibrosis, and blunted tumor growth. There are many studies showing important differences between mouse cancer models and human cancer. Therefore we thought that it was important to investigate the role of this protein in human cancer. As a first step and taking advantage of the Oral Cancer Tissue Array Initiative project of the Oral and Pharyngeal Cancer Branch, we studied the expression of the receptor in an array containing head and neck squamous cell carcinoma samples from China, Thailand, India and Japan, by using immunohistochemistry. We were able to study 80 samples and 35 % of them showed expression of the receptor. In order to better study the expression pattern we also studied tumor samples included in the typical paraffin block. We studied the following tumor samples: 10 squamous cell carcinomas (5 were well differentiated and 5 moderately differentiated) from mouth floor, auditive duct, thyroid gland, larynx, palate and tongue; a small cell carcinomas of parotid gland, a papillary carcinoma and an anaplastic carcinoma of thyroid gland. We detected UPARAP/Endo180 expression in six of these tumors. In all these cases the expression was stronger in the tumor stromal cells than in surrounding histologically normal tissue.Surprisingly, we also detected the presence of the protein in neoplastic cells.

CB-C03
CLONING AND FUNCTIONAL CHARACTERIZATION OF Dlg TUMOUR SUPPRESSOR PROMOTER

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Human Discs large (Dlg) was shown to be targeted by HPV E6 oncoproteins and to be down-regulated in HPV-associated cervical carcinomas. The knowledge about Dlg oncosuppressor functions has progressed in the last years, and it was demonstrated its involvement in both cell polarity control and tissue architecture maintenance. However, the mechanisms controlling Dlg expression at the transcriptional level are still unknown. Therefore, we proceeded to clone and characterise the Dlg promoter and analyse its transcriptional regulation. We cloned a 5´flanking region of Dlg ORF that exhibited promoter functions in different cell lines. We further analyzed the activity of a series of 5' deletion constructs of the Dlg promoter and we could determine the minimal essential sequences that are required for promoter activity and domains that activate or inhibit transcription. Using bioinformatics tools, we found binding sites for the Snail family of transcription factors that repress other oncosuppressors and are up-regulated in tumours. By co-transfection experiments and luciferase assays we could demonstrate that Snail proteins repress also Dlg promoter and, in addition, mutations within the consensus sites that bind Snail, consistently diminished the inhibitory effect. The significance and involvement of Snail proteins in regulating endogenous Dlg levels are currently being analyzed.

CB-C04
A NOVEL ONCOLYTIC ADENOVIRUS THERAPEUTICALLY EFFECTIVE IN MELANOMA TUMOR

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SPARC is a matricellular protein that is overexpressed in malignant and stromal components of human melanomas and SPARC promoter could be a good candidate for generating a conditional replicating oncolytic adenovirus (CRAd). By using luciferase expression as a reporter gene, we selected the promoter sequence F512 (–513/+35) that showed the best ratio of activity vs. specificity. We constructed three adenoviral vectors in which the E1A gene was driven by F512. The lytic capacity of viruses was initially tested in vitro on a panel of malignant cells, melanoma (n=4), colon (n=4), breast (n=3) and cervix (n=1); and normal cells (melanocyte, colon, mesenchymal, fibroblasts and endothelial cells). We found that CRAds based on SPARC promoter seem to replicate specifically in cancer cells while preserving normal cells. Finally, nude mice harboring SB2 melanoma tumors (mean 120 mm3) were treated intratumoraly with three administrations of 10 10 vp/mouse of adenoviruses. The treatment resulted in a potent antitumor effect. In 3 out of 5 mice, the tumor was completely eliminated, and one mouse showed a slight tumor growth, while in mice treated with control virus we observed no therapeutic benefit. These results indicate that CRAds based on SPARC selectively replicate in and eliminate a panel of cancer cells and subcutaneous tumors, suggesting that they might be useful as pan-oncolytic viruses.

CB-C05
A NOVEL SPECIFIC ONCOLYTIC ADENOVIRUS DRIVEN BY HUMAN A33 PROMOTER FOR COLORECTAL CANCER THERAPY

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A33 antigen is a membrane-bound protein expressed only in intestinal ephitelium and over-expressed in most colorectal cancers (CRC). A33 is found in 95% of primary and metastatic colon cancer cells but is absent in most normal tissues and other tumor types. We hypothesized that A33 promoter might be useful in the design of a conditional replicative adenovirus (CRAds). For this purpose we cloned an A33 promoter fragment (A33Pr) that extends from –105 bp to +307 bp upstream of the luciferase gene. Using luciferase activity as a gene reporter we demonstrated that A33Pr was in average 10-fold more active in colon carcinoma cells than in melanoma or breast cancer cell lines. We next constructed a CRAd where E1A was placed under the control of A33Pr (AV22EL). The adenovirus lytic capacity of AV22EL on different cell lines was examined by crystal violet staining. AV22EL induced specific oncolysis of human CRC lines that expressed A33 and have negligible lytic capacity on cells that lacked or had minimal A33 expression. AV22EL was active in CRC cells even at a MOI of 1. AV22EL efficiently reduced tumor size when injected subcutaneosly within the tumor mass. Moreover, AV22EL was also very efficient in tumor cell killing when delivered by intravenous injections in a liver metastatic tumor mouse model. These data demostrates that AV22EL is a potential oncolytic agent for the treatment of CRC.

CB-C06
CHARACTERIZATION OF A SPECIFIC SPARC PROMOTER SUITABLE FOR CANCER GENE THERAPY

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The success of transcriptional targeting in cancer gene therapy depends on the activity of a particular promoter inside the malignant cell. We hypothesized that targeting the entire tumor mass (malignant and stromal cells) might provide better therapeutic effect than conventional tumor cells directed strategies. SPARC is a matricellular protein overexpressed in human malignant melanomas both in tumour and stromal cells (fibroblasts and endothelial cells). For this purpose, we cloned different SPARC promoter fragments that were isolated considering specific motifs such as two GGA boxes, the presence of a TATA-like box and potential transcription initiation sites and a putative downstream promoter element (DPE) that we discovered in the first exon. We tested the strength and specificity of different SPARC promoter fragments by luciferase assay. A fragment of 548 bp (including –513/+35) presents the best ratio, determined as the activity produced between SPARC expressing and SPARC non-expressing cells. Finally, by different mutations we have demonstrated that both elements, TATA and DPE, are involved in transcriptional initiation. In addition, we also demonstrated that dexamethasone reduces SPARC promoter activity. Taken together, our data suggest that our SPARC promoter is a good candidate for transcriptional targeting in cancer gene therapy.

CB-C07
CNBP BINDS PURINE-RICH ssDNA AND ITS ACTIVITY DEPENDS ON THE RGG BOX

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Cellular nucleic acid binding protein (CNBP) interacts with single-stranded (ss) nucleic acids. It was implicated in diverse cellular mechanisms of gene expression control during embryo development. Our goal is to identify CNBP nucleic acid targets and characterize its biochemical activity and biological functions. Recombinant *Bufo arenarum b*CNBP and zebrafish *z*CNBP were used for gel-shift assays. ssDNA probes were designed by rational mutations of reported targets to identify sequences or secondary structures recognized by CNBP. We conclude that CNBP recognizes probes with ss stretches rich in purine nucleotides.
We previously showed that CNBP promotes annealing of complementary DNA strands through the Gly/Arg rich motif (RGG box). Here we show that CNBP is also able to promote nucleic acids melting trough the RGG box. Melting and annealing of nucleic acids are characteristic activities of nucleic acid chaperones, suggesting that CNBP may have this biochemical activity.
Finally, CNBP function was analyzed *in vivo* by microinjecting *Xenopus* embryos with mRNA coding for wild-type *b*CNBP and RGG-deletion mutants. Expression of the putative CNBP targets *c-Myc* and *FoxD3* was analyzed by *in situ* hybridization. Wild-type *b*CNBP overexpression caused an increase in the expression of analyzed genes while RGG-deletion mutants overexpression decreased the expression of the analyzed genes.

CB-C08

CNBP IS REQUIRED FOR CRANIOFACIAL SKELETON DEVELOPMENT IN ZEBRAFISH

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Striking conservation in various organisms suggests that Cellular Nucleic acid Binding Protein (CNBP) plays a fundamental biological role across different species. Recently, it was reported that CNBP is required for forebrain formation during chick and mouse embryogenesis. In this study, we have used the zebrafish model system to expand the basic understanding of CNBP activity during vertebrate head development. We show that zebrafish *cnbp* is expressed in a similar fashion as has been observed in early chick and mouse embryos. Using antisense morpholino oligonucleotide knock-down assays we show that CNBP depletion causes forebrain truncation while trunk development appears normal. A substantial reduction in cell proliferation and an increase in cell death were observed in the anterior regions of *cnbp* morphant embryos. *In situ* hybridization assays show that CNBP depletion does not affect CNS patterning while it does cause neural crest derivative depletion. Our data suggest an essential role for CNBP in mediating neural crest expansion by controlling proliferation and cell survival rather than via a cell fate switch during rostral head development. This possible role of CNBP may not only explain zebrafish craniofacial anomalies but also those ones reported for mice and chicken and, moreover, demonstrate that CNBP plays an essential and conserved role during vertebrate head development.

CB-C09

UNRAVELLING THE INTERPLAY BETWEEN CHROMATIN AND THE CELL CYCLE INHIBITOR p19INK4d

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INK4 proteins are a family of CDK inhibitors that regulate the progression through the cell cycle. Among them, p19INK4d has been implicated in DNA repair, but the mechanisms by which this protein exerts this new function remain elusive. It has been shown that p19 is induced in cells irradiated with the DNA damaging agent UVC. However, transfection of BHK cells with UVC damaged DNA is not enough to achieve its induction. It is also known that DNA lesions lead to changes in chromatin structure, so we wondered if these rearrangement in chromatin organization might be the stimulus that triggers p19 induction upon DNA damage. Treatment of BHK cells with chloroquine or TSA, both chromatin modifiers, specifically induced the expression of p19. Then, we wanted to analyze the role p19 might have on chromatin structure, in an effort to explain what the function of this protein is in DNA repair. In vitro DNA repair assays show that a nuclear extract enriched in p19 is more capable of repairing a reconstituted damaged DNA template than one in which p19 has not been overexpressed. Nevertheless, this difference is not seen when the extracts are incubated in the presence of a naked template, suggesting that p19 might actually be involved in making chromatin more accessible during the DNA repair process. Together these results point to a bidirectional regulation existing between chromatin and p19.

CB-C10

KLF6 BEHAVES AS AN ANTI-APOPTOTIC FACTOR IN MAMMALIAN CELLS

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KLF6 is an evolutionary conserved and ubiquitously expressed mammalian transcription factor that has been involved in cell cycle control mechanisms in normal and cancer cells. This work aimed at characterizing the role of KLF6 in apoptosis regulation in response to DNA-damaging agents commonly used in anticancer therapies. Here, we report that during apoptosis triggered by different DNA-damaging drugs the KLF6 protein levels were drastically downregulated in Cos-7 and HepG2 cells. Moreover, we showed during apoptosis a strong downregulation of the KLF6 mRNA and its transcriptional activity determined by Real-Time PCR and reporter assays, respectively. Additional studies established that KLF6 was silenced in apoptotic cells after DNA damage in a p53-independet manner. Thus, the absence of the KLF6 protein proposes that it might be an inhibitor of apoptosis-induced by DNA-damaging drugs. To test this possibility RNA interference was successfully employed to knockdown KLF6 in HepG2 cells by using small interfering RNAs (siRNAs) targeted to different regions of KLF6 mRNA. Interestingly, KLF6 loss by siRNA sensitized cells to apoptosis triggered by DNA-damaging agents. Altogether our results in which KLF6 protein levels decreased during DNA damage-induced apoptosis and that siRNA-mediated loss of KLF6 favoured cell death, support the notion that KLF6 behaves as an anti-apoptotic factor.

CB-C11

TWO CRM1-DEPENDENT NUCLEAR EXPORT SIGNALS ARE INVOLVED IN THE REGULATION OF HIF1/SIMA

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The heterodimeric transcription factor HIF (Hypoxia Inducible Factor) has a central conserved role in oxygen homeostasis and is composed of two basic-helix-loop-helix (bHLH)-PAS protein subunits, HIFα and HIFβ. HIFα is regulated by oxygen levels through several different mechanisms that include protein stability, transcriptional co-activator recruitment and subcellular localization. We have previously reported that the *Drosophila* HIF-α protein, Sima, is mainly nuclear in hypoxia and accumulates in the cytoplasm in normoxia, but so far the molecular basis of this regulation is unclear. We show here that Sima shuttles continuously between the nucleus and the cytoplasm. Removal of the bHLH domain led to Sima nuclear accumulation, consistent with the occurrence of two nuclear export signals (NES) in this region that promote CRM1-dependent Sima nuclear export, both in cell culture and in vivo. Site directed mutagenesis of either NES provoked Sima nuclear retention and increased transcriptional activity of a LacZ reporter and of endogenous target genes, suggesting that nuclear export contributes to Sima regulation. These NES are conserved and functional in the bHLH domain of several other proteins of the bHLH-PAS family and therefore, we propose that these NES are important for rapid nuclear clearance of bHLH-PAS proteins upon cessation of the external stimulus.

CB-C12
RHO FAMILY PROTEINS ARE INVOLVED IN THE FORMATION OF *COXIELLA BURNETII REPLICATIVE COMPARTMENT*

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Q fiber is a disease caused by the intracellular pathogen *C. burnetii*. This bacterium generates a large replicative compartment in the host cell, called parasitophorous vacuole (PV). We have previously observed that actin is involved in the PV formation. It is known that actin dynamics are regulated by Rho family of GTPases. To test if these GTPases play a role in the PV biogenesis, HeLa cells were infected 16 h with *C. burnetii* and then transfected with pEGFP: Cdc42, RhoA or Rac1 wild types, constitutively active or inactive mutants. *C. burnetii* was detected by indirect immunofluorescence and the cells were analyzed by confocal microscopy. We observed that PVs were decorated by Cdc42WT and the active V14 but not by the inactive N17 mutants. The number of PVs increased only in cells expressing the active mutant V14. Interestingly, whereas the inactive mutant of RhoA (N19) remained diffuse in the cytoplasm and its expression induced the formation of small vacuoles containing *C. burnetii*, the active one (RhoAV14) associated with large PVs. In contrast, the formation and the number of PVs were not modified in cells expressing Rac1WT or Rac1 mutants and these proteins were not recruited to the PV membrane. These results suggest that CDC42 and RhoA are involved in PV biogenesis.

CB-C13
RAB3A TRIGGERS CORTICAL GRANULE EXOCYTOSIS IN MOUSE EGGS

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Cortical granule (CG) exocytosis in the egg is one of most important steps, which occurs after sperm-egg fusion at fertilization. CG exocytosis, which is induced by the spermatozoon, plays a role in blocking polyspermy. Induction of CG exocytosis depends on intracellular calcium mobilization; however little is known about the signal transduction pathway downstream of calcium mobilization. Rab3A, a member of the small GTP-binding protein family, has been implicated in exocytosis regulated by calcium. In mouse egg, Rab3A is specifically distributed at the cortical region where CGs are localized; nevertheless it is unknown if Rab3A has a role in CG exocytosis in mouse eggs. In order to investigate the effect of Rab3A in intact cells, we have designed a membrane permeant by adding a polyarginine peptide (R) at the N terminus of Rab3A (R-Rab3A) and we tested the effect of R-Rab3A in CG exocytosis. Metaphase II eggs were incubated in presence of either A23187, a calcium ionophore, or R-Rab3A. Then, CG were fixed, visualized by staining luminal glycoproteins with FITC-Lens Culinaris Agglutinin, and quantified using Image J program. We found that R-Rab3A stimulated CG exocytosis in the same extent that the calcium ionophore A23187. These results show that Rab3A participates in CG exocytosis signal transduction pathway in mouse eggs.

CB-C14
PREFERENTIAL TRANSFER OF THE COMPLETE GLYCAN IS DETERMINED BY THE OLIGOSACCHARYLTRANSFERASE COMPLEX

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Most eukaryotic cells show a strong preference for the transfer in vivo and in vitro of the largest dolichol-P-P-linked glycan ($Glc_3Man_9GlcNAc_2$) to protein chains over that of biosynthetic intermediates lacking the full complement of glucose units. The oligosaccharyltransferase (OST) is a multimeric complex containing eight different proteins, one of which (Stt3p) is the catalytic subunit. Trypanosomatid protozoa lack an OST complex and only express this last protein. Contrary to the OST complex from most eukaryotic cells, the Stt3p subunit of those parasites transfer in cell free assays glycans displaying $Man_{7\text{-}9}GlcNAc_2$ and $Glc_{1\text{-}3}Man_9GlcNAc_2$ compositions at the same rate. We have replaced *Saccharomyces cerevisiae* Stt3p by the *Trypanosoma cruzi* homologue and found that the complex thus formed preferentially transfers the complete glycan both *in vivo* and *in vitro*. Preference for $Glc_3Man_9GlcNAc_2$ is thus a feature determined by the complex and not by the catalytic subunit.

CB-C15
IDENTIFICATION OF ER UDP-Glc TRANSPORTERS IN YEAST: THE SEARCH CONTINUES

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The UDP-Glc:glycoprotein glucosyltransferase (GT) labels incompletely folded glycoproteins in the ER lumen with a Glc tag. Its donor substrate, UDP-Glc, is synthesized in the cytosol. Several nucleotide sugar transporters (NST) have been identified, but only AtUTR1 from *Arabidopsis thaliana* has been proposed as an ER UDP-Glc transporter. To identify an ER UDP-Glc transporter gene in yeasts we looked for NST homologues bearing the canonical ER retention signals in the genomes of *S. cerevisiae* and *S. pombe*. Two of such genes occur in each yeast: *hut1*$^+$ (an orthologue of AtUTR1) and *yea4*$^+$. We disrupted both genes in *S. pombe* and *S. cerevisiae alg6* or *alg5* mutant backgrounds, and transformed the last yeast with *S. pombe* GT cDNA (*gpt1*$^+$). The use of *alg* mutants allows assignation of protein-linked $Glc_1Man_9GlcNAc_2$ formation to GT activity in the ER, and thus entrance of UDP-Glc into the lumen. Both *S.p. alg6 gpt1* and *alg6 hut1* double mutants, showed similar aberrant morphology and thermal sensitivity, thus suggesting glycoprotein misfolding as the common defect. Nevertheless, *in vivo* labeling of *S.c. alg5 hut1*, *S.c. alg5 yea4*, *S.p. alg6 hut1*, *S.p. alg6 yea4* and *S.p. alg6 hut1 yea4* resulted in $Glc_1Man_9GlcNAc_2$ synthesis, thus suggesting UDP-Glc entrance into the ER lumen through a NST not bearing the classic ER retention signal or, alternatively, by a totally novel mechanism.

CB-C16
PHYSICAL AND FUNCTIONAL INTERACTION BETWEEN GalT2 AND CALSENILIN/CALP

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UDP-Gal: GM2 Galactosyltransferase (GalT2) is a Golgi resident, type II membrane protein, involved in the synthesis of glycosphingolipids. The determinants for Golgi localisation are still uncharacterised. In order to identify putative binding partners involved in this process, we carried out a yeast two-hybrid screen using elements of the N-terminal domain of GalT2 (sufficient for Golgi localisation) as bait. We identified Calsenilin, and its close homologue CALP (Calsenilin-like protein), both members of the Neuronal Calcium Sensor (NCS) family of calcium binding proteins. Calp and Calsenilin are involved in the trafficking of potassium channels of the Kv4 family to the plasma membrane, and they also interact with Presenilins, proteins involved in the pathogenesis of Alzheimer disease. The physical interaction between GalT2 and Calsenilin was confirmed by coimmunoprecipitation experiments in cotransfected CHO-K1 cells. In cells, the expression of Calp or Calsenilin shifts a fraction of GalT2-GFP and Mannosidase II (ManII), from the Golgi to the endoplasmic reticulum. This was not observed for the Golgin GM130. In addition, the expression of GalT2 and ManII decreases the half-life of CALP and Calsenilin, in a proteosome dependent fashion. Results suggest that Calp/Calsenilin are involved in the trafficking of subsets of Golgi resident proteins.

CB-C17
PHOTOACTIVABLE MOLECULES GENERATE TISSULAR DAMAGE BY ROS ON IMMATURE STAGES OF DIPTERANS

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Photoactivable substances react upon absorption of visible radiation with the subsequent formation of reactive oxygen species. We previously demonstrated the photochemical toxicity of Phloxine B (PhB) (a red xanthene eosin) on immature stages of the Medfly, *Ceratitis capitata*. This dye represents an interesting option to traditional insecticides. Hematoporphyrin (HP) and the lactone Santonin, were also possible alternatives. We found that Hematoporphyrin is approximately 3 times more toxic than PhB (LC50: 0.143 mM) whereas Santonin does not showed significant effects. To understand the toxic effects of photoactivable substances at the tissular level we have examined the level of macromolecular damage (lipid peroxidation and DNA damage) in different tissues of *Ceratitis capitata*. Lipid peroxidation was found in larvae fed with three photoactivable substances under light and dark conditions. We have further determined the activation of celular mechanisms such as an increase of the PKB/ AKT pathway by inhibition of PTEN and an increase in nuclear localization of a Rel-like transcription factor in intestine nuclei of larvae fed with PhB as evidenced by EMSA. [*These authors equally contributed to this work].

CB-C18

NOVEL BIOLOGICAL ACTIVITIES OF BENZNIDAZOLE: EFFECT ON THE ACTIVATION AND GROWTH OF RAW 264.7 CELLS

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Benznidazole (BZL) is an antiparasitic drug used in Chagas' disease. We previously showed that BZL inhibits the synthesis of proinflammatory mediators (e.g. nitric oxide, NO) by LPS-stimulated RAW 264.7 macrophages through the inhibition of the activation of NF-κB, which regulates inflammation and cell proliferation. Here we aimed at understanding how BZL affects different aspects of macrophage activation and growth. First, we analysed its effect on ROS production, measured by NBT reduction, in PMA or opsonised zymosan-stimulated RAW 264.7 cells. BZL did not inhibit ROS production by stimulated cells but it decreased LPS-induced NO production. We also found that BZL (1 mM) arrested cell growth, evidenced by trypan blue exclusion and MTT reduction. This effect was also observed when cells were incubated with different NF-κB inhibitors, showing a link between NF-κB basal activation and proliferation in this cell line. Next, we studied NF-κB activation by transient transfection. We found that BZL (1mM) H_2O_2)., inhibited its induction by all stimuli tested (LPS, PMA, IL-1β, TNF-α Gel shift assays with nuclear extracts from LPS-stimulated cells showed that BZL decreased NF-κB but not AP-1 activation. We conclude that BZL specifically affects NF-κB regulated processes in RAW 264.7 cells, in basal state and after stimulation, while it does not affect other pathways of macrophage activation.

CB-C19

MITOCHONDRIAL ATP SYNTHESIS AND ITS ROLE IN ARACHIDONIC ACID RELEASE AND METABOLISM IN THIS ORGANELLE

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It has been shown that prevention of electron transport in mitochondria with antimycin A (ActA) and inhibition of mitochondrial ATP synthesis with oligomycin (Oly) significantly reduces cellular ATP, potently inhibits cholesterol transport, and reduces a protein involved in cholesterol transport (StAR). Since cholesterol transport and the expression of the StAR are dependent of Arachidonic Acid (AA) release in a specific compartment of the cells (the mitochondria), the purpose of this work was to study the effect of ActA and Oly on AA release and metabolism induced by cAMP in steroidogenic cells. MA-10 Leydig cells were incubated with cAMP (1 mM) alone or in combination with AA, in the presence or absence of ActA and Oly (1 uM). After 3 hours cholesterol transport, AA release and StAR induction were determined. ActA and Oly significantly reduced cholesterol transport and StAR induction induced by cAMP. AA was able to overcome this inhibition. ActA and Oly produced an increase in the accumulation of AA release into the mitochondria. Since the induction of cholesterol transport and StAR protein are dependent on AA release and metabolism to lipoxygenated products, these results suggest that disrupting ATP content abolishes the access of AA to lipoxygenase enzyme with the consequent inhibition of cholesterol transport and StAR induction in mitochondria.

EN-C01
INHIBITION OF THE MITOCHONDRIAL ATP SYNTHESIS BY POLYGODIAL AND OTHER DRIMANE ANALOGUES

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Polygodial, a natural sesquiterpene dialdehide, uncouples ATP synthesis from electron transport without increasing the proton permeability of the inner mitochondrial membrane. It also can penetrate phospholipid monolayers increasing the surface potential.
To get insight into the mechanism of action of polygodial, we studied its interaction with submitochondrial particles (SMP). We determine its effect on the 8-anilino-1-naphtalene sulphonate (ANS) fluorescence enhancement that accompanies the binding of ANS to the membrane. Polygodial decreased the affinity of ANS for SMP under uncoupled and energized conditions. It also decreased the number of ANS binding sites, but only under uncoupled conditions. We extended the studies to natural and synthetic polygodial analogues. Drimanial and 1-β-(-*p*-Cumaroyloxy)polygodial (that retain C9 and C8 sustituents and configuration) are as effective inhibitors of ATP synthesis as polygodial. Conversely, Isopolygodial (that differs from polygodial only in the C9 configuration) was 20 times less efective as ATP synthesis inhibitor. Accordingly, the ANS fluorescence enhancement was similarly decreased by the former analogues and polygodial, whereas it was practically not affected by Isopolygodial. These results are another evidence that the effects of polygodial on ATP synthesis and on the affinity of ANS for SMP are closely related phenomena.

EN-C02
TRANSESTERIFICATION ACTIVITY OF A MYCELIUM-BOUND LIPASE FROM *Aspergillus niger* MYA 135

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Introduction: Lipases (EC 3.1.1.3) can be used for the synthesis of esters by transesterification with acyl donors in organic solvents. These enzymes have moved far from their original application to the biotechnological processing of fat and other lipids. Transesterification is especially useful for the preparation of optically active compounds, which are formed by resolution or asymmetrization of racemic or prochiral suitable substrate. The aim of this work was to determine the transesterification activity of a mycelium-bound lipase from *Aspergillus niger* MYA 135. Methods: Enzymatic transesterifications were performed in n-hexano using p-nitrophenyl palmitate and the assayed alcohol as substrates. Mycelium-bound lipase was directly used as a source of lipase. The released *p*-nitrophenol was extracted with Na_2CO_3 before measurement at 405 nm. Reaction mixtures without the assayed alcohol were used as a hydrolysis control. Qualitative analyses of alkylpalmitates were done by thin layer chromatography using chloroform as developing solvent. Result and Conclusion: Under our experimental conditions methyl, ethyl, propyl, buthyl, hexyl and hepthyl palmitate were synthesized. The higher specific transesterification activity (mU per g of dry weight) was observed in presence of hexanol (60.8 ± 1.2 mU/g). This work was supported by grants PICTO 761 and PIP 6062.

EN-C03
STRUCTURE-FUNCTION RELATIONSHIP OF UROPORPHYRINOGEN DECARBOXYLASE (UROD)

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The aim of this work is to study the UroD dimerization equilibrium, and characterize kinetically the dimeric as well as the monomeric form of UroD. This will allow us to determine if the enzymatic activity is dependent on the oligomerization state of the enzyme and if there are differences in the action mechanism, with different substrates. UroD has been expressed in E. coli BL21, induced with IPTG for 5 hours at 37°C. The purification was made through a Ni^{2+} column that retains poliHis-UroD. By Gel Filtration and Cross-Linking, all the results obtained confirm the hypothesis that UroD has a reversible dimerization equilibrium. We have obtained a Kd of 0,27 ± 0,06 μM by Gel Filtration. Tests of Enzymatic activity vs Enzyme concentration were made in addition. Specific activity with Pentagen I remain constant within the range of protein concentration studied; however, using Urogen III as substrate, the specific activity increases with the enzymatic concentration in the same protein range. Then, UroD would behave in a different way according to the substrate that is used: with Urogen III the dimer displays a greater activity than monomer, but with Pentagen I, both of them are equally active. Other tests are being made to verify the hypotheses raised by this work, using diverse methodologies to measure the activity of UroD affecting its dimerization state (effect of salts, pH, etc).

LI-C01
MODULATION OF UNSATURATED FATTY ACID BIOSYNTHESIS BY INSULIN AND NUCLEAR TRANSCRIPTION FACTORS

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Monoenoic and polyenoic fatty acids play leading roles in human and animal physiology. Their biosynthesis is mainly regulated by Δ9, Δ6 and Δ5 desaturases. Currently, we are investigating the crucial roles played by the interaction of insulin, SREBP-1c, PPAR-α, LXR and RXR in the transcription and activity of the three desaturases in the rat liver. Normal and streptozotocin diabetic rats were investigated. The modification of blood parameters: insulinemia, glycemia, triglyceridemia and cholesterolemia was measured as well as hepatic mRNAs and enzymatic activity of the desaturases and liver fatty acid composition before and after treatment with LXR agonist T0901317 (for 6 days) and PPAR-α agonist fenofibrate (for 9 days). It was found that PPAR-α activated insulin secretion in the normal animal and LXR did not modify this effect. Besides, PPAR-α and LXR activated the three desaturases mRNAs and activity. In the diabetic rats insulin and LXR showed a strong interaction that led to a many fold increase of the desaturases mRNAs, apparently through a cooperative effect on nuclear SREBP-1c increase. However, little variation was shown in this short-time experiment on liver fatty acid composition except for 18:1/18:0 ratio.

LI-C02

HORMONAL REGULATION OF ARACHIDONIC ACID RELEASE IN A SPECIFIC COMPARTMENT OF THE CELL

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The mechanism involved in the compartamentalization of long-chain acyl-CoA esters and free fatty acids are important unresolved issues. The simple structure of arachidonic acid (AA) and the natural occurrence of so many close chemical analogues are associated with a lack of specificity. The selective actions of free AA may be explained simply by its specific release under physiological conditions and by the absence of such mechanism for releasing other long-chain fatty acids. Recently, using steroidogenic cells as experimental system, we have described an alternative releasing mechanism of AA with the participation of an acyl-CoA synthetase (ACS4) and a mitochondrial acyl-CoA thioesterase (Acot2). Moreover, we have described a direct effect of AA on cholesterol transport into the mitochondria. Therefore, the purpose of this work was to study the hormonal regulation of AA release into the mitochondria. MA-10 Leydig cells were labeled with [1-^{14} C] AA and then incubated in the presence or absence of 8Br-cAMP (1mM) for 30 minutes. 8Br-cAMP increased the accumulation of AA into the mitochondria 3 times compared with no stimulated conditions. Inhibition of the activity or expression of Acot2 significantly reduces AA accumulation into the mitochondria. In conclusion, we show for the first time that cAMP can release AA in special compartment of the cell, e.g. the mitochondria.

LI-C03

EFFECT OF MELATONIN AND STRUCTURAL ANALOGS ON LIPID PEROXIDATION OF TRIGLYCERIDES RICH IN ω-3 PUFAs

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The lipid peroxidation of triglycerides rich in C20:5 ω3 and C22:6 ω3 was investigated by photoemission techniques and the malondialdehyde (MDA) assay. We have compared melatonin [MLT] antioxidant activity with that of several structural analogs: 5-OH tryptophan [5-OHTRY], n-acetyl serotonin [NAS] and 5-methoxytryptamine [5-MTA]. Butylated hydroxytoluene [BHT], a classical antioxidant, 5-OHTRY and NAS, intermediaries in the synthesis of MLT, inhibited light emission and MDA formation in a concentration dependent manner. The total relative luminic units was found to be lower in those systems incubated in the presence of either BHT, NAS or 5-OHTRY; this decreased proportionally to the concentration of the compound tested. The order of inhibition was 5-OHTRY > NAS > BHT. The free radical scavenger activity of the indoles was also analyzed by the 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) method. The results indicate that 5-OHTRY and NAS exhibited a dose-dependent free-radical scavenging ability of 73.5 % and 84.71 % at the 10 μM level, compared to 51 % activity of BHT. MLT and 5-MTA showed only 1.85 % and 5 % activity. MLT and 5-MTA have very low scavenging activity, enhance photoemission and are unable to inhibit the lipid peroxidation of triglycerides in solution. Melatonin's o-methyl and n-acetyl residues appears to be decisive for its properties in this system.

LI-C04

ENHANCED CYTIDYLYLTRANSFERASE TRANSCRIPTION AND TRANSLATION BY NS398 IN HYPERTONIC MDCK CULTURES

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Papillary collecting ducts have to work in the highest renal interstitial osmolality. We have showed that papillary cells increase their phospholipid synthesis as a protective mechanism. We also showed that renal phosphatidylcholine (PC) synthesis is regulated by cyclooxygense 2 (COX2) a survival gene. To evaluate the molecular mechanism involved, we studied PC synthesis in MDCK cultures submitted to high NaCl to mimic physiological conditions, and found that hypertonicity increases PC de novo synthesis and turnover. In order to determine if PC synthesis in MDCK is regulated by COX2 prostaglandin (PG) synthesis, as occurs in tissue, we studied PC synthesis in the absence or presence of NS398, a COX2 inhibitor. By contrast to that observed in renal tissue, NS398 did not block PC synthesis but caused its increased. In order to explain such an effect we studied the relationship between COX2 activity and CT alpha, PC rate-controlling enzyme, genetic regulation. Thus, MDCK cells were grown in physiological or hypertonic medium, with or without NS398, and PG synthesis and, CT protein and mRNA expression were determined. Under hypertonic conditions, NS398 completely blocked PG synthesis but increased PC synthesis, CT protein and mRNA suggesting that CT transcriptional and translational modulation could be due to a COX2-non prostaglandin mediated effect.

LI-C05

SP1 BINDS ELEMENTS REGULATED BY RB AND REGULATES CTP:PHOSPHOCHOLINE CYTIDYLYLTRANSFERASE EXPRESSION

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The retinoblastoma (Rb) protein is implicated in transcriptional regulation of at least five cellular genes. Co-transfection of Rb and truncated promoter constructs has defined a discrete element (retinoblastoma control element; RCE) within the promoters of each of these genes as being necessary for Rb-mediated transcriptional control. In the present report we demonstrate that two RCE elements identified within the CTP:phosphocholine citydylyltransferase alpha (CTa) proximal promoter region are essential to promote transcription. Mutations that abolish each of the RCE elements abruptly decreased CTa-transcription. Co-transfection of Rb and truncated promoter constructs demonstrated that Rb regulates CTa-expression by different mechanisms depending on the phase of the cell cycle. The regulatory effect caused by Rb on CTa expression required both the Sp1 and the RCE elements. Maximum expression was reached when both Rb and Sp1 were over-expressed and RCE elements are required for Rb to associate with the DNA. This is the first report demonstrating not only that surrounding Sp1-binding sites alter regulation mediated by Rb, but also the expression of a gene involved in PC biosynthesis shares a common regulatory pathway with genes responsible for cell growth and differentiation.

LI-C06

BIOSYNTHESIS OF PHOSPHATIDYLCHOLINE DURING NEURONAL DIFFERENTIATION

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Phosphatidylcholine (PtdCho) is the major phospholipid in membranes cells and is made by the Kennnedy pathway were CTP:phosphocholine citidylyltransferase (CCT) catalyze the slow step. Two genes encode CCT activities: Pcyt1a encodes CCTα (ubiquitously expressed in mouse tissues) and Pcyt1b encodes two transcripts that differ at the 5´end: CCTb2 and CCTb3 (expressed in brain and gonads). In the present work, using the Affymetrix Mouse Chip U74CV2, we analyzed the expression patterns of genes involved in phospholipid metabolism in differentiating Neuro2A cells. We detected many upregulated genes after retinoic acid (RA) treatment (differentiation condition), including those that encode CCTα and CKα choline kinase (the first enzyme of the pathway). The results were confirmed by real time RT-qPCR. By RT-PCR assays, we could also detect increased levels of CCTb mRNA after RA treatment. To identify the mechanisms that regulate the expression of CCTb and CCTα, we transfected Neuro2A cells with reporter construct harboring 5´ deletions of the promoters. We observed that CCTb2 and CCTα expression increased after RA treatment. To evaluate the transcriptions factors involved in such regulation, we performed Band Shift and ChiP assays. Our results suggest that the expression of CCT could be coordinately regulated to provide the PtdCho required for neurite outgrowth.

LI-C07

PHOSPHOLIPIDS INVOLVED IN THE SIGNAL TRANSDUCTION CASCADE LEADING TO ACROSOMAL EXOCYTOSIS

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The acrosome is an exocytic granule that overlies the spermatozoan nucleus. The acrosome reaction (AR) is a tightly regulated calcium-dependent exocytic event leading to a massive fusion between the outer acrosomal and the plasma membrane. We have developed a model of SLO-permeabilized spermatozoa capable of undergoing AR upon stimulation. By using this approach, we have characterized a sequence of events occurring downstream the calcium influx through SOC channels. Phorbol esters (PMA), activators of PKC, promote AR in intact sperm. Here, we demonstrate that PMA and diacylglicerol (DAG, the physiological PKC activator) also triggers exocytosis in permeabilized sperm. The effect was not abrogated by chelating the extracellular calcium, suggesting that these compounds are interacting with factors acting downstream the increase of cytoplasmic calcium. PMA and DAG-induced AR was blocked by PKC (cheleritrine) and PLD inhibitors (butan1ol, antiPLD antibodies) suggesting that PKC is upstream in the exocytic signaling cascade. The fact that phosphatidylinositol4,5-biphosphate (PIP2) was able to recover exocytosis after inhibitors treatment, remarks its essential role in exocytosis. Our results suggest that DAG, phosphatidic acid and PIP2 are part of the signaling cascade activated downstream the opening of SOC channels.

LI-C08

UNEVEN DISTRIBUTION OF LIPIDS AND FATTY ACIDS BETWEEN HEAD AND TAIL IN RAT SPERMATOZOA

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Spermatozoa are structurally and functionally polarized cells. The aim of this work was to study how lipids are distributed between sperm tails and heads. Rat spermatozoa isolated from cauda epididymidis were subjected to ultrasound and these two parts were purified using sucrose density gradients. The cholesterol / phospholipid (PL) ratio was twice larger in the head than in the tail. The head showed a choline / ethanolamine glycerophospholipid (CGP, EGP) ratio larger than the tail, both PL being richer in plasmalogens in the head than the tails. The tails contained virtually all of the cardiolipin, and the heads virtually all of the sphingomyelin (SM) that can be isolated from the cells. The fatty acid composition of all PL also differed between parts. The main head and tail PL polyunsaturated fatty acids (PUFA) were 22:4n-9 and 22:5n-6, respectively, the former being a major PUFA of the head CGP, and the latter of tail CGP. EGP were rich in 22:5n-6 in both parts. An important part of the head CGP 22:4n-9 was associated to plasmenylcholine. Head sperm SM was characterized by a large proportion of very long chain PUFA, the main one being 28:4n-6. The lipid and fatty acid regionalization, unusual for a single cell, associates specific lipids with physiological events distinctive of the head or the tail of spermatozoa.

LI-C09

2-HYDROXY FATTY ACIDS WITH VERY LONG CHAINS IN SPHINGOLIPIDS FROM RAT TESTES AND SPERMATOZOA

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Hydroxylation is an important modification of ceramide (Cer), the structural backbone of glycosphingolipids (GSL) and sphingomyelin (SM). In mammals, GSL with 2-hydroxy saturated and monoenoic fatty acids are known to abound in myelin. In this work we studied this type of fatty acids in rat testicular and spermatozoal lipids. The only 2-hydroxylated fatty acids found in rat testis were very long chain polyunsaturated fatty acids (VLCPUFA) bound to SM and Cer, the most abundant ones being OH-28:4n-6 and OH-30:5n-6. SM and Cer were precisely the lipids previously shown to contain normal, non-hydroxylated VLCPUFA. The small pool of testicular GSL was devoid of OH-VLCPUFA and had only minor amounts of VLCPUFA. In rat testis, OH-VLCPUFA-containing SM and Cer increased steadily from 35 to 55 days of postnatal life and remained constant thereafter. Treatment of adult rats with doxorubicin, an antitumor drug whose administration leads to selective death of germ cells, resulted in the disappearance from the testis of the molecular species of Cer and SM that contain both types of VLCPUFA. The sphingolipids with VLCPUFA and OH-VLCPUFA specifically accompany cells of the germinal lineage from their formation in testis to their final stages of maturation as spermatozoa in caput epididymis. It is in mature spermatozoa where these lipids are likely to play a role in reproductive physiology.

LI-C10
INHIBITION OF SK1 STIMULATES SPHINGOLIPIDS DE NOVO SYNTHESIS, ENHANCING CERAMIDE ACCUMULATION

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We reported that Sphingosine Kinase 1 (SK1) inhibition with D,L,-threo-dihydrosphingosine (tDHS), in subconfluent MDCK cultures, causes cell number and viability decrease (33% and 16%). Incubation with 14C-palmitic acid (PA) shows an increase of de novo synthesized Ceramide (Cer) (39% of labeling sphingolipids (SLs) vs. 26% in control). Since Cer synthesis inhibition with Fumonisine B1 attenuates the apoptotic effect, we concluded that such an effect is due to Cer accumulation. On the other hand, reduction of SK1 expression by using SK1-siRNA, also decreases both cell number and viability (60% and 85%) and raises Cer synthesis (43% vs. 35%). Labeling assays with 14C-PA show that both tDHS and siRNA increase total 14C-PA-SLs by 60% and 43%, respectively. To evaluate if the increase in total SLs synthesis raises Cer production thus contributing with SK1 inhibition effect on cell viability, we studied serine-palmitoyl transferase (SPT), which catalyzes the rate-limiting step of de novo synthesis, by treating MDCK cells with SPT inhibitor, L-cicloserine, plus tDHS. We found attenuation in tDHS effect: a raise in cell number and viability and a decrease in Cer production. These results indicate that SK1 inhibition abrogates a negative control on SLs de novo synthesis (possibly on SPT activity) thus enhancing Cer production, which could not be clearing by its conversion to Sphingosine-1P.

MI-C01
***IN VIVO* LOCALIZATION OF THE *SALMONELLA ENTERICA* PHOP/PHOQ TWO-COMPONENT SYSTEM**

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The PhoP/PhoQ two-component system controls the expression of essential virulence traits in the pathogenic bacteria *Salmonella enterica* serovar Typhimurium. Environmental deprivation of Mg^{2+} activates the PhoP/PhoQ signal transduction cascade, which results in an increased expression of genes necessary for survival inside the host. Recent work showed that constituents of prokaryotic two-component systems such as *P. aeruginosa* PilS/PilR and *E.coli* OmpR/EnvZ show preferential localization. In order to investigate the localization of *S. enterica* PhoP- PhoQ and the effect of the input signal on their spatial distribution, we designed translational fusions to GFP and we also set up the recently developed FlAsH labelling technique, which just adds a hexa-aminoacid motif to the target protein. We demonstrate that, using both approaches, PhoP and PhoQ are recruited to the cell poles when extracelular Mg^{2+} is limiting. This polar localization is abrogated by high extracellular Mg^{2+} and when the fusion proteins are expressed in the Δ*phoPQ* background. This indicates that Mg^{2+} exerts a modulatory effect on PhoP and PhoQ localization provided both components are present in the cell. Besides, we show that the biarsenical-tetracysteine label is a convenient method for the *in vivo* fluorescent protein localization in prokaryotic cells, avoiding bulky tagging techniques and fixation procedures.

MI-C02

E. COLI MALIC ENZYMES: A NAD(P)-ME AND A MULTIMODULAR NADP-ME WITH DUAL ENZYMATIC ACTIVITY

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Malic enzyme (ME) catalyses the oxidative decarboxylation of malate to yield pyruvate, CO2 and NAD(P)H. Distinct isoforms of ME are expressed in both prokaryotic and eukaryotic organisms, where they play diverse metabolic roles. By sequence homology, two isoforms of ME have been detected in E. coli genome: sfcA and maeB. Both genes present high degree of homology with ME from different sources, having the product of maeB an N-terminal region that presents homology with ME and a C-terminal region of approximately 320 amino acids, which shows homology to phosphotransacetylase enzymes (PTA). The aim of the present work was the cloning and expression of both enzymes and the products from the two regions composing maeB. Complete and truncated proteins were purified and kinetically and structurally characterized. The product of the maeB N-terminal region retained ME activity, although the properties of this truncated enzyme were different from that of the complete maeB protein. The MaeB protein as well as the product of the C-terminal region presented PTA activity. In this way maeB is a bifunctional enzyme which activities are reciprocally regulated. The results indicate that the two E. coli ME may fulfill different metabolic roles in vivo and that their activities are highly regulated by different key metabolic compounds.

MI-C03

A NEW DRUG TARGET: ACCASE6, AN ESSENTIAL ACETYL-COA CARBOXYLASE OF *MYCOBACTERIUM TUBERCULOSIS*

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The hallmark of mycobacteria is their lipid-rich cell wall. Much work has been done solving the structures of these unique lipids and their biosynthetic pathways. However, almost no information is available regarding the biosynthesis of the precursors for this complex molecules. Our working hypothesis is that the alpha-carboxy acyl-CoAs utilized in the biosynthesis of the membrane and cell-wall fatty acids are the product of the Acyl-CoA Carboxylase complexes (ACCase) present in *M. tuberculosis*. These enzymes, whose gene structure appears to be unique within actinomycetes, are an attractive target for the development of new and specific anti-mycobacterial agents. We successfully reconstituted the essential ACCase6, whose main role appears to be the synthesis of malonyl-CoA. The kinetic properties of this enzyme showed a clear substrate preference for acetyl-CoA, suggesting that ACCase6 could provide the substrate, malonyl-CoA, for the biosynthesis of straight chain fatty acids in this microorganism. The ligand NCI 170233 inhibited ACCase6 at low µM concentrations. Moreover, this compound inhibited growth of *M. smegmatis* at µM concentrations. Our results shed light on the biological roles of the key ACCases in the biosynthesis of cell wall fatty acids, as well as providing a new target for tuberculosis therapeutic development.

MI-C04

ROLE OF RND EFFLUX SYSTEMS IN THE *BRUCELLA SUIS* PHYSIOLOGY

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Transport processes by RND/MFP systems are one of the main causes of antibiotic resistance and were also implicated in physiological processes such as resistance to bile salts and steroid hormones or transport of N-acyl homoserine lactones.The roles of the RND-MFP systems in *Brucella* spp. remain unexplored. Genome analysis revealed the presence of two putative RND efflux pumps named *bepE* y *bepG*, with high identity to *Escherichia coli* AcrB (40%), each associated with genes encoding proteins from the MFP family (*bepD* and *bepF*). In order to understand the relevance of these transporters, both MFP/RND *loci* were amplified and cloned in a pBBR derivative vector and *Brucella suis* deletion mutants were constructed. Minimal inhibitory concentration using the agar dilution test showed that the *B. suis* Δ*bepE* mutant was significantly more sensitive to DOC, ethidium bromide and crystal violet. Furthermore, overexpression of *bepDE* in *B. suis* increased resistance to the same drugs and also to SDS, ampicillin, tetracyclin, novobiocin and thiamphenicol. In contrast, resistance profiles remain unaltered when the *bepFG locus* was overexpressed or deleted. Surprisingly, the B. suis Δ*bepG* mutant was attenuated in the HeLa cells infection model while the *B. suis* Δ*bepE* remains virulent. These results show that both BepDE and BepFG efflux systems are functional and relevant in the *B. suis* physiology.

MI-C05

CHARACTERIZATION OF THE CITRATE TRANSPORTER AND REGULATION OF CITRATE METABOLISM IN *E. FAECALIS*

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In *Enterococcus* citrate fermentation contribute to aroma development in traditional raw milk cheese manufacture. The expression pattern of the cit locus showed two divergent operons, *citHO* and *oadDBcitCDEFXoadAcitMG*. The genes *citD, citE* and *citF* encode the three citrate lyase subunits, with the genes *oadABD* and *citM* encoding two alternative oxaloacetate decarboxylases were identified. The *citH* gene encodes a membrane protein homologous to Me^{2+}-citrate transporters, to characterize the citrate uptake in *E. faecalis*, the *citH* gene was functionally expressed in *Escherichia coli* and studied using right-side-out membrane vesicles. The transporter CitH catalyzed proton motive force driven uptake of the Ca^{2+}-citrate complex (K_M 3.5 μM). Citrate in complex with Sr^{2+}, Mn^{2+}, Cd^{2+} and Pb^{2+} is substrate of CitH while complexes with Mg^{2+}, Zn^{2+}, Ni^{2+} and Co^{2+} are not. On another hand, the *citO gene* product has high homology to GntR transcriptional regulator proteins. We constructed a CitO defective strain and it was unable to metabolize citrate. CitO was expressed in *E. coli* and purified; band shift experiments showed that CitO could bind to the divergent promoter region. This supports the idea that CitO is a new positive regulator involved in the regulation of the citrate fermentation pathway in *E. faecalis*.

MI-C06

REGULATION OF *PCHP* GENE IN *PSEUDOMONAS AERUGINOSA*

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Taking advantage of the recent identification of the gene encoding for phosphorylcholine phosphatase enzyme, named *pchP*, we proposed to elucidate the molecular mechanisms governing its expression in *P. aeruginosa*. We showed that this gene is principally transcribed as a monocistronic mRNA. Consistently, a functional promoter was found in the intergenic region immediately upstream of *pchP*. β-gal activity measurements showed that *pchP* promoter is induced by choline and derivatives, and it is shut down by addition of succinate plus ammonium. By fusing different fragments of the *pchP* upstream region to a promoter-less *lacZ* reporter gene, the region from –74 to –55 was shown to include the most likely *pchP* promoter. PA5293, a putative LysR-type regulator-encoding gene, is located usptream of *pchP*. A deletion mutant of this gene, which will henceforth be called *pchT*, was constructed. Δ*pchT* mutant produced greatly reduced levels of PChP activity in periplasmic extract, but the deletion of this gene did not affect *pchP* promoter activity. The Δ*pchT* mutant also showed up to 8-fold increase in *pchP* transcription, relative to the wild-type strain, atributed to a constitutive increase of transcription from *pchT* promoter. This indicates that this LysR regulator is not involved in the regulation of *pchP* promoter at the transcriptional level, at least not under the conditions employed in this study.

MI-C07

REGULATION OF THE *ESCHERICHIA COLI* NADH DEHYDROGENASE-2 GENE EXPRESSION BY PHOSPHATE AND GLYCEROL

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NADH dehydrogenase-2 of *Escherichia coli*, encoded by *ndh* gene, is used during aerobic and nitrate respiration. The expression of *ndh*, is subject to a complex network of regulatory controls at the transcriptional level. It was reported that the gene, is expressed mainly in exponential phase of growth and that its expression decays in stationary phase by the regulation of several transcriptional factors (i.e.: FNR, IHF, Fis, etc.). However, in our current studies of NDH-2 activity, we found that expression was maintained up to stationary phase. These contradictory observations are based on the different culture media used: M9 minimal medium in our assays or LB complete medium in previous reports. By comparative expression of transcriptional fusions in different minimal media, we determined that the maintenance of NDH-2 activity in stationary phase depends on the presence of at least 25 uM phosphate. Besides phosphate, glycerol was also required. No correlation between the differential expression and the identified transcriptional factors was found by analysis with several chromosomal mutants. Deletion analysis of the *ndh* promoter region allowed us to recognise nucleotides from -255 to -205 as essential for the regulation by phosphate and glycerol. Our results suggest that glycerol-phosphate is a key compound in the regulation of *ndh* gene.

MI-C08

SELECTIVE DETERMINANTS INVOLVED IN THE CueR-LIKE REGULATORS/TARGET PROMOTERS INTERACTION

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Transcriptional regulators of the MerR family respond specifically to a variety of environmental stimuli, such as heavy metals. *Salmonella* has two MerR homologs that respond to monovalent metal ions: CueR, which is involved in copper homeostasis, and GodS, that we demonstrated previously to be the main responsible for gold detoxification. Both regulators share similarities not only at protein level, but also in the sequence they recognize at the promoters of their target genes. Therefore, we analyzed whether these regulators can functionally replace each other. Using single and double mutant strains we demonstrated that in the absence of GodS, CueR induces small level expression of GodS-controlled genes, and vice versa. By electrophoretic mobility shift (EMSA) and DNAse I footprinting assays we found differences in the binding affinity to the promoters that could explain the low-level cross regulation observed. An *in silico* analysis of the GodS and CueR controlled promoters revealed the presence of distinctive bases that could account for the regulator's discrimination. The characterization of a series of mutant promoters allowed us to identify the selective bases directly involved in selective recognition by the specific regulator. Our results can contribute to better understand the molecular bases of the interaction between MerR like regulators and their target promoters.

MI-C09

TWO TRANSCRIPTIONAL REGULATORS COMPETE WITH IHF FOR THE BINDING TO THE *B. ABORTUS* VIRB PROMOTER

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Type-IV secretion systems (T4SSs) are multicomponent machineries that are present in many bacterial intracellular pathogens. They are involved in the translocation of effector proteins from the bacterium to the host cell cytoplasm. In *Brucella abortus*, the etiological agent of brucellosis, the *virB* operon encodes a T4SS that is essential for virulence and intracellular multiplication. The nucleoid protein IHF plays an essential role in transcriptional regulation of the *virB* operon during intracellular infection of J774 macrophages. Recently, we identified two proteins that bind specifically to different regions of the *virB* promoter. These proteins belong to the GntR and MarR families of negative transcriptional regulators. In this work both transcriptional regulators were cloned and expressed as recombinant proteins. Using Electrophoresis Mobility Shift Assays we determined specificity of binding, identification of binding regions and apparent dissociation constants for each protein. It was observed that incubation of the transcriptional regulators with recombinant IHF and the *virB* promoter did not produce a ternary complex, indicating that the three factors compete for the same binding site. These results suggest that both transcriptional regulators could be acting as a double check point for the control of transcription of the *B. abortus virB* operon.

MI-C10

THE ACID-INDUCED AUTOLYSIS IS COME-MEDIATED BY A QUORUM SENSING-INDEPENDENT PATHWAY IN *PNEUMOCOCCUS*

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In *pneumococcus*, a quorum-sensing system controls the development competence at pH 7.8. The competence-stimulating peptide (CSP) is a cell-density signal secreted by an ABC transporter (ComAB), and its accumulation in the extracellular space is sensed by a transmembrane histidin kinase (ComD). Upon CSP-binding, ComD phosphorilates a response regulator (ComE), activating the transcription of the competence genes. We demonstrated that autolysis is triggered by acidic stress at pH 5.6 and mediated by ComE. However, competence is abolished at pH < 6.8, suggesting that ComE has another function in the acid-induced lysis. Here, we studied the connection between ComE and the quorum-sensing system under acidic conditions. To block the CSP circuit, we constructed the *comA::km* strain, which lysed at pH 5.6 as the wild-type strain (WT), but the lysis of the *comA::ery/ comE::km* mutant was inhibited, indicating ComE regulation by a quorum sensing-independent pathway. To investigate the putative phosphorylation state of ComE at pH 5.6, we generated the hyperphosphorylating *comD*^{T233I} mutant, which lysed more rapidly than WT, but the lysis of the *ComD*^{T233I}*/ comE::km* mutant was not blocked at pH 5.6. These results suggest that ComE-P is necessary for the acid-induced lysis, but if ComE is absent, ComD could phosphorylate another protein (by a signal pathway known as “crosstalk”) to induce lysis.

MI-C11

A LOV-DOMAIN HISTIDINE KINASE IS INVOLVED IN *BRUCELLA ABORTUS* INFECTION

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Brucella abortus is a facultative intracellular pathogen that causes brucellosis in domestic animals and and humans. *Brucella* invade and replicates inside professional and non-professional phagocytes. Two-component proteins are widely recognized as environmental sensors in bacteria. Light, oxygen or voltage (LOV) belongs to the PAS domain superfamily. LOV domains bind a single molecule of FMN and undergo a self-contained photocycle that is dependent on the presence of a highly conserved cysteine residue. We identified a gen coding for a LOV domain protein in *B.abortus* (LOV-HPK). In addition to LOV domain this gene also has a PAS domain and an histidine kinase domains. The gene of LOV-HPK was cloned and expressed in *E.coli*. Illumination of recombinant purified LOV-HPK protein in presence of ATP conduce to autophosphorylation of kinase domain, showing that LOV-HPK is an active histidine kinase. In order to investigate the in vivo function of LOV-HPK, this gene was knocked-out and mutation was checked by PCR. Cell infection assays in macrophages J774 shown that LOV-HPK mutant bacterias has a attenuated phenotype as compared with control. However, in HeLa cells, LOV-HPK mutant shown similar infection as compared with control. Taking together these results suggest that LOV-HPK protein from *B.abortus* is a virulence factor involved in the defense against oxidative killing in macrophages.

MI-C12

THE ERROR-PRONE DNA POLYMERASE IV IS INVOLVED IN *PSEUDOMONAS AERUGINOSA* MUCOID CONVERSION

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Pseudomonas aeruginosa colonizes the respiratory tract of Cystic Fibrosis patients, where a high proportion of hypermutable variants along with mucoid, alginate-overproducing variants, emerge leading to chronic infection and poor prognosis. Conversion to mucoidy is usually caused by a G deletion within a homopolymeric run of G in the *mucA* gene (*mucA22*). Studying the emergence of mucoids in wild type and hypermutable *mutS* deficient strains of *P. aeruginosa* PAO1, we observed a 37 fold increase in the frequency of mucoid variants emerged from the *mutS* mutant. While only 12% of mucoids from the wild type strain carried a *mucA22* allele, this proportion reached 64% for the *mutS* strain. Since in *Escherichia coli* Pol IV (*dinB*) is involved in the generation of -1 deletions in homopolymeric runs, we analyzed emergence of mucoid variants in strains of *P. aeruginosa dinB* and *mutS dinB* double mutants. Emergence of mucoids suffered a 3 fold decrease in the *dinB* strain, and only a 2 fold increase was observed in the *mutS dinB* double mutant respect to the wild type strain. Furthermore, over-expression of Pol IV in the *mutS* strain resulted in a 110 fold increase in the emergence of mucoid variants. Thus we provide the first evidence of the mutagenic activity of Pol IV in *P. aeruginosa* and show that in mucoid conversion, hypermutability of *mucA* is mainly due to Pol IV activity.

MI-C13

ISOLATION OF BIOEMULSIFIER-PRODUCING SPORE-FORMING MICROORGANISMS FROM PETROLEUM CONTAMINATED SITES

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Bioemulsifiers play an important physiological role in hydrocarbon degrading organisms. Their biodegradability is one of the most important assets because it prevents toxicity problems and accumulation in natural ecosystems. Their potential industrial applications include enhanced oil recovery, crude oil drilling, food processing, and cosmetic formulations. The aim of this work was to isolate bioemulsifier-producing spore-forming microorganisms from petroleum contaminated sites. Methods: Nutrient broth agar (NBA), NB, SPI medium containing different sugars (sucrose, starch, glucose and glycerol) and NB containing kerosene were used for isolation, selection and partial characterization assays. Emulsion forming and stabilizing capacity were evaluated using kerosene as immiscible liquid. Results and conclusions: Hydrocarbon contaminated soil samples from NW Argentina had an average of $2x10^{8}$CFU (g of soil)$^{-1}$. Three emulsifier producer strains were selected. The emulsifier capacity was located mainly in the supernatant. Interesting, two of them (BS18 and BS38) also have this activity associated with the cell. No effect was observed with either the addition of protease or the addition of sugar to the culture medium. Except for glycerol that it has a positive response. Promising emulsifiers activities were also very stable This work was supported by grants PIP 6062 and PIP 6203.

MI-C14

STUDY OF PARASPORAL INCLUSIONS BY NUMERICAL ANALYSIS OF THEIR SDS-PAGE PROTEINS PROFILES

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Bacillus thuringiensis(Bt) is a Gram-positive, endospore-forming bacterium that produces proteinaceous parasporal inclusions during sporulation. These inclusions often contain δ-endotoxin proteins that cause rapid and fatal intoxication of several insect orders. It has been established that the inclusions proteins, encoded by cry genes, are highly heterogeneous in structures and biological activities. We previously reported a native Bt RT3 that shows a strong toxicity against *Spodoptera frugiperda* larvae. The aim of this work was to study parasporal inclusions by numerical analysis of their SDS-PAGE proteins profiles. Standard *B. subtilis* (1A571) and Bt strains (BGSC): 4A4, 4D3 and 4D1 as well as native strains were used throughout this study. Proteins were prepared by a rapid washing procedure. SDS-PAGE of standards and inclusions proteins was performed according to Laemmli method. Gels were stained with silver reagent and they were analyzed by using NTSYS program (SM coefficient and UPGMA). Two main groups of *Bacillus* strains can be differentiated showing a similarity of 90 % between them. Protein profiles from native strains were different from those of standard strains. Bt RT3 displays a similarity of 92% with Bt 4D1. This method was more sensitive to differentiate Bt strains than a set of biochemical tests. This work was supported by grants PICTO 761 and PIP 6062.

NS-C01

MOLECULAR DYNAMICS OF ACETYLCHOLINE RECEPTOR ION CHANNEL BLOCK BY THE NEUROLEPTIC CHLORPROMAZINE

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A large series of pharmacological agents, distinct from the typical competitive antagonists, block non-competitively the nicotinic acetylcholine receptor (AChR). Taking the neuroleptic chlorpromazine (CPZ) as an example of such agents, the blocking mechanism to the ion channel pore of the AChR has been explored at atomic level using both conventional and steered molecular dynamics (MD) simulations. Repeated steered MD simulations have permitted calculation of the free energy (~36 kJ/mol) of CPZ binding and identification of the optimal site in the region of the serine and leucine rings, at ~4 Å from the pore entrance. Coulomb and the Lennard-Jones interactions between CPZ and the ion channel as well as the conformational fluctuations of CPZ were examined in order to assess the contribution of each to the binding of CPZ to the AChR. The MD simulations disclose a dynamic interaction of CPZ binding to the AChR ionic channel. The cationic ammonium head of CPZ forms strong hydrogen bonds with various AChR residues. The conventional MD simulation of CPZ at its identified binding site demonstrates that the binding of CPZ not only blocks ion transport through the channel but also markedly inhibits the conformational transitions of the channel, necessary for AChR to carry out its biological function.

NS-C02

PRESENCE OF A GLOBIN mRNA AND MIGRATION OF BONE MARROW CELLS AFTER SCIATIC NERVE INJURY

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Wallerian degeneration is a well characterized process in which one of the hallmarks is myelin breakdown. We have previously reported that in the distal stump of ligated sciatic nerves, there is a change in the distribution of the major peripheral myelin proteins: P0 and myelin basic protein (MBP) immunoreactivities. These results agreed with the studies of myelin isolated from the distal stump of injured sciatic nerve, showing a gradual increase in a 14 kDa band. The aim of the present study is to dilucidate the identity of the 14 kDa band and its participation in the degeneration-regeneration process. The 14 kDa band was resolved by a 16% gel in two bands containing a mixture of MBP fragments and peptides with great homology with α- and β-globins. The presence of α globin mRNA was demonstrated by real time PCR in the proximal and distal stump of the ipsilateral sciatic nerve. The presence of CD34+ and ED1+ cells was also demonstrated by western blot in isolated myelin from the ipsilateral nerve as well as by immunohistochemistry in the nerves. Labeled cells isolated from normal adult rat bone marrow which were injected intraortically were found to migrate to the injured area by immunofluorescence. These cells could contribute to the remyelination of the damaged area participating in the removal of myelin debris in the distal stump of the injured sciatic nerve.

PL-C01

ATP MODULATES PEROXIDASE ACTIVITY AND ELICITS AUTOPHOSPHORYLATION OF RAPESEED 2-CYS PEROXIREDOXIN

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2-Cys peroxiredoxins (2-Cys Prx) are ubiquitous peroxidases devoid of selenium- and the heme-prosthetic group that have been implicated in cell proliferation, differentiation, apoptosis, and photosynthesis. Although the contribution to the cell redox status has been characterized and the chaperone capacity was recently reported, the precise control of functional characteristics remain unknown. We found that ATP impair reversibly the peroxidase activity associated to chloroplast rapeseed 2-Cys Prx. In particular, the noncovalent interaction with ATP not only modifies the catalytic activity but also changes the structural features of the recombinant protein. More importantly, ATP triggers the autophosphorylation of the chloroplast 2-Cys Prx when the protein is successively treated with a reductant and an oxidant. Contrary to the peroxidatic activity, the conserved Cys175 and Trp179 residues are essential for the incorporation of radioactivity from [γ-^{32}P]ATP. Despite significant differences, the process of autophosphorylation is also observed with orthologs from a helminth parasite (Schistosoma mansoni 2-Cys Prx1) and bacteria (Salmonella typhimurium AhpC). These results uncover a previously unknown function of ATP in the regulation of 2-Cys Prx and give a new insight not only to the removal of obnoxious reactive oxygen species but also to the control of signal transduction pathways

PL-C02

NADP-MALIC ENZYME FAMILY FROM *A. THALIANA*: FUNCTIONAL CHARACTERIZATION OF T-DNA INSERTION MUTANTS

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The A. thaliana genome contains four genes encoding NADP-malic enzymes (me1-4). ME4 is localized to plastids, whereas the other three isoforms (ME1-3) are cytosolic. ME2 y ME4 are the only proteins showing a constitutive pattern of expression. Furthermore, ME2 is responsible for the major part of NADP-ME activity in mature plant tissues. In order to elucidate the particular role of each family member in Arabidopsis metabolism, several T-DNA mutants defective in the me genes were analyzed. In addition, homozygous double and triple mutants were generated by crossing. The distribution of NADP-ME activity obtained in different organs was in agreement with expected results. However, none of the single, double or triple mutant combinations showed an obvious phenotype under normal growth conditions. Thus, different stresses were imposed to insertion lines. After high light treatment, the knock-out mutants without ME2 are more sensitive than the wild-type and exhibited severe photo oxidative damage. In view of this result, the me2 gene under control of CaMV35S promoter was introduced into these lines by A. tumefaciens mediated transformation. Transgenic lines were analyzed for NADP-ME activity, native PAGE electrophoresis and western blot. It could be envisioned that ME2 is required in cases where the export of malate to the cytosol is increased, probably contributing to the malate valve.

PL-C03

DOMAINS IMPLICATED IN TETRAMERIZATION AND MALATE INHIBITION OF MAIZE NADP-MALIC ENZYME

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In plants, C4 photosynthetic NADP-malic enzyme (NADP-ME) has evolved from non-C4 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 C4- and non-C4 NADP-ME several reciprocal chimeric enzymes between these isoforms were constructed and analyzed. By this approach, a region between aminoacid residues 102 and 247 of the C4 NADP-ME was found to be implicated in the oligomerization state, being responsible for the tetrameric state of this isoform. In this way, the strategy for oligomerization of these NADP-ME isoforms differs markedly from the one that present non-plant NADP-ME crystallized to date. On the other hand, the region from residue 248 to the C terminal of the C4 isoform was implicated in the inhibition by high malate concentrations at pH 7.0. The inhibition pattern of the C4-NADP-ME and some of the chimeras suggested an allosteric site responsible for such inhibition. In this way, this inhibition may be important for the regulation of the C4 isoform in vivo; presenting maximum activity when photosynthesis is in progress.

PL-C04

HIGH LEVEL OF NADP-MALIC ENZYME IN PLASTIDS REDUCES PROLONGED DARKNESS TOLERANCE IN *A. THALIANA*

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The full-length cDNA encoding maize C4 NADP-malic enzyme was expressed under the control of the CaMV35S promoter in Arabidopsis thaliana (MEm plants). An increase in the plastidic NADP-ME activity led to a green pale phenotype in plants growing in short days and low to moderate irradiance. In these conditions, MEm plants have a decreased fresh weight/area ratio and thinner leaf sections. Measurements of chlorophyll content, chlorophyll fluorescence and CO2 assimilation indicated that the expression of highly active NADP-ME altered photosynthetic metabolism. This phenotype can be reverted by transferring the plants under high light. Moreover, all these features are absent in plants growing in long-day conditions. Metabolite levels of rosettes from transgenic plants indicated that plants grown in both photoperiods have a disturbed metabolic profile. Additionally, dark-induced senescence progressed more rapidly in MEm plants compared to the wild type. While four week-old wild-type plants became pale green five days after the onset of darkness, MEm plants showed this characteristic already after two days with severe yellowing after three days in darkness. Since MEm plants accumulate lower levels of intermediates used as respiratory energy, these metabolites are consumed faster than in the wild-type in prolonged darkness and, as a consequence, MEm plants enter senescence more rapidly.

PL-C05

THE BIOGENESIS OF THE PHOTOSYNTHETIC APPARATUS IS REPPRESED BY THE TRANSCRIPTION FACTOR HAHB-4

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Expression of the sunflower Hahb-4 transcription factor (TF) is regulated by ethylene, drought and ABA as previously reported. Here we show that the expression of this TF is strongly induced also in dark. Such light-dependent regulation was only observed on photosynthetic tissues. In order to locate the promoter cis-acting elements responsible for this regulation we obtained constructs (deletions, chimeras) of the Hahb-4 promoter controlling the reporter gus activity. Analysis of transgenic plants bearing these constructs allowed us to locate sequences involved in the response to dark between positions -319 and -419. Using site directed mutagenesis we found out a GT-1 binding site in the promoter responsible for its response to dark. Aiming to elucidate the significance of these events we performed a microarray experiment of Hahb-4 overexpressing plants. The analysis of the obtained data revealed that a large number of genes involved in the photosynthetic machinery biogenesis is repressed. The results, together with transpiration rates and CO2 fixation measurements, suggest that Hahb-4 functions unleashing a mechanism able to turn off the photosynthetic machinery. We conclude that Hahb-4, also up regulated during hydric stress, can reduce the photo-oxidative stress produced by photosynthesis during periods of drought and

consequently contributes to the observed drought tolerance.

PL-C06
MicroRNA EXPRESSION IN MAIZE AND WHEAT
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MicroRNAs are tiny RNAs of 20-24 nucleotides which are processed from long RNA precursors transcribed from the plant genome. These miRNAs regulate by traductional repression or mRNA cleavage the expression of several genes, mostly transcription factors. 767 miRNAs were identified in plants and 24 families of them were shown to be conserved among Arabidopsis thaliana, rice and other species. Here we studied 17 conserved miRNAs by Northern blotting of low molecular weight RNAs isolated from 6 different tissues from maize and wheat. The expression of these miRNAs differed widely among these tissues. Interestingly the expression of miR167 and miR171 was higher in maize endosperm of 14-27 days after pollination (DAP) than in the embryos while the expression of miR159 and miR396d in maize endosperm was highest at 7 DAP and decreased sharply afterwards. In contrast, the expression of several miRs (miR156, miR159, miR167 and miR171) was higher in immature wheat embryos than in endosperm. The putative targets in these cereals of miRNAs of interest were predicted with the miRU software and their expression in the same tissues confirmed by RT PCR. Experiments of 5¢ RACE were done to determine which is the target that is really being modulated by the miRNAs. [*This authors equally contributed to this work].

PL-C07
TOBACCO TRANSCRIPTOME ANALYSIS IN RESPONSE TO *XANTHOMONAS AXONOPODIS* PV. *CITRI*
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Citrus canker is an endemic disease caused by *Xanthomonas axonopodis* pv. *citri* (Xac) which produces serious economic losses. There are not resistant citrus cultivars to citrus canker but the bacteria is able to induce hypersensitive response (HR) in non-host plants. In this context, we have studied the tobacco-Xac interaction to identify plant genes that modify their expression in the response to Xac. We used the cDNA-AFLP technique to analyze changes in transcript levels at 2, 8 and 24 hs post-inoculation (hpi). We used forty primers combinations (which represent 20 % of the whole tobacco transcriptome) and obtained about 2000 transcript derived fragment (TDFs). Differential expression was observed in 316 TDFs (2.8 %): 178 TDFs (1.6 %) were up-regulated, 56 at 2 hpi (0.5 %), 101 at 8 hpi (0.9 %) and 21 at 24 hpi (0.2 %); 138 TDFs (1.2 %) were down-regulated, 25 at 2 hpi (0.2 %), 76 at 8 hpi (0.7 %) and 37 at 24 hpi (0.3 %). Our results indicate that the main variations at the transcriptional level in tobacco-Xac interaction occurs at 8 hpi, with a similar number of genes up and down-regulated. In addition a significant percentage of genes are up-regulated at 2 hpi. Analyses of TDFs sequences may reveal insights about tobacco defense genes in HR response.

PL-C08
IDENTIFICATION OF PROTEIN-PROTEIN INTERACTIONS BETWEEN *XANTHOMONAS AXONOPODIS* PV. CITRI AND ORANGE

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Xanthomonas axonopodis pv. *citri* (Xac) causes citrus canker. Xac is a bacteria Gram (-) and uses different secretion systems for the translocation of pathogenicity and avirulence proteins to the plant cells. There are many genes characterized as elicitors of the host plant response and/or of the hypersensitive response (HR) in non host plants. Several genes in the sequenced genome of Xac may be considered as effector proteins that exert their functions in the plant cell. XAC3090, avrXacE1 and avrXacE2 are among these genes, with similarity to avirulence proteins from other plant pathogens. In this context, a XAC3090 mutant was constructed by marker exchange. However, the mutant strain showed no differences to the wild type Xac in its interaction with host and non host plants, possibly due to redundant functions. In order to further characterize the function of these proteins, two-hybrids assays in yeasts were carried out with XAC3090, AvrXacE1 and AvrXacE2 and a cDNA library of orange leaves. In total, 192 positive clones were sequenced and analyzed, from which 33 were positive preys for AvrXacE1, 28 for XAC3090 and 58 for AvrXacE2. A variety of proteins with different functions were found with a great prevalence of transcription factors, DNA binding proteins and heat shock proteins. The results suggest that these proteins may have a role in the regulation of the disease process.

PL-C09
HEME OXYGENASE UP-REGULATION IN UV-B IRRADIATED SOYBEAN PLANTS INVOLVES REACTIVE OXYGEN SPECIES

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We have previously reported that heme oxygenase (HO) plays a protective role against oxidative stress in plants, as it occurs in mammal tissues. In the present study we investigated the mechanism of HO-1 up-regulation in leaves of soybean (*Glycine max* L.) plants subjected to UV-B radiation. Under 7.5 and 15 kJ m^{-2} UV-B doses, HO, catalase (CAT, EC 1.11.1.6) and ascorbate peroxidase (APX, EC 1.11.1.11) activities were increased and the production of thiobarbituric acid reactive substances (TBARS) regain control values after 4 h of plant recuperation. Semi-quantitative RT-PCR demonstrated an enhancement of HO-1 transcript levels (77%), while protein expression, assessed by Western-blotting, increased up to 4-fold respect to controls. Plants pre-treated with the antioxidant ascorbic acid did not show the UV-B-induced up-regulation of HO-1 mRNA, but hydrogen peroxide treatment could mimic this reaction. Treatment with 30 kJ m^{-2} UV-B diminished the antioxidant enzyme activities and provoked a significant decrease in HO-1 transcript levels as a result of ROS overproduction. Our data indicates that HO is up-regulated in a dose depending manner as a mechanism of cell protection against oxidative damage and that such response occurred as a consequence of HO-1 mRNA enhancement involving ROS

SB-C01

CRYSTAL STRUCTURE OF PIMA, AN ESSENTIAL MANNOSYLTRANSFERASE FROM *MYCOBACTERIUM SMEGMATIS*

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The unusual architecture of mycobacterial cell envelopes is responsible for many physiological and disease-inducing aspects of *Mycobacterium tuberculosis*. The cell wall core is composed of a covalently bound structure, the mycolyl-arabinogalactan-peptidoglycan (mAGP) complex, interspersed with a variety of free lipids and lipoglycans that complement the mycolate residues to form an asymetric bilayer. Phosphatidyl-*myo*-inositol mannosides (PIMs) provide the lipid anchor to various cell wall lipoglycans in mycobacteria that play intriguing roles in host-pathogen interactions and whose biosynthetic pathways are poorly understood. Here we report structural and functional studies of *Mycobacterium smegmatis* PimA, the essential enzyme responsible for the transfer of the first mannosyl group to phosphatidylinositol. The structure shows the two-domain organization typical of GT-B glycosyltransferases, with the donor substrate GDP-mannose bound at a deep fissure in the interface between the domains. The overall structure and surface charge distribution suggest putative binding sites for the acceptor substrate. These results shed light on the early stages of PIM biosynthesis prior to membrane insertion and provide a structural framework for the development of specific inhibitors with potential anti-mycobacterial activity.

SB-C02

CRYSTALLOGRAPHIC AND ENZYMATIC STUDIES ON LUMAZINE SYNTHASES RIBH1 AND RIBH2 FROM *Brucella spp.*

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Lumazine Synthase (LS) catalyses the penultimate step in the biosynthesis of riboflavin. It is found in plants, fungi and microorganisms showing different quaternary assemblies. The enzyme can exist as free pentamers in solution (~90 kDa), as well as decamers (~180 kDa) and dodecamers of pentamers (~1 MDa). *Brucella spp*, the bacteria that cause the disease brucellosis, express RibH1 and RibH2, two proteins that bear sequence and structural homology with known LSs. In this work we present the three-dimensional X-ray crystallographic structure of both enzymes bound to a substrate analogue inhibitor, together with their enzymatic parameters. Results lead to the hypothesis that both proteins belong to different LSs families: RibH1 (pentameric) is a member of Type I eubacterial LSs, which are present in all organisms that synthesize riboflavin, whereas RibH2 (decameric) is associated with Type II eubacterial LSs, a group with unusual quaternary structure and a very high *Km* for one substrate of the reaction. This work is complemented with crystallographic and enzymatic analyses on RibH2 from *Mesorhizobium loti*, an α2-proteobacterium related to *Brucella*, as well as the comparison of the 3D-structures of RibH1 from two close *Brucella* species, namely *B. abortus* and *B. melitensis*. Overall, this analysis shows that Type II eubacterial LSs may have evolved for a different yet unknown function.

SB-C03
CRYSTAL STRUCTURE OF GUMK, A BETA-GLUCURONOSYLTRANSFERASE INVOLVED IN XANTHAN BIOSYNTHESIS

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Prokaryotic glycosyltransferases are enzymes involved in the synthesis of polysaccharides. The bacterial glucurono-syltransferase GumK is involved in the transfer of a glucuronic acid residue from UDP-glucuronic acid to mannose-α-1,3-glucose-β-1,4-glucose-P-P-polyisoprenyl, a intermediate step in the synthesis of xanthan, an exopolysaccharide produced by Xanthomonas campestris. Here we describe the molecular structure of this membrane-associated protein at 1.9 Å resolution. The molecule has overall dimensions of 45 x 45 x 60 Å. The structure of this enzyme shows a Rossmann-type fold, consisting primarily of α/β/α sandwiches. It is arranged in 2 globular domains connected by a linker loop. This loop is the bottom of a deep cleft that separates both domains. The cleft has a maximum width of 25 Å and a depth of 15 Å. Putative catalytic aminoacids, which are now being mutagenized, lie on loops on the inner surface of this cleft with their reactive side-chains pointing inside it. To study the binding of substrates we performed co-crystallization and soaking of crystals in crystallization solutions containing the donor or the acceptor substrate. In 2Fo-Fc electronic density maps we found the position were a portion of the donor substrate binds. We describe the molecular contacts and interactions involved in this binding, as well as the possible implications for catalysis.

SB-C04
CRITICAL ACTIVE-SITE RESIDUES IN *PSEUDOMONAS AERUGINOSA* PHOSPHORYLCHOLINE PHOSPHATASE

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Pseudomonas aeruginosa phosphorylcholine phosphatase (PChP) is synthesized when the bacteria are grown with choline or metabolic derivatives. With Mg^{2+}, PChP catalyses the hydrolysis of both phosphorylcholine (PCh) and *p*-nitrophenylphosphate (*p*-NPP). PChP contained three conserved motifs characteristic of the haloacid dehalogenases (HAD) superfamily. In PChP, motifs I, II and III correspond to the residues ^{31}DMDNT^{35}, ^{166}SAA^{168} and K^{242}/^{261}GDTPDSD^{267}, respectively. The catalytic importance of the conserved residues in these motifs on the enzyme activity was analyzed by site-directed mutagenesis. The substitution of D^{31} and D^{33} by glutamate caused a complete loss of activity whereas substitution of D^{262} and D^{265} by glutamates caused depletion in K_{cat} of two-three orders of magnitude, but conserves the catalytic sites involved in the hydrolysis of PCh or *p*-NPP. S^{166} is also important to catalyze the hydrolysis of both substrates. The substitution of lysine (K^{242}) by arginine or glutamine revealed the importance of the positive charged group, either from the amino or guanidinium groups. Therefore, D^{31} may be the nucleophile that gets phosphorylated during phosphoryl transfer reaction. The phosphorylated intermediate may be stabilized by D^{33}. In motif III, D^{265} and D^{267} may be involved in coordination of cofactor Mg^{2+} together with D^{31} and D^{33} of motif I forming the Mg^{2+} binding pocket.

ST-C01
EGF RELEASES AA THROUGH ACS4 AND ACOT2 ACTIONS IN STEROIDOGENIC CELLS

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AA plays a pivotal role in cell signaling and proliferation and its release has been shown in response to EGF and other growth stimuli. AA is also the precursor for several eicosanoids with potent effects on inflammation and cell growth. In steroidogenic cells EGF promotes an increase in the steroidogenesis and releases AA although the proteins involved in this event are unknown. We previously have shown that steroidogenic hormones release AA for steroids synthesis by a concerted action of an acyl-CoA synthetase (ACS4) and a mitochondrial Acyl-CoA thioesterase (Acot2).The aim of this study was to determine whether EGF produces AA release through ACS4 and Acot2 actions. For this purpose, MA-10 Leydig cells or Y1 adrenal cells were incubated in presence of EGF and ACS4 and Acot2 expressions were analyzed by Western-blot. We determined a doses dependent increment of both enzymes with a maximal expression at 10 nM EGF. We studied the temporal response pattern of ACS4 and Acot2 expressions, which reached their maximum at 30 min and then gradually decreased. The intramitochondrial AA release was studied by TLC using [^{14}C]-AA. EGF stimulated the AA mitochondrial content 29%± 3. These results show the EGF induction of both ACS4 and Acot 2 for the first time and the participation of these enzymes in the EGF-AA release, which, in turn, regulates the steroidogenesis and cell proliferation.

ST-C02
EXTRA-NUCLEAR LOCALIZATION OF α AND β ESTROGEN RECEPTORS IN C2C12 SKELETAL MUSCLE CELLS

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There is evidence for extra- nuclear localization of estrogen receptors (ERs). We present biochemical, immunological and molecular data supporting non-classical localization of α and β ERs in C2C12 murine skeletal muscle cells. We first established binding characteristics in whole cells employing [3H]17β-estradiol and an excess of 17β-estradiol, 17α-estradiol, estradiol impeded ligands, agonists and antagonists.Saturation assays and blocking experiments with specific antibodies, performed in subcellular fractions, showed predominant specific binding activity in mitochondria and microsomes. Protein purification, Western and Ligand blots confirmed these results. Low molecular weight estrogen receptor a-like proteins representing a population of binding and immunoreactive sites were also detected. Immunocytological studies by conventional and confocal microscopy revealed that ERβ was localized in mitochondria and ERα exhibited a perinuclear and a granular cytoplasmic distribution (Golgi apparatus, mitochondria and endoplasmic reticulum). The specific binding and the immunoreactivity detected by confocal microscopy and Western blots were reduced when cells were transiently transfected with specific ER α and β siRNAs. The non-classical distribution of native pools of ERs suggests an alternative mode of ER localization whose functions are under study (Vasconsuelo & Boland, SAIB 2006).

ST-C03
MULTIPLE KINASES ARE INVOLVED IN p19INK4d INDUCTION AND PHOSPHORYLATION IN RESPONSE TO GENOTOXICS

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p19INK4d belongs to a family of cyclin-dependent kinase inhibitors that arrests cells in G1 interacting with CDK4/6. This protein participates in the cellular response to genotoxic stress, enhancing DNA repair and lowering apoptosis. Upon genotoxic insult, p19 is induced and subject to phosphorylation. As p19 phosphorylation is necessary for its role in DNA repair, we sought to characterize the signal transduction pathways leading to p19 activation and induction in WI38 diploid human fibroblasts exposed to the following genotoxic agents: UVC irradiation, the antitumoral cisplatin, or β-amyloid peptide. Northern blot analysis and immunoprecipitation assays following metabolic labeling with [^{32}P]orthophosphate revealed that inhibition of ATM/ATR by caffeine 5 mM or specific inhibition of Chk1 blocks p19 induction and phosphorylation. Depending on the type of DNA lesion, impairment of Chk2 activity suppressed p19 phosphorylation without affecting its mRNA levels. None of the studied kinases MAPK, PI3K, PKA o CDKs seem to be implicated in the regulation of p19 in response to DNA damage. However, PKA or CDKs activity abrogation, partially or totally, respectively, blocked its phosphorylation. Our results demonstrate that, apart from ATM/ATR activation, different mechanisms would exist for the control of the expression and activity of p19 that vary according to the type of injury.

ST-C04
DIFFERENTIAL INVOLVEMENT OF SAC INFLUX IN ATP MODULATION OF MAPKs IN OSTEOBLASTS AND BREAST CELLS

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Extracellular nucleotides increase intracellular calcium concentration ([Ca^{2+}]i) in breast cancer MCF-7 and osteoblastic ROS 17/2.8 osteosarcoma cells. This effect is mainly due to Ca^{2+} release from inner stores. As previously seen in osteoblasts, using spectrofluorimetric [Ca^{2+}]i measurements, stimulation of MCF-7 cells with ATP or UTP activated calcium (SAC) influx sensitized them to a mechanical stress. ATPgama-S >ATP > UTP >> ADPß-S = ADP elevated [Ca^{2+}]i, in MCF-7 cells, suggesting the presence of P2Y2 receptor (P2Y2R) subtype and that SAC influx is dependent on P2Y2R activation. Gd^{3+} (10µM) inhibited the ATP-dependent SAC influx. U73122 and neomycin, PI-PLC inhibitors, and 2-APB, an IP_3 receptor antagonist, abolished the ATP dependent- Ca^{2+} release and SAC influx. Western blot analysis revealed that P2Y2R stimulation induced ERK1/2, p38 and JNK1 MAPK phosphorylation in both cell lines. Cell treatment with neomycin, Gd^{3+} or a Ca^{2+} free medium (plus EGTA 0.5 mM) reduced this effect induced by ATP in ROS 17/ 2.8 cells, whereas in MCF-7 cells, U73122 and 2-APB completely inhibited the ATP-dependent MAPKs activation while EGTA or Gd^{3+} did not. The results show the presence of ATP-dependent SAC influx in both cell lines. Different from ROS17/2.8 cells, MAPK phosphorylation induced by ATP in breast tumor cells is dependent on PI-PLC/IP_3/Ca^{2+} release but not on SAC influx.

ST-C05

BCL-XL EXPRESSION IS REGULATED BY EPIDERMAL GROWTH FACTOR (EGF) IN MAMMARY EPITELIAL CELLS (HC11)

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EGF activates signalling pathways associated with cell proliferation and apoptosis. The HC11 mouse mammary epithelial cell line is a useful in vitro model system for studying mammary cell proliferation and differentiation. It was reported that EGF reverts confluent HC11 cells apoptosis and increases Bcl-2 (anti-apoptotic protein) levels. The bcl-x gene (a member of the bcl-2 family) codifies different protein isoforms, i.e: Bcl-XL (anti-apoptotic) and Bcl-XC (pro-apoptotic). We have studied the regulation of Bcl-X by EGF. DNA ladder and violet crystal staining showed revertion of starvation induced apoptosis of confluent HC11 cells with EGF (100ng/ml) treatment. This effect was totally blocked by JNK inhibitor (SP), partially blocked by PI3K inhibitor (LY) and not affected by a MEK/ERK1/2 inhibitor (PD). Western Blot analysis of Bcl-XL at confluent HC11 cells showed a 1,9 fold increase of the protein with EGF (100ng/ml, 5 hours treatment). This activation was impared with SP and LY but not with PD. bcl-XL/bcl-XS ratio increases upon EGF tratment (23,1 fold induction vs control) according to Real Time PCR. While SP and LY totally revert this effect, PD does it only partially. Conclusion: EGF reverts confluent HC11 cells apoptosis, increases Bcl-XL protein expression and the bcl-XL/ bcl-XS mRNAs ratio, being JNK and PI3K the kinases involved.

POSTERS

Biotechnology (BT-P1/BT-P33)
Cell Biology (CB-P1/CB-P85)
Enzymology (EN-P1/EN-P22)
Lipids (LI-P1/LI-P31)
Microbiology (MI-P1/MI-P93)
Neurosciences (NS-P1/NS-P7)
Plant Biochemistry and Molecular Biology (PL-P1/PL-P76)
Structural Biology (SB-P1/ST-P21)
Signal Transduction (ST-P1/ST-P30)

BT-P01

CLONING AND EXPRESSION OF UV-SPECIFIC DNA REPAIRING PROTEIN WITH POTENTIAL TRANSDUCING ACTIVITY

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UV radiation can produce mutations in skin cells and correlates strongly with the onset of actinic keratoses and basal and squamous cell carcinomas. Xeroderma pigmentosum (XP) is a heritable disease characterized by an extreme sensitivity of skin to UV radiation. Recently in our lab, we have described the expression of an UV-specific DNA endonuclease from Micrococcus luteus (alfaUVE-A) into XP fibroblasts, which greatly increased UV resistance and, in consequence, cell survival. The aim of this work was to obtain a DNA repairing protein fused to a peptide with transducing ability called TAT. DNA sequence from TAT-UVE-A fusion protein was built in two successive PCR rounds employing two 5' specific primers which codified for TAT sequence. Amplification products were cloned into pGEM-T-Easy vector (PROMEGA) and subcloned into a prokaryotic expression vector (pEX) containing a sequence for a peptide (alfa), wich confers stabiltiy to the UVE-A protein. Clones were analyzed by Colony PCR and enzimatic digestion, the identity of selected colonies was confirmed by automated sequencing. Western blot analysis confirmed the expression of a 27 kDa protein, as expected. In the near future we will analyze enzimatic activity and transducing capability of alfaTAT-UVE-A, in order to determine the possibility to administrate this protein topically.

BT-P02

IMPROVEMENT OF AN *E. coli* STRAIN FOR THE HETEROLOGOUS EXPRESION OF dTDP-SUGAR BIOSYNTHETIC PATHWAYS

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Heterologous expression has become an important tool for polyketide production and combinatorial biosynthesis. Many natural occurring polyketides contain unusual sugars which are essential components of the molecules to be bioactive. Recently we have been able to functionally express in *E. coli* the biosynthetic pathways of dTDP-mycarose and dTDP-desosamine, using a plasmid-based system, resulting in the production of a fully-glycosylated polyketide antibiotic, Erythromicin C, although in very low levels. These sugars, like many others dTDP-sugars, are synthesized via dTDP-4-keto-6-deoxyglucose (dTKDG) intermediate. In *E. coli*, the biosynthesis of dTDP-rhamnose, dTDP-VioNAc and dTDP-Fuc4NAc, which are precursors of cell wall components, occurs through the same intermediate dTKDG. Here we have developed an *E. coli* B host by deleting key genes involved in the biosynthetic pathways of these dTDP-sugars, to improve the expression of new heterologous dTDP-sugar pathways. Deletion of five genes from the biosynthetic pathways of dTDP-rhamnose, dTDP-VioNAc and dTDP-Fuc4NAc resulted in increased intracellular levels of dTKDG. Bioconversion experiments in this new strain expressing the heterologous dTDP-desosamine pathway resulted in highly increased glycosylation levels compared to the parental strain.

BT-P03
PRODUCTION OF A BIOLOGICALLY ACTIVE RECOMBINANT GROWTH HORMONE FROM PEJERREY

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Since economical equation in fisheries is conditioned on fish growing rates, growth hormone based studies became a key factor in aquaculture development. Current pejerrey (*Odontesthes bonariensis*) cultures do not provide adequate growing rates for commercial applications. Oral administration of active growth hormone is a valid tool to promote growth in teleost fish. The aim of this work was to obtain an active recombinant pejerrey growth hormone (pjGHr) by optimization of the solubilization and folding of pjGHr produced in a highly efficient prokaryotic system. Hormone cDNA inserted in pET-24 vector was expressed in E.coli BL21 CodonPlus. Cells were induced with 2 g/l lactose rendering 0.4 g/l pjGHr with a purity of 83%. To achieve an efficient folding, inclusion bodies were solubilized in 8M Urea, Tris 100mM pH 10.5. The optimal folding condition was obtained by dilution of the protein solution in ten volumes of Tris 40mM pH 10,5 with Arginine 50mM used as an intermolecular interaction inhibitor. Finally the dilution was dialyzed 24 hs against Tris 20mM pH 9 followed by 12 hs against sodium bicarbonate 0,05%. Juvenil pejerrey injected with 0.5 µg of pjGHr per gram of body weight exhibited significant increase ($P<0.05$) of hepatic IGF-I mRNA measured by semi-quantitative RT-PCR. Active pjGHr production is the first step to achieve an improvement of growing rates of this specie.

BT-P04
CLONING AND YEAST EXPRESSION OF *BRUGMANSIA CANDIDA* HYOSCYAMINE 6 β-HYDROXYLASE

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Hyoscyamine and scopolamine are tropane alkaloids widely used in the pharmaceutical industry. Scopolamine is more useful for medicinal purposes. The conversion of hyoscyamine into scopolamine is carried out by Hyoscyamine 6 β-hydroxylase (H6H, EC 1.14.11.11). In this work we report the cloning and expression of *B. candida h6h* gene in *Saccharomyces cerevisiae* as the first step in the development of a biological catalyst for the production of scopolamine. The *h6h* cDNA was cloned into the yeast pYES2.1-TOPO TA expression vector. The enzyme expression was induced by changing the carbon source and it was shown that the protein was expressed after 4 hour-induction. The recombinant and wild type *S. cerevisiae* strains were assayed for their tolerance to both alkaloids by growth inhibition assays. Both strains tolerated the maximum scopolamine concentration tested (150 mM) but only the recombinant strain tolerated 150 mM of hyoscyamine. In order to perform the in vitro enzyme activity assay, different cell disruption methods were tested using recombinant yeast harbouring the *lacz* gene instead of the *h6h* gene. Of them, the mechanical lysis gave the best results compared to the liquid nitrogen lysis, permeabilization with toluene and sonication. Processes of conversion of hyoscyamine into scopolamine by the recombinant strain carrying the *h6h* gene are currently under study.

BT-P05
PURIFICATION CHALLENGE IN BACULOVIRUS-INSECT LARVAE EXPRESSION SYSTEM

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Horseradish peroxidase (HRP) is widely used in immunology diagnosis kits. As its source is the Armoracia rusticana root (horseradish), a poor developed culture in Argentina, this enzyme must be imported. Our goal was to express HRP as a recombinant enzyme and purify it up to the analytical purity level. Due to its structural complexity (highly glycosylated heme protein, stabilized by four disulfide bonds), its expression in active form in prokaryote systems is not possible. A low-cost alternative is the baculovirus-cell insect system. Previously, recombinant HRP was obtained in insect cell cultures and, for the scale-up of the process, the same virus was used to infect Rachiplusia nu larvae. Though high peroxidase levels were reached (115.9 ± 8.2 mg/kg larvae), the contamination by larva proteins was the main problem to solve, in particular catalase (CAT), enzyme that competes with HRP for hydrogen peroxide. Since the recombinant enzyme has a 6xHis and a 6xArg tags, immobilized metal ion affinity chromatography (IMAC) and ion exchange chromatography (IEC) were assayed. As HRP accumulates in haemolymph, but haemolymph is difficult to withdraw quantitatively, total larvae extract was chosen as the starting material. SDS-PAGE shows that both methods are able to separate HRP from most of larvae contaminating proteins and CAT, with enzyme yields over 97 %, being IEC more economic.

BT-P06
DEVELOPMENT OF RECOMBINANT BACULOVIRUS FOR PEROXIDASE PRODUCTION IN ORALLY-INFECTED INSECT LARVAE

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For recombinant protein production, Rachiplusia nu (R. nu) larvae can be infected with Autographa californica virus (AcMNPV) by injection or orally. Oral infection is less laborious and time consuming. Most recombinant baculovirus are constructed by replacing the polyhedrin gene with the foreign gene but, as these viruses do not produce polyhedra (occ-), they are not able to efficiently infect larvae orally. In this study we assess two different approaches to produce recombinant baculovirus for oral infection. 1) Mixed polyhedra obtention by co-infection of wild type AcMNPV and AcMNPV HRP+/occ-: A Sf9 subconfluent monolayer was inoculated with both viral populations at a different multiplicity of infection and various proportions. 2) AcMNPV HRP+/occ+ construction: HRP gene was cloned under the polyhedrin promoter, and polyhedrin gene under p10 promoter. A similar monolayer was co-transfected with transfer vector pBacPack-HRP and bAcGOZA. Fourth instar R. nu larvae were starved for 6 h and then they were fed with artificial diet contaminated with 1x106 pol/100 mg of both polyhedra suspensions. At 4º day post-infection hemolymph was collected and peroxidase activity was measured. Polyhedra baculoviruses obtained by both strategies resulted in successful HRP production in orally- infected insect larvae.

BT-P07

HIGH PRODUCTIVITY OF AN INSECTICIDE VIRUS IN CELL CULTURES INFECTED AT LOW CELL DENSITY AND LOW MOI

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Anticarsia gemmatalis is one of the main plagues in soybean crops. This insect is controlled with polyhedral inclusion bodies (PIBs) of an insecticide baculovirus, the Anticarsia gemmatalis multicapsid nucleopolyhedrovirus (AgMNPV). At current, the production of AgMNPV is based on the in vivo propagation in infected larvae. However, a growing demand has spurred an interest in developing alternative processes based on the viral propagation in insect cell cultures. The virus inoculum is an expensive raw material in the production of baculovirus in insect cell cultures, in such a manner that infection of low cell density cultures at low multiplicity of infection could be an attractive alternative for a large-scale process. In order to evaluate the feasibility to use such infection conditions, serum-free suspension cultures of the UFL-AG-286 cell line were infected, following a full factorial experimental design with center point, in a limited range of low initial cell densities (ICD) and low multiplicities of infection (MOI). The PIBs productivities obtained at the several infection conditions ranged from 1 to 1.5x106 PIBs.ml-1.h-1, the same value as obtained in cultures infected in optimal conditions at high MOI. These results demonstrate that is possible to reach an optimal PIBs productivity infecting at low ICD and low MOI, with a significant economy (30-300) of virus inoculum.

BT-P08

TESTING AN ALTERNATIVE EXPRESSION SYSTEM FOR PROTOZOAN PROTEINS

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Crithidia fasciculata is a non-pathogenic parasite of insects, which can be cultivated in high yields using inexpensive undefined serum-free media. The evolutionary proximity of C.fasciculata to protozoan pathogens, makes it an ideal vehicle for the expression of genes from lower eukaryotes. In this work we used the C.fasciculata pNUS-H1 expression vectors (E. Tataud et al., Mol. Biochem. Parasitol. 120, 2002) for expressing the msa 2c gene from Babesia bovis, the causative agent of bovine babesiosis. The obtained constructs bore alternatively a poly-hystidine tag or a secretion peptide signal. To improve transfection efficiency different electroporation conditions were evaluated with GFP constructs. Transfected parasites were selected with Hygromycin B and resistant clones were obtained after 3 weeks. Up to now, stable lines were obtained and expression of MSA 2c was detected by Western blot. The expression of genes in environments that allow the production of functional proteins constitutes a main goal in order to improve the current expression tools for obtaining recombinant proteins. Concerning Msa2c, the post- translational modifications should improve the antigenic efficacy in comparison with the same protein expressed in prokaryotic systems.

BT-P09
HETEROLOGOUS EXPRESSION OF THE RECOMBINANT ANTIGEN 5 OF *Polybia scutellaris* IN *Pichia pastoris*

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Allergy caused by vespid sting is an important cause of morbility and mortality. Antigen 5 (Ag5) is the main allergen found in vespid venoms. However, Ag5 from *P. scutellaris*, a southamerican wasp, is a hypoallergenic variant. Our aim is to evaluate if the *P. scutellaris* Ag5 might be useful for specific immunotherapy. In the present work we report the expression conditions of *P. scutellaris* Ag5 in *Pichia pastoris*. The cDNA of *P. scutellaris* Ag5 was extended in its 3´end with a sequence coding for a His-Tag and cloned into PICZαA. This plasmid was linearized by SacI and integrated into *P. pastoris* GS115 genome by electroporation. Different final concentrations of methanol were assayed (0.5% - 2%) to optimize Ag5 secretion. The His-tagged protein was purified by affinity chromatography. Although Edman degradation unambiguously showed the 10 first amino acid- expected sequence, two major components were visualized in SDS-PAGE and Western-blots (25 and 29 kDa). The presence of two components was also confirmed by mass spectrometry analysis. Mass homogeneity of Ag5 in SDS-PAGE was only reached upon digestion with PNGase F showing that one of the components was glycosylated. The highest protein production, 30 mg/L, was obtained when 2% methanol was used. The high yields of *P. scutellaris* Ag5 obtained will allow us to conduct detailed structural and immunological studies.

BT-P10
CONSTRUCTION OF A GENETIC CASSETTE TO EXPRESS AN IMMUNOGENIC DENGUE VIRUS PROTEIN IN A PLANT SYSTEM

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Dengue virus genome encodes three structural (C, M and E) and seven non-structural proteins, being E the most immunogenic. E protein needs to be directed to the secretory pathway through the signal peptide in the N-terminus, where is N-glycosilated at Asn67 and Asn153. This N-glycosilation is essential for E protein folding and viral morphogenesis in human cells. The presence of an ER retention signal in the C-terminus of some proteins increases their stability and the production level of the recombinant protein targeted to the secretory pathway in plant systems. The aim of this work is to obtain a genetic construct with the E protein downstream of the rice Glycine-Rich Protein signal peptide (GRP) and upstream of the KDEL retention sequence. The protein will be expressed in a plant system and the effect of the KDEL sequence on the expression levels of the E protein will be assessed. An overlapping PCR was performed to obtain the construct containing GRP-E-KDEL and GRP-E with two different restriction sites (Kpn I and Xho I) for its further cloning into the cassette expression system. We used four different PCRs and five different primers designed specifically to assemble the correct constructs. The intermediate constructs were cloned into pGEM T Easy vector and sequenced. We are currently evaluating the expression levels of the E protein using a binary vector in a plant system.

BT-P11
PEPTIDE IMPRINTED POLYMER SYNTHESISED BY RADIATION-INDUCED GRAFT POLYMERISATION

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The design of polymer surfaces with molecular recognition capability is an exciting subject with potential application in the analytical and biotechnological fields. Molecular imprinted polymers (MIPs) are still in their infancy and new methodologies are required to broaden their application. In this work, MIPs were synthesised by simultaneous radiation-induced graft polymerisation in a polar medium using 4-vynilpyridine as specific monomer and dyethylenglycol dimethacrylate as the filler monomer. Bacitracin, a cyclic decapeptide, was used as the target molecule to develop MIPs grafted on macroporous membranes. Soluble ternary complexes of vinyl pyridine, bacitracin and copper (II) were obtained and characterised by visible spectroscopy in an aqueous medium. These complexes were grafted on macroporous polyethylene membranes. MIP materials showed ability to adsorb bacitracin (1.4 µmol/ml) in a higher amount than control polymers(0.4-0.8 µmol/ml). In addition, these materials showed a 5-fold greater selectivity to bacitracin than that of chemically modified bacitracins (acetyl-bacitracin and TNP-bacitracin).

BT-P12
SHORT PEPTIDE LIGANDS FOR AFFINITY PURIFICATION OF RECOMBINANT ERYTHROPOIETIN

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Recombinant erythropoietin (rhEPO) is used for therapeutics of anemia associated with chronic renal failure and for AZT-induced anemia of AIDS. Monoclonal antibody (mAb) affinity chromatography and dye affinity chromatography are alternative techniques nowadays in use for its purification. mAb are expensive, thus increasing the cost of the final product. Affinity chromatography with Cibacron Blue as the ligand is widely used, but the selectivity is not high. The use of short peptides would result in a more economic process than with mAb and a more selective process than with dyes. Divide-couple-recombine (DCR) method allows obtaining a library with all possible combinations of the amino acids in the form of "one bead-one peptide". Peptide ligands can be selected from immunoaffinity screening of the library. A combinatorial library containing the tetrapeptides XXXX (X=all the natural amino acids except cysteine) was synthesised by the DAR method using Fmoc chemistry. The immunoaffinity screening of the solid-supported library for EPO was performed. Beads showing a positive reaction to rhEPO were isolated and peptide sequenced. Eight of the peptides were synthesised and immobilised on Sepharose. All the peptide ligands showed affinity for rhEPO. In future work the purification method will be optimised.

BT-P13
AN EFFICIENT METHOD FOR PURIFICATION OF LUMBROKINASE FROM THE EARTHWORM EISENIA FOETIDA

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Fibrinolytic agents are widely used for thrombosis prevention and treatment. Lumbrokinases (LK) are thrombolytic enzymes from earthworms suitable for oral administration due to their high stability in the gastrointestinal tract. In this work we report a novel purification method for LK from the earthworm Eisenia foetida. Earthworms were homogenised with water. Polyethyleneglycol (PEG), monosodium phosphate and dipotassium phosphate were added to build an aqueous two-phase system. The clear top phase, enriched in PEG, contained all the LK activity together with other proteins and the bottom phase contained the homogenised tissue and the earth. The upper phase was loaded on a Q-Sepharose column equilibrated with 20 mM sodium phosphate buffer, pH 5.8. LK elution was achieved by addition of NaCl up to 0.5 M to the equilibrating buffer. The fraction containing LK was loaded to a Benzamidine-Sepharose column equilibrated with the elution buffer of the previous column. The LK was eluted with 0.1 M sodium acetate. The yield was 90 % and the purification factor 90. SDS-PAGE showed a single band corresponding to the LK molecular weight.

BT-P14
PURIFICATION OF BOVINE SEROALBUMIN BY AFFINITY METHOD

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This study is oriented to the development of particles or adsorbents of affinity with appropriate characteristics to be used in proteins purification by affinity ultrafiltration method. Affinity microparticles from yeast cells modified by chemicals and with the Cibacron blue 3GA ligand molecule immobilized to the wall cell by covalent bond were prepared. The amount of ligand immobilized to the cells was determined by spectrophotometric method after acid hydrolysis and high temperature of adsorbent material. The binding of bovine seroalbumin (BSA) on these particles were investigated in batch system and the adsorption capacity of the affinity microparticle (macroligand) was analyzed by adsorption isotherms assays. Results indicate that the adsorbent had a high capacity of immobilization of ligand (240 µmol ligando/gr cell). The ligand molecule bond to the cell increased the capacity of adsorption of the microparticle by BSA (up to 31 mg of BSA /gr adsorbent). The adsorption of BSA to the pigment is attributed to the structural similarity of bilirrubine with Cibacron blue 3GA, thus the ligand occupies the site that the protein (BSA) has to binding and transport of bilirrubine. From these results, we propose the use of this affinity macroligand in micro or ultrafiltration systems with membranes and the study of conditions for the design of separative process.

BT-P15
PURIFICATION AND PROPERTIES OF POLY(3-HYDROXYALKANOATE) DEPOLYMERASE FROM STREPTOMYCES SP

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The ability to degrade extracellular polyhydroxyalkanoates (PHAs) in the environment and the capability to use degradation products as sources of carbon and energy depends on the secretion of specific PHAs depolymerases. A bacterial strain with this activity was isolated from a compost sample. This organism, which was identified as Streptomyces sp., secreted poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) depolymerase into the culture medium when it was cultivated with PHBV as carbon source. The culture supernatant containing extracellular PHBV depolymerase was concentrated 95 fold by high pressure ultrafiltration (Stirred Ultrafiltration Cells Device) through a membrane YM 10, then was purified to electrophoretic homogeneity by ion exchange column chromatography on DEAE-Sephadex A-50 and gel filtration on Sephadex G-150. The molecular mass of the PHBV depolymerase was estimated to be 24.0 kDa by SDS-PAGE technique on 12% gel. The maximum activity was observed near pH 7.0 and optimum temperature 32ºC. The enzyme retained full activity between pH 4 and 6 and lost activity at temperatures above 45ºC. The activity of the enzyme were assayed with various metal ions and chemical reagent. Inhibition by these compounds showed a dependence on metal ions and both sulfhydryl and disulfide bonds. It was significantly inactivated by Tween-80 and not inhibited by ethylenediaminetetraacetic acid.

BT-P16
ISOLATION OF A PHOSPHOLIPASE A_2 FROM *Bothrops alternatus* VENOM

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Snake venoms toxins are frequently isolated by chromatographic methods. Aqueous two-phase systems have been successfully used for separation and purification of macromolecules because exhibit multiple advantages: good resolution, high yield, low cost and the protein remain their biological activity. In this work we applied a polyethyleneglycol (PEG)-phosphate-water system to isolate a phospholipase A_2 (PLA_2) from *Bothrops alternatus* (yarará) venom. A two-phase system forming by PEG 3350 - potassium phosphate, pH 7.0, was used where the partition of crude venom proteins were assayed. After mixing through inversion and leaving it to settle, the system was centrifuged for the two-phase separation. The top phase was removed and replaced by pure top phase; then, the partition was carried out again. Samples of each phase were withdrawn and their protein content and phospholipase, coagulant and proteolitic activities were assayed. Three partitions, that included the corresponding top phase renovations, were required to isolated the PLA_2 in the bottom phase. Another protein presents in this phase was removed by ultrafiltration (30 kDa). SDS-PAGE electrophoresis of the isolated enzyme showed a single typical PLA_2 band of 14,8 kDa. We concluded that this partition procedure constitutes a viable procedure for isolation and purification of PLA_2 from bothropic venom.

BT-P17

IMMOBILIZATION OF HYBRIDOMA CELLS IN SOL-GEL SILICA MATRICES

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The sol-gel process is an inorganic polymerization taking place in mild conditions, allowing the association of mineral phases with organic or biological systems. The possibility to immobilize drugs, enzymes, antibodies and even whole cells without loss of their biological activity led to the development of diagnostic tools, drug delivery carriers as well as new hosts for artificial organ design. Encapsulated cell technology has the potential to treat a wide range of diseases by the controlled and continuous delivery of biological products to the host. The aim of this study was to assess the influence of sol-gel immobilization on the function of hybridoma cells producing an anti-peptidoglycan receptor protein I-α monoclonal antibody (mAb). In order to immobilized hibrydomas, we resuspended cells in 50% of sol-gel precursor and 50% of complete medium (RPMI 1640, PS/PG, BFS), obtaining between $1.5x10^5$ to $12x10^5$ immobilized cells/ml. Higher mAbs yield were obtain with sol-gel containing $6x10^5$ cells/ml. Cell morphology was conserved as it was observed by optical and fluorescence microscopy. Further studies are being performed in order to improve cell viability and mAbs production. The design of such novel devices with significant added value when compared to current products is probably a key factor when foreseeing industrial developments of sol-gel materials in medicinal science.

BT-P18

IMMOBILIZATION OF BACTERIA IN SILICA MATRICES USING CITRIC ACID IN THE SOL-GEL PROCESS

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The aim of this work was to use citric acid in the sol-gel process to generate an inorganic polymer that allows bacterial survival for long periods of time and to study the influence of different storage temperatures. We compared gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus*, immobilized and preserved at different storage temperatures in silica matrices prepared by the method proposed. Immobilized *E. coli* and *S. aureus* in silica matrices were stored in sealed tubes at 20ºC, 4ºC, -20ºC and –70ºC for 4 months during which the number of viable cells was analyzed. Results show that the immobilization in silica matrices using citric acid, to neutralize the alkalinity of the silica precursors, makes the technique not only biocompatible but also easier to perform since polymerization does not occur immediately as it does when hydrochloric acid is utilized. Encapsulation of bacteria to design recombinant biosensors is one of the most active fields of biocers design. This work contributes to the study of new matrices with an ability to preserve different bacterial strains with possible applications in whole cell-silicate sensors.

BT-P19
SOLUBILIZATION OF INSOLUBLE INORGANIC PHOSPHATES BY IMMOBILIZED *Dark septate endophytes*

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Dark septate endophytes (DSE) are filamentous fungi found in the soil. They are known as pseudomycorrhizal fungi because of their capability to form symbiotic associations with plant roots which provide plants access to additional nutrient sources through hyphal enzyme activity or modifications of the rhizosphere, in exchange for photosynthetic carbon. Sol-gel encapsulation of microorganisms, in silicate matrices, is a well-documented technique to obtain chemically inert bioceramics. These matrices are useful to preserve viable cells for long periods of time and to protect them against adverse environmental conditions. In this work we isolated DSE ICFC-650/06 to demonstrate their solubilizing activity over insoluble phosphate -$CaHPO_4$ and $Ca_3(PO_4)_2$- before and after immobilization in silicate matrices. Immobilized and non-immobilized fungi were grown in a minimal culture media, including the insoluble phosphate source. After 7, 15 and 30 days of incubation, phosphate concentration in the solution was determined by capillary electrophoresis. We found that, after 7 days, immobilized DSE solubilized inorganic phosphate sources with higher activity over $CaHPO_4$ (0,14 g/l) than $Ca_3(PO_4)_2$ (0,02 g/l). Encapsulated microbial systems could be applied in preparation of microbial inoculants for introduction in soils enriched with insoluble phosphates.

BT-P20
BIOCHEMICAL BIOMARKERS OF AGROCHEMICAL EXPOSURE IN TADPOLES USED FOR WATER BIOMONITORING

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Biomarkers are appropriate tools for pesticide environmental impact evaluation. In *Bufo arenarum* larvae, glutathione (GSH)-S-transferase (GST), and carboxylesterase (CE) activities were proposed as biomarkers of pesticide effect. We tested biomarker responses in caged tadpoles in water channels during fruit production season, and compared them with those elicited by a transient exposure to azinphosmethyl (AM) (0.02-2 mg/ml; 4 h), the main pesticide applied in the region. Larvae showed a significant decrease of GSH (58-45%) at 1-2 week-exposure in water channels, correlated with a significant increase in GST activity (51-20%). CE was significantly decreased (26-37%), while the activity of acetylcholinesterase (AChE) was increased (24%) in the first week but then inhibited (34%) in the second week. These responses were emulated to a varying degree by laboratory assays: AChE and GST were induced, CE was inhibited and GSH was decreased immediately after 4 h exposures to AM; only GST and GSH showed changes during the recovery at 2 d. Biomarker responses are compatible with pesticide applications registered during the experiments. GST is known to be induced by oxidative stress through GSH depletion. CE acts as a buffer sequestering pesticide to avoid AChE inhibition until it is overloaded, and AChE induction at mild exposures may be related to the recently described resilient isoenzyme.

BT-P21
BIODEGRADATION OF STERILIZED BIO-PLASTIC

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Replacement of non-degradable polymers by biodegradable plastics is of major interest for polymer industry. Of special interest is the development of polymers from agricultural products that can be biodegraded by composting, Plastic material are frequently sterilized for industrial, clinical or research investigation. In this study, a protein-starch plastic product made in this GIB Laboratory, from Phaseolus vulgaris beans, was sterilized by gamma radiation, and it biodegradation capacity was analyzed. After irradiation the plastic conserved its mechanical properties. The degree of degradation was determined by carbon dioxide production trapped by alkaline medium and by weight loss. The study was performed in comparison with non irradiated control in a closed microcosm based on natural top soil and mature compost, under controlled temperature and water content conditions. Periodically the carbon dioxide production was determined. After two months of incubation, the sterilized and non-sterilized control were degraded until at least 60% of their total carbon content was oxidized. At the 15th day of incubation, a loss of about 30 % in weigh was detected. We conclude that the plastic materials processed by irradiation can be degraded as the non-irradiated control in similar periods of time. Sterilisation process not altered the biodegradation capacity of tested material.

BT-P22
BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF THE ANTARCTIC HYDROCARBON-DEGRADING CONSORTIUM M10

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Antarctica is inhabited by psychrophilic microorganisms, adapted to thrive under harsh environmental conditions. In bioremediation of soils, bioaugmentation (addition of bacterial inocula previously cultured in the laboratory) is an alternative to improve the in-situ processes. The inoculum has the advantage of adaptation to both, contamination and climate conditions. When a bacterial consortium is used, it is important to characterize their components, in order to optimize the scale-up of the inoculum. The M10 psychrotolerant consortium, previously isolated from hydrocarbon contaminated soil from Marambio Station, is being used for bioremediation in Antarctica due to its hydrocarbon degradation capacity. In this work, the culturable components of M10 grown in two different carbon sources (phenanthrene and gas oil) were isolated and characterized using two strategies: 1) evaluation of their morphologic and metabolic features, 2) analysis of the partial sequence of the 16S-rDNA gen. Most of the isolates belonged to the genus *Pseudomonas* and *Stenotrophomonas*. Members of the *Sphingobacteriaceae* family were also identified. A “consensus consortium” was proposed to reconstruct M10 in the laboratory. The evaluation of the hydrocarbon biodegradation effectiveness of this “consensus consortium” represents an important step for their applications in full-scale bioremediation processes.

BT-P23
INFLUENCE OF AGRICULTURAL PRACTICES ON SOIL BACTERIAL COMMUNITIES STRUCTURE

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In the last decades, the implementation of the no-till system became a widespread agricultural practice in argentinean "Rolling Pampa" region. It implies the use of herbicides, which are an irreplaceable tool for weed control. The objective of this work was to analyze the composition of the bacterial communities of three soils with different histories of crop cultures and herbicides regimes. In this way, PCR-single strand-conformation polymorphism (SSCP) of partial 16S rRNA genes was used as a genetic profiling technique followed by DNA sequencing of the highly abundant or specific bands of each profile. The dendograms obtained revealed a higher degree of similarity between the two soils with pasture, either a wild one (CC soil) or a seeded one (CPDB), regardless the fact that only CPDB soil has a history of herbicides use (2,4-DB). The third soil (CMA) had a smaller degree of similarity with the two pastures, but it can not be said if this is attributable to the different crop use (planted alternatively with maize and soy bean), to the different herbicide regimes (atrazine and glyphosate vs. phenoxy herbicides) or to both factors. Nevertheless, these results suggest that the cultured crop would be the main variable affecting the microbial communities composition in these soils, and that the presence or not of an herbicide treatment would not significantly affect soil biodiversity.

BT-P24
SPERMICIDAL ACTIVITIES OF *Solanum tuberosum* MEMBRANE INVOLVE (StAPs) PROTEASES ASPARTIC

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We have previously reported the purification of two Potato Aspartic Proteases (*St*AP1 and *St*AP3) which have antimicrobial activity towards two potato pathogens, *Fusarium solani* and *Phythophtora infestans* explained by interaction with the microbial surfaces and subsequent membrane permeabilization. Taking into account that spermicidal activity has been reported for other antimicrobial peptides, the aim of this study was to evaluate if *St*APs exert this activity on bovine and human sperm. The effect of *St*APs on sperm motility was studied by incubation of sperm suspensions with different doses of these proteins. The binding of *St*APs on spermatozoa surface was monitored by incubation with FITC-labelled *St*APs and membrane integrity was analyzed by SYTOX green uptake. Besides, the lytic effect of *St*APs towards human erythrocytes was also evaluated. The results showed that *St*APs reduced sperm motility in a dose-dependent manner, and 25 µM *St*APs completely abolished progressive motility. StAPs bind on postacrosomal and midpiece region of bovine sperm, and cause agglutination of spermatozoa. *In vitro* cell toxicity was observed by a dose-dependent increase in HOS negative sperm and SYTOX Green uptake. However, no toxic effect was observed on erythrocytes. In conclusion, *St*APs have spermicidal activity involving plasma membrane permea bilization by specific interaction with spermatozoa membrane.

BT-P25
ELECTROPHORETICAL ANALYSIS OF BOVINE WHEY HYDROLYSATES PRODUCED BY PLANT ENDOPEPTIDASES

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Enzymatic hydrolysis of proteins allows to eliminate unwanted fractions or to modify functional properties. Three cysteine plant endopeptidases with basic optima pH isolated by us were used to partially hydrolyze bovine whey proteins and their peptide patterns are reported. Plant proteases were isolated from fruits of *Bromelia hieronymi Mez* (*Bromeliaceae*) (I), fruits of *Bromelia balansae* (*Bromeliaceae*) (II) and latex of *Carica quercifolia* (*Caricaceae*) (III). Hydrolysis was carried out at different temperatures and pH 6.6. Hydrolysates were subjected to denaturing electrophoresis in tricine gels composed of a stacking gel (4 %T), a separating gel (10 %T) and a resolution gel (16,5 %T), which is especially suitable to resolve the mixture of peptides produced. The electrophoretic profiles were analyzed by densitography. Characteristic and differential proteolytic patterns on the main Whey components were obtained for each protease. After 60 minutes maximum degradation rate is observed for β-lactoglobulin (I, II, II), and a partial degradation for α-lactalbumin (I, II, III). New peptides appeared in all hydrolysates in a range of 5-15 kDa. The patterns can be correlated with the modification of functional properties in several industrial processes.

BT-P26
POLYPHENOL OXIDASE DOWN-REGULATION IMPARTS ENHANCED RESISTANCE TO *P. infestans* IN POTATO

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Phytophtora infestans, the causal agent of late blight is the most devastating disease of potato and to a lesser extent also a problem in tomato cultivation worldwide. During the 1840s, late blight disease was responsible for the Irish potato famine causing over one million fatalities. Nowadays, grown potato cultivars lack adequate blight tolerance and disease management is currently based on the application of continuously increased amounts of fungicides due to the pathogen ability for developing resistance. It's estimated that late blight cause annual loses of over U$S 2.750 million to developing countries and that these countries invest about U$S 750 million annually in fungicides to its control. In our laboratory we developed transgenic potato plants with reduced enzymatic browning activity and reduced bruising susceptibility by knocking down the polyphenol oxidase (PPO) genes and evaluated their resistance to *P. infestans*. *In vitro* infection assays with *P. infestans* sporangia displayed an enhanced disease resistance in the transgenic potatoes. We hypothesize that this resistance increase to potato late blight could be a consequence of the incapacity of PPO to inactivate defence molecules like phenolic compounds normally formed against fungal infections. In order to evaluate this hypothesis we are measuring phenolic compounds kinetic synthesis, accumulation and half-life.

BT-P27

KNOCKOUT OF STEROL DESATURASE GENES BY SOMATIC TRANSFORMATION OF *TETRAHYMENA THERMOPHYLA*

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The non-pathogenic ciliate Tetrahymena has a unique set of desaturases including C5, C7 and C22 sterol desaturases. This set of desaturases has potential for biotechnology purposes, particularly the C7 sterol desaturase, as it may be used for decreasing cholesterol content in foodstuffs of animal origin with simultaneous enrichment in pro vitamin D3. Previous in vitro characterization of C7 and C22 cholesterol desaturases established unambiguously oxygen, cytochrome b5 and NADH requirements. In order to identify the genes encoding for these desaturases, a somatic knockout out approach was selected. As a first attempt the sequences 51.m00243, 270.m00036 and 124.m00110 were chosen (TIGR Data base). The constructs were made by overlapping PCR, using flanking regions inserted into a neo 3 cassette, which confers paromomycin resistance, under a cadmiun inducible metallothionein (MTT) promoter. Around 20 transformants for each sequence were obtained by somatic transformation on T. thermophila CU 428 strain. After phenotypic assortment the sterol pattern of the mutants grown in different sterols were analyzed. One of the mutants (270.m00036 KO) showed the expected pattern of sterols corresponding to a KO of a C-5 sterol desaturase. By this way the first sterol desaturase gene could be identified in ciliates.

BT-P28

ENHANCEMENT OF IFN-α2 PHARMACOKINETIC PROPERTIES THROUGH GLYCOENGINEERING

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Interferons are a large family of closely related cytokines that block viral infection, inhibit cell proliferation and modulate cell differentiation. IFN-α2 has been approved for the treatment of a number of diseases, including chronic hepatitis B and C, renal cell carcinoma and chronic myelogenous leukemia, among others.
However, the clinical use of this cytokine has been restricted due to limitations of the standard interferon alfa formulations, which include rapid absorption from the injection site, large volume of distribution, rapid clearance by the kidney, short serum half-life and significant acute and chronic side effects. Several strategies, including pegylation, have been used to enhance IFN activity by decreasing its clearance and prolonging the duration of its action. For this purpose, a glycoengineering approach was used to construct fourteen one site N-glycosylated IFN-α2 analogs which were expressed in CHO cells. The specific biological activity, as well as the degree and pattern of glycosylation, were variable according to the position of the N-glycosylation site. The pharmacokinetic profile of a one-site glycosylated mutein showed a 1.4-fold increase of the area under curve after subcutaneous administration compared to the non glycosylated cytokine. This strategy may be used in order to obtain hyperglycosylated variants with increased therapeutic activity.

BT-P29
SINGLE-DOMAIN LLAMA ANTIBODIES INHIBIT TRANS-SIALIDASE ACTIVITY OF *TRYPANOSOMA CRUZI*

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Trypanosoma cruzi trans-sialidase (TcTS) constitutes a key molecule in both the establishment of the infection and the development of pathologic abnormalities related with Chagas disease.
Llamas produce, additionally to conventional antibodies, unusual immunoglobulins devoid of light chains. Their binding site is formed solely by one variable region (VHH). The binding strategies of these VHH are very particular, their CDR3 region form long extensions that can protrude into cavities on antigens, e.g. the active site crevice of enzymes being suitable for the development of enzyme inhibitors. The cDNA isolated from lymphocytes of llamas immunized with TcTS was used to generate by PCR a VHH library composed of $1x10^6$ clones. Phage display was used for panning and screening of the library. Preliminary TcTS inhibition screening allowed us to identify four strong inhibitors clones (SI), which have the same CDR3's length sharing 16 out of 17 CDRs residues, and few differences along the rest of the sequences. Inhibitory capacity, affinity measurements and native/denature recombinant enzyme recognition assays are shown. We present different evidences suggesting that the SI could be recognizing an epitope probably overlapped to the active site of the enzyme.

BT-P30
GENERATION OF LLAMA SINGLE DOMAIN ANTIBODIES THAT INHIBIT AN ADP-RIBOSYLTRANFERASE BACTERIAL TOXIN

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Llamas produce unusual antibodies composed only of heavy chains. The antigen combining site of these antibodies is formed solely by the heavy-chain variable domain (VHH). VHHs are small, easily produced, highly soluble and very stable. Their CDR3 form long finger-like extensions that can protrude into cavities on antigens, e.g. the active site crevice of enzymes. Salmonella enterica is an intracellular bacteria pathogenic for humans and animales, causing gastroenteritis and typhoid fever. This bacteria express one enzyme called SpvB, that is essential for Salmonella virulecia. SpvB catalyzes the transference of the ADP-ribose moiety from NAD to actin, causing the depolymerization of actin filaments and cell death.
We generated VHHs with inhibitory capacity against SpvB. We isolated cDNA from lymphocytes of llamas immunized with SpvB, generating a VHH library of $4x10^7$ transformants. Phage display allowed an enrichment of binders through consecutive rounds of panning. Five positive clones classified according to the length and variability of their CDR3 inhibited SpvB in an enzymatic test. Two of these clones, VHH1 and VHH5, showed the strongest inhibition. These VHHs were subcloned in an eukaryotic expression vector and their cytoplamic expression will be analyzed. Transfected cells expressing VHHs will be infected with Salmonella enterica to test their effect on virulence.

BT-P31
RESPONSABILITY OF IgG ANTI-PLA2 IN THE REACTION BETWEEN *P. PATAGONIENSIS* VENOM & ANTI-CROTALIC SERUM

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Cross reactivity among viperid venoms and their commercial sera is perfectly known, whereas there is little information about immunoreactivity between colubrid venoms and anti-viperid sera. Herein, the aim of the present work was to detect if the anti-crotalic serum is able to react with the venom of the opisthoglyphous colubrid snake *Philodryas patagoniensis*. In order to analize this, immunodiffusion assay was carried out, which showed a single precipitation line when *P. patagoniensis* venom was tested with commercial anti-crotalic serum. In order to investigate if IgG anti-PLA2 was responsible for this cross reaction, immunodiffusion and western blotting assays were carried out with this antibody. Both tests demonstrated that IgG anti-PLA2 does not participate in this cross reactivity. Moreover, *P. patagoniensis* venom showed to be devoid of PLA2 activity. These preliminary studies confirm that proteins from *P. patagoniensis* venom do not have common antigenic sites with the main component of crotalic venom, the PLA2 from the crotoxin complex. The identity of those antigens shared by both venoms will be further investigated.

BT-P32
USE OF DIGE FOR THE DISCOVERY OF MOLECULAR PATHWAYS AFFECTED BY THE TUMORIGENIC PROTEIN SPARC

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SPARC is a secreted glycoprotein related to tumor progression and metastasis and overexpressed in different tumors. However, little is known about the molecular mechanisms affected by SPARC during tumor growth. We have developed stable cell clones of human melanoma cells (L3B9 and L2F6) in which SPARC expression was downregulated by the use of 2 different RNAi. SPARC downregulation diminished (L3B9) or abolished (L2F6) tumor growth in a murine in vivo model. In order to identify putative secreted proteins that may mediate SPARC biological function, we performed a proteomic analysis of conditioned media of clones L3B9 and L2F6 and the control cell line LBLAST. For this purpose we applied the novel technology of DIGE (DIfferential Gel Electrophoresis) that proved to be superior to traditional silver-stained 2D electrophoresis in many aspects. Along with a thorough description of processing and normalization steps applied to our DIGE data, we will show that unsupervised multivariate analysis was able to identify a set of spots that could distinguish the three different treatments used in the study. These results define a set of proteins potentially related to SPARC role in tumor progression. Further work is in progress to validate our candidates both technically and biologically.

BT-P33
EVALUATION OF A COLORIMETRIC PCR SYSTEM FOR THE DETECTION AND TYPING OF HUMAN PAPILLOMAVIRUSES (HPV)

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In developing countries, the introduction of HPV DNA testing as an adjunct to cytological screening programs has been delayed due to the lack of high performance and cost effective diagnostic nucleic acid methods. In this study we report the development and evaluation of the L1HPVPCR, a PCR-based method for the detection and typing of five of the most prevalent high risk HPV types. The technique is a user-friendly system that allows inexpensive and accurate HPV DNA detection and typing. Different cutoff points for HPV detection were determined using reproducibility analysis and receiver operating characteristic curves to ensure good analytical sensitivity and clinical effectiveness. We used the L1HPVPCR assay to estimate the prevalence of HPV infection in 127 women at risk of cervical cancer from the city of Rosario (Argentina), where no epidemiological data has been previously reported. Further, we explored the clinical utility of the L1HPVPCR assay respect the Pap smear using a combined diagnosis of cytology, histology and colposcopy as gold standard. Our results indicate the assay described here provides a tool for accurate HPV DNA testing and could be applied in regions where no commercial tests are available. Besides, this assay will allow the identification of HPV16-positive women to analyze the viral physical status as a marker for malignant progression.

CB-P01
LOCALIZATION OF NEW COMPONENTS OF THE EGG EXTRACELLULAR MATRIX OF AMPHIBIANS AND FISH

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The importance of the oocyte extracellular matrix during fertilization has been studied in several animal species. However, many of its roles have not been elucidated, being sometimes controversial. Four major glycoproteins have been reported in the Vitelline Envelope (VE) of *Bufo arenarum* egg: gp120, gp75, gp41 and gp38. Recently, we identified two proteins of 115 kDa (p115) and 106 kDa (p106), that are also present in the VE but possess different biochemical characteristics. The present work is focused on the characterization and localization of these new components and analyzes the existence of homologous counterparts in other animals. Antisera were raised against each protein. P106 and p115 were detected both in oocyte plasma membrane and VE extracts. Same results were obtained by inmunohistochemistry (IHQ) studies of *B. arenarum* ovary. The plasma membrane inmunoreactivity was lost after fertilization, but fertilization envelope (FE) remained positively marked. IHQ studies of *X. laevis* and *Danio rerio* ovaries indicated positive inmunoreactivity in both cases with same localization that in *B. arenarum*. The roles of p115 and p106 have not been elucidated yet, although it seems that both components are not exclusive of *B. arenarum* specie. Our preliminary results suggest that they could be involved in the VE adhesion to plasma membrane which is lost after fertilization.

CB-P02
HOMOLOGY OF A GLYCOPROTEIN OF B.arenarum EGG VITELLINE ENVELOPE WITH ZONA PELLUCIDA COMPONENTS

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In previous work, the morphological and biochemical changes in *B. arenarum* eggs envelopes following passage through the oviduct were reported. The transformation of coelomic envelope (CE) into vitelline envelopes (VE) leads to the acquisition of fertilizability and involves the cleavage of glycoprotein components. The differences between electrophoretic patterns of CE and VE indicated that an oviducal protease (oviductin) digests CE glycoproteins gp84 and gp55; decreasing the relative concentrations of gp48 and gp42 with a noticeable increase in gp39. The aims of this work were to know gp39 identity and evaluate its expression pattern in the oocytes during oogenesis. In order to localize gp39 in the egg we performed immunohistochemical studies with polyclonal antibody (AB) obtained in rabbit using purified gp39 as antigen. The specificity of AB determined by Western Blot showed that AB specifically reacts with gp39 of the envelopes obtained from eggs after their passage along the oviduct. However AB recognized also gp55, 48 and 42 on envelopes obtained from eggs previous their transit through the oviduct. On the other hand three peptides from trypsin digestion of gp39 were obtained by preparative HPLC and sequenced. Bioinformatic analyses showed that these peptides have 100% identity with a deduced sequence of ZPC homologue cDNA extracted from *B.arenarum* ovary.

CB-P03
CLONING AND EXPRESSION OF N-Ac GLUCOSAMINIDASE OF XENOPUS LAEVIS

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Sperm-egg recognition events involve carbohydrate-protein interactions in mammals. Among amphibians, sperm binding in Xenopus laevis is mediated at least by N-Ac Glucosamine residues of the vitelline envelope (VE). We have shown that N-Ac glucosaminidase is the most important glycosidase activity in Bufo arenarum sperm and that binds to components of the VE. The aim of the present work was to produce a specific N-Ac Glucosaminidase antiserum as a prerequisite for its biochemical and functional characterization, since antibodies of other animals are not available. By in-silico searching on EST´s from Xenopus laevis embryos, we identified clones codifying for the cDNA of the enzime that were gently provided by the National Institute of Basic Biology of Japan. A sequence with the complete ORF was subcloned in four different bacterial expression vectors and the resulting product shown to be toxic for E. coli in all the cases. To overcome this problem, a sequence coding for an amino terminal fragment (18.6 kDa) of the enzime was cloned and the expression conditions optimized. The recombinant protein, GST fused, was purified with Glutathione-Sepharose followed by SDS-PAGE and electroelution, and used to produce antibodies in rabbits. The antiserum (titer 1/750 for 0.15 µg of the fusion protein) specifically recognized the recombinant protein but not GST.

CB-P04
PHYSIOLOGICAL SPERM MODIFICATIONS PRIOR TO FERTILIZATION

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Mammalian sperm acquire fertilizing capacity after residing in the female tract, where physiological changes named capacitation take place. In animals with external fertilization as amphibians, gamete interactions are first established between sperm and molecules of the egg jelly coat (JC). Since dejellied oocytes are not normally fertilized, the aim of this study was to determine if the JC of the toad promotes a "capacitating" activity on homologous sperm. We found that preincubation of sperm in Egg Water (diffusible substances of JC, named EW) for 90-180 sec is sufficient to render sperm transiently capable of fertilizing dejellied oocytes. The fertilizing state was related to an increase of tyrosine phosphorylation in sperm proteins from 50 to 200 kDa. Incubation of sperm with Tyrphostin A25 20 μM for 30 min prior to insemination, which inhibited phosphorylation at IC50 of 2 μM, significantly decreased fertilization rates (3.79±2.76%) compared with controls (28.1±3.83%) ($p<0.001$). Moreover, after incubation of sperm in EW for 15 min, the sperm cholesterol content decreased to 78.9 ± 3.8 %, and preincubation of gametes in cholesterol-3-sulfate 200 nM for 90 min significantly decreased fertilization rates (12.6±2.45%) compared with controls (64.8±0.74%) ($p<0.001$). The acquisition of sperm fertilizing capacity was shown to be, as in mammals, related to physiological modifications.

CB-P05
EFFECT OF CALTRIN PROTEIN ON RAT SPERM PHYSIOLOGY

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Caltrin (calcium transport inhibitor), the small and basic protein from seminal vesicle secretion binds to the head of rat epididymal sperm covering specifically the acrosomal region, and inhibits extracellular calcium uptake (Coronel et al., JBC 265:6854, 1992). We also demonstrated that rat caltrin inhibits the activity of trypsin and acrosomal proteases as well as the discharge of acrosomal enzymes probably by inhibition of spontaneous acrosomal exocytosis (Winnica et al., Biol Reprod 63:42, 2000). To obtain more information about the functional role of caltrin on sperm physiology, we examined the effect of this protein on rat sperm capacitation and acrosomal exocytosis (AE) by analyzing protein tyrosine phosphorylation and acrosomal hyaluronidase release of epididymal spermatozoa incubated under capacitation conditions. Percentages of acrosome reacted sperm were also evaluated. The presence of caltrin in the incubation medium did not modify the pattern of sperm protein tyrosine phosphorylation but inhibited hyaluronidase release and reduced the percentage of AE. Viability and motility were not affected by the protein. Data suggest that rat caltrin prevents acrosomal exocytosis without affecting the mechanisms of sperm capacitation that involve protein tyrosine phosphorylation.

CB-P06

EFECT OF SPERM BINDING GLYCOPROTEIN ON THE SPERM PHYSIOLOGY

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Millions of spermatozoa are deposited in the female genital tract at ejaculation and only few thousand reach the oviduct and bind the oviductal epithelium. The oviductal reservoir formed in this way may modulate sperm functions. In pig, we have identified and characterized a glycoprotein from oviductal epithelial cells, able to bind to spermatozoa, SBG (sperm binding glycoprotein). SBG may be involved in the binding of sperm to the oviductal epithelium. The aim of this work was to study the influence of SBG on the physiology of sperm. The physiological status of sperm was assessed through Wells-Awa (W-A) staining. The acrosomal reaction (AR) was evaluated with W-A directly and the capacitation was estimated by progesterone induction followed by W-A staining. Sperm suspensions were incubated in capacitating and non-capacitating media, with different concentrations of SBG. The percentage of sperm that underwent AR at different time intervals was estimated. We observed that SBG increased the rate of AR sperm in capacitating medium. Such effect was not present in non-capacitating conditions. The ability of SBG to promote AR could be related to a selection effect, deleting those sperm that reach the oviduct in an inappropriate physiologic condition.

CB-P07

MARCKS PROTEIN PARTICIPATES IN ACROSOMAL EXOCYTOSIS IN HUMAN SPERM

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Although PKC has been proposed as a key component of the acrosomal exocytosis, the targets for its phosphorylation activity are unknown. MARCKS is a prominent substrate of PKC in many cell types; nevertheless the presence of MARCKS in sperm, as a possible PKC substrate has not been investigated. Using a specific antibody against MARCKS, Western blot analysis reveled the presence of MARCKS in human sperm. Furthermore, immunocytochemistry assays showed that MARCKS localized at the acrosomal region in human sperm. This localization prompted us to investigate if MARCKS might participate in acrosomal exocytosis. To test this hypothesis, we expressed MARCKS effector domain (ED) as a GST- fusion protein. Then, using the Streptolysin O-permeabilized sperm model, we investigated the effect of GST-MARCKS ED on the acrosome exocytosis stimulated by a PKC activator, phorbol 12-myristate, 13-acetate (PMA). GST-MARCKS ED inhibited specifically acrosomal exocytosis stimulated by PMA in a concentration-dependent manner. In addition, GST-MARCKS ED also inhibited the acrosomal exocytosis stimulated by calcium and Rab3A -two well known activators of this secretion process. These results show that MARCKS is expressed in human sperm and suggest that MARCKS might be an important component in acrosomal exocytosis signal transduction pathways in human sperm.

CB-P08

DEPHOSPHORILATION OF SYNAPTOTAGMIN VI DURING ACROSOMAL EXOCYTOSIS IS MEDIATED BY CALCINEURIN

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Acrosomal reaction (RA) is a calcium regulated exocytosis that is essential for fertilization. Synaptotagmins (syt) are transmembrane proteins with two cytoplasmatic domains that binds calcium and phospholipids (C2A y C2B). Syt have been involucrated in exocytosis as calcium sensors. We have demonstrated that syt VI is required for RA. This protein is phosphorilated in resting sperm, and become dephosphorilated after stimulation. Our aim is to unveil the phosphatase involved in this process. We have used the streptolysin O permeabilizated sperm model and have evaluated RA by staining with fluorescein-isothiocyanate-coupled Pisum sativum (FITC-PSA). To detect the phosphorylated state we have used an antibody antiphosphosynaptotagmin (antiPP). The antiPP inhibition is lost after calcium stimulation that indicate dephosphorylation of endogenous syt. When sperm were stimulated with Rab3A, antiPP conserved the inhibiting effect like in resting sperm. This indicate that dephosphorylation of syt requires calcium. So, calcineurin, a calcium/calmodulin-activated serine/threonine protein phosphatases, could be implicated in this process. We incubated sperm with Cyclosporin A and FK 506, two calcineurin inhibitors, and we observated that both inhibited the RA. Our results indicate that dephosphorylation of syt require calcium and this process is mediated by calcineurin.

CB-P09

EFFECT OF GLUCOCORTICOIDS ON TESTOSTERONE PRODUCTION IN TOAD TESTES

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In mammals, glucocorticoids (GC) inhibit testosterone (T) synthesis through the glucocorticoid receptors (GR). In toad testes, a GR similar to rat receptor was also described. The aim of this work is to analyze the effect of GC on spermiation and steroidogenesis by culturing testes with dexamethasone (DEX), corticosterone (B), with or without RU486 and glycyrrhetinic acid (GA), an 11β-hydroxysteroid dehydrogenase type 2 (11βHSD-2) inhibitor. After 24-hr incubation, spermiation and T production were assayed. T was measured by RIA while hCG-induced spermiation by counting spermatozoa in the media. GC decreases T secretion, the effect being abolished by RU486. However, the ratio hCG-induced/basal T production was not modified. The effect of low doses of GC was enhanced by GA, suggesting that 11βHSD-2 modulates the effect. After 24-hr pre-incubation, hCG-induced spermiation decreases but in the presence of 15 nM DEX or 15 and 150 nM B spermiation is similar to control. These results suggest that physiological concentrations of CORT revert the negative effect of pre-incubation on spermiation. The presence of GA does not modify the effect of CORT on hCG-induced spermiation. As with T biosynthesis RU486 completely abolished the effect of CORT suggesting that the action of GC on spermiation and T production is due to its interaction with the type 2 or GC receptor on different cell types.

CB-P10
CONFORMATIONAL ANALYSIS OF THE VITELLINE ENVELOPE GP75 FROM *BUFO ARENARUM* EGGS

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Bufo arenarum eggs envelope possess at least four mayor glycoproteins designated gp120, gp75, gp41 and gp38. Post-fertilization, glycoprotein gp75, a *Xenopus laevis* ZPA homolog, undergoes limited proteolysis by cutting 28 amino acids at the N-terminus and this modification seems to be involved in the prevention of the polyspermy and hardening of the EV. The sequence identity between *Bufo* and *Xenopus* ZPA at the N-terminal domain is 50%. The aim of this work was to study comformational changes in *Bufo arenarum* ZPA upon fertilization. In order to determine whether the N-terminal peptide of ZPA cleaved by the cortical granule proteases released during fertilization, was lost or retained from the EV structure, specific antibodies that recognize a peptide at the N-terminal domain of ZPA were used in a Western blot of egg envelope samples. Under reducing conditions, EV showed strong reactivity to the antibodies but the fertilization envelope (FE) showed no reactivity. However under non-reducing conditions both EV and FE showed the same level of antibody binding. These antibodies were probed with fixed embryo sections and showed strong reactivity. The N-terminal peptide fragment of *Bufo arenarum* ZPA remained disulfide bond linked to the ZPA glycoprotein moiety following proteolysis caused by cortical granule proteases during the fertilization process.

CB-P11
POST-TRANSLATIONAL REGULATION OF CELLULAR NUCLEIC ACID BINDING PROTEIN DURING EMBRYOGENESIS

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The zinc-finger cellular nucleic acid binding protein (CNBP) is a single-stranded nucleic acid binding protein essential for normal mouse and chicken forebrain development. We had reported that in amphibian and fish CNBP has a dual sub-cellular localization. CNBP amino acid sequences show putative proteolytic and phosphorylation sites. Phosphorylation and proteolysis are important post-translational modifications in embryogenesis. We showed that zebrafish CNBP (zCNBP) is differentially phosphorylated *in vitro* during embryogenesis by a cAMP-dependent protein kinase, reaching a maximum at 24 - 48 hours post-fertilization. Noteworthy, in these *in vitro* assays we saw that zCNBP protein level decreases at the time the phosphorylation begins to arise, suggesting that both phenomena could be related. Here, we analyzed by western blot assays the *in vivo* zCNBP proteolysis during zebrafish embryogenesis, observing the same pattern than in the *in vitro* assays. To analyze the possible relationship between both processes, we generated a zCNBP mutant not able to be phosphorylated by embryonic extracts. This mutant protein was proteolysed as the wild type zCNBP. This suggests that phosphorylation status of zCNBP may not be the signal to induce zCNBP proteolysis.

CB-P12
CTP: PHOSPHOCHOLINE CYTIDYLYLTRANSFERASE AND CNBP EXPRESSION IN TROPHOBLASTIC CELL AND PLACENTA

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In normal human pregnancies, a villous trophoblast in the placenta consists of a population of proliferating cytotrophoblasts that differentiate and individually fuse into a syncytiotrophoblast. In the first trimester, the cytotrophoblast proliferation rate is high and the proportion of proliferative villous cytotrophoblasts decreases thereafter. In spontaneous abortions, the trophoblasts are less hyperplasic. CNBP stimulates cell proliferation and induces the proto-oncogene c-myc expression. On the other hand, the phosphatidylcholine (PC) requirement increases for membrane biosynthesis during cells growth and differentiation. Thus, we investigated the expression profile of CNBP and different isoforms of CTP:phosphocholine cytidylyltransferase (CCT; enzyme that catalyze the slow step of the PC-biosynthetic pathway). We measured the expression of CNBP and CCT by RT-PCR and Western Blot. We analyzed samples from human and mouse placentas and cultivated human choriocarcinoma cell line (Jeg-3) with and without metotrexate treatment (differentiation condition). The results demonstrated that CCTβ and CNBP have differential expression among the analyzed tissues contrary to the ubiquituosly expressed CCTα. Thereafter, trophoblasts from placentas of normal pregnancies, abortions, and Jeg-3 cells seem to differ in terms of their respective rates of proliferation and degeneration.

CB-P13
INVOLVEMENT OF HSP70 AND INTEGRIN β1 IN THE FERTILIZATION OF *BUFO ARENARUM* OOCYTES

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We previously provided evidence suggesting a role for HSP70 and a RGDS-sensitive integrin in sperm-oocyte plasma membrane interaction. The aims of this work are to: (i) analyze the effects of a RGDS peptide and the antibodies against HSP70 on the fertilization process; and (ii) study the presence and subcellular localization of β1 integrin in oocytes. We performed in vitro fertilization assays with increasing concentration of sperm (10^4, $5x10^4$, 10^5, $3.3x10^5$ y 10^6cells/ml) in the presence of RGDS 1mM or a-HSP70 antibody (1:16 dilution) and in the absence of both (control). At all sperm concentrations the presence of RGDS or a-HSP70 antibody decreased fertilization efficiency. At $3.3x10^5$ cells/ml, the differences were maximum and significant (in fertilization percentage, mean±SEM: control=78.2±4.5, RGDS=43.4±10.9 a-HSP70=51.5±5.0, n=4, $p<0.05$). By using differential centrifugation of oocytes, we isolated plasma membranes, microsomes and the cytosolic fraction. By Western blot using the 8C8 antibody (Hybridoma Bank, against the *Xenopus laevis* homolog), we detected only in plasma and microsomal membranes, four major bands of 33, 51, 104 and 133 kDa, corresponding to mature β1 integrin and its precursors. Our results suggest that β1 integrin expresses in *B. arenarum* oocytes and that both, β1 integrin and HSP70, are involved in the oocyte-sperm interaction at the plasma membrane level.

CB-P14
EXPRESSION OF EBAF IN PREGNANT RAT OVIDUCT. SINTHESYS AND TISSUE DISTRIBUTION

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Ebaf, a member of the TGF-β family, is involved in the regulation of other citokines of this family such us nodal, activin, BMPs, TGF-β1 and Vg1. Previously we had reported the identification, isolation, cloning and characterization of the temporal expression levels of *ebaf* in the oviduct during the early pregnancy of the rat. The aims of the present work were to confirm the synthesis of Ebaf protein in the oviduct and to study its tissue localization. The analyses of the deducted aminoacidic sequence (Accession No. AAV31601.1) revealed a proprotein of a 40,92 kDa with two putative cleavage sites, which could origin two processed protein forms of 32,86 and 26,09 kDa. As *ebaf* mRNA levels were higher during the fourth day of pregnancy, compared to the estrous cycle and other days of pregnancy, we studied samples at this stage. A western blot assay with 100 ug of oviduct total protein was performed, a positive (testicle), a negative (bowel) and a secondary antibody controls were included. We found two bands one of 41 KDa (precursor protein) and other of 26 kDa (processed form). We localized Ebaf in the stroma of the oviduct and mesosalpinx (the peritoneum of the broad ligament) by immunohistochemical techniques. Taken into account these results we suggest that Ebaf is synthesized and processed in the oviduct were it might have a relevant function during the early stages of pregnancy.

CB-P15
EFFECT OF MODERATED ZINC DEFICIENCY ON TESTOSTERONE AND ANDROGEN RECEPTOR IN EPIDIDYMIS

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In previous studies we have analyzed the change in antioxidant defense system under two months of treatment undergoing a moderate zinc deficiency. In all cases we obtained differences in epididymis in the Zinc deficiency (ZD) group. Here we present the effect of Zinc deficiency on testosterone level (T), androgen receptor (AR) and TGF-beta1 expression and histology in epididymis. Wistar male rats were separated in three groups and fed a Zinc-deficient diet (ZD), a Zn-adequate control diet (Co). Some ZD rats received control diet ten days before sacrifice (Ral). Pieces of caput and cauda epididymis were processed for light microscopy. Proteins were resolved by SDS-PAGE and were identified by Western blot and normalized using the values obtained for β-actin. Testosterone decrease in ZD vs Co and Ral ($p<0.01$ and $p<0.05$, respectively). In epididymal caput AR and TGF-beta1 were not modified but in cauda AR and TGF-beta1 increased significantly in ZD vs Co and vs Ral ($p<0.05$ in both cases). In epididymal caput nucleus of principal cells appeared lightly altered, but in the cauda epididymal epithelium is disorganized and present infiltration in the perivascular zone as well as in the basal lamina. Perhaps the increased expression of TGF-beta1 contributes to modify the expression of AR especially in cauda. In this region the changes are more important, such as was reflected by the histology.

CB-P16

RAB1 GTPase MODULATES THE EXPRESSION OF GENES INVOLVED IN PROTEIN TRAFFICKING

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The GTPase Rab1 is essential for Endoplasmic Reticulum to Golgi transport. By using genome-wide microarray analysis on HeLa cells expressing Rab1 and mutants we have identified (SAIB 2005) specific Rab1 dependent changes of gene transcription. Results showed that about 300 probe sets were significantly regulated by expression of Rab1. Here, different non-related criterions were used to select interesting genes, from the reported microarray results, to be validated by quantitative RT-PCR (TaqMan). Genes that code for proteins involved in membrane traffic (6), transcription factors (2), and two proteins used as controls, were selected. Our results showed that quantitative RT-PCR results showed very good correlation with Affymetrix values (Pearson´s r > 0.6 in most cases). Moreover, western blot analysis (using inducible-stable transfected Rab1 cell line) indicated that quantity of proteins coded for three of the selected genes, was also increased in a Rab1-dependent manner. Finally, to define if expression of Rab1 modulated-genes is achieved by signaling pathways involving the proximal promoter region, they were cloned into a luciferase reporter plasmid and the promoter activity was evaluated in Rab1 inducible-stable transfected cell line. Our study aims to understand the molecular mechanisms that may explain how Rab1 modulates expression of genes related to protein trafficking.

CB-P17

PTP1B IS REQUIRED FOR THE TRAFFICKING AND EXPRESION OF N-CADHERIN AT THE CELL SURFACE

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N-cadherin function at the cell surface depends upon binding of β-catenin to its cytosolic domain, event that also requires PTP1B association with cadherin. β-catenin binds to the precursor of N-cadherin (preN-cad) at the endoplasmic reticulum (ER), and both travel as a complex to the cell surface. Since PTP1B is associated to the cytosolic face of the ER we asked if it binds to the preN-cad and its role in cadherin trafficking. To identify and isolate the preN-cad we inserted the HA tag in the propeptide sequence of the N-cadherin. Colocalization analysis and immunoprecipitation assays using anti-HA antibodies indicate a lack of association between PTP1B and preN-cad. Furthermore, in cells with/without PTP1B, similar proportions of β-catenin associate with the preN-cad, and these are not affected by pretreatment with pervanadate. These results suggest that PTP1B targets to the mature form of N-cadherin and that β-catenin/preN-cad association is not regulated by tyrosine phosphorylation. Combination of pulse-chase and biotinilation assays show that cells with/without PTP1B express similar levels of total N-cadherin; however, the levels at the plasma membrane (PM) are lower in the PTP1B-deficient cells. This is consistent with a delayed arrival of N-cadherin to the PM in the same cells. Our results uncover a new role of PTP1B in the trafficking of N-cadherin. Supported by ANPCyT.

CB-P18
THE GTPase ARF6 REGULATES HeLa INFECTION WITH *COXIELLA BURNETII*

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ARF6 is a GTPase involved in vesicular trafficking processes such as phagocytosis. Also, it regulates the actin dynamics through PIP5K and Rac proteins. It is known that actin cytoskeleton dynamics is critical for phagocytosis. After internalization, bacteria are transported to lysosomes for their degradation. The intracellular pathogen *C. burnetii* replicates in a parasitophorous vacuole (PV), a compartment with lysosomal characteristics. Our interest is to study the role of ARF6 and actin in the phagocytosis process of *C. burnetii*. HeLa cells were transfected with plasmids that encode ARF6WT (wild type), Q67L (constitutively active) or T27N (constitutively inactive) mutants fused to hemaglutinin (HA) or green fluorescent protein (GFP) tags. After 16 h infection, the cells were washed to remove extracellular bacteria and incubated for another 24h. Cells were processed by indirect immunofluorescence to detect *C. burnetii* or HA tag using specific antibodies and analyzed by fluorescence and confocal microscopy. We observed that infection was significantly inhibited in cell expressing the T27N mutant comparing with cells expressing the WT or Q67L mutant of ARF6. Interestingly, we observed that ARF6 WT and Q67L mutant were recruited to the PV together with actin. These results suggest that *C. burnetii* infection of host cells is regulated by ARF6 and actin.

CB-P19
VAMP7, A POSSIBLE SNARE INVOLVED IN EXOSOMES RELEASE IN K562 CELLS

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Multivesicular bodies (MVBs) are membranous structures, which accumulate small vesicles in the lumen of the vacuole. These structures are an intermediate state between the early endosome/lysosome compartments. Fusion of MVBs with the plasma membrane results in the release of the internal vesicles called exosomes. The aim of this work is the identification of the SNAREs (soluble N-ethylmaleimide-sensitive factor attachment protein receptors) involved in the fusion of MVBs with the plasma membrane. We show that TI-VAMP (tetanus neurotoxin-insensitive vesicle-associated membrane protein)/VAMP7 colocalize with the MVBs marker N-Rh-PE (N-lisamina rhodamine B sulfonyl-phosphatidylethanolamine). VAMP7 also colocalized with Rab11, a small monomeric GTPasa that participates in homotypic fusion of MVBs. Overexpression of the dominant negative amino-terminal domain of VAMP7 (NT-VAMP7) markedly reduced exosomes release. Furthermore we used N-ethylmaleimide (NEM), an inhibitior of NSF (N-ethylmaleimide-sensitive factor), and we observed an accumulation of Rab11 positive vesicles labeled with N-Rh-PE at the plasma membrane. Our data suggest that Rab11 likely participates in the transport of MVBs to the plasma membrane and VAMP7 is involved in this docking/fusion event.

CB-P20
GAP-43 TRAFFICS FROM GOLGI TO PLASMA MEMBRANE BY A BREFELDIN A-INSENSITIVE EXOCYTIC PATHWAY

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GAP-43 is a dually palmitoylated (C3,4) protein that mostly localizes in plasma membrane (PM) and is enriched in growth cones in developing neurons. To elucidate mechanisms for intracellular transport of GAP-43, the N-terminal domain (N13GAP-43) and the full length sequence of GAP-43 (GAP-43full) were fused to GFP. Biochemical experiments demonstrated the membrane association of these constructs expressed in CHO-K1 cells. Using confocal microscopy analysis we found that at steady state N13GAP-43 and GAP-43full are associated with the recycling endosome and TGN in addition to PM. By selective photobleaching we demonstrated the dynamic nature of N13GAP-43 association with PM and endomembranes. Depalmitoylation of GAP-43 by treatment with an inhibitor of protein palmitoylation drastically reduced its TGN and PM localization. In addition, a double mutant of GAP-43 (C3,4S) displayed a cytosolic diffuse pattern. In synchronized experiments of protein expression we found that a fraction of newly synthesized N13GAP-43 localized at the TGN. It was also demonstrated by using live cell confocal microscopy that N13GAP-43, but not a GPI-anchored protein, trafficked from Golgi to PM by a BFA-insensitive pathway. Together, these results suggest that newly synthesized GAP-43 is acylated early in the secretory pathway and then transported to PM by a mechanism independent of clathrin-coated vesicles.

CB-P21
PROTEIN PHOSPHATASE 5 (PP5) REGULATES THE GLUCOCORTICOID RECEPTOR NUCLEOCYTOPLASMATIC SHUTTLING

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The glucocorticoid receptor (GR) exists as a heterocomplex with hsp90, p23, hsp70 and a high MW immunophilin (IMM). Without steroid, GR is primarily cytoplasmic and it rapidly moves to the nucleus upon steroid binding. Regardless of its localization, the GR shuttles continuously between both compartments. PP5 is a Ser/Thr-phosphatase that possesses an FK506 binding site, so it belongs to the IMM family. PP5 also has three tetratricopeptide repeats (TPR), through which PP5 binds to hsp90 and becomes one of the major components of the GR-hsp90 complex. The biological role of PP5 associated to GR is still unknown. To study whether PP5 affects GR subcellular localization, we measured the hormone-dependent nuclear import rate of GFP-GR in 293T cells overexpressing PP5, as well as the nuclear export rate after steroid withdrawal. In the absence of steroid, the nuclear/cytoplasmic ratio of GR is higher in PP5 overexpressing cells, whereas both nuclear import and nuclear export rates of GR were delayed when the steroid was added or washed out, respectively. Okadaic acid did not affect these results nor the GR nuclear reentry when the cells were reincubated with steroid. This indicates that the intrinsic phosphate activity of PP5 is not required for GR trafficking. Nonetheless, our observations strongly suggest that PP5 plays a role in the molecular mechanism of GR nucleocytoplasmic shuttling.

CB-P22
ARGINYLATED CALRETICULIN: A NEW ISOSPECIES OF CALRETICULIN COMPONENT OF STRESS GRANULES

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The post-translational arginylation consists in the covalent union of an arginine into an acidic amino acid at the N-terminal position. Using mass spectrometry, we demonstrated in vitro the posttranslational incorporation of arginine into calreticulin (CRT). To further study arginylated-CRT we raised an antibody against the peptide RDPAIYFK, which contains an arginine followed by the first 7 N terminal aa of mature CRT. This antibody specifically recognizes CRT obtained from rat soluble fraction that was arginylated in vitro and also recognizes endogenous arginylated CRT from cells in culture, indicating that CRT arginylation takes place in living cells. We found that arginylation of CRT is Ca2+-regulated. In vitro and in cells in culture, the level of arginylated-CRT increased with the addition of a Ca2+ chelator, whereas a decreased arginine incorporation into CRT was found in the presence of Ca2+. The arginylated-CRT was observed in the cytosol, in contrast to the non arginylated CRT which is in the ER. Under stress conditions, arginylated-CRT was found associated to stress granules. These results suggest that CRT arginylation occurs in the cytosolic pool of mature CRT which is probably retro-translocated from the ER. [This work was supported in part by grants from the CONICET- FONCyt, SECYT UNC and from SECyT (Argentina)-ECOS (France)].

CB-P23
PROTEIN TRANSPORT TO LYSOSOME IN EUKARYOTES: A SIMPLIFIED MODEL IN THE PROTOZOAN *GIARDIA LAMBLIA*

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Giardia lamblia is an earlier branching protist that possesses a unique secretory system. This parasite lacks compartments that resemble early/late endosomes and lysosomes; instead, it possesses peripheral vacuoles (PVs) that seem to function as endosomes and lysosomes at the same time. The long-term goal of this work is to determine the molecular mechanisms involved in intracellular protein trafficking from and to the PVs in this organism. Lysosomal trafficking is achieved by adaptins (APs) that recognize specific tyrosine-based motifs in the cytoplasmic tail of membrane proteins. We found that *Giardia* has a tyrosine-based sorting system, which mediates the targeting of membrane and soluble lysosomal hydrolases to the PVs in a clathrin-adaptin dependent manner. Nevertheless, the nature of the hydrolases receptor remains elusive. Here, by using pull-down assays, we show that a putative hydrolase receptor (gHR) interacts with the soluble acid phosphatase and possesses a similar localization pattern. It also contains a tyrosine-based motif that could be recognized by AP1. On the other hand, we show that gAP2 is likely involved in receptor-mediated endocytosis by using a knock-down strategy and uptake experiments. Further studies will provide new insight into the minimal machinery necessary for intracellular transport in higher eukaryotes.

CB-P24
THE INSULIN RECEPTOR, c-fos AND PCNA EXPRESSION IN RAT MAMMARY CARCINOGENESIS

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We have developed experimental malignant mammary tumors in normal rats and benign lesions in diabetic ones by three ip injections of N-Nitroso-N-Methylurea (NMU) at 50, 80 and 110 days of animals life. The aim of this work was to study expression of insulin receptor (IR), PCNA and FOS protein in the mammary gland during the carcinogenic process in these animals. Eight groups of rats were used: 1) control; 2) tamoxifen (Tam) treated; 3) NMU injected; 4) NMU injected and Tam treated; 5) diabetic; 6) diabetic and Tam treated; 7) diabetic and NMU injected; 8) diabetic, NMU injected and Tam treated. All gland mammary tissues were processed at 60, 90 and 120 days. Western blot and immunohistochemical methods were employed. The results indicate: A) IR expression is increased in group 3 vs 1 during all the observation period. Group 4 discloses IR expression similar to 3, while group 2 is positively stained only at 120 days. IR is lower in all groups of diabetic rats compared to non diabetic-ones. B) PCNA expression in diabetic rats is lesser than in non diabetic ones. NMU injected groups shows higher PCNA staining than non injected ones. C) FOS expression is slighter in diabetic groups. In summary, the differences observed in proteins expression can be correlated to the more benign histological pattern found in tumors of diabetic vs non diabetic rats.

CB-P25
THE INSULIN GROWTH FACTOR-I RECEPTOR EXPRESSION IN RAT MAMMARY CARCINOGENIC PROCESS

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In previous experiments we demonstrated that three i.p. injections of N-Nitroso-N-Methylurea (NMU) at 50, 80 and 110 old of life, induce the development of malignant mammary tumors on normal rats and benign mammary lesions on diabetic ones. The objective of this study was to investigate the expression of the insulin growth factor type-I receptor (IGF-IR) in mammary gland of diabetic and non-diabetic rats during carcinogenic period. Four group of rats were randomly separated: a) control (C); b) injected with NMU; c) diabetic rats (STZ); d) diabetic rats injected with NMU (NMU+STZ). All mammary gland samples were processed at 85, 115 and 145 days. For western blot IGF-IR cuantification, microsomal fraction were separated and immunoblotted with the IGF-IR antibody. All samples were also fixed in formalin and tested using anti-IGF-IR specific antibody. The results indicate that IGF-IR content was significantly increased in NMU rats vs. C ones along the carcinogenic period ($P<0.05$, Anova and Tukey post-test). Interestingly, NMU+STZ samples at the same times showed a significant lower content of IGF-IR than NMU ($P<0.05$). Immunohistochemical results are in concordance with these observations. The present results correlates with the hypothesis that IGF-IR expression is a crucial factor in malignant transformation and its expression is early modified during mammary carcinogenesis.

CB-P26

THE *IN VIVO* AND *IN VITRO* ROLE OF NITRIC OXIDE IN TUMOR PANCREATIC GROWTH

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The role of free radical nitric oxide (NO) in tumor biology is controversial but it has been demonstrated that exerts an antiproliferative action in certain tumoral cells. The aim of this work was to assess the NO involvement in the in vivo and in vitro growth of the human pancreatic carcinoma cell line PANC-1. Cell proliferation was evaluated by the clonogenic assay. A dose-dependent inhibition on cell growth was observed when cell cultures were treated with NO synthase (NOS) inhibitors (L-NAME and Aminoguanidine, AG) and the NO donnor (SIN-1). Endothelial and inducible NOS (eNOS and iNOS) isoforms mRNA expression was determined by RT-PCR. eNOS expression was only detected, being positively modulated by L-NAME and negatively by SIN-1. A significant augmentation of intracellular NO level was demonstrated by flow cytometry after L-NAME exposure. Nude mice were sc inoculated with PANC-1 cells. The developed xenografts were undifferentiated adenocarcinomas. When tumour volume reached 65 mm^3, animals were divided in two groups: control and AG (po 2 mg/ml daily in drinking water). AG group showed a lower growth rate, PCNA and antioxidant enzymes SODs, GPx and Cat expression. In conclusion, NO levels modulate in vitro eNOS expression and cell growth. Moreover, free radical NO toxicity and the decrease of the antioxidant enzymes may be involved in the in vivo diminished tumor growth.

CB-P27

COMPARISON BETWEEN THE EFFECTS OF FLAVONOIDS ON ARSENITE-TREATED TUMORAL AND NON-TUMORAL CELLS

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Objective: To study the effect of flavonoids on membrane of arsenite treated breast adenocarcinoma cells (MCF-7, ZR-75-1). Methods: Cells were incubated for 2 hours, with and without recovery (2 hours). Treatments: 200 µM NaAsO2 (As), 5 µM silymarin (S), 50 µM quercetin (Q), As+S, As+Q and controls (C). They were assayed for gamma-glutamyltranspeptidase activity (GGT), conjugated dienes measurement (CD) and sialic acid content (SA). Significant correlation coefficients were <-0.5 (indirect) or >0.5 (direct). Results were analyzed by ANOVA followed by the Tukey test ($p<0.05$). Results: MCF-7 had significant lower GGT activity than ZR-75-1 and did not response to treatments. After recovery, S, As, As+S and As+Q treatments raised it respect to controls; this behavior was opposite to that in CHO-K1 cells. GGT in ZR-75-1 cells was increased by flavonoids, mainly Q. After recovery, As and As+S inhibited GGT. On the other hand, As, S, Q and As+S decreased SA values in MCF-7 cells respect to C, while As+Q increased membrane SA content independently of the tumor line. SA in CHO-K1 cells did not change. SA presented an inverse relation with CD levels in tumoral cell lines, which were directly associated with GGT activity. Conclusions: The studied breast cancer cells responded dissimilarly to As, and were not protected by flavonoids as occurred in non-tumoral CHO-K1 cells.

CB-P28

ACETALDEHYDE ACCUMULATION AND OXIDATIVE STRESS IN RAT BREAST. THEIR ROLE IN ETHANOL INDUCED CANCER

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In previous studies we reported the presence of several pathways of activation of ethanol to acetaldehyde (AC) and hydroxyl free radicals as well as the promotion of oxidative stress. In the present studies we tested the possibility that after alcohol drinking, AC accumulated in mammary tissue to reach concentrations higher than in blood. Three different doses of alcohol were tested and AC concentrations in mammary tissue, liver and blood were measured at times ranging from 1 to 24 hours. We also determined ADh; AlDh and CYP2E1 activities. Oxidative stress induced hydroperoxides formation; depletion of alpha-tocopherol and GSH contents for the higher dose at different times of exposure. Hydroperoxides levels led to increased values at 6 hours for the higher dose tested also. The obtained results showed that AC concentrations at the three doses tested were always higher than in blood. Limited ADh and AlDh activities in mammary tissue were observed. The microsomal CYP2E1-mediated p-nitrophenol hydroxylase in mammary tissue was several times smaller than in liver. In summary, results suggest that the mutagen AC, either formed in situ or, even in small amounts, arriving via blood, tends to accumulate in mammary tissue as a consequence of a limited capacity of it for detoxification. [Supported by ANPCyT (PICT 05-6045) and UNSAM (PIB S05/03)].

CB-P29

ETHANOL TOXICITY ON RAT PROSTATE AND ITS ROLE IN PROSTATE CANCER RELATED TO ALCOHOL DRINKING

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The correlation between alcohol (EtOH) consumption and prostate cancer is controversial. It would be important to know whether prostate tissue leads to similar alterations to those attributed for EtOH promoted liver injury or cancer. These studies were performed in Sprague Dawley male rats fed with a nutritionally adequate liquid diet containing EtOH for 28 days and compared against adequately pair fed controls. Prostate microsomal fractions showed a CYP2E1-mediated EtOH metabolism and that activity was induced by repetitive EtOH drinking. Cytosolic activation of AC led to acetyl radical, as detected by spin trapping and GC-MS. Low activities of ADh and AlDh were observed in prostate tissue and AC accumulation occurred after EtOH administration. An increased oxidability of lipids was detected by chemiluminiscence emission and by increased levels of lipid hydroperoxides. Alterations in epithelial cells involved condensation of chromatin, dilatation of ER and some cells undergoing apoptosis. In summary, alcohol drinking leads to the formation of mutagenic AC and to tumor-promoting oxidative stress. However, it exerts direct and undirect proapoptotic effects in the prostate epithelial cells. The balance between both actions might explain, at least in part, many controversial results observed in epidemiological studies. Supported by CONICET (PIP 02323) and UNSAM (PIDA UF013).

CB-P30
MUCINS EXPRESSION IN COLON CARCINOGENESIS RODENT MODEL

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Administration of 1,2-dimethylydrazine to rodents is known to cause adenocarcinomas of the colon similar to those seen in cancer patients, and it has been widely used as a colon cancer model. We have developed a similar animal model of chemical-induced intestinal cancer for studying mucins expression. The mucins are secreted in normal/abnormal epithelium by the globet cells. Different studies have demonstrated a change in the transcript level as well as an increase in inmunoreactivity of the mucin along the colon carcinogenesis steps. The main objective of this study was to determine the secretion of sulphated acid mucin (SAM), non- sulphated acid mucin (NSAM) and neutral mucin (NM) in the lesions produced by 1,2-DMH in Wistar rats. Thirty animals were injected with 15 mg/kg of 1,2-DMH once a week during six consecutive weeks. Twenty-eight weeks after the first inoculation, rats were killed by CO2 asphyxiation. Then the mucin was studied with Alcian Blue 8GX (pH 1 and pH 2,5%) and peryodic acid shiff (PAS) staining. The SAM was found in 60% of the polyps and 40% of the malignant tumors. On the other hand, the NSAM was found in 55% of the polyps and in 46% of the malignant tumors. The NM was detected in 60% of the polyps and 40% of malignant tumors. No statistically significant difference was found in the secretion of NSAM, but we observed differences in the secretion of NM and SAM in both polyps and neoplastic tumors.

CB-P31
SPARC MODULATES MELANOMA CELL - ECM INTERACTION THROUGH SPECIFIC INTEGRINS AND RAC GTPase

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Cancer progression is characterized by the dynamic interaction of the malignant cell's cytoskeleton with the extracellular matrix (ECM). SPARC is a secreted non-structural protein component of the ECM, with a yet unclear role in its physiology. However, it has been postulated that SPARC might act as a de-adhesive protein promoting focal cell-adhesion disruption in normal cells. Data from different groups have demonstrated that SPARC expression is associated with aggressive behavior in most human carcinomas. Here we show that knocking-down SPARC expression in melanoma cells using different approaches induced a cytoskeletal rearrangement that was accompanied by relocalization of focal cell adhesion contacts and changes in integrins expression. Moreover, this effect was reversed either by the expression of plasmidic and adenoviral vector-encoded SPARC, or by addition of SPARC protein purified from melanoma. In order to define whether specific members of the Rho small GTPases family are involved in SPARC-induced cytoskeletal rearrangement, we constitutively expressed either their constitutively active or dominant negative forms in SPARC-depleted melanoma cells. Here we show that Rac could act as a potential downstream mediator of SPARC effects on melanoma cells. These results suggest that SPARC enhances melanoma aggressiveness by signalling through specific integrins and small GTPases.

CB-P32

SPARC PROTEIN INHIBITS PROLIFERATION OF FIBROBLASTS AND ENDOTHELIAL CELLS BUT NOT CANCER CELLS

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The contribution of the extracellular matrix to cell proliferation has not been clearly elucidated. SPARC, a matricellular component of the ECM, both impairs the proliferation of different cell types in vitro and promotes tumor aggressiveness. This apparent paradox could result from differential biochemical properties in SPARC produced by normal or tumor cells. Alternatively, the apparent contradiction could be the consequence of differential responses of diverse cell types to SPARC. To test this, SPARC secreted by melanoma cells and recombinant SPARC obtained from different sources were compared and no evidence for dissimilar properties was observed on endothelial cell proliferation, adhesion and migration capacity. Important differences were detected instead when the effect of hMel-SPARC was tested on additional cell types. While hMel-SPARC greatly impaired the proliferation of endothelial cells, it exerted a moderate biphasic effect on human fetal fibroblasts proliferation but had no effect at all on the proliferation of several human cancer cell lines. Importantly, downregulation of SPARC levels in melanoma cells sensitized melanoma cells to hMel-SPARC. Thus, SPARC overexpression by the tumor mass might serve to modulate stromal cell behaviour without affecting the malignant cells themselves. *These authors contributed equally to this work.

CB-P33

HISTAMINE MODULATES PROLIFERATION IN MCF-7 HUMAN BREAST CANCER CELL LINE

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We have reported that histamine (HA) regulates differentially signaling processes in normal and malignant mammary cells. The aim of the present study was to investigate the biological responses triggered by HA in breast cancer cells MCF-7 (ERα+). For this purpose we determined the expression of the HA receptors subtypes, H1R, H2R, H3R and H4R by RT-PCR; histidine decarboxylase (HDC) by western blot; intracellular polyamines by HPLC; HA content by immunostaining; cAMP production by RIA and cell proliferation by clonogenic assay. Results indicate that MCF-7 cells expressed the four HA receptors, HDC and presented a moderate level of intracellular HA. HA at 10 µM produced a 2-fold increase in cAMP levels and reduced cell proliferation (30%) and spermine levels (8,3±0,7 vs.10,6±0,4 pmol/µg protein). Similar effects were observed with lower HA doses. By using specific HA agonist and antagonist, we determined that HA decreased proliferation through the stimulation of the four HA receptors subtypes however, the most significant effect was exerted via H4R (70%). We conclude that the inhibitory effect of HA on proliferation is associated with an increase in cAMP levels that induces differentiation and a reduction of the intracellular spermine that is involved in cell growth. In addition, we observed that HA reduces the mitochondrial transmembrane potential suggesting a pro-apoptotic action.

CB-P34

HISTAMINE AS A POTENTIAL FACTOR INVOLVED IN BREAST CANCER CELLS PROGRESSION

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Transformed epithelial cell progression to metastasic state is characterized by diminished cell adhesion and increased motility, and expression of extracellular proteinases. Histamine (HA) was reported as an angiogenic and growth factor in many neoplasms. We studied the action of HA on cell adhesion and gelatinolytic activity in tumorigenic (MCF7 and MDA-MB-231) and non tumorigenic (HBL100) transformed mammary cell lines. We evaluated the effect of HA on cell growth by clonogenic assay and assessed cell adhesion in cultures treated with HA, agonists and antagonists for 24 hours, using Methylene Blue staining. MMP2 and MMP9 metalloproteinases gelatinolytic activity was studied by zymography. Results showed that 10 μM HA negatively modulates cell growth in tumorigenic cells, but not in HBL100 cells. In MCF7 cells, MMP2 basal activity is higher than in MMP9 and increased by HA and agonists. MDA-MB-231 cells showed MMP9 basal activity higher than MMP2, being positively modulated by HA and agonists. This increase correlates to a diminished cell adhesion after these treatments. In HBL100 cells, adhesion significatively increases with HA and agonists, while gelatinolytic activity was undetectable. In conclusion, HA selective modulation of proliferation,adhesion and gelatinolytic activity in these cell lines signals HA as a potential factor involved in breast cancer cells progression.

CB-P35

MODIFIED SIALYL RESIDUES AS A TOOL TO IDENTIFY CELL ACCEPTORS OF TRYPANOSOMA CRUZI TRANS-SIALIDASE

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Trypanosoma cruzi expresses a unique catalytic activity known as *trans*-sialidase (TS), able to transfer sialyl residues among glycoconjugates. The enzyme is shed to the milieu, being detected in the blood of infected mammals, where it constitutes a virulence factor that induces apoptosis in cells from the immune system by surface sialylation. It is therefore of interest to identify these acceptors to understand the apoptotic induction mechanisms. Here we used a recently developed approach that involves an azide-modified sialic acid (N-Azidoacetyl sialic acid, SiaNAz), which can be obtained from glycoproteins of cells fed with N-Azidoacetyl mannosamine as precursor. The SiaNAz is detected by its reaction with a phosphine-based molecule associated to a FLAG epitope, followed by an HRP-labeled anti-FLAG mAb in a WB assay. TS successfully hydrolyzed and transferred the SiaNAz residue from Jurkat cells. The transfer of SiaNAz to glycoproteins was tested with asialofetuin as acceptor and competed with lactose. Then, thymocytes and splenocytes were assayed with TS in the presence of Jurkat cell extracts and, after extensive washing, lysates were reacted with the phosphine-FLAG and WB was performed. TS transferred SiaNAz to lymphocytes and therefore, this approach provides a tool to identify the surface molecules that are acceptors of the sialyl residue.

CB-P36

STAGE-SPECIFIC EXPRESSION OF *TRYPANOSOMA CRUZI TRANS*-SIALIDASES IS MODULATED BY THEIR 3'UTRs

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Regulation of gene expression in trypanosomes is achieved through post-transcriptional mechanisms since they are unable to modulate expression at the transcriptional level. In this work we analyzed trans-sialidases (TS) mRNAs as a model of stage-specific expression regulation. TS genes from *Trypanosoma cruzi* can be divided in two groups. Members of one group are translated in the mammalian stages of the parasites (msTS) while the second group is translated in the epimastigote stage (isTS). Analysis of the 3'UTR of msTS and isTS mRNAs, revealed an identity higher than 78% among members of each group. On the other hand, the 3'UTRs from each TS mRNAs group displayed an identity lower than 25%. 3'UTRs were fused to GFP reporter gene and their expression in the different parasite stages was then analyzed. Sequences in the 3'UTR of msTS allowed GFP expression in all mammalian parasite stages. Conversely, constructs containing the is3'UTR were highly expressed in the insect parasite stage. Regulation of GFP expression was due to modulation of the translation efficiency. Using CAT as another reporter gene, we also found that these 3´UTRs are able to modulate transcript stability. Taking together, these results strongly support that the 3'UTRs are involved in the regulation of the stage-specific expression of TS genes.

CB-P37

INVOLVEMENT OF AUTOPHAGY IN DIFFERENTIATION AND RESPONSE TO NUTRITIONAL STRESS IN TRYPANOSOMA CRUZI

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Trypanosoma cruzi, the parasite causing Chagas Disease, has a complex life cycle, involving an insect vector and a mammal, and four major developmental stages. The differentiation steps, and the response to the nutritional stress suffered inside the vector, may involve autophagy. This process has not been studied in detail in the parasite, and nothing was known about their molecular mechanism. Data from the Genome Project suggest that a pathway involving proteins homologous to yeast Atg4 (autophagin) and Atg8 may be operative in T. cruzi. Two Atg4 and two Atg8 homologues have been cloned and expressed in Escherichia coli. Both T. cruzi autophagins are cysteine peptidases able to process the Atg8 homologues at the conserved Gly residue, and to complement the autophagy defect observed in a yeast strain defective in Atg4. In addition, one of the two T. cruzi Atg8 homologues (Atg8.1) was able to complement Atg8 deficiency in yeast. Atg8.1 is a suitable autophagosomal marker during nutritional stress in T. cruzi, as shown with specific antibodies. Using this tool, autophagy was demonstrated to take place during differentiation of epimastigotes to metacyclic trypomastigotes at the end of the insect vector gut. If autophagy is also involved in parasite remodeling inside the mammal, its inhibition might represent a novel strategy in searching for new antiparasitic drugs.

CB-P38
Rab1b IS RECRUITED BY THE *COXIELLA BURNETII* REPLICATIVE VACUOLE

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Coxiella burnetii, the ethiological agent of Q fever, is a Gram-negative obligated intracellular bacterium. Once inside the cell, this bacterium replicates in a large parasitophorous vacuole that shares characteristic of phagolysosome-autophagolysosomal compartment. We have previously demonstrated that the activation of the autophagic pathway increases the infection with *C. burnetii*. Rab1b is a small GTPase responsible for the anterograde transport between Endoplasmic Reticulum and Golgi Apparatus. We have evidence that Rab1b is involved in autophagosome formation. In the present work, CHO cells overexpressing GFP-Rab1bwt or the active GTPase defective mutant were infected with *C. burnetii* for different periods of time. Using confocal microscopy, we observed that at early times after infection no colocalization with this Rab protein was detected. In contrast, at later times after infection (i.e. 24-48h) the large replicative vacuoles were decorated by Rab1b, in particularly by the active mutant. These results suggest that membranes from the early secretory pathway contribute to the development of the *Coxiella*-replicative compartment.

CB-P39
CYTOTOXICITY OF ACIDIC PHOSPHOLIPASE A2 FROM *BOTHROPS ALTERNATUS* CULTURED IN VENOM

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Snake venom phospholipases (PLA_2s) display an amazing profile of toxicological activities and in addition to their normal digestive action, a wide variety of pharmacological activities has been described for PLA_2s isolated from *Bothrops* snake venoms. Species from this genus inflict the vast majority of snakebites in Latin America, and in spite of several works published on this topic, the cytotoxic activity of *B. alternatus* PLA_2 from North-eastern Argentina has not yet been described. In this study we report the cytotoxic activity of an acidic PLA_2 isolated from *B. alternatus* venom, as determined on a murine mammary tumor cell line (LM_3) in comparison with the toxicity exhibited by the crude venom. Briefly, different concentrations of PLA_2 or venom (0.01-100 µg/ml) were diluted in culture medium and then added to the cells. After 3 h of incubation, citotoxicity was quantitatively assayed by crystal violet method. The percentage of viable cells in the monolayer culture was registered. Results indicate that acidic PLA_2 induces dose-dependent cytotoxicity. This effect was already observed with very low toxin concentrations (0.01 µg/ml) and was predominant at higher enzyme levels (10-100µg/ml); while crude venom showed major percentage of lethality at the same range concentrations. It is concluded that this acidic PLA2 isolated from the *B. alternatus* venom contributes to its cytotoxicty.

CB-P40

INHIBITION OF TNF-α BY *LACTOBACILLUS REUTERI* IN LOW CHOLESTEROL CELLS

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Several pathogen microorganisms and microbial-derived toxins interact with their target cells via cholesterol enriched plasma membrane microdomains or lipid rafts. We investigated the role of lipid rafts in the action of a no pathogen microorganism, *L. reuteri* CRL 1098, on TNF-α production. To disrupt the lipid rafts of peripheral blood mononuclear cells (PBMC), cholesterol was depleted to 45% by extraction with 10 mM methyl-ß-cyclodextrin (MßCD). Control (no treatment) and low cholesterol PBMC were incubated with *L. reuteri* at different MOI and times of incubation at 37°C. TNF-α value detected by chemiluminiscence assay in the supernatant of $1x10^6$ cells was 149 pg /ml. No modification was observed in TNF-α production when control and low cholesterol cells were incubated with *L. reuteri* at 10 MOI up to 4 h incubation. However, an inhibition of 27% and 51% of TNF-α produced was found in control and low cholesterol cells, respectively, incubated with *L. reuteri* at 20 MOI for 4 h, while no difference was detected in presence of heat-killed *L. reuteri.* The inhibitory bacterial product was partially identified as a <10 kDa peptide. TNF-α production by control and low cholesterol PBMC was inhibited by this peptide by 41 and 63%, respectively. Studies are currently in progress to further define the role of lipid rafts in action of this peptide.

CB-P41

EXPRESSION OF VCAM-1 AND APO J IN AORTAS OF VITAMIN A-DEFICIENT RATS

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Vitamin A reduces the degree of lipoperoxidation, regulates the expression of antioxidant enzymes and inhibits atherosclerosis protecting LDL from oxidation. We have previously found increased TBARS levels in serum and aorta, higher NF-kB binding activity and increased iNOS, COX-2 and TNF-alpha expression in aorta of animals fed on a vitamin A-deficient diet. We also communicated disturbances in lipid metabolism in serum and aorta of these animals. In the present work we inform the expression levels of VCAM-1 and Apo J in aorta of vitamin A-deficient rats. Wistar male 21 d old rats were fed during three months with free vitamin A diet (-A) and the same diet plus 8 mg of Retinol palmitate/kg of diet (+A). Some -A rats received control diet fifteen days before sacrifice (-A refeeded). Vitamin A deficiency was confirmed in plasma by HPLC. Total RNA was isolated by using TRIzol. The –A group showed increased RNAm expression levels of VCAM-1 and Apo J in aortas compared with +A ($p< 0.01$ and $p< 0.001$, respectively) and –A refeeded ($p< 0.001$ and $p< 0.05$, respectively). Alterations in redox status and lipid metabolism in aorta could induce changes in the expression levels of VCAM-1 and Apo J in response to the nutritional vitamin A deficiency.

CB-P42
VITAMIN A DEFICIENCY MODIFIES THE DAILY EXPRESSION OF ANTIOXIDANT ENZYMES IN HIPPOCAMPUS

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Vitamin A deficiency is known to lead to oxidative stress and some evidence points out retinoids as regulators of clock genes activity through retinoid nuclear receptors (RARs and RXRs). Our objective was to investigate the effect of vitamin A deficiency on the circadian expression of antioxidant enzymes in the hippocampus of Wistar rats fed during 3 months with a vitamin A-deficient diet. Plasma and liver retinol concentration were determined by HPLC. Total RNA from hippocampus was extracted using the Trizol reagent (Invitrogen). Transcript levels of RARα, RXRβ, CAT, SOD and GPx were determined by RT-PCR and normalized to β-actin as endogenous control. Relative quantification by Real-time PCR using SYBR Green I dye was performed to measure the mRNA levels of RARα and RXRβ. Subclinical plasma retinol concentration and negligible liver retinol stores confirmed the vitamin A deficiency. Significant lower mRNA levels of RXRβ, CAT and GPx were observed in the hippocampus of vitamin A-deficient compared to control and vitamin A-recovered rats. Vitamin A deficiency modified the daily rhythmicity in the expression of CAT and GPx. Those changes might occur as a consequence of a reduced availability of RXR. Establishing a role for vitamin A in the circadian regulation of antioxidant environment in the hippocampus might contribute to get an improved cognitive daily performance.

CB-P43
PROTECTIVE ROLE IN LIVER RAT OF FLAVONOIDS PRESENT IN PROTEIN CONCENTRATE FROM AMARANTHUS CRUENTUS

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Flavonoids are phenolic compounds that represent substantial constituents of the non-energetic part of the human diet. They are naturally found in vegetables, exert multiple biological effects. We evaluated the presence of flavonoids in protein concentrate from Amaranthus cruentus seeds (PC) and their effect as antioxidant on the histoarchitecture of Wistar liver rats. Flavonoids were determined spectrophotometrically. Twelve rats were separated in two groups: one control fed with casein, and other with PC as protein source. All diets had 11.9% of protein and were supplemented with 1% of cholesterol. Total glutathione, reduced glutathione and total non protein thiols were measured. The experience lasted 28 days. For the histological study the sections were fixed by liquid of Bouin, colored with H-E and MG-G and observed to the optical microscope. The concentration of flavonoids was of 45.15 ± 1.87 (mg/100g). Increased of total glutathione ($P<0.001$), reduced glutathione ($P< 0.001$), total non protein thiols ($P<0.05$), compared to casein we observed. The histopathological diagnosis indicates that rats fed with casein present the liver with moderate fat infiltration, whereas this was not observed in rats fed with PC and liver has structural integrity. The presence of flavonoids would provoke an increase of the antioxidant defenses and would produce a protective role in liver.

CB-P44

EFFECT OF THE ANTINEOPLASTIC AGENT TAMOXIFEN IN EXPERIMENTAL PORPHYRIA INDUCED BY HEXACHLOROBENZENE

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Hexachlorobenzene (HCB) exerts its porphyrinogenic action through metabolization of the drug by Cytochrome P450 isoenzymes, which is increased by estrogens then exacerbating this experimental porphyria. A reported carcinogenic activity of HCB can be associated with different types of cancer. The antiestrogenic drug tamoxifen is used for treatment of breast neoplasia, being under discussion its porphyrinogenic action. In the present study, different parameters related to porphyria were measured in normal and HCB experimental porphyric rats, after treatment with tamoxifen. Although urinary porphyrin contents were increased from 8 weeks after tamoxifen treatment in normal rats, this level is diminished when performed in HCB porphyric animals. In normal and porphyric rats, diminished liver porphyrin contents were found at 10 weeks after treatment with tamoxifen. It was found an increase in tryptophan pyrrolase holoenzyme activity, and a decrease in the apoenzyme, only in porphyric rats treated with tamoxifen respect to HCB-treated ones. These results allow us to suggest that the antiestrogenic action of tamoxifen produces a decrease in metabolization of HCB, in ROS generation and thus in URO-decarboxylase inhibitor formation and in its derived porphyria, what is shown by a decrease in hepatic and urine contents of porphyrins in rats treated with HCB and the antineoplastic.

CB-P45

ALTERATION IN TRYPTOPHAN SEROTONINERGIC PATHWAY IN AN EXPERIMENTAL ACUTE PORPHYRIA

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Human acute porphyrias are diseases caused by alterations in heme synthesis. In its clinical expression, neurological disturbances are reported, like anxiety and depression. So, we study the metabolism of tryptophan in different tissues, to determine the level of tryptophan and its metabolites with the purpose to understand human clinical manifestations. Females Wistar rats were treated with 2-allyl-2-isopropylacetamide; (AIA 100, 300 or 500 mg/kg bw) and 3,5-diethoxycarbonyl 1,4-dihydrocollidine (DDC 50 mg/kg bw). As porphyric parameter, hepatic 5-aminolevulinic-synthase (ALA-S) was measured, obtaining an increase of 4-fold for the highest dose. Content of tryptophan and serotonin were evaluated in brain, liver and blood, using HPLC method. The results showed dose dependent alterations in tryptophan and serotonin levels. In brain tissue an increase of serotonin was observed (21 % for the highest dose) and in hepatic tissue an increase of tryptophan (23 % for the highest dose). Metabolites content in blood was variable respect to the control. These results suggest that the brain increase of serotonin, observed in this model could be related to the human neurological manifestations; whereas the hepatic increase of tryptophan could be related to the negative effect produced on the gluconeogenesis, and a consequent deficit in glucose availability that triggers crises in patients.

CB-P46

SOME ASPECTS OF THE RESPONSE AGAINST OXIDATIVE STRESS-INDUCED TOXICITY IN AN ACUTE PORPHYRIA MODEL

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Oxidative damage to biomolecules has been implicated in the pathology of a number of diseases including acute human porphyries. Our group already has been informed that in acute porphyria model the overproduction of 5-aminolevulinic acid (ALA) generates oxidative stress with an increase of reactive oxygen species (ROS). Therefore, it seemed interesting to investigate the activities of enzymes glutathione reductase (GR) and glucose 6-phosphate dehydrogenase (G6PDH) that produce reduced glutathione (GSH) and NADPH, respectively, both protective substances of the cell against ROS. Females Wistar rats were treated with 2-allyl-2-isopropylacetamide (AIA, 100, 300 or 500 mg/kg.bw) and 3,5-diethoxycarbonyl 1,4-dihydrocollidine (DDC, 50 mg/kg.bw). As porphyric parameter, hepatic 5-aminolevulinic-synthase (ALA-S) was measured. The enzymatic activities were monitored spectrophotometically and GSH in accordance to Ellman method. The obtained results indicated increases of GR activity about 50% and 40% for G6PDH respect to the control for the highest dose. The levels of GSH did not demonstrate significant variations with the administered doses. It is concluded that in the developed model some glutathione metabolizing enzymes such as GR and pentose pathway enzyme G6PDH activity exhibit a protective behavior against oxidative stress promoted by drugs.

CB-P47

PHYTOPOLYPHENOLS OF ROSEMARY: EFFECT ON PROLIFERATION AND DIFERENTIATION IN NORMAL AND TUMORAL CELLS

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Currently traditional chemotherapeutic agents have serious disadvantage and new compounds are been investigated. Antioxidant phytopolyphenols from rosemary have antimutagenic, analgesic, microbicide [1] and anticancer properties. The broad spectrum of research on rosemary has been concentrated on its biological action [2]; the mechanisms are not established yet. In this work we study of functional properties of *Rosmarinus Officinalis* in order to identify bioactive compounds affecting proliferation and/or differentiation of mammalian cells. Cell viability, cytotoxic and metabolic activity were tested. No significantly cytotoxicity on epithelial normal cells with up to 40 mg/ml of plant extract and up to 4 mg/ml using isolated rosmarinic acid and carnosic acid, was found. The viability of 3T3-L1 fibroblast increased notably after 7 days of plant extract treatment (20 mg/ml). On the other hand, we also found a considerably decrease on the proliferation of human colorectal cancer cells by the extract (ED50=25 mg/ml) in a concentration-dependent manner after 5 days of treatment. Therefore, rosemary compounds have inhibitory effect on tumor cell lines but not in normal fibroblast suggesting a potential used as new chemotherapeutic agents. [1] Moreno, S. et al., Free Radical Research, 2006, 40: 223-231. [2] Lo et al., Carcinogenesis, 2002, 23, 983-991. This work was supported by CONICET

CB-P48

TIME COURSE AND RECOVERY OF LEAD-INDUCED CHOLINESTERASE INHIBITION IN TWO FRESHWATER INVERTEBRATES

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Cholinesterase (ChE) activity is widely used as biomarker for organophosphates and carbamates. However, the inhibition of ChEs from several species by other environmental contaminants has been reported. The aim of this work was to study ChE inhibition by lead in *Biomphalaria glabrata* and *Lumbriculus variegatus*. The organisms were exposed for 48 h to several concentrations of lead (0.2-2.5 mg Pb/ L) and ChE was assayed according to the method of Ellman. The NOEC level was 0.2 mg Pb/ L in both invertebrates. At 2.5 mg/ L, ChE inhibition was 36% and 27% for *B. glabrata* and *L. variegatus*, respectively. For the time-course studies, organisms were exposed to 0.5 mg Pb/ L, for 24, 48 and 72 h. Our results showed that ChE activity from both species was inhibited by lead exposure at 48 and 72 h. The recovery process in uncontaminated media was very fast in both species. The activities were reestablished to control values within 24 h with the exception of *B. glabrata* snails exposed to 2.5 mg Pb/ L. In this case, ChE recovery was observed at 48 h. Previously, we have reported that both invertebrate species showed a very different susceptibility to ChE inhibition by the organophosphate azinphos-methyl. In this work, we show that lead, a non-specific anti-cholinesterase agent, induces similar and transient inhibition on ChE activity in both invertebrates.

CB-P49

REGULATION MECHANISMS OF INTESTINAL NA/CA EXCHANGER BY $1,25(OH)_{2D3}$

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Calcitriol regulates gene expression and induces rapid non-transcriptional responses in the intestine. However, intestinal Na/Ca exchanger (NCX) activity and its regulation by calcitriol remains unknown. The aim of this study was to determine calcitriol mechanisms triggered on the intestinal NCX activity. Normal controls, rachitic and rachitic chicks treated with calcitriol were used. Normal chicks fed a low calcium diet were employed in some experiments. Enterocytes were isolated and NCX activity was determined by Ca uptake experiments. The data show that NCX activity was lower in enterocytes from rachitic animals than that of normal controls. Either vitamin D treatment or low Ca diet increased the activity of the carrier. Enterocytes incubated with calcitriol also showed an increment of NCX activity. This effect was abolished by previous treatment with cycloheximide, which indicates that protein synthesis is involved in the hormone mechanism. Short exposure of the intestinal cells to calcitriol (3-15 min) resulted in a rapid induction of the NCX activity, peaking at 5 minutes. Forskolin, adenilil cyclase activator, stimulated the NCX activity, suggesting that PKA may be a mediator in the signalling cascade that regulates the intestinal carrier activity. In conclusion, calcitriol stimulates intestinal NCX activity by genomic and non genomic mechanisms.

CB-P50
THALLIUM EXPOSURE LEADS TO CELL DEATH THROUGH THE INDUCTION OF APOPTOSIS

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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+} (10-100 µM) (24 hs). Previous results showed that both Tl^+ and Tl^{3+} decrease mitochondrial membrane potential, effect that was accompanied by an increase in H_2O_2 content. A significant decrease in GSH content was also observed. To study the possible mechanisms of cell death involved in Tl toxicity nuclear morphology was characterized using the probes acridine orange and ethidium bromide. An increase in the number of apoptotic cells was found. Western blot analysis showed higher PARP cleavage, the release of cytochrome c into the cytosol, and a diminished bcl-2 content. Besides, an increase in caspase 8 activity was observed when the cells were incubated in the presence of Tl^{3+}. Neither autophagic cell death as evaluated by the incorporation of the probe MDC into autophagic vacuoles, nor necrosis as evaluated by LDH activity, were involved in Tl toxicity. In summary, Tl caused a decrease in GSH, and altered mitochondrial functionality increasing H_2O_2 content, effect/s that could trigger cell apoptosis. This work was supported by grants of UBA. (B072) and ANPCyT (PICT 12285), Argentina.

CB-P51
CHOLESTEROL DEPLETION ACTIVATES RAPID INTERNALIZATION OF AChR DOMAINS AT THE CELL MEMBRANE

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Novel effects of cholesterol on nicotinic acetylcholine receptors (AChR) cell-surface stability and function (single-channel behavior) are reported. AChR are shown to occur in the form of diffraction-limited (240-280 nm) domains that remain stable at the cell-surface membrane of CHO-K1/A5 cells over a period of hours. Acute (30 min, 37°C) exposure to methyl-β-cyclodextrin, commonly used as a diagnostic tool of endocytic mechanisms, is shown here to enhance AChR internalization kinetics in the receptor-expressing clonal cell line. This treatment drastically reduced (~50%) the number of plasma membrane domains and accelerated internalization ($t_{1/2}$ decreased from 1.5 to 0.5 h). In addition, cholesterol depletion produced ion channel gain-of-function of the remaining cell-surface AChR, whereas cholesterol enrichment had the opposite effect. Homeostatic control of cholesterol content at the plasmalemma may thus modulate cell-surface organization and stability of receptor domains, and fine tune receptor channel function to temporarily compensate for acute AChR loss from the cell surface. * Collaborative work with Max-Planck Inst. biophys. Chem., Göttingen, Germany (T.L.) and Drexel Univers. College of Med., Philadelphia, USA (M.M.W.)

CB-P52
NICOTINIC ACETYLCHOLINE RECEPTOR INTERNALIZATION VIA A NOVEL ENDOCYTIC PATHWAY

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Endocytosis of the nicotinic acetylcholine receptor (AChR) is proposed to be a major mechanism of neuromodulation at neuromuscular junctions and in pathology of many diseases of cholinergic synapses in the central nervous system, but it is still poorly understood. We have characterized the endocytic mechanism of the AChR in CHO-K1/A5 cells, a Chinese hamster ovary (CHO) cell line heterologously expressing murine adult-type receptor. Fluorescence derivatives of the competitive antagonist α-bungarotoxin (αBTX) were used to label the AChR in living cells. AChR was mostly distributed at the cell-surface; binding of αBTX shifted the distribution of the AChR to an internal pool coincident with Golgi and late endosomal markers. Internalization takes place via a clathrin- caveolin- and dynamin-independent pathway that depends on the integrity of the cytoskeletal network. The ligand-induced endocytic processes revealed here in a simple heterologous mammalian cell system may shed light on the dynamics of receptor internalization at the cholinergic synapse.

CB-P53
SPHINGOMYELINASE MODULATES ACETYLCHOLINE RECEPTOR TRAFFICKING IN CHO-K1/A5 CELLS

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Sphingolipids and cholesterol are important for the function of the nicotinic acetylcholine receptor (AChR). We have previously demonstrated that incubation of CHO-K1/A5 cells with ceramides (Cer), biosynthetic precursors of sphingomyelin (SM), decrease the number of cell-surface AChR concomitantly with an increase in intracellular receptor levels. Here we studied the effects of endogenous Cer generated by sphingomyelinase (SMase) hydrolysis. SMase efficiently generated Cer, as assessed by a fluorescence assay using the fluorescent SM analogue C_5-BODIPY-SM. One-hour incubation of CHO-K1/A5 cells with 100 mU/ml SMase diminished cell-surface AChR by 35%. SMase treatment also decreased (~55%) surface fluorescence of the non-toxin SM-marker lysenine. Upon 1 h treatment with SMase followed by 3 h incubation in SMase- and serum-free medium a 60% decrease in cell-surface AChR fluorescence was observed. The t½ of AChR internalization decreased about 30% in SMase-treated cells. The mechanism by which short treatment with SMase decreases cell-surface AChR may thus involve increased receptor internalization, and this in turn appears to be due to depletion of plasma membrane SM. Additionally, endogenous Cer accumulation may also reduce AChR exocytic trafficking, synergically contributing to cell-surface receptor depletion

CB-P54

TRANSMEMBRANE CHARGED RESIDUES INVOLVED IN ASSEMBLY/SURFACE TARGETING OF THE ACETYLCHOLINE RECEPTOR

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The αM4 transmembrane (TM) domain of the acetylcholine receptor (AChR) is flanked by two amino acids (His^{408} and Arg^{429}) located at its cytoplasmic and extracellular facing extremes, respectively. A series of single and double mutant constructs of these motif were produced and subunits as stable clones in a mammalian co-expressed with wild-type β δ and ε heterologous expression system in CHO-K1 cells. The mutants were studied by α-bungarotoxin ([^{125} I]αBTX) binding, fluorescence microscopy, and equilibrium sucrose gradients centrifugation. Cell-surface [^{125} I]αBTX binding diminished by ~40% in His^{408}Ala, ~80% in Arg^{429}Ala and ~95% in His^{408}Ala/Arg^{429}Ala. Reversing amino acid charge in Arg^{429} (e.g. Arg^{429}Glu) abolished cell-surface expression of the AChR. Fluorescence microscopy disclosed that Arg^{429} AChR mutants were retained at the endoplasmic reticulum, with an enhanced content of unassembled α subunit. Centrifugation analysis confirmed the lack of fully assembled AChR pentamers in all Arg^{429} mutants. We conclude that His^{408} and Arg^{429} in αM4 are involved in assembly and cell-surface targeting of muscle AChR. Arg^{429} appears to play a more decisive role in these two processes, suggesting an asymmetric weight of the two charged motifs at each extreme of the transmembrane segment. α subunit M4.

CB-P55

THE FORMATION OF CYTOPLASMIC STRESS GRANULES UPON STRESS REQUIRES THE RETROGRADE MOTOR DYNEIN

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Under cellular stress protein translation is inhibited by the phosphorylation of the eukaryotic initiation factor 2α. This leads to the accumulation of stalled translational preinitiation complexes that agregates in the cytoplasm along with a number of RNA-binding proteins forming novel structures named Stress Granules. SGs are proposed to serve as a triage site that controls the fate of untranslated mRNAs. The change in the subcellular distribution of the translational machinery and ribonucleoparticles that occurs upon stress implies an active transport that we are aimed to investigate. To asses the participation of molecular motors in this phenomenon, the effect of drugs that specifically disassemble microtubules or microfilaments was assessed. We found that the absence of cytoskeletal structures affects SG size and distribution. These results suggest that microtubule and microfilament-dependent molecular motors may be involved in their formation. Consistently whits this, we found a strong immnuofluorescence signal in stress granules for Myosin Va, Dynein and Kinesin I. The presence of dynein and the requirement of microtubules is compatible with a role for this minus-end motor. We found that impairment of dynein function using different strategies prevents the formation of stress granules. How the retrograde transport is activated upon stress remains to be investigated.

CB-P56

TUBULIN AND Na+,K+-ATPase: ITS ASSOCIATION IN HUMAN ERYTHROCYTES MEMBRANES

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Acetylated tubulin is associated with plasma membrane Na+,K+-ATPase inhibiting its enzymatic activity, in neural and non-neural cells. We studied the presence of this association in human erythrocytes membrane (HEM). We determinated in HEM: 1) different tubulin isotypes, 2) hydrophobic properties of membrane tubulin, 3) capacity of the membrane to convert hydrophilic in hydrophobic tubulin, 4) effect of exogen tubulin on Na+,K+-ATPase activity in membranes. Results indicated that HEM contain 4-fold less tubulin that brain plasma membrane and is 50% less acetylated. As others cells, HEM's tubulin is a peripherical membrane protein, probably by its association with integral membrane protein. HEM were able to associate hydrophilic tubulin of rat brain and convert it in hydrophobic component, inhibiting its Na+,K+-ATPase activity. Double inmunofluorescence observed by confocal microscopy indicated that tubulin and Na+,K+-ATPase co-localize at the periphery of human erythrocytes. Biochemical experiment using isolated human erythrocytes demonstrated that L-glutamate increase the Na+,K+-ATPase activity and decrease of the amount of acetylated tubulin of the HEM. This effect is reverted by glucose addition. These result show that the acetylated tubulin may be involved in the modulation of the control of Na+ and K+ transport in human erythrocytes by its interaction with ATPase.

CB-P57

DECORIN EXPRESSION IN ENDOTHELIAL CELL LINES

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Decorin is characteristically present in vascular endotelial cell (EC) basement membranes and takes part in their migration. We have examined decorin secretion and its intracellular production by different vascular EC lines. Supernatants of confluent H5V, REC-A4 and 1G11 EC lines were partially purified by ion exchange chromatography and analyzed by Western blot, as well as microsomal fractions from every cell lines lysates. Zymography was performed to evaluate metalloprotease activity. Both H5V and 1G11 secrete a ~30 kDa decorin isoform but elute from DEAE column from 0.9 to 1.2 M and at 1 M NaCl, respectively. However, different isoforms were detected intracellularly: while H5V produce ~20.6 kDa isoform, 1G11 presents ~124 kDa. REC-A4 microsomal fraction only expresses the 30 kDa isoform but no secretion was detected. All isoforms produced by 1G11 would be glycosylated by chondroitin sulfate as was seen by AB chondroitin lyase digestion results. Different intracellular and secreted metalloprotease activity was detected among EC lines. Chemotaxis assays suggest that different number of migrated cells were triggered by the same stimulus. These results suggest that, decorin is differentially produced and secreted among these EC lines, and a balance between decorin and biglycan is required for cell movement. Studies are going on to understand their rol in EC migration.

CB-P58
EXPRESSION OF T MYOSIN ISOTYPES IN PERITUBULAR MYOID CELLS OF SEMINIFEROUS TUBULES IN DEVELOPMENT

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Peritubular myoid cells (PMC) produce the contraction of the seminiferous tubules (ST) facilitating the transport of spermatozoa and testicular fluid. The PMC proliferates in the testes during the embryonary state and since birth are in continuous remodelling, without dividing, in order to cover the ST, which grows in diameter. In our laboratory we have described a new isotype of myosin II (T-myosin), which is only present in PMC. We analyzed the expression of the myosin II isotypes in ST of prepuber and adults rats (12-50 days of age). ST were isolated and T-myosin purified using the method of temperature assembly and ATP disassembly. The presence of T-myosin isotypes were analyzed by SDS-PAGE and the bands were compared. We observed that rats of 12 days express 3 isotypes of T-myosin and have a molecular weight (kDa) of A: 205, B: 200 and C: 195 with a ratio of 1: 1: 1. Rats of 27 days express 3 isotypes in a relation of 1: 1: 1,5 . Rats of 35 days express a relation of 1: 1: 0,5 and rats of 50 days only express the isotypes A and B (0.5:1). We observed that in the development of ST it express the 3 myosin-T isotypes and in rats of 35 days the C isotype diminishes. The changes of T-myosin isotypes expression are produced in the major days of the remodelling of the PMC. We conclude that T-myosin isotypes can take active part in the molecular process of remodelling of PMC.

CB-P59
EARLY EXPRESSION OF ANG II RECEPTORS IN RAT FETAL TISSUES

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Angiotensin II (Ang II) elicits many physiological effects through binding to membrane receptors, classified by their pharmacological characteristics as AT_1 and AT_2 receptors. Ang II receptor expression is highly modulated during development suggesting a role of these receptors in growth and organogenesis. In the present work we analyze gene expression of the AT_1 and AT_2 receptors by RT-PCR and Northern blot analysis in rat fetal tissues. mRNAs of rat hindbrain and whole fetuses at different embryonic stages (E13 to E20) were analyzed by RT-PCR and Northern blot. A good correlation was observed between both methods. Both AT_2 and GAPDH gene expression was determined in a multiplex RT-PCR, allowing us to semi-quantify the expression level. In whole fetal tissues the expression level of AT_1 R is lower and less abundant than that of AT_2 R. mRNA of AT_2 receptor was detected at early developmental stage (E13), and increases during development showing maximum level at day E20. In contrast, in hindbrain we did not find expression of AT_1R, while low expression of AT_2R is present in E19 and increases during development. RT-PCR results correlate with those from Northern blot and are in agreement with previous observations by binding autoradiography and *in situ* hybridization. The present results support the hypothesis of a potential role of these receptors on development and organogenesis.

CB-P60

ESSENTIAL ROLE OF GLYCOSPHINGOLIPIDS DURING *Xenopus laevis* EMBRYOGENESIS

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Eukaryotic cells contain a heterogeneous class of lipids called glycosphingolipids (GSLs), these molecules are composed of ceramide backbone and a sugar headgroup. A pharmacological agent termed 1-phenyl-2-palmitoyl-3-morpholino-1-propanol (PPMP) is an important inhibitor of GSL biosynthesis, and is thus a useful tool for the study of the involvement of GSL in many cellular processes during embryo development. In this work we report the involvement of PPMP in cell behaviour of mesodermal and ectodermal layers in the amphibians *Xenopus laevis*. In order to analize the expression of specific mesodermal (*brachyury, goosecoid, paraxis*) and neural (*Sox2*) markers, resin microspheres soaked in PPMP were implanted in the right side embryos at gastrula stage (st.10). We analysed by whole mount *in situ* hybridization the morphogenetic movements during gastrulation in normal and treated embryos. Our results show that specific inhibition of glycosphingolipid biosynthesis produces embryos with abnormal mesodermal cell migration and altered ectodermal radial interdigitation. The alterate morphogenetic movements are coupled with dynamic changes in gene expression. Further studies are necessary to determine if the changes in the expression of the genes analyzed turn the cell fate in the ectodermal and mesodermal layers.

CB-P61

EXPRESSION OF MANNOSE-6-PHOSPHATE RECEPTORS DURING PERINATAL DEVELOPMENT IN RAT ORGANS

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Lysosomal enzymes (LE) are transported to lysosomes via mannose-6-P receptors (MPRs). Two MPRs have been described; the cation-dependent (CD-MPR) and the cation-independent (CI-MPR), although their co-existence is still unclear. We are interested in knowing whether CD- or CI-MPR (or both) participate in maturation of lysosomal apparatus (LA) during development. We have described that expression of both MPRs varies in rat brain and liver during perinatal development. Here, we extended this study to other organs to determine if variations of MPRs are related with functionality of the organ, and with maturation of LA. By western blot analysis, we measured expression of both receptors in rat organs at different perinatal ages. We observed that variations in both MPRs depend on the organ, as the CD-MPR in kidney (K), lung (L), hearth (H), and thymus (T) was increased at the day 10th after birth, meanwhile in spleen (S) did not change and interestingly this receptor increased in pancreas (P) of 90 day old rats. In turn, CI-MPR expression decreased progressively from newborns to adult rats in the studied organs, except in P where a dramatic increase was observed. The activity of two LE correlated with variations in expression of CD-MPR. We concluded that CD-MPR may be involved in maturation of LA, and CI-MPR is mostly devoted to regulate cell differentiation at early stages of development.

CB-P62
EFFECT OF AZYNPHOS METHYL ON POLYAMINE LEVELS DURING *BUFO ARENARUM* EMBRYONIC DEVELOPMENT

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The polyamines (PA) putrescine (Pu), spermidine (Sd) and spermine (Sm) are ubiquitous polycations involved in cell growth and differentiation. On the other hand, azynphos methyl (AM) is an organophosphate insecticide widely applied in the Alto Valle region. Our objective is to determine the effects of AM on PA levels during the embryogenesis of the common toad (*Bufo arenarum*). Embryos were exposed to 9 mg/l of AM since fertilization and until complete development (11 days), with medium replacement every 48 h. PA levels were determined by HPLC at different days of development. The three PA showed a continuous increase during development until complete operculum, being Pu the most relevant followed by Sd. Sm was detectable only in the last stages. AM induced a significant increase on Pu levels (131%) on late developmental stages, while caused a significant drop in Sd (30%) and Sm (below detection limit) levels. AM might block Sd and Sm synthesis, increase their oxidation by polyamine oxidase, or both thus increasing Pu levels and disturbing normal embryonic development. Pu might also increase as a secondary response to stress. Oxidation hypothesis is in agreement with the oxidative stress due to AM exposure during *B. arenarum* embryonic development suggested by the responses of superoxide dismutase, catalase, glutathione reductase and glutathione transferase activities.

CB-P63
ABNORMAL HEME DISTRIBUTION IN MUTANT YEAST CELLS

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Heme is a critical cofactor required for many biological processes. In eukaryotic cells, the last steps of heme biosynthesis are carried out in the mitochondria, but how it is delivered to the target proteins still unknown. In Saccharomyces cerevisiae, deletion of the genes *YLR201C* and *YOR205C* results in respiratory deficient strains. Both are nuclear genes that encode mitochondrial proteins. Jonhnson *et al.* named the *YLR201C* gene product as Coq9, because this protein is involved in CoQ biosynthesis (*JBC*, 2005, 280, 42627-35). In our hands, those mutant strains have shown lower total heme content compared to the WT. This lack of heme is not a result of a blockage in the heme biosynthetic pathway or as a result of loss of mitochondrial DNA. In *ylr201c* cells the heme deficiency was restricted to the mitochondria being the cytosolic levels comparable to the WT. The deficiency of CoQ could affect assembly and/or maintenance of complex III and its absence the cause of heme-depleted mitochondria. Preliminary results on *yol205c* mutant cells show similar phenotype that *coq9* mutant, although, it is not involved in heme or CoQ biosynthesis. The absence of this protein evidences a general effect over different components of respiratory chain of yeast mitochondria.

CB-P64

LEUCINE UPTAKE BY THE GENERAL AND SPECIFIC YEAST PERMEASES IN BREFELDIN A PRESENCE

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Amino acid transport in *Saccharomyces cerevisiae* is mediated by the general amino acid permease (GAP1) and various permeases with more restricted specificities. GAP1 catalyzes the transport of all the D- and L-isomers of amino acids. BAP2 (S1) and S2 are group-specific systems, which transport all three branched-chain amino acids. S1 has a high affinity for the substrate while S2 has a low affinity. Brefeldin A is a commonly used antifungal agent that reversibly blocks protein transport from the endoplasmic reticulum to the Golgi complex. In this study, we aimed to characterize Leucine uptake in *S. cerevisiae* in the presence of brefeldin A. For this purpose, we used a synthetic medium, containing L-proline and the detergent SDS, which allows the agent to permeate into the yeast cell. We studied the kinetics of the process in the wild type strain MMY2 and in its derived mutant gap1 MMY2/H3. We evaluated the kinetic parameters for the uptake of L-citruline, an amino acid that is only transported by the general system The results obtained indicate that BFA causes either a direct or indirect modification of the transport and/or processing of Leucine permeases. The presence of BFA affects the kinetic parameter values for L-leucine uptake and decreases not only the uptake mediated by the general system (GAP1), but also that through the specific BAP2 (S1) and/or S2 systems.

CB-P65

TRANSPORT OF LEUCINE, THREONINE AND GLUTAMINE IN YEAST, THREE SUBSTRATES AND ONLY ONE PERMEASE?

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In *Saccharomyces cerevisiae*, *LET2* gene controls the low-affinity system of L-leucine permease. Its mutant allele, *let2*, causes a major deficiency in utilization as sole nitrogen source not only of leucine but threonine as well. Presumably, it encodes or regulates a permease capable of transporting both amino acids. Our goal was to investigate the threonine transport itself. We isolate mutants deficient in threonine transport by selecting strains resistant to the threonine analog, β-hydroxynorvaline (BHNV). These mutants showed a low growth on threonine as sole nitrogen source, which suggest that could contain alleles of *let2*. We also examined if glutamine permease, Gnp1, was the threonine permease; and gnp1 another possible allele of *let2*. All the new mutants obtained and the original *let2* strain showed a modest degree of resistance to the glutamine analog, L-glutamic acid-g-monohydroxymate (GHX). Therefore, it appeared that Gnp1p was a candidate for the major threonine permease. We used appropriate primers to amplify GNP1 from total DNA from three normal and two mutant strains. Taking together these results we concluded: a) a major threonine system appears to have a relatively broad specificity, capable of transporting branched-chain amino acids and glutamine in addition to threonine, and b) it appears likely that one can isolate *let2* mutants by selecting for BHNV resistance.

CB-P66

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

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c-Fos, a component of the AP-1 family of transcription factors, is expressed at very low levels in resting cells. However, its expression is rapidly up regulated in cells undergoing G0 to S phase transition leading to AP-1-dependent gene transcription responses. In addition, cytoplasmic c-Fos associates to the endoplasmic reticulum (ER) membranes and activates phospholipid synthesis during cell growth and differentiation. Herein, it is shown that in T98G cells, c-Fos/ER association and consequently phospholipid synthesis activation is regulated by the phosphorylated state of c-Fos tyrosine residues. The small amount of c-Fos present in quiescent T98G cells is tyrosine-phosphorylated and not ER-membrane bound. In growing cells, it is dephosphorylated, associated to ER membranes and promotes phospholipid synthesis activation. Impairing tyr-dephosphorylation abrogates phospholipid synthesis activation and reduces proliferation rates to those of quiescent cells. Substitution of tyrosine residues 10, 30, 106 and 337 evidence tyr 10 and 30 as relevant for this regulatory phenomenon. It is concluded that phosphorylation of tyrosine residues 10 and 30 of c-Fos regulate the rate of synthesis of phospholipids by regulating c-Fos/ER association.

CB-P67

CADMIUM AFFECTS ANTERIOR PITUITARY CELLS VIABILITY BY AN INCREASE IN CYTOSOLIC CALCIUM

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INTRODUCCION: Previously we showed that Cd induces apoptosis of anterior pituitary cells (APC). This effect was partially dependent on mitocondrial damage by ROS. RESULTS: In cultures of APC, 25 µM Cd time-dependently increased cytosolic calcium concentration ([Ca]i), as measured by flow cytometry using the fluorescent probe Ca-green (Relative fluorescence, control: 8.77+0.03, Cd 6 h: 9.64+0.03*, Cd 12 h:12.1+0.04*, Gmean+SEM, N=10.000,*p<0.001). This increase in [Ca]i affected cell viability since BAPTA, a specific Ca chelator, was able to reverse the cytotoxic effect of Cd (Cell activity (abs 600nm), control: 0.26+0.02, BAPTA 50 µM: 0.27+0.02, Cd: 0.16+0.02*, Cd+BAPTA: 0.22+0.02^, mean+SEM, n=8, *p<0.05 vs. control, ^p<0.05 vs. Cd). Calpains are proteases activated by high [Ca]i. The cytotoxic effect of Cd was dependent of calpains activation since the type-1 calpain inhibitor (CI) reduced the metal toxicity (Cell activity (abs 600nm), Control: 0.19+0.02, CI 0.5 µM: 0.21+0.02, Cd 0.11+0.01*, Cd+CI: 0.17+0.02^, mean+SEM, n=8,*p<0.01 vs. control, ^p<0.01 vs. Cd). The elevation of [Ca]i was not modified by two antioxidants (TROLOX and N-acetyl cystein) (Data not shown). CONCLUSION: The cytotoxic action of Cd is mediated by an increase in [Ca]i. This effect is independent on Cd effect on ROS, suggesting that at least two mechanisms are involved in the cytotoxic effect of Cd on APC.

CB-P68
BONE MORPHOGENETIC PROTEINS MEDIATE DIABETIC INTESTINAL DISFUNCTION

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Bone morphogenetic proteins (BMPs) have been shown to be involved in a wide range of cellular behaviors from embryogenesis through organogenesis in the gut. In previous work we have demonstrate that this developmental pathway remains in adult life and could be altered in diseased tissue. Gastrointestinal disorders are common complications in diabetic state. In this study we have investigated the implications of BMP/Smad pathway in diabetic intestinal disorders. Our results demonstrate a significative alteration of mRNA profile for BMP ligands, receptors and Smad cytoplasm effectors during diabetic state. We observed change in levels of BMP2, BMP4 and BMP7 mRNA. In contrast no variation for BMP6 mRNA was detected. An increased of BMP type I, II receptors and Smad 1 mRNA was also reported. Interestingly similar results were obtained at protein level by western blotting. Inmuno-histochemical labelling reveals altered distribution of BMP ligands BMP2, BMP4 and BMP6 under hyperglycaemia conditions. Also, we have evaluated the level of phosphorilated Smad1 indicative of active BMP/Smad signalling. This protein is down regulated in diabetic animals. In the present work we observed an association between the diabetic intestinal dysfunction with alterations in BMP/Smad signalling, suggesting a role for BMPs in the adult intestinal homeostasis.

CB-P69
ROLE OF A NEW PITUITARY GENE IN POST-TRANSLATIONAL PROTEIN MODIFICATION

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Conjugation of the small ubiquitin-like modifier SUMO regulates cellular processes including protein trafficking and degradation, chromatin structure and regulation of transcription. In this work, we characterize R-SUME (for RWD-containing Sumoylation Enhancer), a new RWD domain-containing gene cloned from a pituitary cell line with an increased tumorigenic potential. In cultured cell lines, R-SUME localizes to both nucleus and cytoplasm and enhances SUMO-1, 2 and 3 conjugation as observed by Western blot analys. This effect is dependant of Ubc9, the SUMO conjugase, as it is abrogated whern a dominant negative form of Ubc9 is co-expressed. R-SUME co-localizes with Ubc9 in the nucleus when analyzed by fluorescent confocal microscopy. Moreover, in pull-down experniments, recombinant R-SUME interacts with a GST-Ubc9 fusion protein, even in the absence of SUMO suggesting a direct interaction which is also observed when GST-Ubc9 is used to precipitate a cell extract containing R-SUME. In addition, R-SUME increases the sumoylation of IκB, a known SUMO target, in vivo and in vitro (whereas a structural mutant of R-SUME inhibits sumoylation), leading to an inhibition of NF-κB transcriptional activity (κB-LUC reporter assay; 40% inhibition $p < 0,05$). Together, these results indicate an important role of R-SUME regulating SUMO conjugation, and therefore many critical regulatory pathways.

CB-P70

AQP9 PROTEIN EXPRESSION IN HUMAN PLACENTA IS REGULATED BY HYPOXIA

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Normal fetal growth and development is critically dependent on sufficient transport of nutrients, metabolites, ions and water across the placenta. Previously we have reported the expression of aquaglyceroporins (AQP3, AQP7 and AQP9) in human placenta. In particular, we observed over expression of AQP9 in preeclamptic placentas. We hypothesized that generated hypoxia-ischemia and/or the augmented hypoxia inducible factor-1α (HIF-1α) observed in this syndrome could be responsible for the up-regulation in AQP9 protein. We have recently reported that AQP9 mRNA expression increased after the induction of HIF-1α, but water uptake decreased. Here we investigate whether AQP9 protein is regulated by HIF-1α or by other conditions associated to hypoxia. Explants from normal placenta were cultured in normoxia, hypoxia, and chemical hypoxia generated by CoCl2, a HIF-1α inducer. Semiquantitative Western blot and immunohistochemistry were performed. The biochemical viability of the explants was determined by β-hCG. In hypoxia conditions AQP9 protein expression was higher that in normoxia. Interestingly after CoCl2 treatment AQP9 protein level significantly decreased.Our results provide new evidence to suggest that hypoxia but not HIF-1α would be the responsible for AQP9 over expression in preeclampsia. Further studies are required to clarify the regulation of AQP9 in human placenta.

CB-P71

HORMONAL REGULATION OF AQP9 MOLECULAR EXPRESSION IN SYNCYTIOTROPHOBLAST OF HUMAN TERM PLACENTA

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The syncytiotrophoblast of human term placenta (hST) is not only involved in the transport of water and solutes between mother and fetus but it also produces the majority of the placental hormones throughout pregnancy. Because of its syncytial nature the mechanisms of transport should take place primarily via transcellular routes. Previously, we have reported the expression and localization of AQP3, AQP7 and AQP9 in hST. In pathological conditions like preeclamptic pregnancies, we have also observed an over expression of AQP9 correlating with an increase of human chorionic gonadotropin hormone (hCG). The aim of this study is to determine if hCG or steroid hormones as progesterone (P4) may regulate AQP9 molecular expression. Normal placental explants were cultured and treated with P4 or hCG. Semiquantitative RT-PCR and Western blot and Immunohistochemistry were performed to evaluate AQP9 expression. After P4 treatment, AQP9 protein expression decreased and it is localized in the apical membrane of hST. On the other hand, after hCG treatment we showed that AQP9 levels were not modified but it is also localized in the cytoplasm region. Our results suggest that P4 and hCG may be involved in the regulation of AQP9 expression in hST. However, much further work is needed to clarify its role in human placenta.

CB-P72
DROSOPHILA eIF4E-BP IS NECESSARY FOR ADAPTATION TO LOW OXYGEN STRESS

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eIF4E-binding protein (4E-BP) is an important negative regulator of translation in eukaryotic cells. 4E-BP binds the translation initiation factor eIF4E, thereby impairing recruitment of the 40S ribosomal subunit to translation initiation complex. The activity of 4E-BP is negatively controlled by the kinase TOR and therefore, it is one of the final effectors of the Insulin/PI3K signalling pathway. It has been recently demonstrated that 4E-BP is critical for survival under dietary restriction and oxidative stress conditions because of its function as a metabolic brake. Microarray comparison of hypoxia vs. normoxia expression profiles in Drosophila S2 cells suggested that 4E-BP is induced in hypoxia, opening the possibility that it is required for hypoxic adaptation. In this work, we show that in living embryos 4E-BP is upregulated in hypoxia in a defined expression pattern. Homozygous 4E-BP mutants have increased lethality under prolonged hypoxia and a reduced capacity to recover upon exposure to severe acute hypoxic stress. Thus, our results show that 4EBP is necessary for adaptation to both prolonged and acute hypoxia, suggesting that general silencing of protein translation is necessary for the induction of specific genes that mediate survival in low oxygen conditions.

CB-P73
INHIBITION OF PROLIFERATION BY SCAVENGING H2O2 IN NEUROBLASTOMA CELLS IS MEDIATED BY G1/S ARREST

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A role of H2O2 in the induction of cell proliferation has been demonstrated. In a previous report we demonstrated the inhibition of proliferation in tumor cells treated with exogenous catalase (CAT) or transfected with cDNA of CAT. The aim of the present study was to evaluate the mechanisms by which scavenging of H2O2 inhibits cell proliferation in neuroblastoma cells. For this purpose, the modulation of cell proliferation was evaluated after treatment with CAT (0-1000 U/ml) in the neuroblastoma PAJU cell line. Proliferation was evaluated by MTT assay and cell cycle analysis was performed by flow cytometry. To analyse the cell cycle arrest at G1/S, the levels of cyclins D1 and E, CDK2, CDK4 and p27 were determined by western blot. The levels of reactive oxygen species (ROS) were measured by flow cytometry using DCFH assay. Results demonstrated a dose-dependent inhibition of proliferation by CAT and a significant ($p<0.05$) cell cycle arrest at G1 phase. A decrease in cyclin D1 and an increase in p27 levels were observed with CAT treatment, e.g. 42 % and 38 % respectively, after 6 h with 1000 U/ml. A significant decrease ($p<0.05$) in the levels of ROS was detected in cells treated with CAT, e.g. 72 % with 1000 U/ml. In conclusion, the scavenging of H2O2 by CAT induced G1/S arrest by modulating specific regulatory proteins of early to mid G1 (cyclin D1) and transition G1/S (p27).

CB-P74

ANTIPROLIFERATIVE EFFECT OF GLIBENCLAMIDE IN MDA-MB-231 CELL LINE

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Glibenclamide (Gli) is a sulphonylurea widely used for treatment of non-insulin-dependent diabetes mellitus, and signaled as antitumoral in several cell lines. We previously demonstrated the antitumoral effect of Gli on mammary tumors induced in rats. The aim of this work was to study the in vitro effect of Gli alone or combined with tamoxifen (Tam) on MDA-MB-231 breast cancer cells. Cell growth was determined using the clonogenic assay and results indicate that Gli inhibited it in a dose dependent manner (IC50=25 μM). Gli also produced a significant cell-cycle arrest in G0/G1 phase (89% vs. 50% in controls), analyzed by flow cytometry. In agreement, the doubling time showed a significant increase after Gli treatment (34.6 h vs. 24.9h in controls) and PCNA expression decreased significantly (66% vs. 100%), evaluated by flow cytometry. However, neither differentiation nor apoptosis was detected by nile red staining and Annexin V, respectively. Furthermore, the cell proliferation inhibition upon Gli addition was reverted after treatment removal. The combination of Gli plus Tam (0.1, 1, and 5 μM) produced similar results to Gli alone. We conclude that Gli inhibits the G1/S phase progression without inducing apoptosis suggesting a novel use of Gli as an alternative drug to treat breast cancer however, further studies are necessary to elucidate the mechanism involved in growth arrest.

CB-P75

CHARACTERIZATION OF A SR-RELATED PROTEIN FROM T. CRUZI AND ITS BEHAVIOUR UNDER STRESS CONDITIONS

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TcSR62 is a RNA Binding Protein (RBP) from Trypanosoma cruzi belonging to the SR-related RBP family. SR and SR-related proteins have multiple roles in mRNA metabolism, particularly as regulators of pre-mRNA splicing. Molecular characterization of TcSR62 showed that it is encoded in a single-copy gene expressed both at the RNA and protein level in all parasites stages. The protein localizes mainly to the cell nucleus in a speckled pattern, and it is also present, although in lesser amount, in the cytoplasm in a fine punctuated pattern. Taking into account that TcSR62 and its orthologue in T. brucei (RRM1) have been putatively implicated in cell cycle/ differentiation and that the parasite's differentiation might be trigger by different environmental conditions we were interested in studying TcSR62 involvement in these processes. We have evaluated TcSR62 behavior under oxidative stress and heat shock. We found that there is an increment on the relative abundance of TcSR62 in a precipitable fraction after subcellular fractionation induced by oxidative stress or heat shock treatment. This behavior could be reverted after removing the stressor. In order to explore this issue further, we are currently performing inmunofluorescence assays on parasites submitted to different stresses to investigate the subcellular localization of the precipitable fraction.

CB-P76

SERINE 76 PHOSPHORYLATION AND THE FOURTH ANKYRIN DOMAIN ARE INVOLVED IN p19INK4d DNA REPAIR ACTIVITY

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We have previously reported that p19INK4d (p19), a member of INK4 cell cycle inhibitors, enhances DNA repair ability and increases the capacity to survive after UV damage. However, the mechanism by which p19 confers this ability remains unknow. p19 has 4 putative serine phosphorylation sites, 2 of which, serine (ser) 66 and ser76, were found to be phosphorylated in vivo. Besides, p19 has 4 ankyrin domains, the fourth (ANK4) markedly differs in DNA sequece from the other 3 INK4 family members. This work aims to study if p19 phosphorylation at ser 66 and/or 76 are required to increase DNA repair activity upon DNA damage and if the ANK4 has a role in this process as well. First, we made a time course phosphorylation curve applying UV, cisplatin and beta amyloid protein as damaging agents. The three types of damages promotes p19 phosphorylation beginning within 2hs upon damage. Four p19 mutations were made, replacing ser for alanine at ser66 or ser76 or at both sites and truncating the last ankyrin domain. For all of them, cell cycle arrest ability, DNA repair capacity and caspase activity were assayed. We conclude that ser76 but not ser66 is necessary to increase DNA repair activity and diminish apoptosis although neither of them are involved in cell cycle arrest ability. As for ANK4, it´s found to be essential to enhance DNA repair but also to exert an efficient cell cycle arrest.

CB-P77

TRANSCRIPTIONAL REGULATION OF THE STARD7 GENE BY STEROIDOGENIC FACTOR 1

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We have previously cloned and characterized a novel gene up-regulated in choriocarcinoma JEG-3 cell line. This gene, denominated StarD7 encodes a protein that belongs to the StAR-related lipids transfer proteins involved in intracellular transport and metabolism of lipids. To understand the molecular mechanisms that regulate the expression of StarD7 gene, we have cloned and characterized the 5´flanking region of the gene. Transient transfections of several 5´deleted StarD7-promoter-firefly luciferase constructs into JEG-3 and COS-7 cells suggest that the -312/+157 region contains the gene minimal promoter. In addition, sequence analysis of a 1,6 kb gene fragment revealed the presence of a TATA-less promoter as well as multiple regulatory motifs, including one steroidogenic factor 1 (SF-1) binding site at -796/-784 and three cAMP-responsive binding elements at -512/-509, -465/-462 and -115/-111. Electrophoretic mobility shift assays using nuclear proteins from JEG-3 show DNA-protein specific complexes. Co-transfection experiments of JEG-3 and COS-7 cell lines demonstrated that the human StarD7 promoter is activated by SF-1 transcription factor and this effect is increased by cAMP. These studies provide novel insights into the regulation of the human StarD7 gene at the transcriptional level by SF-1. [Supported by CONICET, FONCyT and SECyT-UNC].

CB-P78

C/EBPβ AND HP1 ASSOCIATION DURING 3T3-L1 CELL DIFFERENTIATION

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Obesity is one of the most prevalent health problems in the world, considered in relationship to the prevalence of associated diseases such as type II diabetes, dyslipemia and hypertension. Obesity is, in part, the resultant of increased adipogenesis, a process triggered by a cascade of genetic events in which the transcription factors CCAAT/Enhancer Binding proteins (C/EBP) play a key role. Intriguingly, C/EBPβ localizes in heterocromatic areas upon induction of 3T3-L1 to differentiate into adipocytes. To elucidate the molecular mechanism that regulates such nuclear distribution, we investigated whether C/EBPβ interacts with putative C/EBP sites present in satellite DNA. Chromatin immunoprecipitation assays show that binding of C/EBPβ to satellite DNA increases as cells differentiate. However, it cannot be ruled out that localization in heterochromatin may be also regulated by protein–protein interactions. Consistent with this hypothesis, C/EBPβ co-immunoprecipitates with HP1α, a protein enriched in heterochromatin. Moreover, HP1 restrains C/EBPβ transcriptional capacity in a concentration dependent manner. In summary, upon induction of adipogenesis C/EBPβ localizes in heterochromatin possibly driven by its interaction with HP-1α and binding to C/EBP sites present in satellite DNA. C/EBPβ-HP1 complexes may participate in the control of C/EBP target genes during adipogenesis.

CB-P79

ANTAGONIC FUNCTIONS OF GALECTIN-8 ON CELLS OF THE IMMUNE SYSTEM

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Galectins are mammalian lectins that bind galactosyl residues. Galectin-8 (Gal-8) is a tandem repeat-type galectin, presenting two carbohydrate binding-domains joined by a linker peptide. In contrast to Gal-1 and 3, which were extensively studied in their roles in the immune system, little is known about Gal-8. We have determined by RT-PCR and western-blot the presence of Gal-8 in thymocytes, thymic epithelial cells and splenocytes. Thymocytes and splenocytes were incubated with 0,5μM of recombinant Gal-8. In thymocytes, apoptosis induction triggered by caspase-3 activation was observed, as assessed by substrate cleavage and flow cytometry. In contrast, Gal-8 induced proliferation of splenocytes as determined by ^{3}H thymidine incorporation. This effect was also seen with splenocytes from *nude* mice, indicating that B cells were activated directly. Apoptosis and proliferation were fully prevented when a galactose analog (TDG) was included in the assays, demonstrating that these effects were caused by the Gal-8 sugar-binding ability. In order to identify potentials ligands for Gal-8 in the spleen, a pull-down assay was performed. Biotinylated membrane proteins of splenocytes from *nude* or *wt* mice were employed and defined bands were revealed in both cases. These data support a divergent role for Gal-8 among different immune system compartments.

CB-P80
DEVELOPMENT OF OLIGOBODIES (APTAMERS) AGAINST THE BOVINE PRP (PRION)

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Oligobodies (Obs) are a new generation of aptamers, which possess higher specificity than their predecessors. The objective of this work was to develop polyclonal Obs against synthetic peptides corresponding to three different regions of bovine PrP (PrPc, non-infective), and to test them in their ability to recognize cellular PrP (PrPc) and recombinant PrP (rPrP), as a first approach in the development of Obs against the infective prion (PrPsc). Three synthetic peptides were designed as temporary targets for PrP. Selections were performed incubating membranes saturated with each target peptide and with the single strand library. The bound and eluted oligonucleotides were then amplified by PCR and the selection repeated until a clear binding to the target was obtained. Then, the peptide targets were changed for the entire PrP protein present in a complex mixture. This procedure adds a pressure of selection towards high specificity, instead of high affinity, which distinguishes our method from SELEX, the method to produce aptamers. The polyclonal Obs obtained were able to recognize rPrP (50 ng) in Western blots. Even thought the specificity was not optimal yet, they might be used in a blood assay if a previous treatment with proteinase K is performed. We will now introduce random mutations on the monoclonal Obs to attempt to improve their specificity. Acknowledgments: CONICET and UBA.

CB-P81
ZCD1 IS A NEW MITOCHONDRIAL-LOCATED PROTEIN DOWN-REGULATED IN CYSTIC FIBROSIS

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We have characterized a CFTR-dependent gene, ZCD1, which codifies for the first member of a new Zn-finger family. The function is unknown yet. ZCD1 mRNA is negatively modulated in CFDE cells (Cystic Fibrosis, CF) and its levels are recovered in CFDE cells ectopically expressing wt CFTR. Confocal FISH, in situ hybridization, and semi-quantitative RT-PCRs confirmed these results. Cells treatment with glibenclamide (50 μM, 24 h), produced a reduction in the ZCD1 mRNA levels. The protein sequence of ZCD1 has a motive for mitocondrial translocation, as predicted by using the program PSORT II. Transfecting CFDE cells with a chimera of ZCD1 and EGFP, and comparing the results with cells treated with TMRM (a mitochondrial specific dye), we have observed by confocal microscopy that the mitochondrial prediction for its location was correct. At present, we are performing additional studies by using a more specific inhibitor than glibenclamide (CFTR(Inh)-172), recently developed by other researchers, and using CFTR and ZCD1 siRNAs to attempt to define its possible biological function, most likely related to the mitochondrial activity. Its downregulation in cystic fibrosis might explain mitochondrial failures reported many years ago in CF, for which the mechanisms are still unknown. Acknowledgements: University of Buenos Aires, CONICET, and ANPCyT.

CB-P82
DOWN-REGULATION OF THE MITOCONDRIAL GENE ND4 IN CYSTIC FIBROSIS

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Cystic Fibrosis (CF) is a frequent genetic disease produced by mutations in the CFTR chloride channel. We have previously identified the subunit ND4 of the mitochondrial Complex I (mCx-I) as a CFTR-dependent gene down-regulated in CF. Our objective is now to confirm its CFTR-dependency and to determine whether the failure in the Cl- transport by CFTR induces a reduced mCx-I activity. Blue Native-PAGE was used to measure the mCx-I activity on mitochondria extracted from cells derived from a patient with CF (CFDE cells), the same cells ectopically expressing wt CFTR, and T84 cells (expressing wt CFTR). A significant decrease in the activity of the mCx-I was observed between CFDE and CFDE/6RepCFTR cells, an effect that could also be obtained on T84 cells by using glibenclamide, a CFTR inhibitor. We are now confirming these results with the new CFTR inhibitor (CFTR(inh)-172), which is more specific and potent than glibenclamide. The expression of ND4 could also be reduced by using the CFTR inhibitor glibenclamide or CFTR(inh)-172, confirming that ND4 expression can be modulated by the CFTR chloride transport activity. These results might explain the mitochondrial abnormalities reported by other authors in CF, which mechanisms remain unknown yet. Acknowledgments: University of Buenos Aires, CONICET, ANPCYT and Ministry of Health (Carrillo Oñativia Fellowship).

CB-P83
MODULATION OF CELL MATRIX ADHESION PLASTICITY BY BRADYKININ (BK) IN RENAL COLLECTING DUCT CELLS

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Focal adhesions (FA) are structures of cell attachment to extracelular matrix. We previously found that BK induces a restructuration of FA by mobilizating FA protein vinculin (V), but not talin (T), by a mechanism that involves phosphatidylinositol 4,5 biphosphate hydrolysis by PLC. Now, we performed a detailed quantitative morphometric analysis of FA, which include size, axial ratio (AR) and average length (AL). After 1', 5' or 10' (minutes) BK incubation, cultured cells were immunostained and analyzed by confocal microscopy. BK induced a decrease in the number of V-stained FA per cell 5.1; p±9.1, 30.6±9.8, 29.4±14.4 vs 25.2±(Control vs 1',5',10': 72.0<0.05), 6.1, pNS). Although±10.7, 43.5±9.5, 50.6±4.2 vs 41.2±but not T-stained FA (41.6 V- and T-stained FA shape remained round to ellipsoid in all groups (AR<3), m2) between cells (Control:μBK induced a great variation in V-stained FA size (0.3-7, 1': 0.3-25, 5': 0.3-12, 10': 0.3-9), but not in T-stained FA. Also induced a great variation in V-and T-stained FA AL. BK induced the formation of "transversal stress fibers" and migratory structures. So, BK by modulating the plasticity of V-containing cell matrix adhesions and the actin cytoskeleton, could be inducing changes in cells morphology to assume the irregular shapes, perhaps to induce cell motility.

CB-P84
IN VITRO STUDIES OF GLYCOLIPID GLYCOSYLTRANSFERASE INTERACTIONS

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Glycolipid glycosyltransferases are type II membrane proteins resident of the Golgi complex (GC). Their N-terminal domains (Ntd) participate in protein-protein interactions in vivo, forming at least two multienzyme complexes: one formed by GalNAcT/GalT2, and another by GalT1 /SialT1/ SialT2. To study the molecular determinants of these interactions in vitro, we designed molecular constructs of GalNAcT(Ntd)-YFP and GalT2(Ntd)-HA-YFP fused to several tags, and expressed the recombinant proteins in E. coli. Among these, fusions to intein were found convenient. GalNAcT(Ntd)-YFP-intein was linked to a chitin matrix, and was then allowed to interact with GalT2(Ntd)-HA-YFP. As the constructs contain a hydrophobic transmembrane domain, the binding assay was optimized by adding lipids or Triton-X100 at different concentrations. The interaction was determined by monitoring the amoGunt of GalT2(NTd)-HA-YFP specifically bound to the matrix by Western blot, using an anti-HA specific antibody. Among other conditions, the presence of lipids and/or Triton X100, was found necessary for the interaction to occur. This method will allow to perform in vitro studies of the effects of point or cluster amino-acid mutations on binding properties of glycolipid glycosyltransferases.

CB-P85
FLUORESCENCE IN ROOT EXUDATES OF SOYBEAN SEEDLING IS INFLUENCED BY PHOSPHATE STARVATION

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Phosphorus is one of the essential macronutrients in plants. Phosphate starvation leads to changes in morphological, physiological and biochemical processes. Some roots fluoresce when irradiated with UV light. This trait has been useful as a marker in genetic studies. We studied the influence of phosphate starvation on the amount of root and seed exudates and leaves fluorescence in two soybean genotypes (FN-4.85 and FN-4.10). Plants were grown in hydroponic cultures. The fluorescence was determined with a spectrofluorometer in 21 days-old plants. Our results indicated that fluorescence emission (F420nm) of root exudates was significantly higher ($p<0.05$) in FN 4.85 and significantly lower in FN 410 ($p<0.05$) in phosphate deficient plants. In seeds, fluorescence had a different maximum emission band (F503nm) than root exudates, but was identical between both genotypes. Fluorescence in leaves showed the same emission spectrum (F420nm) of root exudates, but no effects of phosphate limitation was detected. We also determined that the fluorescence ratio (F684/F737) for chlorophyll emission did not vary under phosphate starvation. This is an indication that growth for 21 days under the latter condition had not yet induced quali/quantitative changes in photosystems. These results could reflect the genetic variability in soybean which could perhaps be extended to other soybean genotypes.

EN-P01
KINETIC CHARACTERIZATION OF *A. TUMEFACIENS* GLYCOGEN SYNTHASE

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Glycogen and starch are the most widespread energy storage compounds in living organisms. Glycogen and starch synthases (GS or SSs) are glycosyltransferases that catalyses the transfer of glucosyl residues from ADPGlc to the non-reducing end of a growing alpha-1,4-glucan chain. The structural, kinetic and regulatory properties of GSs from mammals or SSs from plants were well characterized. Furthermore, it has been recently described that the 3D structure of A. tumefaciens GS posseses a fold common to other glycosyltransferases. However, the regulatory properties from bacterial GSs were less studied. In the present work, recombinant His-tagged A. tumefaciens GS was expressed in E. coli BL21 RIL cells and purified to homogeneity by one-step purification using a Ni-chelating colum. We determined that A. tumefaciens GS is active in the presence of different glucosyl donors. Indeed, the enzyme is inhibited by malate, succinate and citrate, three metabolites from the TCA cycle. These results allow us to propose the different residues that determine glucosyl donor specificity and suggest that the GS activity is determined by the levels of different compounds that participate in energetic metabolism.

EN-P02
CHARACTERIZATION OF GDP-MANNOSE PYROPHOSPHORYLASE FROM *Leptospira interrogans*

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Leptospirosis is a widely spread disease of global concern. The genome project of *Leptospira interrogans* serovar Copenhageni (http://aeg.lbi.ic.unicamp.br/world/lic) was recently finished, which is of great help in the characterization of relevant proteins related to the adhesion, the invasion and the haematological changes that characterize leptospirosis. Exopolysaccharides and other glycoconjugates play a key role in the pathogenicity of many microorganisms. The biosynthesis of these compounds includes sugar activation by nucleotide diphosphate-sugar pyrophosphorylase enzymes. We report the molecular cloning of the gene encoding for GDP-mannose pyrophosphorylase (GDP-Man PPase) from *L. interrogans*, as well as the expression, purification, and functional characterization of the recombinant enzyme. We used genomic DNA of *L. interrogans* serovar Copenhageni as template for PCR. The amplified DNA was cloned into pGEM-T easy vector. The identity of the cloned gene was confirmed by complete sequencing. The expression system selected was *E. coli* BL21(DE3)/ pRSETB vector for expression. The purified recombinant protein reversibly synthesized GDP-Man and PPi from GTP and Glc-1P. The kinetic characterization of the recombinant enzyme included analysis of alternative substrates, allosteric effectors and K_m values for GDP-Man (0.20 mM), PPi (0.10 mM), Man-1P (0.06 mM), and GTP (0.36 mM).

EN-P03

AN UDP-GLUCOSE PYROPHOSPHORYLASE MUTANT EXHIBITS A PARTICULAR KINETIC BEHAVIOR

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Xanthomonas campestris is the microbial source for the hetero-exopolysaccharide xanthan gum, a natural product with key industrial applications. The enzyme UTP:a-D-glucose-1-phosphate uridylyltransferase (EC 2.7.7.9; UDPGlcPPase) is of central importance for the polysaccharide synthesis, which metabolism begins with UDP-glucose. We purified a recombinant UDPGlcPPase mutant from *X. campestris* pv *campestris* displaying a single amino acid mutation (Y41H) that exhibited high specific activity (25 U/mg), and distinctive kinetic properties. It followed sequential hyperbolic kinetics respect to both susbtrates, but displayed Glc-1P reversible and time dependent inhibition, in both forward and reverse reaction directions. The enzyme appears to exist in equilibrium between monomeric and oligomeric forms, wich are affected by substrate. These results were observed by gel filtration chromatography analysis and SDS-PAGE of oligomeric structures stabilized by corsslinking with the bifunctional reagent bisuberate. Comparative studies of this mutant with other bacterial UDPGlcPPases are critical to understand the relevance of residues involved in substrate binding and enzyme oligomerization.

EN-P04

MOLECULAR CLONING AND CHARACTERIZATION OF UDPGLUCOSE PYROPHOSPHORYLASE FROM *Trypanosoma brucei*

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Trypanosoma brucei is the etiological agent of african trypanosomiasis. The metabolism implicated in Glc-P partitioning and synthesis of structural polymers has been poorly characterized in parasites. In trypanosomatids, the production of structural oligo- and polysaccharides occurs via UDP-Glc; thus, being UDPGlcPPase (EC 2.7.7.9) the enzyme that catalyzes the first step in the pathway of carbohydrates interconversion. We report the molecular cloning of the gene coding for UDPGlcPPase from genomic DNA of *T. brucei*. The identity of the amplified gene was confirmed by complete sequencing. We used BL21(DE3)/pRSETB for expression of the recombinant UDPGlcPPase. The purified recombinant protein exhibited typical hyperbolic saturation kinetics for substrates. Values of Km for UDPGlc (0.22 mM), PPi (0.28 mM), UTP (0.30 mM) and Glc-1P (0.12 mM) were determined. The enzyme activity was affected by redox modification of thiol groups. A similar study was performed for the enzyme from *T. cruzi*. Results suggest the occurrence of a physiological redox mechanism for regulation of UDPGlcPPase in trypanosomatids. A comparative analysis with the enzyme from bacteria and plants is rendering relevant information for the understanding of evolution and structure to function relationships of this key protein in carbohydrates metabolism. Granted by: UNL, CAI+D 2006; ANPCyT, PICT´03 01-14733, PAV´03 137.

EN-P05
SOLVING THE EXPRESSION OF ADP-GLUCOSE PYROPHOSPHORYLASE FROM *Mycobacterium tuberculosis*

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In Gram negative bacteria, the biosynthesis of glycogen have been extensively characterized, specially the key enzyme ADP-glucose pyrophosphorylase (ADPGlcPPase, EC 2.7.7.27). *M. tuberculosis* is a human pathogen of very slow growing velocity and consequently the isolation of the enzyme from the microorganism is a time consuming practice. Expression of the gene encoding the *M. tuberculosis* ADPGlcPPase in *Escherichia coli* resulted recalcitrant to obtain the soluble protein. We solved this problem through the expression of the gene in *M. smegmatis*. Thus, the *M. tuberculosis glgC* gene was cloned into the shuttle pMIP12 vector. Competent *M. smegmatis* mc^2155 cells were transformed with the recombinant construct (pMIP12/Mt*glgC*), the protein was expressed with a C-term HisTag and purified chromatographically. Eluted fractions were tested by Western blotting and enzymatic activity. Functional characterization was performed in both ADPGlc synthesis and pyrophosphorolysis. Values for *Km* of 0.53 mM (ATP) and 0.52mM (ADPGlc) were determined. The enzyme was sensitive to allosteric effectors, different to that found for *Bacillus stearothermophilus*, the unique studied recombinant enzyme of Gram positive bacterium. The developed recombinant system is being useful not only for the functional characterization of the enzyme, but also for generation of *glgC* null mutants.

EN-P06
CAPILLARY ELECTROPHORESIS AND INTRINSIC FLUORESCENCE BINDING STUDIES ON ADPGLUCOSE PYROPHOSPHORYLASE

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ADPGlc pyrophosphorylase is a key regulatory enzyme in the pathway of glycogen synthesis in bacteria. Site directed mutagenesis and kinetic studies were performed in order to understand allosteric mechanisms of activation of the enzyme from *Escherichia coli*. Capillary electrophoresis and emission fluorescence techniques were employed for the analysis of effector binding affinities. As they are based on different principles, sensibility and information obtained from each technique were different. We used electrophoretic mobility shifts to determine the dissociation constants of the activator (fructose1-6- bisphosphate, FBP) to the wild type enzyme (25 µM), and mutants W113A (71µM) and Q74A (81µM). The degree of specific chemical modification by the activator pyridoxal-5-phosphate (PLP) was also determined by assuming constant hydrodynamic radius. Intrinsic fluorescence was not sensible enough to quantify effectors affinities, but a blue shift in the lambda peak of emission denoted a conformational change after FBP binding to the wild type enzyme at optimal concentration. This change was not observed with the single mutants that were insensible to FBP and PLP activation, although both of them bound FBP and PLP. Kinetic and ligand binding results are in agreement with the hypothesis that mutated residues are important to elicit a conformational change after FBP binding.

EN-P07
INHIBITORY EFFECTS OF SUBTRATES AND PRODUCTS ON CYCLODEXTRIN GLUCANOTRANSFERASE ACTIVITY

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Cyclodextrin glucanotransferase (CGTase) catalyses starch degradation to cyclodextrins (CD), cyclic oligosaccharides consisting of six (α-CD), seven (β-CD) or eight (γ-CD) glucose units. Besides, catalyses a coupling reaction, where the CD ring is opened and transferred to a linear dextrin and a disproportionation reaction, where part of a linear oligosaccharide is transferred to another. As a result of these reactions, CD, glucose, maltose, maltotriose and other linear maltooligosaccharides are produced. CGTase activity can be inhibited by high starch concentration and product accumulation. In this work, we study these inhibitory effects on the activity of the CGTase from Bacillus circulans DF 9R in order to optimize the CD production process. The initial reaction rate was determined as a function of the potato starch α, β and γ-CD. The optimal starch concentration was determined by measuring 2% and a substrate inhibition kinetic was observed. Furthermore, α, β and γ-CD showed a competitive inhibition kinetic on cyclation activity when the CD production from starch was measured. Low molecular weight saccharides from maltose to maltoheptaosa at 1% w/v concentration inhibited the β-CD production from 1% starch. Maltose had the greatest inhibitory effect on initial reaction rate (56%). Glucose had inhibitory effect at higher concentration (above 2%).

EN-P08
ENZYMATIC ACTIVITY OF A *Escherichia coli* NADH DEHYDROGENASE-2 LACKING THE CARBOXI-TERMINAL REGION

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NADH dehydrogenase-2 (NDH-2) of *Escherichia coli* is a membrane flavoprotein with cupric-reductase activity. By bioinformatics, we proposed four functional domains in NDH-2, one of them, domain IV, could be involved in the enzyme anchorage to the membrane. However, other authors have proposed that this interaction could occur by multiple anphipatic turns along the enzyme. The aim of this work was to obtain a mutant protein without the carboxi-terminal region or domain IV and to analyze the effects over NDH-2 enzymatic activities. We used a NADH dehydogenase deficient strain complemented with either the wild-type *ndh* gene or the mutant one. The results obtained with all the cellular fractions tested showed that the enzyme without carboxi-terminal region was less active respect the wild-type enzyme. Furthermore, the NDH-2 mutant was not able to grow in mannitol as it was described for NADH dehydrogenase null mutants. Our results suggest that the carboxi-terminal region of NDH-2 could be implicated in its enzymatic activities. The lack of activity could be caused by abnormal cellular localization and improper interaction with quinones.

EN-P09
CLONING AND CHARACTERIZATION OF THE FERREDOXIN-NADP REDUCTASE FROM *Xanthomonas axonopodis* pv. *citri*

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Xanthomonas axonopodis pv. *citri* (Xac) is the phytopathogen responsible for citrus canker. In plant-microbe interactions, increased production of reactive oxygen species (ROS) is an important component of the active plant defense response, which is thought to function directly in growth inhibition of pathogens. In order to survive and proliferate, bacteria evolve enzymatic and non-enzymatic mechanisms to rapidly detoxify ROS. One of the enzymes involved in the oxidative stress response in bacteria is ferredoxin-NADP(H) reductase (Fpr/FNR), which mediate reversible redox reactions between NADP(H) and one-electron carriers, such as ferredoxin or flavodoxin. The Fpr activity is thought to keep NADPH at tolerable levels during the progress of the stress condition. Recently, the genome of Xac has been sequenced and the *fpr* gene identified. The sequence analysis of the Fpr protein revealed characteristic features of the subclass I of bacterial FNRs, represented by the *Azotobacter vinelandii* prototype. We present the molecular cloning of the *fpr* gene from the bacterial genome and an efficient expression and purification system of the recombinant protein. We determined the spectral properties and kinetic parameters of Xac Fpr and found that they are very similar to those reported for other type I bacterial FNRs.

EN-P10
MOLECULAR BASIS FOR THE HIGH CATALYTIC EFFICIENCY OF PLANT FNRs

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FNRs are ubiquitous flavoenzymes that participate in a broad range of redox metabolic pathways. FNRs consist of two domains; one involved in the binding of the prosthetic group FAD and the other in the binding of $NADP^+$. An aromatic residue (Tyr308 in pea FNR) has been suggested to be responsible for high enzyme catalytic efficiency and is conserved in all plant-type FNRs. Tyr308 stacks coplanar to the re-face of the isoalloxazine moiety making extensive interactions with it. Analysing single or double mutants of aliphatic amino acids facing the other side of this Tyr308 by circular dichroism we observed that all mutants were properly folded. Thermal conformational analysis showed that increasing the amino acid volume decreases the stability of the FNRs mutants. Reducing the amino acid volume produces equally or more stables FNRs than the WT enzyme but, with lower catalytic efficiencies, probably due to an increase of the Y308 - isoalloxazine interaction. Our results exemplify how the evolutionary success of enzyme is a compromise between high activity and high stability and show that in the FNR the volume of some aliphatic amino acids have been evolutively selected to maximize enzyme catalytic efficiency.

EN-P11
MOLECULAR STUDIES OF GLYCEROL-3-PHOSPHATE DEHYDROGENASE FROM *TRIATOMA INFESTANS* THORACIC MUSCLES

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Triatoma infestans, Chagas disease vector, acquires wings and the ability to fly after the last molt from fifth instar nymph to adult. The ability to fly is important for dispersion. In previous studies, in our laboratory, it was demonstrated that glycerol-3-phosphate dehydrogenase (GPDH), involved at glycerophosphate shuttle, increases its activity 30-fold in adults thoracic muscles. Adult muscles should have higher glycolytic and respiratory capacity to support fly activity. Electrophoretic studies from thoracic muscles showed two GPDH isoenzymes. Nymphs predominant isoform has less mobility. The aim of this work is to begin GPDH molecular studies. Using cDNA pools with degenerated *primers*, we amplified a 200 bp RT-PCR product from 1 to 3 days old *T. infestans* adults thoracic muscles. Upon sequencing and database alignments the fragment was shown to be part of GPDH cDNA. Based on the sequence obtained, specific *primers* were designed for RACE experiment. We successfully amplified the 5´ and 3´end of the GPDH cDNA. Sequencing results allow identity, homology and conserved domains comparative studies with others species.

EN-P12
2-CYS PEROXIREDOXIN GLUTATHIONYLATION IS INVOLVED IN CHLOROPLAST FRUCTOSE-1,6-BISPHOSPHATASE ACTIVATION

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2-Cys Peroxiredoxins (2-Cys Prx) are ubiquitous heme-free peroxidases that possess two conserved cysteines involved in the detoxification of reactive oxygen species and redox signalling. In particular, oxidized derivatives of the Cys in the N-terminal region are implicated in the peroxidase activity and cell redox regulation. We found that rapeseed 2-Cys Prx stimulates the activity of chloroplast fructose-1,6-bisphosphatase (CFBPase) through a mechanism different from the reductive activation. In order to analyze the action of cysteine chemical modifications on the activity of CFBPase, we prepared the reduced, the S-glutathionylated; the superoxidized (Cys53-SO3H) and the phosphorylated forms of rapeseed 2-Cys Prx and subsequently confirmed the derivatization by MALDI-TOF. Only 2-Cys Prx bearing a glutathione moiety exhibited the capacity to enhance CFBPase activity. Remarkably, the polypeptide was glutathionylated on both Cys53 and Cys175 when incubated at pH 8 but only in Cys175 when incubated at pH 8.5. Of these preparations, the former stimulated the activity of CFBPase. These results suggest that the incorporation of a glutathione moiety to Cys53 may be essential for the mechanism of CFBPase activation. Hence, our results provide insights about signalling via the S-glutathionylation of proteins, an alternative well-studied in mitochondria but yet unexplored in chloroplast.

EN-P13
THIOREDOXIN LINKED METABOLISM IN *Entamoeba histolytica*

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Entamoeba histolytica, an intestinal parasitic protozoan, is the causative agent of amoebiasis. The parasite usually lives and multiplies within the human gut, under reduced oxygen pressure. During tissue invasion, it is exposed to increased amounts of reactive oxygen species (ROS), which are highly toxic for the parasite. The metabolic pathway used by this organism to cope with such environmental changes is a matter of some controversy. In this work, we present the cloning and expression of the genes *ehtrxr* and *ehtrx41*, coding for thioredoxin reductase (TRXR) and thioredoxin (TRX), respectively. The *Eh*TRXR was evaluated in its ability to catalyse the NADPH dependent reduction of DTNB, *Eh*TRX41 and *Ec*TRX. This work strongly supports the occurrence in *E. histolytica* of a metabolic pathway, the *Eh*TRXR/*Eh*TRX41 system functioning coupled to peroxiredoxin, which was not previously described in the parasite. The redox system could be operative for NADPH-dependent detoxification of ROS, and to metabolize low molecular weight thiols (glutathione and cystine). In addition, it is shown that *Ec*TRX can replace *Eh*TRX41 in this antioxidant system, which might be another tool for coping with the oxidative stress through an association between the parasite and the bacterium. Results add value to the genome and proteome of *E. histolytica*. [Granted by UNL, CAI+D 2006; ANPCyT, PICT´03 01-14733, PAV´03 137].

EN-P14
KINETIC AND CATALYTIC MECHANISMS OF cAMP PROTEIN KINASE (PKA)

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Like all Ser-Thr Protein Kinases, PKA is a ATP-dependent phosphotransferase that delivers a single phospohryl group from the γ position of ATP to the hydroxyls of a Ser or a Thr in protein substrates. It is a heterotetramer composed of a regulatory subunit dimer (R_2) and two catalytic subunits (2C).The inhibitory site (IS) of R subunit docks to the active site of C subunit. The kinetic pathway for substrate phosphorylation by PKA is composed of three fundamental events: substrate binding, the phosphoryl transfer step, and irreversible net product release. While the simple core structure is shared within the protein kinases family, the core frequently interacts with regulatory proteins or domains that either enhance or repress catalytic function. For instance, biochemical assays perfomed with synthetic peptides in PKA system have shown that the presence of the 40-residue region N-terminal to the IS enhances the activity of C subunit. To evaluate which of the reaction steps are being affected by this region we have estimated the effect of solvent viscosity on the kcat. kcat was fully sensitive to solvent viscosity using kempide as substrate, indicating the rate-limiting step is the net product release; while when using the peptide that includes the N-terminal to the IS region the effect is intermediate, implying that phosphoryl transfer and net product release are both rate-determining.

EN-P15
ETHYL ACETATE PRODUCTION BY IMMOBILIZED ESTERASES FROM SPORE-FORMING MICROORGANISMS

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Introduction: Ethyl acetate (EAc) is an important raw material for many applications in chemical industry including coatings, adhesives, perfumes, and plasticizers. Enzymatic biotransformation has the advantage of high specificity and much simpler processes in comparison to chemical synthesis and various resolving agents. The aim of this work was to evaluate the production of EAc using immobilized esterases from spore-forming microorganisms. Methods: Standard *Bacillus subtilis* (168) and spore-forming microorganisms isolated from different sources (A14, A55, A60, A62, M2) were used throughout this study. Microorganisms were grown in LB at 37 °C. Both cells harvested by centrifugation and polyacrylamide gel–entrapped supernatant were used as source of enzyme. Esterifications were performed during 24h in n-hexane using acetic acid and ethanol as substrates. Ester synthesis was determined by acid titration with NaOH. Qualitative analyses of EAc were done by thin layer chromatography using chloroform as developing solvent. Result and Conclusion: Good productions of EAc in all systems assayed were observed. The highest yield was obtained with the polyacrylamide gel–entrapped supernatant from the isolation M2 showing a conversion of 93 %. This result indicates that gel entrapment method offers a good way for ester synthesis. This work was supported by grants PIP 6062 and PIP 6203.

EN-P16
PLACENTAL CARBOXYLESTERASES AS BIOMARKERS IN THE INTRAUTERINE EXPOSURE TO PESTICIDES

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The α, β-hydrolase-fold superfamily includes acetyl cholinesterase (AChE), involved in cell growth modulation and carboxylesterases (CE), major determinants of the pharmacokinetic behavior of most xenobiotics. The current work evaluates these enzymes as potential biomarkers of pesticide effects in human placenta. It is included in a prospective study performed in women living in an area of intensive agriculture exploitation. Comparing the average activity obtained in placentas collected during the non-application period of pesticides with samples collected during the application season, an increase in AChE (98%) and a decrease (35 %) in CE1 (substrate: α-naftyl acetate) was observed while no differences were found for CE2 (substrate: α-naftylbutirate). AChE and CE1 activities were lineary correlated. We postulate that switched molecular events include transcriptional responses and overproduction of different AChE isoforms. To examine this hypothesis RT-PCR was conducted and optimal conditions for amplification of AChE-R, S and E isoforms were determined. Nested PCR was required evidencing AChE is a low expression gene. Preliminary results suggest both enzymes represent a valuable approach to evaluate toxicological and pharmacological adverse effect of pesticide exposure during fetal development. Supported by U.N.Comahue, FONCYT and CONAPRIS.

EN-P17
FIRST REPORT OF CARNITINE PALMITOYLTRANSFERASE-I ACTIVITY IN CRUSTACEANS

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Carnitine palmitoyltransferase-I (CPT-I) is a key enzyme in the control of long-chain fatty acid oxidation that catalyzes the conversion of acyl-CoA to acylcarnitine in the outer mitochondrial membrane. In a previous work, we reported an increase in fatty acid activation and β-oxidation in mitochondria of the hepatopancreas of the freshwater prawn *Macrobrachium borellii* after a chronic exposure to water soluble fraction (WSF). The aim of this work was to characterize its CPT-I enzymatic activity as a follow up on the research on the effect of the WSF exposure on lipid metabolism. CPT-I activity was assayed by measuring the release of CoA-SH spectrophotometrically (Ellman's reaction) using palmitoil-CoA and L-carnitine as substrates. We observed a linear relationship between the mitochondrial protein and the enzyme activity up to 67 µg/ml. A great temperature dependence was observed with an optimum value at 34 ^{o}C; the optimum pH value for this prawn CPT I was 8.0. The Hill coefficient, n ≈1 using palmitoil-CoA, indicates a Michaelis-Menten behavior. The kinetics parameters, Km and Vmax were 259.6 µmol.l^{-1}, and 76.8 nmol. min^{-1}. mg protein^{-1}, respectively. Finally, after 7 days exposure to a sublethal concentration of WSF (0.6 mg/l), an increasing trend of CPT-1 activity was observed between control and exposed shrimps. This is the first report of CPT-1 activity in crustaceans.

EN-P18
EFFECTS ON THE DIMERIZATION STATE AND ENZYMATIC ACTIVITY OF UROPORPHYRINOGEN DECARBOXYLASE (UROD)

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In order to determine the structure-function relationship of UroD, we have evaluated the effect of diverse salts (KCl and $(NH_4)_2SO_4$), GdmHCl, and pH on UroD dimerization and the effects on its enzymatic activity. UroD has been expressed in *E. coli* BL21, induced with IPTG for 5 hours at 37°C. The purification was made through a Ni^{2+} column that retains poliHis-UroD. We have studied the behavior of the enzyme using the technique of Cross-Linking, and glutaraldehyde as the linking agent. These allow us to evaluate the degree of dimerization of UroD under different conditions. After completion of the technique, the effect of different conditions on the equilibrium dimerization of UroD was evaluated, and the results obtained indicate that the dimerization state can be altered by the compounds or conditions assayed. All of them produced a variation in the percentage of linked species (dimer). These results show that it is possible to alter the balance of UroD dimerization. These will allow us to evaluate the UroD enzymatic activity under different conditions, where it prevail one or another form of the enzyme, and to evaluate finally if the monomer of the UroD is active or not. According to this, we measured UroD activity varying the concentration of KCl, using Uro III as the substrate, and we could verify that only the dimeric form would be active, consistent with our previous work.

EN-P19
ISOMERIC IDENTITY OF HEPTACARBOXYLIC PORPHYRINS FROM BIOLOGICAL SYSTEMS

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We studied the isomeric identity of the heptacarboxylic porphyrin (Hepta) accumulated in a) uroporphyrinogen III (Urogen III) enzymatic decarboxylation by rat liver Uroporphynogen Decarboxylase (URO-D), and b) hexachlorobenze (HCB)-poisoned rats urines. URO-D carries out the sequential decarboxylation of the 4 acetic acid side chains to methyl groups. 4 Heptas with the methyl group in ring A; B; C or D, (Hepta a; b; c or d) can be obtained. We have synthesized all of them and they were used for comparison with the biologically accumulated Heptas. Wistar rats were treated with HCB and their urines collected. Rat liver URO-D and Urogen III initial concentrations from 1 to 10 µM were used when enzymatic decarboxylation was studied. Porphyrins from the solutions were separated as usual and chromatographied on alumina eluting with toluene-$CHCl_3$. Hepta fractions were chromatographied twice and recrystallised from methanol-$CHCl_3$. Porphyrins were analyzed by reverse-phase HPLC. We could separate the 4 Heptas isomers in only one run. HPLC analysis mixture of the biological Heptas with the synthesized ones has shown that Hepta d was the only isomer accumulated in both cases. 50% mixtures of the two Hepta heptamethyl methyl esters were used to confirm their identity by NMR europium titration which is one of the most important techniques for the elucidation of complex molecules structures.

EN-P20
WHY DOES ISOCOPROPORPHYRIN (ISOCOPRO) APPEAR IN THE FECES OF HEXACHLOROBENZENE (HCB) POISONED RATS?

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Porphyria Cutanea Tarda (PCT) shows hepatic Uroporphyrinogen Decarboxylase (URO-D) deficiency. High carboxylated porphyrins are found in PCT urines. Isocopro is often the dominant porphyrin excreted in PCT and HCB poisoned rat feces. The toxic effect of HCB closely resembles PCT, both clinically and biochemically. We try to elucidate the reasons of Isocopro excreta in PCT patients. Isocopro is produced by hepatic Coproporphyrinogen Oxidase (CO) on pentacarboxylate porphyrinogen III (Pentagen abd) and the vinyl group hydrogenated to an ethyl group by intestinal micro-organisms. CO is located in the inner membrane of mitochondria. We have found that HBC treatment significantly increased rat liver Pentagen abd accumulation and CO activity as URO-D activity decreased. Normal mitochondria rats (N) CO have shown higher Km values for Coproporphyrinogen III than HCB treated rats. N and HCB-disrupted mitochondria have not shown differences in CO activities and Kms and similar to those of whole HCB mitochondria. Respiratory control ratio and electron microscopical studies have not shown significant differences between both mitochondrial preparations. Digitonin sub-fractionated mitochondria and markers enzymes activities demonstrated that CO in HCB treated mitochondria was looser in the inter-membrane space than in N mitochondria, making the access of substrates to the active site of CO easier.

EN-P21
PURIFICATION OF HIERONYMAIN III, AN ACID PEPTIDASE ISOLATED FROM *BROMELIA HIERONYMI* MEZ

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Hieronymain III is the third cysteine endopeptidase isolated from fruits of *Bromelia hieronymi* Mez and shows different characteristics than the other proteases purified from the same species. Crude extracts were obtained by homogenizing frozen unripe infrutescences in buffer containing protectors, and then treated with cold acetone and centrifuged; the obtained precipitate was redissolved with phosphate buffer and used for the next isolation steps. The purification scheme included anionic and cationic exchange chromatography (FPLC). The new protease presented maximum proteolytic activity between pH 8.7 and 9.3. The enzyme was completely inhibited by E-64 and iodoacetic acid and activated by the addition of cysteine, results that strongly suggest than the isolated protease should be included within the cysteine group. Molecular mass (23,713 Da) was determined by MALDI-TOF/MS. The enzime presented two multiple forms with isoelectric points values of 5.6 and 6.4. Hieronymain III showed preference for the Lys derivative within the set of N–a–CBZ–L–amino acid p–nitrophenyl esters available and also degraded. Z-Phe-Arg-pNA and Z-Arg-Arg-pNA substrates. The N-terminal sequence (AVPQSIDWRRYGAVT TSRNQG) showed a high identity degree with different plant cysteine proteases belonging to the papain family. Mass spectra & N-terminal sequences were carried out in IBB, UAB, Spain , by Dr.S.Trejo.

EN-P22
***CAESALPINIA PARAGUARIENSIS* EXTRACT: ITS POTENTIAL APPLICATION AS NATURAL ANTIINFLAMMATORY**

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Hyaluronic Acid is responsible for structural and functional integrity of articular cartilage. When this tissue is target of chronic inflammatory pathologies, e.g. Osteoarthritis, Hyaluronidases (HYAL) are activated and generate low weight molecular fragments. These promote the release of pro-inflammatory cytokines that activates other degrading enzymes, contributing to the progress of the disease. *C. paraguariensis* ritidome infusion is rich in polyphenols (*pf*); these compounds are associated to diverse pharmacologic effects, among them antiinflammatory. The research is focused on the inhibitory activity of this extract on HYAL, and its relation with tannins, other *pf* or both compounds. The Infusion (1) was lyophilized and sequentially extracted with: Ether (2), Chloroform (3), Methanol (4). BSA was added to extract (4), and the supernatant (5) was separated by centrifugation. *Pf* were determined by *Price y Butler* Method. The inhibitory effect on HYAL was evaluated by a colorimetric method based on *Morgan-Elson* Reaction. Reference substances: Aspirin (A) and Gallic acid (GA). The HYAL inhibition percentage obtained were: 75,1; 0,6; 14,9; 73,9 ; 71,3; 34,3; 10,2; for ex-tracts (1), (2), (3), (4), (5), (A) and (GA), respectively. It is concluded that (1) displays inhibitory effect on HYAL and that this activity is associated to *pf* or other compounds different from tannins.

LI-P01

STUDY OF THE ENZYMES INVOLVED IN THE FIRST STEPS OF PHOSPHOLIPID BIOSYNTHESIS IN BACILLUS SUBTILIS

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In *E. coli* two acyltransferases, PlsB and PlsC, are required for phosphatidic acid production. A different pathway has been described in *Streptococcus pneumoniae*. The pathway involves two enzymes, encoded by *plsX* and *plsY* genes, which catalyze the reaction of acylation of G3P using a new intermediate, fatty acyl-phosphate. In *B. subtilis* there are genes with homology to *plsX*, *plsY* and *plsC*, but no *plsB* homolog could be identified. To determine the functions of the enzymes encoded by $plsX_b$, $plsY_b$ and $plsC_b$ in *B. subtilis*, conditional mutants were constructed. The analysis of the mutants indicated that the expression of each gene is essential for the growth of *B. subtilis*. The analysis of the lipids extracted from cultures of the conditional mutants without inducer indicated that phospholipid biosynthesis was blocked in the three mutants, and fatty acids accumulated in the $plsY_b$ and $plsC_b$ conditional mutants. The subcellular localization of $PlsX_b$ and $PlsC_b$ was analyzed by immunofluorescence microscopy (IFM). In these experiments $PlsC_b$ and $PlsX_b$ were found homogeneously distributed in the cytoplasmic membrane, but $PlsX_b$ is a soluble protein as determinated by western blot. These results suggest that *B. subtilis* uses the PlsX/Y pathway for the phospholipids biosynthesis, and that a putative protein attaches in vivo $PlsX_b$ to the cell membrane.

LI-P02

THERMAL REGULATION OF TRYPANOSOMATID METHYL-END FATTY ACID DESATURASES

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We have previously characterized trypanosomatid desaturases involved in the conversion of oleoyl- to linoleoyl-moieties of phospholipids in the endoplasmic reticulum membrane of the parasites. Trypanosoma brucei and Leishmania major oleoyl desaturases share 56% of identity and 75% of similarity, and comparable percentage of conversion (in vivo) of 16:1 and 18:1 fatty acids to the 16:2 and 18:2 ones, when expressed in yeasts at 30 ºC. Interestingly, T. brucei desaturase showed an increased substrate conversion at lower temperatures, more significant for 16:1, whereas L. major enzyme appears to be insensible to temperature downshift. Analysis of primary structure for both enzymes revealed a conserved theoretical membrane topology and consensus sequences for the three clusters of histidines, presumed to be part of the active site. T. brucei desaturase presents a 29 aminoacids larger N-terminus when compared to the L. major desaturase, which appears to be exclusive to the trypanosome enzyme as judged after multialignment with numerous available sequences. Construction of truncated and chimeric desaturases between the orthologous genes allowed us to localize the region for thermal regulation on the first half of T. brucei desaturase, with components present in i) the proximal N-terminus and ii) the catalytic domain located between the first and second hydrophobic domains.

LI-P03
DIVERGENCE ON TRIACYLGLYCEROL ACCUMULATION AND ANTIBIOTIC PRODUCTION IN *STREPTOMYCES COELICOLOR*

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The occurrence of triacylglycerols (TAGs) as reserve compounds is widespread among eukaryotic organisms. However, accumulation of TAGs in prokaryotes has been reported for bacteria mainly belonging to the actinomycetes group. In *Streptomyces*, accumulation of TAGs was propose to be necessary to maintain cell integrity after glucose becomes exhausted, and also to provide the carbon units needed for subsequent biosynthesis of polyketide antibiotics. Here we describe the identification and characterization of proteins from *S. coelicolor* involved in the biosynthesis of TAGs and its role on antibiotics production. Sequence comparison allowed identify three genes in the chromosome of *S. coelicolor* encoding putative acyl-CoA:diacylglycerol acyltransferase (DGAT) enzymes which catalyze the final step in TAG biosynthesis. In vitro experiment using recombinant proteins allowed us to confirm that Sco0958 is a DGAT. Furthermore mutant strains for Sco0958 and Sco0123 genes were generated by genetic manipulation. Characterization of these mutant strains in rich liquid medium showed that they accumulated less amounts of TAGs and produced more polyketide antibiotics than the parent strain. These results suggest that TAG biosynthesis in *Streptomyces* is demanding acetate-derived unit rather than providing carbon units for antibiotics production as previously proposed.

LI-P04
TEMPERATURE-SENSITIVE SYNTHESIS OF EPOXY-MYCOLIC ACIDS IN *MYCOBACTERIUM SMEGMATIS*

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Mycolic acids are essential components of the mycobacterial cell envelope responsible for the low permeability displayed by these micro-organisms and involved in virulence. Based on this fact, we screened a chemically mutagenized culture of *M.smegmatis*, a saprophytic mycobacteria, looking for mutants with increased permeability to lipophylic drugs and a Temperature-Sensitive (TS) phenotype. One of such mutants showed a drastic growth reduction without lysis in liquid medium at 42°C and a TS non – lethal phenotype on solid medium. This mutant displayed a mild (2-fold) increase in the susceptibility to lipophylic drugs, an aberrant sliding motility and an increased Congo Red binding, suggesting alterations of the cell envelope. Growth at non-permissive temperature was partially restored by the addition of long chain unsaturated fatty acids (oleic acid and erucic acid) but not by saturated fatty acid of any chain lenght . Analysis of mycolic acid biosynthesis at 42°C showed the complete loss of the oxigenated epoxy-mycolic acids (which are a minor fraction of the total mycolate content) but normal contents of the α and α' non-oxigenated mycolic acids. This is the first report of a TS mutant deficient in the synthesis of epoxy-mycolic acids. These results suggest that epoxy-mycolic acids have a role on cell envelope architecture that can not be compensated by non-oxygenated mycolic acids.

LI-P05
PRESENCE OF PHOSPHOLIPID-VESICLES IN THE APOPLAST OF SUNFLOWER SEEDS

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The phospholipids(PL)-rich plasma membrane separates the symplast from the apoplast and couples perception of extracellular factors with intracellular biochemical responses. Hence, PL participates in diverse intracellular signaling cascades but the putative role of PL in transferring the reverse information (from a cell to the extracellular context) has not been studied yet. We have recently hypothesized on the participation of PL in plant extracellular signaling events and we have reported the presence of PL in the extracellular fluid (EF) of sunflower seeds. Since PL are insoluble in aqueous fluids the aim of this work was to determine their organization in the sunflower apoplast. The EF was subjected to fractionation by centrifugation steps at 10000g, 40000g and 100000g and the pellets analyzed by transmission electronic microscopy. The observation revealed the presence of particles of 120 nm in the 10000g and 40000g pellets, with apparent membrane organization as multivesicular compartments. The characterization of these fractions indicates the presence of PL and proteins, showing a differential content in each fraction. The organization of specific PL and proteins in vesicular structures resembles those of animal systems which have been proposed to participate in the intercellular communication.

LI-P06
STUDY OF MEMBRANE PHOSPHOLIPID COMPOSITION ON BEWO CELLS

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Since the placenta is the first fetal arising organ that regulates fetal fatty acid homeostasis, the fatty acid/lipids composition in the placenta may serve as an indicator of fetal composition. It is known that Hypoxia inducible factor-1α (HIF-1α) is augmented in preeclampsia. We observed an increase of AQP9 expression with a lack of functionality in this syndrome. The same results were observed when normal term explants were treated with Cl2Co, a HIF-1 inducer. The AQP-9 is a membrane-associated protein. It is well known that membrane protein activity can be modulated by the lipidic environment. Thus, alterations in lipid composition could induce changes in protein activity. Here we evaluated the phospholipid composition of BeWo cell line membranes (accepted as a trophoblast human model). BeWo cells were exposed to a chemical hypoxia with Cl2Co. Lipid were extracted by Bligh-Dyer method, phospholipids separated by thin layer chromatography (TLC) and phosphatidylcholine (PC) and phosphatidylethanolamine (PE) quantified by Fiske-Subarrow. The biochemical viability was determined by β-hCG dosage. The PC/PE for control condition was 3.76 ± 0.32 since the Cl2Co was 4.12 ± 0.028 (NS). These preliminary results can not discard that changes in minor phospholipids or in fatty acid composition cause the lack of functionality of AQP9 in preeclampsia.

LI-P07

CHANGES IN RAT SPERMATOZOA LIPIDS AND FATTY ACIDS DURING EPIDIDYMAL MATURATION

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During their epididymal transit, spermatozoa undergo a series of maturational changes that render them potentially capable of fertilization. Here we focused on the changes undergone by major lipid classes and fatty acids. The content of neutral lipids including cholesterol (Ch) as well as of phospholipids (PL) decreased with epididymal maturation. The Ch/PL ratio, and within PL the choline/ethanolamine glyceroPL (CGP, EGP) ratio, increased significantly from caput to cauda epididymis. The most pronounced change observed in total glyceroPL fatty acids was an increase in the proportion of 22:4n-9. The percentage of this fatty acid increased about 8-fold in the CGP with sperm maturation, whereas the EGP contained 22:5n-6 as their major fatty acid at both stages. Interestingly, mature sperm 1-O-alkyl-2,3-diacylglycerols also had 22:4n-9. The content per cell of sphingomyelin (SM) and ceramide (Cer), also changed with maturation, increasing the Cer/SM ratio in sperm from cauda with respect to caput. Both these lipids contained normal and 2-hydroxylated VLCPUFA, such as 28:4n-6 and 2-OH-28:4n-6. The marked alterations spermatozoa undergo during their epididymal transit in lipid and fatty acid profile may lead to changes in the biophysical properties that are optimal in determining the physiological functions of these gametes.

LI-P08

VARIATION OF LIPID COMPOSITION OF PIG (*SUS SCROFA*) SPERMATOZOA WITH PH

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Mammalian spermatozoa undergo changes on their journey through the female genital tract. The pH also varies along this path. To determine the possible influence of pH over lipid composition of pig sperm, cells were incubated in non capacitating media at pH 6.6 (uterus) and 7.6 (oviduct). Total lipid extracts were assayed for the composition of fatty acids involved in acylglycerides as molar % using GC-FID with an internal standard. The acyl moieties that showed statistical significant differences were: C14:0, C22:6 ($p<0.005$); C18:1, C22:5 ($p<0.01$) and C22:4 ($p<0.0001$)($n=15$). The amounts of total acyl masses differed significatively ($p<0.005$, $n=15$). The profile of sterols (as relative %) and their total amounts were also estimated for samples incubated at both pH, using GC-MS of their trimethylsylyl derivatives, with an internal standard. Cholesterol, desmosterol and a third sterol (probably hydrodesmosterol), not described previously as a component of boar sperm, were found. A significant statistical difference was found in cholesterol relative % ($p<0.05$, $n=16$) when incubated at different pH. Neither desmosterol nor the third sterol showed significant differences. Sterol's masses differed significatively ($p<0.05$, $n=13$). The present results indicate that variations in pH promote changes in the total lipid composition of boar sperm.

LI-P09
EFFECTS OF HYPERTHERMIA ON RAT SEMINIFEROUS TUBULE CELL LIPIDS AND POLYUNSATURATED FATTY ACIDS

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Previous work showed that cryptorchidism selectively damages testicular germ cells at specific stages and rapidly results in an important depletion of the germinal linage. The aim of this study was to examine the long-term effects of short (15 min) periods of relative hyperthermia (43ºC) applied once a day, on seminiferous tubule lipid composition and labeling with [^{3}H]arachidonic acid. After 5 days of this treatment, a progressive decrease in testicular weight and germ cell depletion started, most of the tubules showing a selective decrease of spermatocytes and spermatids. Three weeks later, practically no germ cells remained in the tubules, which consisted mainly of Sertoli cells and spermatogonia. This involution was accompanied by a loss of 22:5n-6-rich glycerophospholipids (GPL), those remaining being instead relatively richer in 20:4n-6. When [^{3}H]20:4n-6 was used as a marker, control seminiferous tubules incorporated similar amounts in the major GPL as in the relatively minor triglycerides (TG). The esterification of [^{3}H]20:4n-6 into GPL per unit weight was as active in control as in germ cell depleted seminiferous tubules, but the latter showed a marked reduction in TG labeling. This difference points to an important role of TG in the metabolism and distribution of PUFA among lipids of cell populations within the seminiferous tubule.

LI-P10
CHANGES WITH SEXUAL MATURATION AND CRYPTORCHIDISM OF GERM CELL-LINKED POLYUNSATURATED FATTY ACIDS

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The major polyunsaturated fatty acid (PUFA) of mature rat testis is known to be 22:5n-6. The aim of this work was to study how this fatty acid is accrued from postnatal to sexually mature rat testis as an acyl group of glycerophospholipids (GPL), and to determine in adult rats how these GPL are affected by cryptorchidism. Before the onset of spermatogenesis, 20:4n-6 was the most abundant PUFA in testicular choline and ethanolamine glycerophospholipid (CGP, EGP) in both their major subclasses (phosphatidyl and plasmenyl). Both EGPs contained a significant proportion of 22:6n-3. This high 22:6n-3 was restricted to the prepuberal age of the testis, gradually decreasing with sexual maturation. Concomitantly with the population of the testis with germ cells, the amount of 22:5n-6 increased in both CGP and EGP, to become the major PUFA of testis and remain so throughout the adult life of the rats, 20:4n-6 remaining in a modest second place. With cryptorchidism, germ cells were selectively and rapidly lost from mature, fertile testis (10 days). The amount of CGP and EGP subclasses decreased to similar extents. This was accompanied by a dramatic elimination of 22:5n-6 from all GPL, 20:4n-6 decreasing less, especially in the plasmalogens. The germ cell-lacking immature testis and the germ cell-depleted adult rat testis were both characterized by a low proportion of 22:5n-6 in all their GPL.

LI-P11
PREVENTION OF HEPATIC FATTY ACIDS ALTERATIONS IN ATHYMIC MICE BY NEONATAL THYMULIN GENE THERAPY

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During adult life athymic (nude) male mice display not only a severe T-cell related immunodeficiency but also endocrine imbalances and a moderate hyperglycemia. We studied the impact of congenital athymia on hepatic lipid composition and also assessed the ability of neonatal thymulin gene therapy to prevent the effects of athymia. We constructed a recombinant adenoviral vector, RAd-metFTS, expressing a synthetic DNA sequence encoding met-FTS, an analog of the thymic peptide Facteur Thymique Sérique (FTS) whose Zn-bound biologically active form is known as thymulin. On postnatal day 1-2 homozygous (nu/nu) nude and heterozygous (nu/+) mice were i.m. injected with 108 pfu of RAd-metFTS or RAd-βgal (control vector). The animals were processed at 52 days of age. Serum thymulin, hepatic phospholipid fatty acid composition and free and esterified cholesterol was determined. The relative percentage of 16:0, 18:1 n-9 and 18:1 n-7 fatty acids was lower whereas that of 18:0, 20:4 n-6 and 22:6 n-3 acids was higher in hepatic phospholipid (PL) of nu/nu animals as compared to nu/+ counterparts. Some of these alterations, like that in the relative content of 22:6 n-3 in liver PL and the unsaturation index, were completely or partially prevented by neonatal thymulin gene therapy. We conclude that the thymus influences lipid metabolism and that thymulin is involved in this modulatory activity.

LI-P12
KEY ENZYMES OF LIPID AND CARBOHYDRATE METABOLISM IN LIVER OF DYSLIPEMIC RATS: EFFECT OF FISH OIL

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Objectives: The present study analyses different enzymes involved in the synthesis and oxidation of fatty acid in the liver and its relationship with glucose metabolism. Male Wistar rats were fed a SRD (w/w: 63% sucrose, 8% corn oil (CO), 17% protein) for 6 months. After that, one half of the rats continued with the SRD up to 8 months, while the other half received a SRD in which the CO was substituted by cod liver oil (CLO) (w/w: 7% CLO plus 1% CO) from months 6 to 8. Control rats received a control diet (CD) (w/w: 63corn-stach, 8% CO, 17% protein) for 8 months. We studied a) plasma: glucose, insulin, Tg, AGNE, b) liver: Tg and glycogen content and enzymatic activities of ME, FAS, FAO, GSa, GK, G-6-PPase. Results: SRD vs CD: Increased plasma Tg, AGNE and glucose levels $p<0.05$. Liver: Increased: 1) Tg and glycogen content, 2) ME, FAS and GSa activities and the rate of G-6-PPase/GK. The addition of CLO normalized plasma Tg, AGNE and glucose levels. The liver Tg content as well as ME, FAS and GSa activities reached normal values, while FAO activities increased. Conclusions: The altered pattern of lipid and carbohydrate metabolism of SRD fed rats tended to be normal with the administration of cod liver oil.

LI-P13
DYSLIPEMIA AND INSULIN RESISTANCE. EFFECT OF THE AMOUNT OF DIETARY SUCROSE

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The present study was designed to further investigate the effect of reduction of the amount of dietary sucrose (63% to 20% w/w) upon the altered lipid and glucose homeostasis present in rats chronically (30 weeks) fed a sucrose rich diet (SRD). In these animals a well stablished dyslipidemia and insulin resistance was observed before the refined sugar was isocaloric replaced by corn starch during 12 weeks. We are interested in study whether changes in this macronutrient are able to improve and/or reverse the hypertriglyceridemia, impaired glucose homeostasis and insulin secretion from beta cells induced by a high sucrose intake.
At the end of experimental period (42 weeks) the following results were obtained: 1) The hypertriglyceridemia induced by high-sucrose diet was completely normalized ($p<0.05$) by reducing the amount of sucrose in the diet. These changes were associated with an improve of plasma FFA levels, adiposity (epididymal and retroperitoneal fat pad) as well as carcass fat content. 2) The impaired glucose tolerance observed in SRD-63% rats was normalized ($p< 0.05$). It was accompanied by an improve of the insulin response to i.v. glucose and glucose stimulated insulin secretion from perifused beta cell. These data suggest that reducing the amount of refined sugars in SRD fed rats improve lipid and carbohydrate metabolism.

LI-P14
EFFECT OF DIETARY SOY PROTEIN (SP) IN THE DYSLIPEMIA INDUCED BY A SUCROSE-RICH DIET (SRD)"

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Normal Wistar rats fed for 3 weeks with SRD develop dislypemia (high free fatty acids -FFA- and triglyceride –Tg- and liver Tg) and insulin resistance. We evaluated if the substitution of the source of protein in the diet (casein -C- for SP) could prevent/improve this abnormalities. To carry out this objective, rats were fed 3 weeks a SRD (63%) or a cornstarch (63%) diet (CD), where the protein source was C or SP. Body weight and total caloric intake was similar in each group. We analyzed: 1: Plasma: Glucose (G), Total and HDL Colesterol (T/HDL-C), Tg and FFA levels; 2) Tg secretion rate (TGSR) and intravenous fat tolerance test (IVFT), 3) euglucemic-hyperinsulinemic clamp as a measure or whole body peripheral insulin sensitivity; 4)Liver: Tg content. Results: G, T-C and HDL-C were similar in all groups. In the SRD-SP group: Tg and FFA were as higher as in the SRD-C group, while the liver Tg were similar to the control group. The IVFT was similar to the SRD-C group , however a decrease of TGSR was observed .The peripheral insulin sensitivity was normalized. This results show that when soy was the source of protein in the SRD, this diet ameliorate some of the metabolic alteration induced by the SRD.

LI-P15
LIPID AND GLUCOSE METABOLISM IN HEART MUSCLE OF INSULIN RESISTANT RATS: EFFECTS OF FISH OIL

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The present work analyzes: 1) The effect of increased triglyceride (Tg) and long chain acyl-CoA (LCACoA) storage on the oxidative and non-oxidative glucose metabolism in the heart of rats fed a sucrose-rich diet (SRD) for 8 months, 2) The replacement of the source of fat in the diet from corn oil (CO) to fish oil (FO) upon the above parameters. Male Wistar rats were fed a SRD for 6 months. After that, a half of the rats continued with the SRD for 2 more months. The other half (SRD+FO) received for 2 months the SRD in which the source of fat was replaced by FO. A reference group was fed a control diet (CD) (corn starch). Parameters evaluated: i) Plasma: glucose, Tg, FFA and insulin, ii) Heart tissue: Tg, diacylglycerol (DAG), LCACoA, glycogen (Gly) and glucose-6-P(G6P) levels and hexoquinase, glycogen synthase (GSa), piruvate dehydrogenase complex (PDHc) and PDH kinase activities. As well the protein mass expression PKCξ and βII in membrane and cytosol fractions. Results: SRD vs DC ($p<0.05$): an increase of: 1)Tg, glucose and FFA plasma levels, and 2)Tg, LCA-CoA, DAG and PKCξ and βII (fraction membrane). G6P levels and GSa, hexoquinase and PDHc activities were reduced. In SRD+FO: all parameters reached values similar to CD. The hypolipidemic effect of FO may play an important role in normalizing the metabolic disturbances in the heart of rats chronically fed with a SRD.

LI-P16
LONG TERM SUCROSE-RICH DIET INDUCES CHANGES IN RAT ADIPOSE TISSUE. EFFECTS OF DIETARY FISH OIL

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Long term sucrose-rich diet (SRD) induces increased plasma free fatty acids (FFA) and triglycerides (Tg), insulin resistance (IR), hyperglycemia and adipocyte hypertrophy in normal Wistar rats. Goal: i) Analyze the morphological and functional changes in adipose tissue (AT) that could contribute to abnormal glucose homeostasis and IR ii) Analyze if dietary cod liver oil (FO) improves or reverses this abnormalities. 40 rats were fed SRD for 6 months. Then, 20 rats continued on SRD and the other 20 received SRD in which fat (corn oil 8%w/w) was replaced by FO (7%w/w + 1%w/w corn oil) (SRD+FO) for 2 months more. The reference group was fed a control diet (CD) for 8 months. We evaluated: i) Plasma: glucose, insulin, Tg and FFA, ii) Adipocytes: basal and stimulated glucose uptake, iii) basal and stimulated lipolysis, iv) antilipolitic action of insulin, v) LPL and G6PDH activities in AT. Results: SRD vs CD: 1) Increased Tg, FFA and glucose levels, 2) Increased LPL and G6PDH activities, 3) Decreased insulin stimulated glucose uptake, 4) Increased basal and stimulated lipolysis, 5) Decreased antilipolitic action of insulin. FO in the SRD normalized these changes. Conclusion: SRD induces metabolic and morphological alterations in AT that contribute to abnormal glucose homeostasis and IR. N-3 fatty acids could play an important role in the management of dislipidemia and IR.

LI-P17

GENETIC VARIANTS OF PPAR- γ 2 AND METABOLIC PROFILE IN OBESE AND LEAN CHILDREN

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Recently, a role for PPAR-γ2 transcription factor in the regulation of adipogenesis has been established. Genetic variants of PPAR-γ2 gene have been identified as Pro12Pro, Pro12Ala and Ala12Ala, being the Pro12Pro genotype the most prevalent in Caucasian populations. Our objective was to study the putative relationship between metabolic parameters and the PPAR-γ2 polymorphism in lean and obese children. Twenty children (mean age 10±5 y) were studied. Serum Cholesterol, HDLc, LDLc, Triglycerides (TG) and Glucose concentrations were determined by available commercial kits. Serum Insulin, TSH and FrT4 levels were measured by EIA. The Insulin Resistance was estimated according to HOMA-IR following the formula: (fasting Insulin x fasting Glucose)/22.5. Analysis of PPAR-γ2 gene polymorphism was performed by Taqman Allelic Discrimination using a Standard 7500 Termocycler (Applied Biosystems). We found higher TG concentration and significant lower HDLc levels in the obese children compared to the lean ones. Four out of the twenty children show the Pro/Ala genotype. The 75% of Pro/Ala children have the lowest HOMA-IR values in the group.These observations might suggest a possible association between Insulin Sensitivity and the Pro/Ala genetic variant of PPAR-γ2 althought a bigger sample size would be necessary to perform an accomplished statistical correlation analysis.

LI-P18

VITAMIN A REGULATES THE EXPRESSION OF GENES INVOLVED IN THE FATTY ACID BETA OXIDATION IN RAT HEART

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We have previously showed in the rat heart that vit A deficiency alters the mitochondrial lipid contents, decreases the Acetil CoA Carboxilasa (ACC) activity, and increases the Carnitina Palmitoiltransferasa I (CPT I) activity, key enzyme in the fatty acid beta-oxidación. Here, we determined by RT-PCR in heart the mRNA expression of ACC2, isoform associated to the mitochondria, CPT I and retinoic acid receptors (RAR). Male Wistar rats at 21 d age were weaned onto either a vit A deficient diet (-A) or the same diet with 8 mg retinol/kg diet (control). They were fed for 3 months. Also, a –A group was refeed by 15 days with control diet (-A refeed). Serum and liver Vit A levels were measured by HPLC. Statistical analysis by ANOVA. In –A rats the mRNA expression of CPT I increased ($p < 0.01$), while that of ACC2 decreased ($p < 0.002$), compared to control. These results were related with the enzyme activities previously determined. The RAR mRNA levels decreased ($p < 0.01$) in –A heart. Vit A restitution to the diet reversed the CPT I and RAR mRNA levels, whitout change in the ACC2 mRNA. In addition to the effects of vit A deficiency on those genes, the possibility that a lower availability of the endogenous inhibitor of CPT I, malonyl-CoA, can be involved in the regulation of the beta-oxidation process could be considered since we have previously determined a decrease of the ACC activity.

LI-P19
EFFECT OF MEMBRANE COMPOSITION ON CHOLESTEROL EFFLUX MEDIATED BY HUMAN APOLIPOPROTEIN A-I

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Cholesterol efflux mediated by apoA-I involves different pathways, including active efflux via the ABCA1 transporter, and passive pathways mediated by scavenger receptor BI. To evaluate the importance of membrane composition and lateral domain phase separation on apoA-I interactions and cholesterol mobilization, we constructed a permanent Chinese Hamster Ovary Cell line (CHO-K1) overexpressing the rat Stearoyl CoA Desaturase gen (SCD). Cells transfected with an expression vector in mammalian hosts (containing or not the gen for the SCD) induced a stable increase in fatty acid unsaturation at membrane phospholipids. SCD mRNA overexpression was characterized by northern blotting. SCD activity was evaluated by analysis of fatty acid composition at the plasma membrane, showing a relative increase of 2.1 times in the ratio 16:1/16:0 and 18:1/18:0. In addition, we proved that, as expected, expression level of ABCA1 was not modified in SCD-overexpressing cells, by western blotting. Incubation of SCD overexpressing cells with ApoA-1, resulted in a decresed cholesterol removal, as well as in the formation of lipid-apoliprotein particles. Furthermore, membrane fluidity was assesed by Laurdan General Polarization (GP) and 2-photon fluorescence microscopy, in control cells and in cells treated with methyl beta ciclo dextrines. Results are discussed in terms of lateral phase separation.

LI-P20
MITOCHONDRIAL GPAT1 IS MOST ACTIVE IN OMM BUT NOT IN MITOCHONDRIAL ASSOCIATED VESICLES (MAV)

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Glycerol 3-phosphate acyltransferase-1 (GPAT1), catalyzes the committed step in glycerolipid synthesis. Both GPAT1 and carnitine-palmitoyltransferase 1 (CPT1) are located on the outer mitochondrial membrane (OMM) and their reciprocal regulation may control acyl-CoA metabolism at the OMM. To determine whether GPAT1, like CPT1, is enriched in both mitochondrial contact sites (CS) and OMM, and to correlate protein location and enzymatic function, we obtained rat liver submitochondrial fractions. Most GPAT1 protein was present in a vesicular fraction associated with mitochondria (MAV) but GPAT specific activity in this fraction was low. In contrast, highest GPAT1 specific activity was present in OMM from purified mitochondria. Contact sites from crude mitochondria (ER-CS) also showed high GPAT1 expression but low specific activity. To determine how GPAT1 is targeted to mitochondria, recombinant protein was synthesized in vitro and its incorporation into crude and purified mitochondria was assayed. GPAT1 was rapidly incorporated into mitochondria, but not into microsomes. Incorporation was ATP-driven and GPAT1 became an integral membrane protein after incorporation. These results demonstrate that two pools of GPAT1 are present in rat liver mitochondria: an active one, located in OMM and a less active one, located in membranes (ER-CS and MAV) associated with both mitochondria and ER

LI-P21
PHOSPHATIDIC ACID METABOLIZATION IN NUCLEI FROM CEREBRAL CORTEX AND CEREBELLUM

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We have reported that in the Central Nervous System phosphatidic acid is metabolized to diacylglycerol (DAG) by the isoforms of phosphatidate phosphohydrolase (namely, PAP1 and PAP 2) and that DAGs generated by PAPs are partially metabolized to monoacylglycerols (MAG) by diacylglyceride lipase (DGL) activity. The aim of the present work was to study which PAP isoforms are associated to nuclei in the cerebellum and cerebral cortex (CC). To this end, adult (4 mo) rat CC and cerebellums were homogenized and highly purified nuclei were isolated by sucrose-density ultracentrifugation. Nuclear preparations were checked for purity by electron microscopy. The different PAP isoforms were assayed according to their NEM sensitivity (Methods in Enzymol., 197, 553-563, 1991). The DGLs activities were assayed using DAG as substrate, which was generated by PAPs action. Our results indicate that NEM-sensitive (PAP-1) is predominantly present in nuclei from CC while NEM-insensitive (PAP-2) evidences a similar activity in nuclei from both CC and cerebellum. In addition, it was observed that nuclear DGL activity is higher in the cerebellum than in CC. The PA metabolization in nuclei is indicative of an important signalling pathway in this fraction.

LI-P22
NUCLEAR ARACHIDONIC ACID UPTAKE AND UTILIZATION

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Regulation of gene expression by fatty acids is an event that takes place inside the nuclei of eukaryotic cells, but the mechanisms of nuclear fatty acids (FA) uptake is still incompletely understood. We have already demonstrated that FA free or bound to L-FABP are incorporated into metabolic active nuclear lipids pools by an acyl-CoA pathway. In these experiments whole nuclei or isolated nuclear matrix (double membrane-depleted nuclei) were incubated in vitro with [14C]FA. The aim of the present work was to determine if exogenous FA could be internalized to endonuclear lipids (nuclear matrix). With this purpose, nuclear 20:4n-6 pools were labeled by in vitro incubation of isolated nuclei from rat liver cells with [1-14C]20:4n-6, ATP and CoA at different times. After nuclear labeling, matrix were isolated removing the double nuclear membrane by incubation with 0.08% TX – 100. Arachidonic acid was incorporated in nuclear matrix lipid pools, as FFA and esterified to PL (PtdCho > PtdEtn > PtdIns) and neutral lipids (TAG >> DAG). These endonuclear 20:4n-6 pools could provide and endogenous source of PPARα – activating ligands, as FFA and / or through an in situ regulated PL hydrolysis. In conclusion, exogenous arachidonic acid not only is incorporated into nuclear membrane, but also is internalized to endonuclear lipid pools as FFA or esterified to PL and TAG.

LI-P23
UPREGULATION OF TONEBP AND COX2 EXPRESSION IN HYPERTONICITY. EFFECT OF COX INHIBITORS

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Renal inner medulla plays a key role in body water and electrolytic homeostasis. Such physiological actions are performed by medullary structures immersed in the highest organism interstitial osmolality. Thus, renal cells need protective mechanisms so as to preserve their integrity. TonEBP, a transcriptional activator protein, stimulates genes whose products drive cellular protection from high salt and urea concentration, osmolytes and urea transporters and Hsp70, among others. On the other hand, COX2 has been reported as a protective gene in renal tubular cells exposed to high NaCl. Thus, the aim of the present work was to evaluate the relationship between both proteins and their contribution to renal cell survival. First, we evaluated COX2 activity inhibition in MDCK cultures exposed to 150 or 250 mM NaCl. NS398 abolished COX2 prostaglandin synthesis but no effect on cell number and viability was observed. NS398 caused a concentration-dependent increase in COX2 protein which was parallel to the increase in cell number, and COX2 siRNA decreased cell survival. NS398 also increased TonEBP expression and TonEBP siRNA abolished COX2 expression and decreased cell survival. These results show that COX2 is involved in cell protection against hypertonicity by a prostaglandin independent mechanism and its expression requires TonEBP activity.

LI-P24
LIPID ANALYSIS OF LOW-DENSITY CAVEOLAE-LIKE MEMBRANES FROM *Bufo arenarum* OOCYTES

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Lipid microdomains (rafts) are dynamic assemblies of cholesterol, sphingolipids and specific proteins involved in cell signaling regulation. We conducted a quantitative analysis of lipids and proteins of detergent-free low-density membranes isolated from *B. arenarum* oocytes in order to elucidate how these domains perform their regulatory functions in the amphibian system. Membrane fractions were separated in the absence of detergent by sucrose gradient centrifugation and characterized by Coomassie staining and Western-blot. Lipids were analyzed by chromatography techniques. The fraction corresponding to "light" membranes (10-22.5% sucrose interface) derived from plasma membrane as suggested by the activity of 5'nucleotidase. In addition, g prot.) and cholesterolμthis fraction is enriched in sphingomyelin (0.12 nmol/ g prot.) with respect to the other membrane fractions. Extra heavyμ(0.48 nmol/ membranes are enriched in cardiolipin, a specific component of the inner mitochondrial membrane and evidence a low level of cholesterol. Staining of proteins showed differential bands mainly between light and the other two membrane fractions. The finding of a single 21-kDa caveolin in light membranes indicates the presence of caveolae-like structures in *B. arenarum* oocytes. In support of this finding, lipid composition of light membrane fraction is consistent with that proposed for lipid rafts.

LI-P25

SPHINGOSINE 1-PHOSPHATE TRIGGERS ACROSOME EXOCYTOSIS IN HUMAN SPERMATOZOA TROUGH A MEMBRANE RECEPTOR

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The acrosome is an exocytic granule that overlies the spermatozoan nucleus. In response to progesterone or zona pellucida, it undergoes calcium-regulated exocytosis. To determine if lipid molecules may activate signaling pathways similar to those triggered by physiological acrosome reaction (AR) inducers, we tested the effect of sphingosine 1-phosphate (S1P). In other cell types different S1P receptors have been described. These receptors are members of the lysophospholipid receptor family and are associated with different G proteins. By using functional assays, we observed that S1P induces AR in intact sperm. Its effect was inhibited by BAPTA suggesting that it depends on the presence of extracellular calcium. This result is consistent with the hypothesis that S1P activates exocytosis by a receptor-mediated mechanism that opens SOC channels. To confirm this hypothesis, we used Ni2+, an inhibitor of SOC channels which blocked progesterone and also S1P triggered exocytosis. As expected, no effect of S1P was observed in permeabilized sperm, where opening of SOC channels is not stimulatory by itself because of the absence of calcium in the medium. Furthermore, voltage-dependent calcium channel inhibitors, such as verapamil and nifedipine, efficiently blocked S1P induced AR. This is the first evidence suggesting that S1PR are present in sperm cells.

LI-P26

THE ROLE OF SPHINGOLIPIDS IN MDCK CELLS POLARIZATION SUBMITTED TO HYPERTONIC STRESS

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Renal papilla plays a key role in water and electrolytic homeostasis since regulates urinary pH and ionic concentration. These functions are carried out by papillary collecting ducts constituted by highly polarized epithelial cells which present two membrane domains, apical and basolateral. Both domains show different lipid composition: high concentration of glycosphingolipids (GSL) in apical domain while basolateral is enriched in sphingomyelin (SM). It has been reported that during renal development hypertonicity induces differentiation of tubular structures which implies cellular polarization, but the mechanism involved is not well understood. In order to explore the role of sphingolipids (SL) in renal epithelial differentiation, we studied SL metabolism and gp135 expression, as polarization parameter, in MDCK cells submitted to hypertonicity. Cultures were grown in high NaCl media (250 mM) for 24 and 48 hs in presence of 14C-Palmitic acid or 14C-Galactose to monitor de novo SL and GSL synthesis, respectively. Total 14C-Palmitic acid incorporation to SLs was significantly higher in cells under hypertonicity, even more at 48 hs. SM labeling registered the mayor increase. Similarly, GSL synthesis was also increased in hypertonic conditions. Increased SLs metabolism preceded gp135 protein in apical membranes suggesting early sphingolipid participation in cell polarization.

LI-P27

EFFECTS OF CAPACITATION AND ACROSOME REACTION ON RAT SPERMATOZOA LIPIDS AND FATTY ACIDS

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Mammalian spermatozoa must mature (capacitate) before they can undergo the acrosome reaction (AR) that enable them to fertilize an egg. The mechanisms underlying these events have not yet been fully elucidated. In this work we focused on the effects of these sperm functions on the amounts and fatty acid composition of the main lipid classes of mature rat spermatozoa. Capacitation was assessed by a protein tyrosine phosphorylation assay, and AR was induced by stimulation of spermatozoa with A23187/Ca2+ in the presence of albumin. Capacitation resulted in a significant loss of cholesterol and phospholipids (PL) from sperm. In acrosome-reacted spermatozoa, part of the PL were hydrolyzed, as reflected in the generation of diacylglycerol, phosphatidic acid, and free arachidonic acid (20:4n-6). The amounts per cell of the male germ-cell specific sulfogalactolipid (seminolipid), as those of sphingomyelin and ceramide, remained similar to controls in capacitated and acrosome-reacted spermatozoa, thus increasing their proportions in relation to PL. The latter two sphingolipids contained high percentages of very long chain polyunsaturated fatty acids, both non-hydroxylated and 2-hydroxylated. The lipid changes may work synergistically with protein phosphorylation to convert an initially stable into a fusion-competent membrane, capable of engaging in fertilization-promoting events.

LI-P28

OXIDATIVE STRESS INDUCES DIACYLGLYCEROL-GENERATING PATHWAYS IN RAT CEREBRAL CORTEX SYNAPTIC ENDINGS

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Neurotoxic agents such as Fe2+ induce lipid peroxidation, impairment of glutamate and glucose transport and mitochondrial dysfunction. These toxic effects on biological membranes are comparable to those of β amyloid peptide (βA) on the brain of Alzheimer's disease patients. Recent studies from our laboratory demonstrated that the exposure of rat cerebral cortex synaptic endings (Syn) to Fe2+ induces a marked increase in tyrosine phosphorylation of several proteins and the activation of PI3K/Akt pathway.Our purpose was to study the effect of oxidative insult on diacylglycerol (DAG) generation from phosphatidylcholine (PC) in Syn. DAG from PC can be generated by phospholipase D (PLD) and phosphatidic acid phosphohydrolase type 2 (PAP2) pathway or by a phosphatidylcholine-specific phospholipase C (PC-PLC).
Free iron stimulated DAG generation from PC as a time-function. Assays conducted with ethanol demonstrated that both PLD/PAP2 and PLC pathways were stimulated by Fe2+. Preincubation of Syn either with the PI3K inhibitor (LY294002) or with Genistein or Herbimycin A, two tyrosine kinases inhibitors, did not modify the DAG increase induced by Fe2+. This work constitutes the first report about the effect of oxidative insult on DAG generating pathways in synaptic endings. DAG formation induced by Fe2+ seems to be independent of PI3K activity and tyrosine phosphorylation pathways.

LI-P29
LIPIDS CONCENTRATION IN RAT LUNG AFTER INHALATION OF BUDESONIDE AND SALBUTAMOL

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Inhaled drugs are utilized in neonato with respiratory alterations. Budesonide (B) is used as a glucocorticoids therapy for treatment of chronic lung disease and salbutanol(S) as a broncodilatador. The purpose of this study was evaluate three dose no citotoxic for human, of both drugs, on the lipids content of total lung, surfactant and alveolar macrophages of adult male rats, in order to determine if there was correlation in the changes of lipids content with the doses used. Fifty six rats were separated in seven groups, one was control, three were inhalated with B and the others three with S using 0.05,0.5 and 5 µg/ml of B and 0.311,3.11 and 31.10 ug/ml of S respectively, each day, twice, during fifteen days in a special box.The rats were sacrificed, the total lung, alveolar macrophages and surfactant were separated. The lipids were extracted and triglycerides, cholesterol total phospholipids and Phosphatidylcholine separated by TLC as described in Lipids, 32,3, 278-285, 2002. The results showed that changes in the lipid content have not correlation with doses of inhaled drugs. Theses results could indicate that the effects of theses drugs are independent of the changes in the lipids content in relation to the controls.

LI-P30
COMPARISON OF THE EFFECT OF DIFFERENT DOSES OF CADMIUM ON PROSTATE LIPID METABOLISM

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The aim was to study the effect of Cadmium (Cd) on lipid metabolism. Wistar male rats were separated in groups: Cd1 and Cd2, exposed to 15 and 100ppm of Cd for 12 weeks, and control without Cd. Lipid contents and FAS were quantified. FAS, GPAT, DGAT1 and 2, FAT/CD36, E-FABP, LPL, ACO, CPT-1 and cytidylyltransferase (CT) expression was measured. Cd1: TG content and DGAT1 mRNA decreased. Cd2: TG and EC decreased FC and PL increased. GPAT and CT mRNA increased while DGAT1 decreased. FAS and LPL remained unchanged in all groups. There would be a redistribution of substrates, prioritizing phosphatidylcholine (PC) synthesis in Cd2. FAT/CD36, involved in the uptake of FA across the membrane decreased in Cd1 and 2. FABP, involved in FA intracellular traffic was increased in Cd2. CPT-1, a mitochondrial beta oxidation enzyme, increased in Cd1 and 2 while ACO, from the peroxisomal process decreased in Cd2. Increased E-FABP and CPT-1 in Cd2 may be related to higher mobilization of FA to mitochondrial beta-oxidation. Exposure to 100ppm of Cd would produce changes in lipid metabolism regulation what would lead to increased FA utilization for PL synthesis in detriment of TG's. This could alter membrane composition and function and signaling through PC. The effects induced by 15ppm would occur only through the oxidative stress generated by Cd.

LI-P31
CIRCADIAN REGULATION OF PHOSPHATYDILCHOLINE BIOSYNTHESIS IN CULTURED FIBROBLASTS

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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. In murine cells, this enzyme show four isoforms (CTα, CTβ1, CTβ2 and CTB3). Total cellular CCT activity is the sum of CCTα and CCTβ. Our results showed that the biosynthesis of PC exhibits a daily oscillation in fibroblasts NIH 3T3 after synchronization with serum shock but not in fibroblasts from mutant mice Clock/Clock. In NIH 3T3, we observed that the CCT activity show daily oscillation ($P<$ 0.009647); with maximum levels at 6.5 h and 35h after serum shock. The period (τ) is ~ 29 h, which is coincident with period previously observed in the 32P-phospholipid labelling of NIH 3T3. Preliminary data in fibroblast Clock/Clock showed that the CCT activity not significative change along the day. For ICC we observed that CCTα, CCTβ2 y CCTβ3 isoforms maintain subcelular localization at different time of day. 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 corresponds to the differential circadian regulation in some isoforms of CCT.

MI-P01
EFFECT OF HEPATITIS C VIRUS (HCV) CORE AND NS5A PROTEINS IN APOPTOSIS OF HeLa CELLS

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INTRODUCTION Both Core and NS5A HCV proteins play important roles in viral replication and pathogenesis. These proteins may also affect many cellular functions, including gene expression, signal transduction and apoptosis. OBJECTIVE To evaluate the effect of transiently expressed Core and NS5A proteins in Staurosporine-induced apoptosis of HeLa cells.METHODS HeLa cells were transiently transfected. Apoptosis was determined in Staurosporine (STR) treated and untreated cells by Anexina V and Hoechst staining. Bax, bcl-2 and telomerase mRNA levels were evaluated by multiplex RT-PCR. RESULTS Transient expression of either Core or NS5A HCV proteins was associated with a slight inducer effect on apoptosis of HeLa cells after 42hs, but not after 30hs. There was an increase in apoptosis of STR-treated cells only when the viral proteins were expressed for 30hs and cell death quantitative analyses allowed discrimination between early and late apoptosis. No variations were detected in the mRNA levels of bax, bcl-2 and telomerase of transfected HeLa cells. Decrease of these mRNA levels induced by STR was not reverted by either viral proteins. CONCLUSIONS Both Core and NS5A proteins appear to accelerate STR-induced apoptosis in transiently transfected HeLa cells, without affecting bax, bcl-2 and telomerase mRNAs levels.

MI-P02

JUNV INFECTS VERO CELLS BY CLATHRIN MEDIATED ENDOCYTOSIS

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Junin virus (JUNV) entry is conducted by receptor mediated endocytosis. Previous results, using compounds that specifically blocked different entry pathways, have suggested that JUNV would use clathrin mediated endocytosis to infect Vero cells. To further explore the cellular entry mechanism of JUNV, assays with 35S-methionine-labeled virions were performed and the kinetics of binding and internalization was determined. We found that with chlorpromazine (CPZ), which inhibits clathrin mediated endocytosis or nystatin (NT), that alters lipid raft microdomains impairing caveolae mediated endocytosis, virion binding does not seem to be affected. In contrast, radioactivity levels that represents internalization of JUNV, showed a significant reduction when cultures were treated with CPZ. To have a direct evidence JUNV entry, transmission electron microscopy (EM) was performed. EM showed JUNV particles of about 60 to 100 nm in membrane depressions that had an electron-dense coating. In addition JUNV particles were found within invaginations of the plasma membrane and vesicles that resembled those of clathrin-coated pits and clathrin-coated vesicles. Taken together our results demonstrate clathrin mediated endocytosis as the main JUNV entry pathway into Vero cells and represents an important contribution to the characterization of arenavirus multiplication cycle.

MI-P03

INIBITION OF VSV INFECTION IN EPITHELIAL CELLS BY CDM, A NATURAL BIOACTIVE COMPOUND

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The tetranortriterpenoid, 1-cinnamoyl-3,11-dihydroxymeliacarpin (CDM), isolated from purified leaf extracts of *Melia azedarach* L., exhibits antiviral activity and nuclear factor kB modulating properties in Vero and human conjuntival cells. To increase our understanding about CDM action, we investigated its mechanism of antiviral function in a human lung epithelial cell line (A549), since these cells serve as the primary site for productive infection of several viruses including vesicular stomatitis virus (VSV), human parainfluenza virus type 3 and human respiratory syncytial virus. CDM induced cytoplasmic alkalinization in A549 treated cultures measured by an acridine orange staining, at concentrations that did not affect cell viability. The antiviral action was studied in A549 cells infected with VSV and treated with 75 µM CDM. At different times post-infection, virus yields were determined by plaque assay, and the intracellular localization of the matrix protein (M) and viral glycoprotein (G) were visualized by immunofluorescence studies. In CDM-treated cells, viral infectivity was reduced with respect to virus control, and the patterns of fluorescence of viral proteins were affected. Therefore, CDM is able to inhibit VSV multiplication in A549 cells by affecting the transport of the G glycoprotein and altering the intracellular localization of M protein.

MI-P04
SEQUENCE ANALYSIS OF A 50 kDa *NATRONOCOCCUS OCCULTUS* PROTEIN THAT REACTS WITH ANTI-UBIQUITIN ANTIBODIES

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Both eukaryotic and prokaryotic organisms contain ubiquitin-like proteins. In most eukaryotic cells, ubiquitin appears as a multiubiquitin chain or as a fusion protein of different ribosomal proteins. We have previously reported data suggesting the presence of ubiquitin–like proteins in halophilic archaea. This work reports the isolation by 2D SDS-PAGE of a 50 kDa and pI 3.1 protein from Natronococcus occultus. It reacted with antibodies against ubiquitin and, like proteins of the ubiquitin family, is heat-stable. After its in-gel digestion with trypsin, the resulting peptides were analyzed by MALDI-TOF. In spite of the scarce data available of protein sequences of haloalkaliphilic microorganisms, alignment of the peptide mass fingerprint showed significant score with two proteins. One is the Signal Recognition Protein 54 (SRP54) from Archaeoglobus fulgidus of 48.2 kDa and pI of 9.03. The other is a Ribosomal Proteins L11p from Pyrococcus abissy of 17.74 kDa and pI 6.02. Likeness to more than one protein of the same spot suggests presence of a conjugation complex even when size and pI of individual proteins not agree with those found. Differences may be linked to either aminoacidic composition or loss of fragments during conjugation. The presence of the L11p ribosomal protein is consistent with our previous studies describing the presence proteins reactive with antiubiquitin antibodies in Ncc. occultus ribosomes. The significance of presented results in protein metabolism must be further studied. [This work was supported by CONICET and UNMdP].

MI-P05
OPTIMIZATION OF 2D GEL ELECTROPHORESIS FOR PROTEOME ANALYSIS IN THE ARCHAEON *NATRIALBA MAGADII*

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The haloarchaeon *Natrialba magadii* grows in 4 M NaCl-pH 10. Its proteins have a high content of acidic amino acids, low hydrophobicity and require high salt to be functional. These features give rise to several problems during the extraction and analysis by electrophoretic methods. The aim of this work was to optimize a protocol for the analysis of proteins by 2D gel electrophoresis for proteomic studies in Nab. magadii. Various protocols for protein extraction were assayed, the selected one included: washing the cells with 4 M NaCl-Na_2CO_3 (pH 10), cell disruption by ultrasound in HCl-Tris (pH 8), 1mM PMSF and 2mM EDTA, acetone precipitation followed by several washing steps to eliminate salts, and suspension in 0.25 M glycerol for protein quantification and electrophoresis. 2D eletrophoresis was performed using isoelectric focusing (IEF) and SDS-PAGE. The pH gradient, pore size and visualization procedures were adjusted. After several trials, the optimized conditions were as follows: proteins in the first dimension were separated in a pH gradient with the following proportion of ampholites: 30% pH 2.5-5, 40% pH 4-6.5 and 30% pH 3-10; the second dimension was performed in 12% SDS-gels and proteins were stained with colloidal Coomassie Blue. This optimized protocol will allow to perform a proteomic approach in *Nab. magadii* under different

conditions. Supported by CONICET and UNMDP.

MI-P06
PHYSIOLOGICAL ROLE OF AN OUTER MEMBRANE PROTEIN OF *ACINETOBACTER BAUMANNII* ASSOCIATED TO CARBAPENEM

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We previously demonstrated that acquisition of resistance to b-lactam carbapenems among multidrug-resistant isolates of the Gram-negative nosocomial pathogen A. baumannii is associated to the loss of an outer membrane protein (OMP) of 29 kDa designated CarO. DNA sequence determination and OM analysis including SDS-PAGE, immunoblotting, and MALDI-TOF revealed two mechanisms responsible for the absence of CarO in carbapenem resistant strains: 1) disruption of the carO gene by insertion elements (IS) and, 2) the existence of allelic variants of CarO with distinctive immunogenic properties. Also, two-dimensional SDS-PAGE and immunoblotting of OM fractions of both carbapenem-sensititive and carbapenem-resistant A. baumannii strains indicated that all CarO variants formed stable oligomers, a property distinctive of OM channels. We also provided evidence that CarO represents a selective channel for basic amino acids. The overall data support the hypothesis that CarO represents a novel and selective OM channel for compounds structurally related to basic amino acids such as the carbapenems through the A. baumannii OM, and that the evolution of resistance to these b-lactams in the clinical setting could result from either the natural deletion of the carO gene by endogenous IS or the accumulation of specific mutations that result in substantial restrictions to the entrance of these antibiotics.

MI-P07
AN sbmA MUTATION IN E. *COLI* TN10–CARRYING STRAINS RESULTS IN HYPERSUSCEPTIBILITY TO TETRACYCLINE

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The *Escherichia coli sbmA* gene product is involved in uptake of microcins B17 and J25, but its physiological role is still unknown. We have found that an *E. coli sbmA*::Tn*5* mutant harboring a Tn*10* transposon does not form isolated colonies in the presence of as low a concentration of tetracycline as 5 mg/ml, in spite of the presence of the Tn*10* transposon. When a culture of the *sbmA* mutant was grown in LB, serially diluted, and aliquots of 10-2 and 10-3 dilutions spread on LB-Tc plates there was confluent growth. However, 10-4 and higher dilutions resulted in no single colonies. One explanation for these findings is that as the plates become increasingly crowded the ability of the mutant to divide normally in the presence of tetracycline is restored. An interpretation of this observation is that cells release a factor that diffuses to other cells and stimulates their growth and division. Thus, a certain number of neighboring cells would be required in order to reach a high local concentration of the factor. Below a critical number of cells they should fail to develop into a colony because they are unable to signal to themselves. What is not yet clear is the mechanism by which the mutation abolishes the resistance conferred by the transposon and how it is restored by the putative factor. Our results could throw light on the natural role of SbmA.

MI-P08

ACTIVITY OF MICROCIN J25 AGAINST INTRACELLULAR *SALMONELLA* AND PHARMACOKINETIC PARAMETERS

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Microcin J25 (MccJ25) is a low-molecular-mass (2,107 Da) peptide antibiotic, active against strains of the genera *Escherichia*, *Shigella* and *Salmonella*. One of the purposes of the present study was to assess its antibacterial efficacy against the intracellular pathogen *Salmonella enterica* serovar Newport. To this end, macrophages were collected from BALB/c mice by peritoneal lavage, infected in vitro with S. Newport, exposed to gentamicin and washed. Macrophages were then treated with different concentrations of MccJ25 and counts of viable bacteria were made after the desired incubation period. Results were compared to a control without treatment. Intracellular bacteria were killed by MccJ25. Coincidentally, this demonstrated that the antibiotic is able to traverse the cytoplasmic membrane of eukariotic cells. We also studied pharmacokinetic parameters for MccJ25 in plasma from BALB/c mice, such as area under the concentration-time curve (AUC), half-life ($t_{1/2}$), clearance (Cl), and volume of distribution (V_d). These values allowed a characterization of both the kinetic of absorption and elimination of MccJ25. Our results could be useful for a potential therapeutic application of this antibiotic.

MI-P09

MICROCIN J25 INDUCES ATP DEPLETION IN *ESCHERICHIA COLI* CELLS

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After entering sensitive cells through the outer membrane receptor FhuA, the 21-residue peptide antibiotic microcin J25 (MccJ25) displays a dual mechanism of action: it inhibits both RNA polymerase (RNAP) and cell respiration. The latter effect is apparently mediated by an overproduction of superoxide radical which, in turn, would affect superoxide-sensitive enzymes from the respiratory chain, leading to a reduction in the intracellular levels of ATP. In the present work, we examined the effect of MccJ25 on *E. coli* ATP levels using a luciferin-luciferase bioluminescence method. Our results show that MccJ25 induces a significant ATP reduction in the MccJ25-hypersensitive strain AB1133, whereas no effect was detected by using strain MC4100, which has an intrinsic partial resistance to the antibiotic. A drop in ATP levels took place also in PA232 (pGC01), a strain that carries a mutated MccJ25-resistant RNAP and overexpress the FhuA receptor (encoded by plasmid pGC01). Previously, we observed that the increase in superoxide radical production caused by MccJ25 was prevented by the addition of the enzyme superoxide dismutase (SOD). Since only a small ATP depletion occurred when cells were coincubated with MccJ25 and SOD, we conclude that the fall in ATP levels is a consequence of superoxide production induced by MccJ25, and that this effect is independent of RNAP inhibition.

MI-P10

ESCHERICHIA COLI CELL-FREE EXTRACTS CONTAIN AN ANTIBIOTIC ACTIVITY WITH THE SPECIFICITY OF MCCJ25

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The 21-residue peptide antibiotic microcin J25 (MccJ25) surges from a precursor of 58 amino acids. Our long term goal is the elucidation of the mechanistic details of MccJ25 maturation. As a first step toward an in vitro maturation system, we are trying to purify the precursor protein. For that purpose, cell-free extracts of *E. coli* cells harbouring a plasmid encoding the MccJ25 precursor were subjected to HPLC analysis. One of the peaks showed antibiotic activity against *Salmonella* Newport (an indicator strain hypersusceptible to MccJ25) and some strain of *E. coli*. A likely explanation is that the precursor itself has antibiotic activity. Surprisingly, however, extracts from plasmidless *E. coli* K-12 cells also showed the same activity, and this substance was inactive against MccJ25-resistant mutants or strains harbouring the MccJ25 immunity gene. Preliminary experiments showed this compound to be a peptide. It is tempting to speculate that the *E. coli* chromosome encodes a product structurally similar to MccJ25 or, at least, with the same specificity of action. Purification of this substance is under way, but is hampered by the fact that it is present in very little amounts in cell extracts.

MI-P11

STRUCTURE-FUNCTION RELATIONSHIP IN MICROCIN J25

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To study structure-function relationships in the peptide antibiotic MccJ25, the structural gene was subjected to site-directed mutagenesis. Antibacterial activity of the peptide mutants was tested against *E. coli* DH5a and MC4100. We obtained 279 mutants, from which 218 were inactive and 61 active. From the latter, 3 have wild-type activity, 48 were less active, and 10 were more active than native MccJ25. When tested against strains hyperexpressing the MccJ25 importers FhuA and SbmA, less active mutants became as active as MccJ25, suggesting that the mutated amino acid is involved in microcin import. Using the same strains, no activity change was observed with the other mutant peptides, indicating that the mutated residue is either important for antibiotic action or is needed for MccJ25 maturation and export. In other experiments, we deleted the entire leader peptide as well as almost all of it, leaving only the three amino acids to the left of the cleavage site. In both cases, we provided a Shine-Dalgarno and an ATG codon. Anyone of these constructs led to the production of extracellular active microcin, suggesting that integrity of the leader peptide is essential for MccJ25 production.

MI-P12

SYNERGISM OF MICROCIN J25 WITH A MEMBRANE-PERMEABILIZING PEPTIDE AGAINST MICROCIN-RESISTANT BACTERIA

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Microcin J25 (MccJ25), a peptide antibiotic produced by *Escherichia coli*, is active on strains from Escherichia, Salmonella and Shigella species. Other gram negative bacteria such as clinical isolates of *Enterobacter cloacae*, *Citrobacter freundii*, *Klebsiella pneumoniae* and *Salmonella typhimurium*, are completely resistant. Entry of MccJ25 into susceptible cells is mediated by the outer membrane receptor FhuA, and the inner membrane proteins TonB, ExbB, ExbD, and SbmA. The aim of the present study was to determine whether the membrane-permeabilizing peptide (MPP) KFFKFFKFFK could sensitize microcin-resistant strains against the antibiotic. When MccJ25-resistant *fhuA* and *sbmA* mutants, as well as the above clinical isolates, were coincubated with MccJ25 and MPP (30 µg/ml) they became sensitive to the antibiotic. MPP has no direct activity when used alone. Using the dye-probe DiSC3[5] we corroborated that MPP increases membrane permeability, and this could allow nonspecific (receptor-independent) entry of MccJ25 into the cells. Interestingly, we found that *E. coli* SBG231, a strain carrying an RNA polymerase mutation that makes it resistant to MccJ25, was also sensitized by MPP. This suggests that the antibiotic is acting on the membrane target. Our results could have implications for a potential therapeutic use of MccJ25.

MI-P13

EVALUATION OF *GYRB* SEQUENCES FOR TAXONOMIC ANALYSES OF HEAVY METAL RESISTANT ACTINOMYCETES

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Actinomycetes are Gram-positive bacteria whit high G+C content. 16S rDNA sequences analysis is a simple and commonly used method for identification of microorganisms, but in the last years it was demonstrated that *gyrB* gene sequences may be also applied to perform phylogenetic analyses of *Streptomyces* and other actinobacteria. In this work it was evaluated the use of *gyrB* sequences for taxonomic analyses of heavy metal resistant actinomycetes and the obtained results were compared with the 16S rDNA procedure. Total gDNA of heavy metal resistant actinomycete strains was amplified using 16S universal and *gyrB* specific primers. DNA and closely related specie sequences from public databases were aligned and compared; while phylogenetic trees were constructed by the neighbor-joining method. According to 16S rDNA analysis ten strains belonged to the *Streptomyces* genus and two to *Amycolatopsis*. *gyrB* gene sequences characterize the same ten strains as *Streptomyces* but failure to identify the *Amycolatopsis* genus. In addition, trees constructed with the related species from public databases were different depending on the taxonomic marker used. We conclude that the *gyrB* public database is still incomplete for *Streptomyces* and has not been developed yet to *Amycolatopsis*. This work contributes to construct a database of *gyrB* sequences to re-classificate *Streptomyces* and *Amycolatopsis* species.

MI-P14
MOLECULAR CHARACTERIZATION OF NEW SEROTYPE 14 VARIANTS OF THE SPAIN9V-3 PNEUMOCOCCAL CLONE

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We have analyzed by MLST the genetic background of 17 invasive pneumococci isolated from pediatric patients in Cordoba City, Argentina. All the penicillin-resistant isolates were serotype 14 variants of the Spain9V-3. To investigate whether our strains derivate from other serotype 14 variants described previously, some specific genes were analyzed. Interestingly, the Cordoba variants were differentiated from the Uruguayan/European variants by the *pbp1a* gene (1473-1922 region), suggesting a different recombinational replacement of the capsular genes. To probe this hypothesis, we tried to identify the recombinational sites and the proximal crossover point was clearly localized into the *spr0309* gene, and the distal site was restricted to the *recU gene*, indicating a different recombinational event. The analysis of the *dexB, cpsB, aliA* and *pbp1a* genes from our strains showed a high homology with the corresponding genes of the Spain14-5 clone, suggesting that the *14cps* genes were provided by this international clone. On the other hand, the PCR-BOX analysis and the capacity to transfer penicillin/cefotaxime resistance by *pbps* genes revealed a divergent local epidemiology of the Cordoba variants, suggesting a non-recent dissemination in our pediatric population. Despite the geographical proximity, the Cordoba variants showed a different clonal origin to those isolated in Uruguay.

MI-P15
MYCOBACTERIOPHAGES AS TOOLS TO DISECT MYCOBACTERIAL CELL ENVELOPE ESTRUCTURE

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Analysis of *Mycobacterium smegmatis* mutants resistant to the mycobacteriophage D29 revealed alterations in an specific glycolipid molecule that has subsequently been chemically characterized (Besra et al. Carb. Res., 251:99). The glycolipid barrier is (along with mycolic acids), a key component responsible for the very low permeability of mycobacteria to drugs. Thus, it is necessary to study their biosynthetic pathways since there is a direct relation between cell envelope composition and cell permeability. This fact makes glycolipid biosynthesis an attractive target for drug development. We report here the isolation and preliminary characterization of four mycobacteriophages. All of them are temperate phages differing in plaque morphology and turbidity. Three of them belong to the same immunity group. *M.smegmatis* lysogenic strains to each phage and mutants resistant to three of the phages were isolated. They showed differences in sliding motility, Congo Red binding and biofilm formation when compared to the wild-type strain, suggesting cell envelope alterations. The chemical nature of the modifications are currently being analyzed. The simplicity and economy of the isolation and characterization of phages makes phage hunting a great learning project for undergraduates while providing new useful information on mycobacteriophage-host cell interactions.

MI-P16
ULTRAVIOLET-A RESPONSE IS MODIFIED BY GROWTH WITH DIVERSE CARBON SOURCES IN *RHODOCOCCUS* SP

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Rhodococci are potentially useful in bioremediation processes because of their ability to degrade a variety of organic compounds. The recognition of environmental agents able to affect bacterial metabolism could help to improve their utilization. In this context, we studied the effect of UV on *Rhodococcus* sp RMB90. Bacteria cultured in minimal medium with 1-butanol, 3-methyl-1-butanol, sodium lactate, sodium succinate, or sodium benzoate as a sole C source were exposed to UV-A (365 nm). Survival of bacteria grown utilizing lactate and 1-butanol were similar to that observed irradiating bacteria grown in nutrient broth. Increased resistance was found with succinate and 3-methyl-1-butanol grown cells, probably due to the low growth rates achieved with these substrates. In contrast, benzoate utilization reduced resistance of RMB90 to UV-A. Growth delay induced by sub-lethal doses of UV-A in cultures of RMB90 using 1-butanol as a sole C source was longer than that induced in cultures grown with lactate. In rhodococci primary alcohols and benzoate are intermediate metabolites in degradation of n-alkanes and biphenyl, respectively. In outdoor environments the utilization of these intermediates by rhodococci, and eventually the degradation of relevant pollutants, could be impaired by sunlight due to an increment in bacterial sensibility to UV-A.

MI-P17
INCREASED RESISTANCE OF *SPHINGOMONAS PAUCIMOBILIS* TO SUNLIGHT AND UV-A RADIATION

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Here we report the high resistance to solar and ultraviolet radiation of a yellow pigmented bacteria isolated in our laboratory. It was characterized as a Gram-negative rod and identified as *Sphingomonas paucimobilis* by light microscopy analysis, physiological and biochemical tests, and by sequencing the 16S RNA gene. It was designated RMB80. *S. paucimobilis* RMB80 displayed an important resistance to sunlight, artificial UV-A radiation, and nutritional stress. The degree of photo-induced damage in RMB80, determined by a chemiluminescence assay, was compared with that found in *Escherichia coli* as a reference organism. The results suggest that the radiation-induced damage in RMB80 is smaller than that in irradiated *E. coli.* The study of a protective role of bacterial pigments strongly suggests their involvement in the prevention of radiation damage, since the impairment of carotenoid synthesis causes an important loss in radio-resistance. The analysis of enzymatic antioxidative defenses showed high levels of catalase activity in RMB80. Even after inactivation by UV-A exposure the residual activity present in *S. paucimobilis* was five times higher than that found in non-irradiated *E. coli*. The results reported herein contribute to the understanding of the ultraviolet radiation resistance in this species and to optimizing its eventual use in outdoor bioremediation processes.

MI-P18

MUTATION OF THE FER GENE, HAS A PLEIOTROPIC METABOLISM-RELATED PHENOTYPE IN *BACILLUS SUBTILIS*

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Ferredoxin are soluble iron-sulfur proteins ubiquitous in nature involved in several membrane-bound electron transport systems as well as the metabolism of carbon, hydrogen, oxigen sulfur and nitrogen. Recently the single fer gene of B. subtilis has been expressed and characterized. It has been demostrated that Fer protein, is a low molecular weight, 4Fe-4S ferredoxin, and supports electron tranfer to cytochrome P450BioI, involved in biotin synthesis. On the other side it has been reported that cyanobacterial desaturases use ferredoxin as an electron donor in the desaturation reaction. We report here the isolation of fer mutant L43 in B. subtilis by gene disruption using a kanamycin resistance cassette. Parent and mutant strains were identical for unsaturated fatty acid biosynthesis as it has been demostrated by GC-MS and TLC analysis. This mutant can not growth in minimal medium but the defect could be overcomed by supplementing the media with methionine, isolucine and valine. Ours results show that ferredoxin is not involved in the desaturation reaction and the ferredoxin gene disruption affects aminoacids metabolism, ressembling oxidative stress condition in Bacillus subtilis cells.

MI-P19

ROLE OF GLUTATHIONE IN THE *RHIZOBIA-PEANUT* SYMBIOSIS

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It is known that glutathione (GSH) is essential in the nodulation process by infection threads in the rhizobia-legume symbiosis. In the case of rhizobia-peanut symbiosis, there are no infection threads, instead, infection through the epidermis involves intercellular penetration. We previously studied the importance of GSH in Bradyrhizobium sp. SEMIA6144 using a GSH-deficient mutant obtained by disruption of the gshA gene, encoding γ-glutamylcysteine synthetase (γECS), which was able to induce nodules in peanut roots. To investigate the involvement of the peanut GSH content in the symbiosis, a strategy aimed at modifying the GSH pool was developed using L-buthionine-sulfoximine (BSO), a specific inhibitor of γECS in plants. Peanut seedlings were grown in the Farhaeus medium either in the presence or absence of BSO (0.1 to 1 mM). The concentration of 0.1 mM BSO did not modify the plant growth and the typical anatomic structure of the peanut roots. However, the GSH content was reduced by 51% after treatment with BSO. This concentration was chosen to further analyze the implication of GSH in the nodulation process. Preliminary results showed that BSO-treated plants and inoculated with wild-type strain formed fewer nodules than control plants suggesting that the peanut GSH content could have a role in the establishment of the symbiosis. [Supported by SECyT-UNRC, CONICET, ANPCyT-PICT].

MI-P20

MODIFICATION OF PHOSPHOLIPID COMPOSITION IN *PSEUDOMONAS* BY CONTACT WITH TETRADECYLTRIMETHYLAMMONIUM

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Quaternary ammonium compounds (QAC) are cationic biocides included in antiseptic solutions. Some strains of *Pseudomonas* are resistant to QAC but little is known about of basis of this tolerance. The aim of this work was to know if the response to tetradecyltrimethylammonium (TDTMA), a QAC, involves changes in the phospholipid (PL) composition in species of the *Pseudomonas* genus. *P. putida* A ATCC 12633, *P. putida* KT2440 y *P. aeruginosa* PAO1, were exposed to 50 mg l^{-1} TDTMA for 15 min and PL composition was analyzed. In presence and absence of TDTMA, the PL pattern was qualitatively the same but the amount of each individual PL, measured by Pi concentration or ^{32}Pi changed. Respect to control values, all strains showed an increase in phosphatidic acid (PA) (140%). Accompanying this change, phosphatidylglycerol increased 100% and cardiolipin decreased 60%. *P. putida* A ATCC 12633 was cultivated in a media containing gradually increasing TDTMA concentrations. Following the last culture grown in the presence of TDTMA (280 mg l^{-1}) successive subcultures without TDTMA were made. In TDTMA-adapted bacteria the most significant change was a 380% increase in PA. This change was reverted after two subcultures without biocide. These results suggest that PL are involved in cellular responses to QAC, utilizing PA principally to neutralize the high positive charge density given for the TDTMA moiety.

MI-P21

HIGH CAPACITY FOR COPPER CAPTURE BY AN *ESCHERICHIA COLI* STRAIN DEFICIENT IN NADH DEHYDROGENASE-2

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We found that an *Escherichia coli* strain deficient in NADH dehydrogenase-2 presented a lower extracellular Cu(II)-reduction and a higher intracellular metal accumulation than the parental strain, after incubations with nontoxic copper concentrations. The aim of the present work was to further analyze that behavior when bacteria are incubated with high copper concentrations. Cells of the strains ANN001 (deficient in NDH-2) and AN387 (wild-type) were harvested in stationary phase and incubated with different copper concentrations, in the presence of a respiratory substrate. ANN001 had higher copper capture ability than its parental strain. The cellular viability decreased in both strains upon copper incubations, although the damage is higher in the mutant strain. These results were corroborated by measuring the oxygen consumption and by analyzing the activity of the respiratory chain components. In addition, the metal concentration in the solution significantly decreased, when it was incubated several times with fresh cells. Even though the results are preliminary, they suggest that bacterial strains deficient in respiratory chain components could be used to reduce copper concentrations in polluted waters.

MI-P22

IDENTIFICATION AND CHARACTERIZATION OF MALIC ENZYMES FROM *STREPTOMYCS COELICOLOR*

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S. coelicolor has been adopted as a model system to study secondary metabolism, which is regulated through a complex cascade of regulatory factors. Primary metabolism, which supplies the building blocks for antibiotic biosynthesis, has received little attention. Since anaplerotic enzymes give rise to branch points between energy-producing and biosynthetic pathways, their activity is likely to be regulated at the transition phase between primary and secondary metabolism. For this reason, we studied anaplerotic malic enzymes from *S. coelicolor*. Malic enzymes catalyze the oxidative decarboxylation of L-malate into piruvate with reduction of NAD or NADP. Here we describe the characterization of two genes encoding putative malic enzymes that where found in the chromosome sequence project of *S. coelicolor*. In vitro enzymatic assays using recombinant proteins allowed validate Sco2951 protein as a NAD- and Sco5261 as a NADP-dependent malic enzyme. Single and double mutant strains were constructed by genetic manipulation. Individual mutations in either Sco2951 or Sco5261 genes resulted in no significant growth phenotype, but the double mutant strain showed severe differences in growth and antibiotic production under different carbon sources tested, indicating a main role of the activity of malic enzyme in primary and secondary metabolism of *S. coelicolor*.

MI-P23

DETERMINATION OF PLASMID ENCODED AS RESISTANCE GENE IN *MICROCOCCUS* SP. ISOLATED FROM ANDEAN WETLANDS

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Andean wetlands are extreme environments where bacteria should develop efficient resistance systems to survive. These mechanisms should include resistance to the high heavy metals levels present in water and soil. *Micrococcus* sp. A7 was isolated from Laguna Azul (14 □g/L arsenic) in Argentinean Andean wetlands. This bacterium (AM235879) is able to growth at high levels of different kinds of antibiotics and resist to high ultraviolet-B irradiation.

In this work, *Micrococcus* sp. A7 showed to be an arsenic resistant bacterium. This bacterium was able to growth in two kinds of arsenic salts at high concentrations: sodium arsenate and sodium arsenite.

The most described arsenic resistance system is encoded by the *ars* operon that functions by reducing arsenate to arsenite and export it. *ArsB* gene is always present in this operon and encode for the arsenite efflux pump. We isolated from *Micrococcus* sp. A7 a low molecular weight plasmid that may provide this arsenic resistance. Specific primers were designed for this *arsB* gene and an 800 bp PCR product was obtained and sequenced. BLAST analysis showed 99% of identities with the arsenical pump membrane protein (*arsB*) from *Staphylococcus haemolyticus* JCSC1435. In this study we postulate that *Micrococcus* sp. A7 is able to growth in arsenic salts due to an efflux mechanism encode in an *ars* operon.

MI-P24

MOLECULAR DETECTION OF ENZYMES INVOLVED IN DEGRADATION OF γ-HCH BY *STREPTOMYCES* SP. M7

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The presence of *lin*B and *lin*C genes, which participate in the γ-hexachlorocyclohexane (γ-HCH) degradation was studied in *Streptomyces* M7. This strain can grow in culture medium with γ-HCH as only carbon source. *Streptomyces* sp. M7 was grown in MM medium with and without γ-HCH 100 µg L-1. A protein band, using SDS-PAGE, of approximately 66,2 kDa was detected in the cell-free extract; this band was not present when the microorganism was grown without the pesticide. Plasmids have not been detected by alkaline lysis procedure and by Pulsed Field Gel Electrophoresis (PFGE). These results suggest that genes encoding pesticide degradation enzymes could be localized in chromosomal DNA. PCR amplifications of *lin*B and *lin*C genes, that codify for γ-pentachlorocyclohexane hydrolytic dehalogenase and 2,5-dichloro- 2,5-cyclohexadiene- 1,4-diol dehydrogenase respectively, were carry out using oligonucleotide designed based of the gene sequences involved in γ-HCH degradative pathway of *Sphingobium japonicum* UT26 and *Microbacterium* sp. ITRC1. The size of amplification fragments obtained were according to the expected. These results show for the first time that *Streptomyces* M7 could has specific enzymes for degrading of γ-HCH.

MI-P25

METABOLIC STATE AND CATALASE ACTIVITY OF *P. FLUORESCENS* BIOFILMS AS RELATED TO CORROSION OF COPPER

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Aiming to determine reciprocal interactions at the biofilm/metal interface, biofilms of *P. fluorescens* (ATCC 17552) were biochemically analysed during the growth on copper, while electrochemical reduction curves were performed to evaluate the formation of the protective oxide layer on the metal. DNA profiles showed an extended lag phase for biofilms grown on copper as compared to those grown on glass. As indicated by the RNA/DNA ratio, the overall metabolic state of copper biofilms was always lower than that of glass biofilms. Also lower was catalase specific activity. When analysed by Western blot hibridization, the expression of at least four catalase isoforms were detected in *P. fluorescens* biofilms, with MW of 77, 63, 50 and 38.4 kDa. The two lower bands being differentially expressed during growth on copper. The growth of the protective oxide layer of copper was inhibited by the presence of biofilms. Moreover, the existing oxide layer was decreasing with biofilms growth, evidencing a biological effect on metal stability. Open biofilm with elevated cell clusters were observed at the reflectance microscope, which are presumed to allow the access of dissolved oxygen to the metal surface. Presented results reinforce the possibility for the action of the catalase mediated mechanism proposed previously as an explanation for microbiologically influenced corrosion.

MI-P26

PA4496 IS ONE OF THE GENES RESPONSIBLE TO ENCODE ACETYLCHOLINESTERASE IN *PSEUDOMONAS AERUGINOSA*

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P. aeruginosa acetylcholinesterase (AChE) is produced when bacteria are grown with choline or betaine as nutritional source. Attempts to identify the gene encoding for AchE by random mutagenesis, were unsuccessful. The difficulty to found an AchE- phenotype led us to purify a protein with AchE activity, and use it to identify the encoding gene. Periplasmic extract obtained from betaine-grown cells, was precipitated with ammonium sulfate, and filtered in a Sephacryl S-300 column. An AChE activity 31,3 fold purified was obtained. The enzyme was found to be a monomer of 60,6 kDa with a pI of 8,5. After 2D electrophoresis AChE was detected with silver stain and by enzymatic histochemical assay. Peptide mass fingerprints (PMF) using MALDI-TOF mass spectrometry followed by in silico analysis showed that this purified protein corresponds to PA4496 gene from *P. aeruginosa* PAO1. Up to this moment, this gene is annotated in the data bank as a probable periplasmic component of the ABC transporter. PA4496 gene has a similarity of 41% with human AChE 1B41. PA4496 full-length gene was PCR amplified, cloned in pTYB12, expressed in *E. coli* ER2566 as an intein-fused protein, and purified by a chitin column. Even though we cannot discard the existence of other proteins with AchE activity, our present results indicate that PA4496 is at least one of the gene responsible to encode an AChE activity.

MI-P27

ROLE OF CONSERVED DOMAINS IN THE ACTIVITY OF *P. AERUGINOSA* HEMOLYTIC PHOSPHOLIPASE C

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Hemolytic phospholipase C (PlcH) of *P. aeruginosa* belongs to the phosphoesterase family. The full-length protein of 82,7 kDa contains a signal peptide (sp) of 2,4 kDa plus highly conserved regions: a phosphoesterase domain (pho), and two tandem repeated DUF756 domains and an unconserved central region (core) domain. The secreted native protein (wt) with 78,5 kDa is not in agreement with the full-length PlcH. The aim of this work was to construct PlcH variants in order to identify the essential regions for enzyme activity. Regions of PlcH were cloned, overexpressed and purified as His-tag fusion to measure the enzyme activity. Full-length PlcH and PlcH without sp, exhibited for *p*-NPPC Km values of 6,6 and 17 mM, respectively. Therefore, this change of affinity was a consequence of the lost of sp. Both enzymes were similarly inhibited by Triton X-100, TDTMA and Zn^{+2}. The pho domain alone, conserves the property to hydrolyze *p*-NPPC but with a catalytic efficiency 30 times less than the wt protein. The same construction of pho plus DUF1 with or without core produced a protein without catalytic activity. It was restored with the addition of DUF2, but the catalytic efficiency was 100 times less compared with the wt. This study revels that the pho region is the essential domain for the catalytic activity; all the others regions would be critical to restore or to control the full activity.

MI-P28
CHARACTERIZATION OF ADPGLUCOSE PYROPHOSPHORYLASE FROM *STREPTOMYCES COELICOLOR*

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Glycogen synthesis in bacteria occurs by the pathway utilizing ADP-Glc as the glucosyl donor, being ADP-Glc pyrophosphorylase (ADP-Glc PPase) the enzyme that catalyzes the key regulatory step. The polysaccharide has been identified to play a critical role in development of *S. coelicolor*, a bacterium exhibiting complex morphological differentiation. ADP-Glc PPase from Gram negative bacteria has been relatively well characterized, but studies concerning the enzyme from Gram positive microorganisms are scarce. We report the molecular cloning of the *glgC* gene (coding for ADP-Glc PPase) from *S. coelicolor* genomic DNA. The identity of the amplified gene was confirmed by complete sequence, and it was cloned in the expression vector pET24b. Successful expression of soluble recombinant ADP-Glc PPase required the use of *E. coli* BL21(DE3) cells also expressing GroES/EL. The enzyme was obtained as a His-tag fused protein. Recombinant ADP-Glc PPase reversibly catalyzed synthesis of ADP-Glc and pyrophosphate from Glc-1P and ATP, and it was activated by pyruvate (4-fold, $A_{0.5}$ of 0.050 mM), phospho-enol-pyruvate (14-fold, $A_{0.5}$ of 0.25 mM), and fructose-6-phosphate (20-fold, $A_{0.5}$ of 0.34 mM). Results represent one of the first characterization of this key enzyme from Gram positive bacteria, and they suggest that regulation of glycogen synthesis in these organisms exhibits distinctive properties.

MI-P29
METABOLOME ANALYSIS OF ACID GROWN *SINORHIZOBIUM MELILOTI* (SME)

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Sme is an alfa-proteobacteria able to fix nitrogen-in symbiosis with Medicago spp. As soil microorganisms, rhizobia must overcome different stress conditions in free-living before entering the plant root. Soil acidity is a challenging environmental stress frequently present in agricultural fields, and Sme is particularly sensitive to the acid media. We approached a metabolome analysis of acid-stressed rhizobia to get a deeper insight into the physiology of the acid growth. Bacterial cells were grown in batch cultures using defined minimal medium (early log phase of growth), both at neutral and moderately acid (pH 6.1) pH. It is already known that during acid cultivation Sme triggers an Acid Tolerance Response. Seventy metabolites from cells in ATR phase were analyzed by GC/MS and compared with the same compounds from the neutral cultures. Significant differences in several amino acid were observed (p=0.05). Analysis of the central carbon metabolism showed that under acid cultivation rhizobia accumulate metabolites that belong to the pentose phosphate pathway (PPP); together with an increase of metabolites that connect PPP with the TCA cycle. The increase in the biosynthetic PPP intermediates correlates with a concomitant increase in exopolysaccharide production, and most likely with a decrease in the aerobic metabolism as shown by lower amounts of the first two TCA intermediates.

MI-P30
OCCURRENCE OF SUCROSE SYNTHASE IN THE UNICELLULAR CYANOBACTERIUM *THERMOSYNECHOCOCCUS ELONGATUS*

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Sucrose in one of the most common nonreducing disaccharides found in nature. Up date, its biosynthesis seems to be limited to oxygenic photosynthetic organisms. In filamentous heterocyst-forming cyanobacteria, sucrose cleavage was reported to take place through the action of sucrose synthase (SuS, UDP-Glucose: -D-glucosyl transferase, EC 2.4.1.13) yielding U/ADP-Glc andαD-fructose 2- fructose. SuS activity was detected in several strains like *Anabaena* sp. PCC 7119 and 7120, *Anabaena variabilis*, *Nostoc punctiforme*, *Nostoc commune*, and in *Calothrix* PCC 7601. Biochemical studies of SuS and the functional characterization of its encoding gene (susA) was particularly performed in *Anabaena* sp. PCC 7119 and 7120 cells. *Thermosynechococcus elongatus* strain BP-1 is a unicellular rod-shaped cyanobacterium. Sequence searches in its genome using as query the amino-acid predicted sequence of *Anabaena* sp. PCC 7120 SusA revealed the presence of the 2427 *orf*, whose deduced protein sequence is 72% identical to that of SusA. The protein product of the expression of the 2427 *orf* as a His-tagged fusion protein in Escherichia coli showed SuS activity, preferentially with ADP than with UDP. The recombinant protein was immunorevealed by polyclonal antiserum raised against SusA of the PCC 7119 strain. This is the first report on the presence of a functional SuS in a unicellular photosynthetic prokaryote.

MI-P31
SUCROSE-PHOSPHATE PHOSPHATASE ACTIVITY IN *SYNECHOCOCCUS* SP. PCC 7942

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The biosynthesis of sucrose (Suc) occurs through the concomitant action of sucrose-phosphate synthase (SPS) and sucrose-phosphate phosphatase (SPP). In unicellular cyanobacteria, the SPS protein exhibits a two domain arrangenment containing a glucosyl-transferase domain (GTD) and a phosphohydrolase domain (PHD), at the Nt and Ct regions, respectively. In the freshwater cyanobacterium *Synechococcus* sp. PCC 7942, we firstly demonstrated the presence of Suc, and we also showed that the open reading frame Selo020095 encodes a protein with SPS activity (named 7942-SPS) by expression in *Escherichia coli*. After sequence analysis of the *Synechococcus* sp. genome, we did not find any independent sequence of a putative phosphohydrolase (corresponding to a SPP-like protein) as there is present in other unicellular cyanobacteria. By in silica analysis of the deduced amino acid sequence of 7942-SPS, we determined that the reported crucial amino acids for a functional SPP (Asp 9-Asp 11) are present in the PHD domain of the protein. Therefore, we used different biochemical approaches to evaluate whether 7942-SPS may have also SPP activity. The results seems to indicate that a unique protein could catalyze not only the glucosyl transferase reaction but also the dephosphorylation of the intermediate (Suc-6P). This finding is very important to understand the evolution of the Suc biosynthesis enzymes.

MI-P32

ANALYSIS OF DNA METHYLATION IN *PSEUDOMONAS AERUGINOSA*

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In prokaryotes, DNA methylation is associated with restriction-modification systems and with the regulation of important cellular events such as gene transcription, DNA mismatch repair, initiation of chromosome replication and nucleoid structure. In *E. coli*, Dam methylation of GATC sequences is the signal to direct the Mismatch repair system (MMRS) to the DNA strand recently synthesized. In order to know the role of DNA methylation in the MMRS of *P. aeruginosa* (PAO1), we first analyzed the content of N6-methyl-deoxyadenosine (6mdA) and 5-methyl-deoxycytidine (5mdC) in chromosomal DNA of this specie (and in *E. coli* DNA as a control), by reverse phase HPLC chromatography. The following values were obtained for PAO1: dC: 34.2%; dG: 32.3%; dA: 18.3%; dT: 14.8%; 5mdC: 0.16% and 6mdA: 0.03%. Since no homologous of *E. coli* Dam or Dcm was found in the genome sequence of PAO1 we carried out experiments in order to obtain a knock-out strain for the gene encoding the synthetase of the methyl donor, S-adenosyl-methionine (*metK* gene). An in vitro mutagenized copy of the *metK* allele (*metK::km*), was delivered to the host chromosome via homologous recombination. Exhaustive molecular screening of clones with the appropriate phenotype showed the presence of partial recombination products with an intact copy of *metK* gene which indicates that probably this gene is essential for PAO1 viability.

MI-P33

STRUCTURAL ANALYSIS OF THE C-TERMINAL REGION OF *ESCHERICHIA COLI* MUTS

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E. coli MutS, an 853 amino acids (aa) protein, is involved in the postreplicative DNA Mismatch Repair System. This is an oligomeric protein with ATPase activity and capacity to bind to heteroduplex DNA. *E. coli* MutS exists as dimers and tetramers. It is known that the deletion of the 53 C-terminal aa of MutS impedes tetramer formation and that the replacement of the *mutS* gene in the *E. coli* chromosome with this deleted version abolishes mismatch repair. Nevertheless, the exact localization and structure of the C-terminal aa sequence responsible for tetramer formation is not known. Here, we show using circular dichroism that a synthetic peptide that contains the 50 C-terminal aa of the protein is largely α-helical. This result agrees with the secondary structure prediction that shows the presence of two α-helix encompassing aa 823-831 and 839-850 respectively, separated by an apparently unstructured region. We have previously established that disruption of the 839-850 α-helix is sufficient to impair MutS oligomerization. We have constructed several C-terminal point mutated versions of MutS in conserved aa within this region, as well as fusion proteins of the maltose binding protein with different portions of the C-terminal region of MutS, in order to clarify the influence of this region in the oligomerization capacity of the protein.

MI-P34

PLASMID MUTABILITY/STABILITY IN *E. COLI DAM* CELLS IS AFFECTED BY MUTS AND/OR MUTL LEVEL

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Escherichia coli MutS, -L, -H, and Dam proteins are the principal components of the DNA postreplicative mismatch repair system (MMRS). We have recently observed that an increased level of MutS, -L or -H in *E. coli dam* cells results in a more active MMRS. In *E. coli dam* cells the MMRS is non-directed, so it can result in double-strand breaks and DNA degradation. Using a plasmid carrying a mutated copy of a gene that confers resistance to tetracycline, we observed that *E. coli dam* cells generate less than 10% of the tetracycline resistant colonies that can be obtained with *E. coli* MutS or MutL-deficient strains. This result shows that the endogenous MutS/L/H proteins are able to act on plasmid DNA eliminating most of the copies in which a mutation occurred. We then analyzed the effect of normal and plasmid overexpressed MutS and/or MutL on plasmid stability in *dam* cells. After 5 days of successive culture in reach media without antibiotic one of the plasmid analyzed (pET) was found in approximately 80% of the *dam* cells or the *dam* cells with increased levels of MutS and/or MutL. The other plasmid analyzed (p5) was found in 100% of the *dam* cells but in less than 40% of the *dam* cells with increased levels of MutS and/or MutL. These results show that extra-chromosomal DNA mutability/stability in the *E. coli dam* cells can be affected by MutS and/or MutL level.

MI-P35

INCREASED MUTS/L/H LEVELS IN *E. COLI DAM* CELLS ENHANCE THE MISMATCH-STIMULATED CELL KILLING

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E. coli dam strains, deficient in DNA methylation, have an active but non-directed mismatch repair system (MMRS) that can cause cell death by making double-strand breaks on unmethylated GATC sequences. Here we show that an increased level of MutS, -L or -H in *E. coli dam* cells enhances the mismatch-stimulated cell killing, resulting in strains with an apparent reduced mutation frequency. These strains have a similar duplication time, percentage of live/dead cells and morphotype to that of the *dam* strain. These strains also showed an increased sensitivity to the base analogue 2-AP, confirming that they have a more active MMRS. Dam methylation also participates in transcriptional regulation of virulent genes in several bacterial species. Therefore, *dam* strains of some of these bacterial species are less virulent and are being tested as live vaccines to generate immune protection against the virulent wild-type bacteria. However, a nondesirable characteristic of these *dam* strains is that they are mutator. Our results suggest that this disadvantage can be considerably reduced increasing the level of MutS, -L or -H.

MI-P36

ANALYSIS OF THE *MUTS-RPOS* CHROMOSOMAL REGION IN *PSEUDOMONAS AERUGINOSA*

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In enterobacteria genomes, *mutS* and *rpoS*, encoding one of the main components of DNA mismatch repair and the alternative stationary-phase σ^S factor respectively, are located in a highly polymorphic region of the chromosome. The extensive polymorphism of this region is postulated to result from cycling between environments in which the functional or non-functional *mutS* and *rpoS* genes provide distinct fitness advantages. Furthermore, in *E. coli* alterations in this region have been correlated with a *mutS* produced enhanced mutation/ recombination rate and implicated in the emergence of new pathogenic clones. In spite of the importance of *Pseudomonas aeruginosa* as a human pathogen, there is no characterization of this segment in this versatile bacterium. By PCR amplification and restriction fragment length polymorphism (RFLP) we first analyze the extent and nature of the *mutS-rpoS* intergenic region in sixteen different *P. aeruginosa* Cystic Fibrosis isolates as well as in the PAO1 and the soil isolated Hex1T strains. Interestingly, we observed that the length of that region was equivalent in all the strains analyzed, which in addition to the RFLP analysis suggest it would be unexpectedly conserved. Further studies are being carried out in order to analyze the structure of this region in *P. aeruginosa* and the consequences associated with *mutS* mutations and loss of DNA mismatch repair.

MI-P37

ENVIRONMENTAL REGULATION OF *MUCR* GENE EXPRESSION IN *SINORHIZOBIUM MELILOTI*

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Environmental conditions and nutritional status of the medium influence *S. meliloti* biofilm formation (Rinaudi et al., 2006). Adhesion of bacteria to different surfaces and between bacteria could be modulated according to environmental changes by regulation of the exopolysaccharide (EPS) synthesis. The promoter of the *mucR* EPS-regulatory gene was amplified and cloned upstream the *lacZ* reporter gene and the expression of the *mucR-lacZ* fusion was analysed under different media conditions. As expected, we observed that the expression of the *mucR* promoter was low in rich media but induced in a minimal media, which favours EPS production and biofilm formation. We found that the activity of the *mucR* promoter correlate with some of the conditions that stimulate biofilm formation. In fact, the presence of 0.3 M sucrose, 0.015 M NaCl and 25 mM phosphate to the minimal medium had a positive effect on the *mucR* promoter activity. However, no induction was observed in the presence of 7 mM calcium, which was previously observed that stimulate biofilm formation. Our data suggest that *mucR* dependent processes modulate biofilm formation, possibly through regulation of EPS production.

MI-P38

TRANSCRIPTIONAL ANALYSIS OF THE GENES INVOLVED IN DIACETYL BIOSYNTHETIC PATHWAY IN *L LACTIS*

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During glucose fermentation *L. lactis* produce and release lactate to the medium. At acidic external pH, lactic acid reenters into the cell acidifying the cytoplasm. *L. lactis* is capable of detecting that unfavorable condition and unchain a coordinated expression of the proteins responsible for citrate transport and metabolism. We have previously demonstrated that CitP removes lactate from cytosol ($citrate^{-2}$–$lactate^{-1}$ antiport) and that H^+ are consumed by an oxaloacetate decarboxylase activity. Finally, the end product pyruvate is derived to the synthesis of four carbon neutral compounds (4CNC). In the present work we studied the influence of external pH on regulation of the genes codifying for the 4CNC pathway. Transcriptional fusions of *als, aldB, aldC* and *butB* promoters were constructed. Moreover, a transcriptional analysis of these genes using RT-PCR was performed and the transcription start sites of the mentioned genes were mapped. We observed that the levels of transcription were higher for Pals, intermediate for PaldC and PaldB, and lower for the *butBA* operon. The expression of these four genes was analyzed in function of pH detecting different levels of induction. These results confirm that CitP, the citrate fermentation enzymes and ALS are induced coordinately under specific acidic stress, conforming a mechanism of resistance to acidic conditions in *L. lactis*.

MI-P39

ROLE OF LIPIDS IN THE ACTIVATION OF ΣE FACTOR DURING THE SPORULATION IN *BACILLUS SUBTILIS*

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In the absence of nutrients *B. subtilis* initiates a differentiation process called sporulation. After an asymmetrical division two compartments are created, the larger one called mother cell and the smaller one, the prespore. σ^E factor, which is essential for sporulation, is proteolitically activated only in the mother cell by the membrane protease SpoIIGA which requires SpoIIR, a protein synthestized in the prespore. We have previously shown that inhibition of fatty acid biosynthesis by addition of the antibiotic cerulenin at the onset of sporulation precludes σ^E activation, which blocks the differentiation process. To understand at which step of σ^E activation lipids are required, we constructed a version of SpoIIR lacking its signal sequence and found that translocation of SpoIIR is not necessary for SpoIIGA activity when both proteins are expressed in the same compartment. The analysis of the sporulation frequency of mixed cultures of *spoIIR* and *spoIIGA* mutant strains in the presence and absence of cerulenin suggested that lipids are required for SpoIIR translocation or its interaction with the protease. Finally, we analyzed σ^E activation in a *plsC* conditional mutant. In this strain, phospholipid biosynthesis is blocked and fatty acids are accumulated. Our experiments indicated that fatty acid and not phospholipids biosynthesis is essential for σ^E activation.

MI-P40

DIFFERENTIAL INHIBITION OF CHROMOSOMAL GENES TRANSCRIPTION BY MICROCIN J25

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Microcin J25 (MccJ25) is an *Escherichia coli* antimicrobial peptide of 21 amino acids which inhibits transcription by binding to RNA polimerase (RNAP) secondary channel. The aim of the present study was to determine whether the inhibition of chromosomal genes transcription by MccJ25 is a global or a differential effect. We generated a library of transcriptional lac fusions in the chromosome of a sensitive strain (RYC1000) and B-galactosidase assays were performed to test the expression levels of these fusions in the presence and in the absence of subinhibitory concentrations of the peptide. From 100 clones studied, 96 showed a moderate repression of B-galactosidase activity (2 times), whereas in 2 clones the enzyme was strongly inhibited (24 times). Finally, 2 fusions were not affected at all by MccJ25. In one of the strains showing strong inhibition (RYC1000-17) the fusion was approximately located using a set of Hfr strains. The inactivated gene mapped somewhere between min 97 and 98 of the *E. coli* chromosome. Interestingly, the growth rate of RYC1000-17 was slower than that of the parental strain, suggesting that the mutated gene is involved in cellular growth. The fusion was inhibited by endogenous as well as by exogenous MccJ25. This effect was not observed in a MccJ25-resistant RNAP mutant strain indicating that the inhibition is mediated by the RNAP.

MI-P41

SUPPRESSION OF AMIKACIN RESISTANCE DUE TO *AAC(6')-IB* BY RNASE P-BASED ANTISENSE TECHNOLOGY

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Antimicrobial resistance is a growing concern especially in thir world countries. Aminoglycoside 6'-N-acetyltransferases are group of enzymes that catalyze the transfer of an acetyl grou from acetyl coenzyme A to a primary amine in a wide variety c acceptor molecules including clinically important aminoglycoside (Ag). Among them, AAC(6')-Ib is commonly found in the clinica environment and catalyzes the inactivation of amikacin and othe important Ag. The aim of our study was to silence the gene codin for this enzyme using an RNase P-based antisense strategy. Fiv antisense oligoribonucleotides (ORNs) were designed to targe single stranded regions of the mRNA. All five efficiently mediate in vitro RNase P degradation of *aac(6')-Ib* mRNA. Selected ORN were then cloned under T7 promoter control. The recombinar plasmids were introduced into *Eschericha coli* BL-21(DE3) alread harboring a compatible low copy number plasmid carrying *aac(6') Ib*. Growth curves showed that the three strains were inhibited b 15 µg/ml of amikacin. These results suggest that antisens technology based on RNAse P can be a viable strategy to dea with the growing problem of resistance to amikacin.

MI-P42

IDENTIFICATION, DISTRIBUTION AND REGULATION OF PhlAEa, A TYPE-A PHOSPHOLIPASE FROM *E. AEROGENES*

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Enterobacter is an opportunistic pathogen associated to nosocomial infections. We selected an *E. aerogenes* isolate from an *Enterobacter* clinical collection strain that exhibited marked extracellular lipolytic activity. We identified that this exoprotein corresponded to a type A phospholipase ($phlA_{Ea}$) with high homology to phospholipases present in *Serratia, Yersinia, Xanthomonas* and *Photorhabdus* (*E. aerogenes* has no available genome database). By southern-blot analysis we showed that this locus was present only in some strains of our *Enterobacter* collection suggesting that this genomic region was subjected to DNA rearrangement processes. We determined the transcription initiation site for phlAEa by primer extension. The putative $phlA_{Ea}$ promoter region showed sequences that matched to predicted cAMP-CRP and σF recognition motifs. We analyzed $phlA_{Ea}$ expression by measuring lipolytic activity and transcriptional regulation by β-galactosidase activity from a *$phlA_{Ea}$::lacZ* fusion. We observed that $phlA_{Ea}$ expression was regulated by catabolite repression and induced when bacteria were grown at 30ºC. $phlA_{Ea}$ transcriptional levels were abolished in an *E. coli flhD* strain consistent with the σF-regulatory-dependence. In addition we showed that the expression of the flagella was required for $PhlA_{Ea}$ to be exported.

MI-P43

GENETIC AND PHYSIOLOGICAL ANALYSIS OF THE *SALMONELLA* COPPER RESPONSE

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Copper is an essential trace metal element for both eukaryotes and prokaryotes. It is associated with several metal enzymes, which are involved in electron transport, reduction of nitrite and nitrous oxides, and electron carriers. Intracellular copper homeostasis results from a very complex ensemble and interaction of a number of cellular processes. We have undertaken an *in vivo* characterization of the extracellular copper response in the pathogenic enterobacterium *Salmonella typhimurium*. This bacterium harbours all the components of the cytoplasmic/periplasmic-copper controlling CueR system, including the MerR-like regulator CueR, and the multicopper oxidase CueO. It also harbours a gene coding for a P-type ATPase responsible for copper transport, *copA*, which we demonstrate to be under direct CueR regulation. Surprisingly, no homologous system to CusR/CusS or its target operon, *cusCFBA*, could be identified in the *Salmonella* genome. Furthermore, *Salmonella* harbours a second CueR-homologous transcriptional regulator that is highly sensitive to gold ions, GodS, which induces the expression of a CopA-homologous protein, GodT. We provide evidences that, in the absence of a functional CopA, GodS induces the expression of GodT, which can substitute for the ancestral copper-transporter for metal-resistance.

MI-P44
FIGHTING COPPER: THE *SALMONELLA* SPECIFIC RESISTANCE

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Copper ions represent a dual challenge for both prokaryotic and eukaryotic cells. It is required for the proper function of several enzymes but can also catalyse harmful redox reactions resulting in cell damage. Cellular systems must respond to changes in the extracellular bioavailability of this element over a wide and dynamic concentration range. *Escherichia coli*, one of the best studied organisms, harbours three systems involved in copper resistance: the ATPase efflux protein CopA, the periplasmic multicopper oxidase CueO, and the multicomplex system CusCFBA. We investigated the presence of similar systems in the pathogenic bacterium *Salmonella enterica* serovar Typhimurium. Although, *copA* and *cueO* ortholog genes are encoded in the bacterial genome, no homolog system to CusCFBA could be found, suggesting that *Salmonella* employs a different mechanism to cope with the metal ion excess. We performed a genomic screening searching for genes involved in copper resistance. We identified a protein that we named CueP, involved in copper resistance both in anaerobic conditions and in environments where CueO is not active. We established that *cueP* expression is controlled by copper ions level in the surrounding media. These results show that *Salmonella* has evolved specific systems to survive in toxic copper environments.

MI-P45
REGULATION OF THE EXPRESSION OF SUCROSE METABOLISM GENES IN *NOSTOC SP*. PCC 7120

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In the heterocyst-forming cyanobacterium *Nostoc* sp. strain PCC 7120 sucrose (Suc) is synthesized in a two-step pathway involving sucrose-phosphate synthase and sucrose-phosphate phosphatase, and cleaved by sucrose synthase. These enzymes are encoded by *spsA* and *spsB*, *spp*, and *susA*, respectively. Importantly, Suc was demonstrated to play a crucial role in N_2 fixation in that strain. Heterocyst development and diazotrophic growth is mediated by a global nitrogen regulator (NtcA). The identification of the direct targets of NtcA is crucial for the functional understanding of the cells of cyanobacterial nitrogen-fixing filaments. Primer extension, RT-PCR, enzyme activity, protein immunodetection and band-shift experiments were performed to provide evidence for a direct role for NtcA as a regulator of the expression of Suc metabolism genes. Thus, *susA* down-regulation and *spsB* up-regulation were shown in a NtcA null mutant. Also, after band-shift experiments, evidence that NtcA induces DNA bending was obtained by analysing its interaction with the promoter regions of Suc metabolism genes. Taken together these results support the hypothesis that Suc and nitrogen metabolisms are coordinated through the action of NtcA. [Supported by ANPCyT, CONICET, UNMdP and FIBA].

MI-P46

DISSECTING THE TRANSCRIPTIONAL REGULATION OF THE ACYL CARRIER PROTEIN PROMOTER IN *BACILLUS SUBTILIS*

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The acyl carrier protein (ACP) plays a key role during 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. Also, by fluorescence microscopy we found that ACP is synthesized only in the mother cell. Our results suggest a transcriptional rather than posttraductional mechanism regulating the compartmentalization of ACP. This expression pattern is specific of the *acp* promoter and not a global behaviour of σA (the vegetative RNA polymerase sigma factor) dependent promoters. In order to understand these observations, we obtained fusions of the *lacZ* reporter gene to several deletions of the *acp* promoter region and determined their activities by beta-galactosidase assay. We found the existence of two important regions that modulate the promoter activity. These results suggest that there is a regulatory mechanism that governs ACP expression during sporulation in *B. subtilis*.

MI-P47

ROLE OF THE RcsC/YojN/RcsB REGULATORY SYSTEM IN LPS MODIFICATION IN *SALMONELLA TYPHIMURIUM*

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Prokaryotic organisms respond to changing environmental conditions regulating the genes expression via two-component systems. The RcsC/YojN/RcsB phosphorelay system consists of the sensor protein RcsC, the cognate response regulator RcsB and the histidin-containing phosphotransfer protein YojN that is apparently used as an intermediary in the phosphoryl transfer from RcsC to RcsB. We studied the role of Rcs system in the control of those genes involved in the Salmonella enterica virulence and determined that RcsC/YojN/RcsB and PmrA/PmrB regulatory systems promote wzzB (O-antigen chain length determinant gene) transcription under different environmental conditions and independently of each other. The transcriptional induction of the wzzB gene increased the amount of O-antigen in the LPS leading to a higher resistance to the serum complement-mediated killing. In addition, we identified a new role of the WzzB protein in the lipid A modification, indicating that the WzzB is required for many biological processes. We also demonstrated that the absence of pbgE2 and pbgE3 genes, which are regulated by the PmrA/PmrB system and involved in the lipid A modification, resulted in an O-antigen without the region containing 1 to 15 O-antigen subunits. These results indicated that both O-antigen formation and the lipid A modification are required at the same time during the Salmonella infection.

MI-P48

ACTIVATION PATHWAY OF THE RCSC/YOJN/RCSB REGULATORY SYSTEM IN *SALMONELLA ENTERICA*

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The RcsC/YojN/RcsB phosphorelay system controls the expression of the colanic acid biosynthesis cps and the master flagellar regulator flhDC operon's. The Rcs system consists of the sensor protein RcsC, the cognate response regulator RcsB, and the histidin-containing phosphotransfer protein YojN, which serve as an intermediary in the phosphoryl transfer from RcsC to RcsB. Although numerous direct and indirect genetic and environmental factors are known to induce the Rcs system, the real signal that leads to activation of this system is unknown. We are interested in defining the signal that is sensed by RcsC and YojN proteins and determining whether of these two proteins can independently activate the RcsB regulator. In this way we found that, in an ack mutant overproducing RcsB regulator, only the RcsC protein is required for cps induction. Here, RcsC use the acetyl-phosphate, accumulated in the ack mutant, as a phosphoryl group donor to activated RcsB. In addition, we are defined that rcsB, located downstream of yojN, is expressed under the controls of two promoters: the yojN promoter and a new one located in the yojN N-terminal region. These results demonstrated that in some Rcs induction conditions, only RcsC or YojN, but not both, are required to RcsB phosphorylation, indicating that one or more than one signals are involved in the activation of Rcs system.

MI-P49

TRANSCRIPTIONAL REGULATION OF THE GENE ENCODING LIPOYL SYNTHASE IN *BACILLUS SUBTILIS*

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Lipoic acid (1,2-dithiolane-3-pentanoic acid) is a sulfur-containing cofactor essential for function of several key enzymes involved in oxidative metabolism. The biosynthetic pathway of this important cofactor is poorly understood in *Bacillus subtilis*. It was recently demonstrated in our laboratory that *yutB* codes a lipoyl synthase in *B. subtilis*, so the gene was renamed *lipA*. The aim of the present work was to study the regulation of this gene under different growth conditions. This was accomplished by monitoring the expression of P*lipA-lacZ* transcriptional fusions and assaying β-galactosidase activity. The results indicate that *lipA* expression is repressed during growth on poor sulfur sources, such as glutathione and sulphate, and increased in the presence of methionine. We also found out that *lipA* expression was temperature-dependent: a decrease in growth temperature induces the expression of the gene. In order to determine the involvement of the *B. subtilis* two component system DesK-DesR and its homologous YvfT-YvfU in the temperature-dependent *lipA* expression, we analyzed the expression in mutants in Δ*desKR*, Δ*yvfTU* and in both systems. We observed that the temperature regulation of *lipA* expression is not modified in these mutants. In addition, in order to characterize the *lipA* promoter we are performing primer extension experiments to map the transcription initiation site.

MI-P50

METABOLIC PROFILE CHARACTERIZATION OF *ESCHERICHIA COLI ARCA* MUTANTS GROWING IN GLYCEROL

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The ArcAB two-component system of *E. coli* regulates adaptive responses under changing oxygen availability by modulating the expression of several operons involved either in respiratory or fermentative metabolism. Metabolic engineering for chemicals (e.g., ethanol, lactic and succinic acid) has focused in the over-expression of the biosynthetic genes; but manipulating the signal transduction pathways that govern their expression could be a more efficient approach. Inactivating or down-regulating the action of the Arc system seems an alternative to relieve the repression of genes for aerobic respiration. In this work we have assessed the effect of *arcA* mutations on the fermentation patterns of *E. coli* growing under microaerobic conditions using glycerol as carbon source. Glycerol is not only abundant but has a higher reduction state than other carbohydrates, and a significant increase in the yield of reduced chemicals should be expected. When compared to *E. coli* K1060 (parental strain of *arcA* derivatives), Arc mutants produced more reduced fermentation products. CT1061 (leaky mutant, carrying the *arcA2* allele) and CT1062 (Δ*arcA*) excreted 5.13 ± 0.23 g/L and 2.05 ± 0.03 g/L ethanol, respectively; whereas K1060 produced 0.21 ± 0.01 g/L. Our results suggest that *E. coli* CT1061 could be used as a biocatalyst for the synthesis of reduced fermentation products of biotechnological interest.

MI-P51

POST-TRANSCRIPTIONAL REGULATION OF A PORIN mRNA BY THE GAC/RSM SYSTEM IN *P. FLUORESCENS* CHA0

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In the rhizospheric biocontrol strain *P. fluorescens* CHA0, the production of antibiotics and exoenzymes is under a strict control by the post-transcripcional global regulatory cascade Gac/Rsm. The GacS/A two-component system activates transcription of three small regulatory RNAs that antagonize the translational blockage exerted by the RNA binding proteins RsmA/E on the RBS of target mRNAs. Using the 14-nt target sequence determined for the regulated operon *hcnABC* as a query for a genomic search, a highly similar motif (13 out of 14 nt) was identified around the RBS in the 5'-UTR of the major outer membrane porin gene *oprF*. However, a translational *oprF'-'lacZ* fusion did not show a strong regulation by Gac or Rsm members of the cascade, under different growth conditions. A single point mutation introduced in the 5'-UTR of the *oprF* gene put the mRNA under the tight control of the Gac/Rsm cascade. A folding algorithm predicts that the mutation may allow a more stable hairpin to form around the *oprF* RBS which in turn may enhance recognition by the RsmA/E repressor proteins. Thus, it seems that secondary structure may be more important than primary structure as a determinant for post-transcriptional reglation by the Gac/Rsm system in *P. fluorescens* CHA0.

MI-P52

THE EXOP N-TERMINAL DOMAIN IS REQUIRED FOR POLYMERIZATION AND/OR EXPORT OF THE EPSI IN *S. MELILOTI*

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ExoP is a protein tyrosine kinase (PTK) required for polymerization and/or export of the succinoglycan (EPSI). ExoP can be divided into an N-terminal domain (N-term) periplasmic and a C-terminal domain (C-term) cytoplasmic with ATPase activity. Another putative PTK, ExoP2, was found in the genome of *S. meliloti*. A deletion in *exoP2* did not affect the distribution of EPSI, indicating that ExoP2 is not associated to EPSI biosynthesis. ExoP was detected by westernblot in membrane fractions of *S. meliloti*, we found that ExoP was not phosphorylated on tyrosine under different conditions tested. On the other hand, amino acid substitutions in the proline-rich motif of ExoP (N-term) resulted in phosphorylation on tyrosine of the C-term, suggesting that phosphorylation could proceed by conformational changes of the N-term. Previous studies showed the presence of a coiled-coil motif in the ExoP N-term which suggests that this domain could interact with another protein and thus modulate ExoP phosphorylation and EPSI polymerization and/or export. We generated a mutant in the N-term with a deletion in the coiled-coil motif. Phenotypic analysis of this mutant revealed that the N-term is required for EPSI polymerization and/or export. In order to dilucidate the molecular mechanism of ExoP in EPSI polymerization and/or export, experiments involving heterologous protein expression are in course.

MI-P53

TRIMERS OF DIMERS AND THE METHYLATION RESPONSE OF CHEMORECEPTORS IN *E. COLI*

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The chemical environment that surrounds *E. coli* cells is sensed by the periplasmic domain of chemoreceptors and the information is transmitted to the histidine kinase CheA, whose activity ultimately determines the frequency with which cells change their swimming direction to seek for optimal environments. An increase in attractant concentration results in a rapid inhibition of CheA while, at the same time, the methylation rate of receptors is increased. This latter effect, together with a reduced rate of demethylation, determines a higher level of receptor methylation and thus a return of CheA activity to its pre-stimulus level. We are interested in the role that the trimer-of-dimer organization of chemoreceptors plays in signaling. We address now the effect that trimers of dimers play in the methylation response specifically. To that end, we analyzed the methylation changes that occur upon addition of attractants and repellents in wild type receptors under conditions of trimer disruption, as well as the response of receptor mutants that have defects in trimer formation. We observed an increase in the level of basal methylation in “trimer-less” conditions. In response to attractants the methylation is slightly increased under these conditions, suggesting that trimers of dimers are not essential for the communication of the occupancy state of receptors to the methylation machinery.

MI-P54

A RECOMBINANT *BRUCELLA ABORTUS* S19 EXPRESSING A MYCOBACTERIUM BOVIS ANTIGEN IS IMMUNOSTIMULATORY

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Due to the strong cellular and humoral immune response that the attenuated *Brucella abortus* strain 19 (S19) elicits, it is an attractive vector for the delivery of heterologous antigens. The aim of the present study was to express antigens of other pathogens that require the same type of immune response elicited by *Brucella* to control the disease they elicit. Recombinant *B. abortus* S19 expressing MPB83 (an immunogenic antigen) of *Mycobacterium bovis* were generated. *mpb83* was PCR-amplified. The amplicon was subcloned under different promoters and signal sequences (lacz and *B. abortus*: bp26 and omp19) to study diverse subcellular localizations for the heterologous protein. The plasmid stability and the immunogenicity of the heterologous proteins were analysed. MPB83, both as a fusion with the first aminoacids of β-galactosidase and with the amino terminal sequence of OMP19, resulted in the expression of MPB83 in association to the membrane of *Brucella*. Furthermore, processing of the fusion protein OMP19-MPB83 is inhibited by globomycin, a specific inhibitor of lipoprotein signal peptidase, suggesting that it is associated to lipids. Mice immunized with these recombinant strains developed specific immune responses to the mycobacterial protein MPB83, shown by lymphocyte stimulation assays. Therefore we concluded that the expression of MPB83 in S19 *B. abortus* is immunostimulatory.

MI-P55

EVALUATION OF CYTOKINE EXPRESSION PROFILE USING QUANTITATIVE REAL-TIME PCR (QRT-PCR) IN CATTLE

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Bovine tuberculosis caused by *Mycobacterium bovis* remains an economically important problem with potential zoonotic consequences. Understanding the role of the host response to the infection and specially the critical involvement of T cells is required if better vaccines and diagnostic tests are to be developed. Quantitative real–time transcriptase polymerase chain reaction (qRT-PCR) is becoming a widely used method to quantify cytokines expression. The method allows for the direct detection of PCR product during the exponential phase of the reaction, combining amplification and detection in a single step. Here we present a system that allows the quantification of four important cytokines of the Th1-type cell-mediated immune response (IL-2, IL-12p35, TNF alpha and IFN gamma), to evaluate this system, peripheral blood mononuclear cells (PBMC) from several animals, some of them positive for the tuberculin test and other negatives, were processed and cytokine expression was determinated. PBMCs from PPD+ animals expressed higher levels of IFN-gamma and TNF-alpha mRNA than similar PBMCs from control uninfected cattle (in some cases higher than 10 folds), together with IL-2 and IL-12 variations are useful for the establishment of characteristics cytokine expression patterns during M. bovis infection.

MI-P56

A *DAM* DELETION MUTANT OF *SALMONELLA ENTERITIDIS*: STUDIES ON VIRULENCE AND PROTECTION

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S. Typhimurium null *dam* mutants have been proposed as ideal vaccine strains. In contrast, *S.* Enteritidis mutants bearing a defective Dam protein are less protective and unable to agglutinate in the presence of antibodies against LPS O9 antigen. Here, we analyze the behaviour of SEΔdam, a *dam* deletion mutant of *S*. Enteritidis. This mutant was constructed by PCR-based mutagenesis. Attenuation and protection studies were performed in BALB/c mice. Epithelial and macrophage cell lines were used for in vitro studies. Nitric oxide (NO) production was assessed using Griess reagent. We found that SEΔdam mutant is moderately attenuated (LD_{50}:10^7 cfu), induces significant delayed-type hypersensitivity and improves bacterial clearance from spleen of immunized mice (range: 44-200 vs. 4 x 10^5 - 2 x 10^8 cfu). However, its protective capacity is reduced (20% survival rate). In vitro SEΔdam induces production of NO in macrophages but not in Hep2; concurrently, we found that it is unable to invade epithelial cells. In addition, SEΔdam LPS presents high proportion of reduced length O antigen polysaccharide. This defect does not affect the mutant serum resistance. Our results suggest that deletion of *dam* gene in *S.* Enteritidis renders a modestly attenuated mutant with reduced protective capacity. Interestingly, we found that Dam protein is involved in the regulation of LPS synthesis.

MI-P57

EXPRESSION AND SECRETION OF SOPA EFFECTOR IN *DAM* MUTANTS OF *SALMONELLA TYPHIMURIUM*

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DNA adenine methylase (Dam) protein plays an important role in the pathogenesis of several bacterial species, including *Salmonella* Typhimurium. Here we examined the effect of Dam protein on the expression and secretion of *Salmonella* pathogenicity island 1 (SPI1) proteins, such as SopA. We obtained a SopA tagged Dam mutant named STD2795. Bacteria were grown under SPI-1 condition. The expression and secretion of SopA was determined in bacterial pellets and culture supernatants, respectively. Invasion and proliferation were studied in Hep-2 cells. Analysis of methylation status showed that STD2795 has unmethylated DNA, this pattern was reverted in the complemented strain. It was found that the *dam* mutant invades epithelial cells 58 % less than the wild type (wt). The proliferation of the mutant within Hep-2 cells was also affected. Proliferation but not invasiveness was restored in the complemented strain. Western blot analysis showed that STD2795 expresses lower levels of SopA ($p < 0.001$) than the wt under SPI-1 conditions. When cultured at 28ºC the mutant expressed similar levels of SopA whereas wt and complemented strains showed no expression. On the other hand, SopA secretion was only observed in the wt under SPI-1 conditions. The partial inability of the *dam* mutant to invade epithelial cells could be related to a reduced secretion of invasion proteins encoded in the SPI1, like SopA.

MI-P58

EXPRESSION OF SIPA AND SOPD EFFECTORS OF *SALMONELLA TYPHIMURIUM* DURING MURINE SYSTEMIC INFECTION

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Salmonella pathogenicity island 1 (SPI1) mediates invasion of epithelial cells. SipA and SopD are required in this step of *Salmonella* infection. We studied the expression of SipA and SopD in vivo. *Sop*D and *sip*A tagged strains (3xFLAG) of *S.* Typhimurium were used during the experiments. Firstly, strains were grown under SPI1 or SPI2 conditions, supernatants and pellets were used to investigate secreted and cell associated proteins, respectively. Expression and secretion were analyzed by SDS-page and immunoblotting with anti-FLAG antibodies. SipA and SopD expression was observed under SPI1 and SPI2 conditions. However, secretion only occurred under SPI1 conditions. Hep-2 cells were infected with these strains at a moi of 10:1. Bacteria recovered from infected monolayers showed SipA and SopD expression at 24 hs post infection (p.i.). In a mouse model of systemic infection, internalized bacteria were recovered from lymph nodes (LN) and spleen after intraperitoneal inoculation. Western blot analysis revealed that SopD and SipA were expressed in vivo 18 hs and 5 days p.i. Moreover, SopD translocation was detected in LN macrophages. These results indicate that *S.* Typhimurium colonizing deep organs expresses effector proteins that may participate during late stages of systemic infection.

MI-P59

BIOCHEMICAL AND FUNCTIONAL STUDIES ON RibH2 OF *Brucella spp*

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Brucella spp. have two paralog genes coding for Lumazine Synthases (LS) named RibH1 and RibH2. Previous work shows that RibH1 is the active enzyme while RibH2 has negligible LS activity. A *B.abortus* strain lacking RibH2 showed attenuated virulence and enhanced sensitivity to H_2O_2. These results indicate that this protein is a virulence factor and our aim is to find its molecular mechanism. Intents to generate a double *ribh1/ribh2* mutant suggest that at least one of these genes must be present for viability. Plasmidic vectors have been constructed with the *ribh2* promoter transcriptionally fused to lacZ in order to determine when this gene is transcribed during macrophage infection and what conditions up- or down-regulate its expression in culture. We have also determined that RibH2 binds the RedOx cofactors riboflavin and hemin with high affinity and specificity *in vitro*. RibH2 from *Mesorhizobium loti*, a closely related bacterium, showed binding to hemin *in vitro* as well. To assess the connection between riboflavin and hemin with virulence we generated a mutant W22A incapable of binding riboflavin to use it in complementation experiments. Hemin-binding mutants will also be done. To appraise the roll of RibH2 in NO detoxification we are also conducting survival experiments with wt, *ribh2*$^-$ and complemented *B.abortus* in activated macrophages and in culture in the presence of a NO donor.

MI-P60

MEMBRANE FUSION ACTIVITY OF THE IIVA VIRULENCE FACTOR OF *BRUCELLA ABORTUS*

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We have previously identified the ORF BAB1_1543 of *Brucella abortus* as contributing to its virulence. It encodes an 11 kDa basic protein of unknown structure and function, named IivA. We have shown that this protein has two structural domains: a carboxy terminal coiled coil domain through which the protein self-associates as a trimer, and a natively unfolded amino terminal domain that undergoes a structural rearrangement in the presence of phospholipid vesicles. To study IivA association to the vesicles we constructed two single-Trp mutants (IivA-L19W and IivA-S114W), that span the amino and carboxy terminal domains, respectively. We found that the fluorescence maximun of IivA-L19W shift to lower wavelength upon mixing with phosplolipid vesicles, while the IivA-S114W mutant showed no shift. These results and the structural resemblance of IivA to viral membrane-fusion proteins prompted us to investigate its fusogenic activity. With this aim, we measured the size increment of phospholipid vesicles after addition of protein using Static and Dynamic Light Sacttering, and also lipid membrane mixing by FRET of phospholipid derivatives. These experiments, demonstrated that IivA has an *in vitro* membrane-fusion activity that is optimal at acidic pH. This activity require the full length protein as the isolated carboxyl and amino terminal domains have none or a moderate activity.

MI-P61

VERSATILE RIVET VARIANTS FOR THE IDENTIFICATION OF TRANSIENTLY EXPRESSED GENES IN BACTERIA

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The availability of the complete genomic sequence of several microorganisms made possible genome-wide high throughput methods, like transcriptomics and proteomics. However, these approaches can be poorly effective in identifying transiently/low expressed genes, or when the collection of the target sample is difficult and/or the amount limiting. In such cases, alternative approaches like RIVET (Recombination-based In Vivo Expression Technology can be attempted to identify specific genes induced under particular conditions. We have constructed three RIVET systems, each one consisting on two components: 1) a promoter-trap plasmid/transposon, consisting on a promoter-less tnpR (resolvase)-lacZ transcriptional fusion; and 2) an independent DNA cassette carrying positive (sacB) and negative (nptII) selectable markers which are excised only upon expression of TnpR. We constructed three variants of the promoter trap element supported in: a) a suicide R6K derivative plasmid, b) a broad host-range stable RK2 derivative, and c) a mini-Tn5 variant for the generation of promoter fusions without the need of a plasmid library as in the first two cases. The three configurations of our RIVET system represent a versatile set of tool for the analysis of transiently expressed transcriptomes during the in vivo/ex vivo interaction of diverse bacteria with their natural environments.

MI-P62

NATURALLY OCURRING ANTI-GM1 IGM-ANTIBODIES: *CAMPYLOBACTER JEJUNI* STRAINS COULD ACT AS IMMUNOGEN

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In contrast with the large body of evidence supporting a primary role of anti-GM1 antibodies in neuropathies, very little information is available about their origin. Based in studies on the antigen structural requirements for their binding it has been proposed that they originate from naturally occurring anti-GM1 antibodies. Anti-GM1 antibodies of the IgM isotype are part of the normal repertoire of human serum. They are absent in day-old babies and can be detected after few months. Their appearance shows a perfect concordance with well characterized anti-bacterial antibodies, an indication that they are part of the defence system toward these organisms. In the present study we develop a strategy to detect bacterium strains that could act as immunogen . Normal occurring anti-GM1 IgM-antibodies were purified from healthy human sera by GM1-affinity chromatography. This highly specific preparation was used to detect GM1-determinants in the lipopolisaccharides (LPS) fraction of several bacteria species. Positive reactions were only obtained with LPS from Campylobacter jejuni (four of twenty strains). These results indicate that subclinic infection with certain strains of these bacterium could trigger the production of naturally occurring anti-GM1 antibodies.

MI-P63

REGULATORY LOCI *SAE* IS REQUIRED FOR *S. AUREUS*-INDUCED APOPTOSIS IN CARCINOMA CELL LINE

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Staphylococcus aureus has the ability to invade and persist within eukaryotic cells. It possesses several cell surface adhesion molecules that allows its binding to non-professional phagocytes. The internalized bacteria are able to induce apoptosis. Global regulatory loci *agr* and *sigB* have been shown to play a role in the induction of apoptosis by *S. aureus* in epithelial and endothelial cells lines. Global regulatory locus *saeRS* consists of a two-component regulatory system that upregulates the synthesis of several exoproteins at the transcriptional level. The aim of the present study was to investigate the role of the *sae* locus on the ability of *S. aureus* to induce apoptosis in the human breast cancer cell line MCF-7. The prococols for these experiments were mainly based on the methods described by Bayles *et al.* (Infect. Immun. 1998, 66: 336). The results revealed that six hours after internalization of *S. aureus* strain RN6390 (wild type) MCF-7 cells displayed morphological changes compatible with apoptosis. In contrast, the isogenic *sae* mutant RC200, which internalized at levels similar to those of RN6390, failed to induce apoptosis. DNA-laddering experiments confirmed the lack of apoptosis by the *sae* mutant. These observations suggest that alpha-hemolysin, as well as other as yet unidentified virulence determinants, might trigger the apoptotic events induced by *S. aureus*.

MI-P64
MOLECULAR CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* VIRULENCE GENES ISOLATED FROM BOVINE MASTITIS

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Staphylococcus aureus is recognized worldwide as a frequent cause of intramammary infections in dairy cows. Produce a variety of exoproteins that contribute to its ability to cause disease in the cow. The aim of this work was to characterize genotypically *S. aureus* strains isolated from milk samples of cows with subclinical mastitis by PCR amplification of coagulase (coa) gene, protein A (spa) gene and *S. aureus* exoprotein expression gene (sae). Twenty four isolates collected from cows in Rio de Janeiro, Brazil, were identified as *S. aureus* according to routine microbiological diagnostics and identified by PCR amplification of species-specific parts of 23S rRNA gene. Presence of coa, spaA and sae genes was investigated using specific primers. Typical polymorphisms of the coa genes amplification were obtained yielding nine different coa types. It is important to note that 8 (38%) strains presented more than one amplicon. Determination of the spaA gene size, revealed two amplicons of about 900 and 800 bp. PCR amplification of the sae gene yielded an amplicon size of about 900 bp for the 15 (71%) of the *S. aureus* investigated. The genotyping results of the present study give information about genotypic properties and the distribution of virulence genes among the *S. aureus* strains isolated from bovine mastitis in Brazil and might be used for development of preventive strategies.

MI-P65
MOLECULAR STUDIES OF VIRULENCE GENES ON *STREPTOCOCCUS UBERIS* STRAINS ISOLATED FROM BOVINE MASTITIS

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Streptococcus uberis is known world wide as an environmental pathogen responsible for clinical and subclinical mastitis cases in lactating cows. Different genetic determinants for putative virulence factors were reported. The aim of this work was to identify, by PCR, predominant genotypic profiles of genetic determinants for putative virulence factors in *S. uberis*. This analysis included plasminogens activator gene (pauA/pauB), streptokinase C gene (skc) and three genes encoding hyaluronic acid capsular (hasA, hasB, hasC). Forty one isolates collected from the central dairy region of Argentina were identified as *S. uberis* by RFLP of 16S rDNAs. The determination of the genes was investigated using oligonucleotide primers derived from published sequences. Amplification of the pauA gene yielded an amplicon of 800 bp for 30 (73,2%) strains. On the other hand, none of the strains harbored pauB gene. Amplification of the hasA and hasB gene yielded an amplicon for 24 (58,5%) and 17 (41,5%) strains, respectively. The gene hasC could be observed for 35 (85,4%) strains. Genotype pauA/hasABC was the predominant profile found for 12 of 41 (29,3%) strains and genotype pauA/hasC was found for 10 of 41 (24,4%) strains.The remaining strains (46,3%) presented 9 different genotypes. The genotyping results of the present study give the first information about the distribution of genetic determinants for putative virulence factors in *S. uberis* strains in Argentina. They enlarge the knowledge on molecular epidemiology and might be the base for mastitis preventive strategies.

MI-P66

SCREENING AND IDENTIFICATION OF *BRUCELLA* EFFECTOR PROTEINS SECRETED TO THE HOST CELL

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Brucella spp. are Gram-negative bacteria that cause a worldwide zoonosis called brucellosis. They are facultative intracellular pathogens that replicate inside professional and nonprofessional phagocytes. A type IV secretion system (T4SS) is essential to avoid lysosome fusion and to create a replicative niche inside cells. A possible role of T4SS is to deliver effector proteins that modulate intracellular trafficking to allow intracellular multiplication. To identify these effector proteins we took advantage of the completed genome of *B. abortus* S2308, and looked for proteins with eukaryotic domains assuming they are good candidates for modulation of host cell functions. All the open reading frames were scanned using web-based programs. Proteins with eukaryotic domains were selected for fusion with *Bordetella pertussis* Adenylate Cyclase catalytic subunit (CyaA), a reporter for protein translocation from bacteria to eukaryotic cells. When translocated to the host cell cytoplasm, the hybrid enzyme is activated by calmodulin and synthesises cAMP. We infected macrophagic cells with bacteria expressing the fusion proteins and analyzed cAMP content at different times post-infection. We identified two proteins that are delivered to the eukaryotic cytoplasm. One of them requires the presence of a functional T4SS and is the first *Brucella* effector protein identified so far.

MI-P67

THE ANIONIC CHARACTER OF *BRUCELLA ABORTUS* CYCLIC GLUCAN IS REQUIRED FOR HYPO-OSMOTIC ADAPTATION

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Large amounts of periplasmic glucans are found in the periplasmic space of *Proteobacteria*. Depending on the species considered periplasmic glucan could be modified to various extents by a variety of substituents; however the role attributed to these substituents remains unclear. The *Brucella abortus* cyclic β-1,2-glucan virulence factor is substituted with O-ester succinyl residues, which confer on the cyclic glucan an anionic character. In a previous work, we demonstrated that *B. abortus* cyclic β-1,2-glucan succinylation is not required for virulence. Data presented here support a role for anionic substituents of cyclic glucan in hypo-osmotic adaptation. First, we observed that the osmolarity affects the substitution of cyclic β-1,2-glucan, with the highest degree of substitution reached when the cells were grown in a low osmolarity medium. Second, mutants in synthesis (*cgs*), transport (*cgt*) and modification (*cgm*) of cyclic β-1,2-glucan were impaired for growth under low osmolarity conditions. Third, raising the osmolarity of the medium with the addition of ionic or nonionic compounds can restore growth of the cyclic β-1,2-glucan mutants. Moreover, *cgm* mutant strain harboring a *S. meliloti* phosphoglicerol transferase is able to restore the *cgm* mutant grows. This is the first report, in which the role of anionic substituents of cyclic glucan was assigned.

MI-P68

ROLE OF CELL ENVELOPE LIPIDS OF *BRUCELLA ABORTUS* IN THE BACTERIA–HOST INTERACTION

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One of the striking peculiarities of the cell envelope (CE) of *Brucella* that distinguishes it from other Gram negatives is the presence of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and ornithine lipid (OL) as the main lipids (around 30 % each one). The membrane of pathogenic bacteria that cause persistent infections usually contains PC and OL, but the function they play in the interaction with the corresponding host is completely unknown. We have identified the genes responsible for their synthesis and generated the corresponding knock-out mutants. In contrast to what has been observed with other bacteria, absence of each of these lipids does not impair Brucella viability whereas affects its virulence traits. Remarkably, lack of PE was compensated increasing the percentage of PC and OL while the absence of OL raised the amount of PE and PC. This suggests that a regulatory mechanism could compensate the absence of each lipid. Absence of PE in *B.abortus* impaired MΦ J774 infection, whereas the lack of OL had not displayed such effect. Notably, a mutant over-expressing OL showed a reduction in MΦ J774 infection similar to what has been observed in the PE mutant, suggesting that level of OL in the CE is critical for virulence. All the above results indicate that lipid composition of the *Brucella* CE influences the virulence performance of the pathogen. The role each lipid could play in the interaction with the host is discussed.

MI-P69

FUNCTIONAL MAPPING OF *BRUCELLA ABORTUS* CYCLIC GLUCAN SYNTHASE BY PENTAPEPTIDE SCANNING MUTAGENESIS

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B. abortus cyclic β-1,2-glucan synthase (Cgs) is a 320-kDa polytopic integral inner membrane protein responsible for the synthesis of the virulence factor cyclic β-1,2-glucan by a novel mechanism in which the enzyme itself acts as a protein intermediate. Cgs functions as an inverting processive β-1,2-autoglucosyltransferase and has the three-enzymatic activities required for synthesis of the cyclic glucan: initiation, elongation, and cyclization. To identify functionally important regions of Cgs, we used random pentapeptide insertion mutagenesis and assessed the function of the resulting mutants by a mobility assay. We analyzed 2464 Cgs mutants and mapped by sequencing the exact localization of the insertion in 330 of them. In this way, we built a functional map of Cgs from which we identified various domains required for overall function and delimited the minimal region of the protein required for the synthesis of cyclic glucan. Then, we characterized *in vitro* and *in vivo* the activity of several selected mutants. Particularly, we found that most of the pentapeptide insertions into the Cgs region between the residues 990 and 1547 inactivated the synthesis of cyclic glucan but the incorporation of glucose residues into the protein through the initiation and elongation reactions was not affected. Therefore, this region of the protein may be implicated in the cyclization reaction.

MI-P70

NDVB PROTEIN GENE IS INVOLVED IN *XANTHOMONAS CAMPESTRIS* (XC) GLUCAN BIOSYNTHESIS AND PATHOGENICITY

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Xc is a major bacterial pathogen of cruciferous plants worldwide. The ability of Xc to incite disease in plants depends in part of the production of extracellular factors such as degradative enzymes, exopolysacharide, etc. An extracellular molecule also produced by Xc, the cyclic glucans, have been shown to be important for a number of plant-microbe interactions. We have identified a gene involved in cyclic beta glucan biosynthesis of in Xc. The orf YP245226 (identified as NdvB protein, an smaller version of the Sinorhizobium NdvB gene) was disrupted through a cassete in a site directed mutagenesis, and the insertion was confirmed by PCR and Southern blot. The insertional inactivation 1,2 glucan, assayedβof NdvB protein compromised the production of the cyclic by size exclusion cromatography and TLC. The mutant strain shows a significative impairment in the bacterial growth in plant respect to the wild type strain.When the plant was inoculated with the mutant it showed an exacerbated defense response (PR1 transcript accumulation, callose deposition) compared with the wild type inoculated plants. The production of cyclic glucan and pathogenicity were fully restored by complementation of the mutant with the Xc NdvB protein gene. This results suggest an important role of the YP245226 orf in xanthomonas glucan biosynthesis and pathogenicity.

MI-P71

THE OUTER MEMBRANE FACTOR OF *BRUCELLA* IS INVOLVED IN ANTIMICROBIAL RESISTANCE AND VIRULENCE

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Brucella spp., like other pathogens, must cope with the environment of diverse host niches during the infection process. In doing this, pathogens evolved different type of transport systems to help them survive and disseminate within the host. Members of the Outer Membrane Factor (OMF) in association with two inner-membrane proteins were shown to be involved in the export of chemically diverse molecules. The role of proteins from this family was little explored in *Brucella*; to analyze its biological relevance we performed heterologous complementation assays: the gene encoding the unique *B. suis* OMF protein (Bep) was cloned and expressed in the *tolC* mutant of *E. coli*. Bep fully complemented the resistance to drugs such as chloramphenicol and acriflavine but was incapable to restore hemolysin secretion in this mutant. An insertion mutant was constructed in *B. suis* and the sensitivity to several drugs was analyzed, as well as the virulence in diverse models; the mutation in the *bep* gene strongly affected the resistance phenotype to bile salts and toxic chemicals and significantly decreased the resistance to antibiotics such as erythromycin, tetracycline and norfloxacin. Moreover, the *bep* mutant of *B. suis* was attenuated in the mice model. Taking together, these results suggest that in *B. suis*, efflux processes that are Bep dependent contribute to survival inside the host.

MI-P72
CHARACTERIZATION OF INVASION PLASMID SPONTANEUS MUTANTS OF *SHIGELLA SPP*
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It is known that the ability of *Shigella* spp. to invade and escape from enterocytes cells is conferred by loci products encoded on a 220 kb virulence plasmid (megaplasmid pINV). In a previous work we described clinical isolates of Shigella which had the ability to invade Hep-2 cells monolayers even thought the absence of the plasmid pINV. In the present work we characterized different spontaneous mutants with deletions in the ipaBCDA and replication regions of the plasmid pINV, and analyzed then the ability to invade Hep-2 cells monolayers. A total of 100 clinical isolates of *Shigella* spp. were employed. All isolates were assayed for the presence of genes encoding in the ipaBCDA and replication region, by PCR using specific primers for each gene with boiled culture as template. Isolates that give negative amplification for one o more genes were analyzed for the ability of invade Hep-2 cells. Twenty mutants were characterized as negative by one or more of the genes. Seven were negative only for the replication region, three for the ipaBCDA region and ten for both regions. Four mutants in the replication region were invasive in Hep-2 cells assay, as well as one for the ipaBCDA region and six for both regions. Invasion of *Shigella* was also confirmed by fluorescent microscopy using green fluorescent protein (gfp) labeled mutants.

MI-P73
CLONING AND CHARACTERIZATION OF POLYPHOSPHATE KINASE 2 (PPK2) OF *BRUCELLA ABORTUS*
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Many bacteria are capable of accumulating Pi as polyphosphate (polyP). PolyP is a linear polymer of inorganic phosphate residues linked by high-energy phosphoanhydride bonds. The principal enzyme responsible for the synthesis of polyP in *Escherichia coli* is the polyP kinase (PPK). PolyP can also be hydrolyzed to Pi by the exopolyphosphatase (PPX). Among the many functions proposed for polyP one is regulating networks essential to respond to environmental stresses and survival during the stationary-phase of growth. Furthermore, some virulence factors are expressed during the stationary-phase. The relationship between polyP level and virulence was suggested recently. PPK is highly conserved in many bacterial species. Important human pathogens included *Brucella* has two ORF coding for putative polyP kinases (PPK1 and PPK2). Like the *E. coli ppk-ppx* operon, the *B. abortus ppx* gene is located immediately downstream the *ppk1* gene. A putative pho box was found in the promoter region of the *B. abortus ppk1*. These features suggest that *ppk1* and *ppx* of *B. abortus* may form an operon, as in *E. coli* and other gram-negative bacteria. To elucidate the role of polyP in *Brucella* pathogenesis ppk null mutants of *B. abortus* were constructed. The *ppk2* mutant show a similar behavior as the wild type in HeLa and J774 cell infections assays, however the survival was reduced in stationary-phase of growth.

MI-P74
EXTRACELLULAR ENZYMES OF *FUSARIUM GRAMINEARUM* ISOLATES FROM DIFFERENT AGROECOLOGICAL REGIONS

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F. graminearum is the causal agent of the Fusarium head-blight (FHB), a destructive disease of wheat. In Argentina, under favorable environmental conditions for the occurrence of spike infection, the producing regions were grouped according to grade of damage attributable to this pathogenic fungus in: slight (IV and V regions), intermediate (II region) and serious (I and III regions). In this work, isolates from IV, V and II regions were examined according to the production of different enzyme activities: cellulase (CMCase and ß-glucosidase), hemicellulase (endoxylanase), and pectinase (PGase and PMGase). The isolates were grown in minimum salt medium supplemented with 0.25% glucose, 0.125% citric pectin and 0.125% oat bran as carbon sources and/or enzyme inducers. PGase activity was produced early (after 2 days of incubation) in all the cultures, it was found to be the highest for all the isolates. PMGase was high only for those isolates of the II region. CMCase and endoxylanase activities were particularly found at late stages (after 4 and 7 days of incubation, respectively) and the maximum values obtained were lower than pectinase activities. The *in vitro* enzyme production of the *F. graminearum* isolates could suggest that although similar enzymes were detected, their sequential and differential production profile may be related to the fungal aggressiveness.

MI-P75
YARROWIA LIPOLYTICA Y1095 LIPASES: PRELIMINARY CHARACTERIZATION OF SYNTHESIS AND LOCALIZATION

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Lipases (triaylglicerol acylhydrolases) are important industrial enzymes. Microbial strains of the same species may produce distinct lipases that can be exploited for several industrial applications. Here, we report the pattern of lipase production by *Y. lipolytica* Y1095, their relationship with cell growth and substrate consumption, and the effect of different carbon and nitrogen sources. Profiles of cell growth, extracellular and cellular lipase activity (LA), and residual substrate for the cultivation of this yeast in olive oil medium were determined. LA was found in a period from 6 to 78 h of incubation, and the maximum LA was found between 30 and 36 h, when the substrate was completely consumed. Both extracellular and cellular LAs were detected, and the maximum LAs obtained were 4.08 and 2.33 U mL^{-1} culture, respectively. Cellular LA seems to be divided into a citoplasmatic and a cell-bound LA. Carbon and nitrogen sources strongly influenced lipase production. LA was not observed in culture media containing glucose or glycerol until carbon source was entirely consumed, and olive oil was necessary to induce LA. Biomass and LA was significantly increased by complex nitrogen sources, when compared to ammonium salts. Thus, we postulate the use of this strain for industrial applications considering both the high growth rate and the lipase synthesis in olive oil.

MI-P76

TWO-STAGE CULTURE FOR DHA PRODUCTION BY *S. LIMACINUM* SR21

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Production of ω-3 fatty acids, especially docosahexaenoic acid (DHA) by microbial fermentation has attracted considerable attention in relation to their benefic effects on human health. *Schizochytrium limacinum* SR21 is a marine microheterotroph that can be cultured to produce high biomass with high DHA yields. We had applied statistical experimental designs to optimize the propagation medium for that strain, as a first step to optimize DHA production. In the present study, conditions for a two-stage culture suitable for DHA production by *S. limacinum* SR21 were investigated. 2, 3.5 and 5 days cells growth in the optimized propagation medium were washed and inoculated into 50 ml flasks containing 10 ml production medium with 10% glucose and ammonium acetate to reach 10:1, 50:1 and 100:1 C:N relationships. The inoculum growth stage and the harvest time (3 and 5 days) did not have significant effects on the dry biomass, lipids and DHA production. The highest values of dry biomass (40 g/l approx.), lipid content (50% of the biomass) and DHA yields (7 g/l) were found in C:N 50:1. The effect of glucose addition (with and without ammonium acetate) to 3 days cultures with optimized propagation medium was tested too, but low values of biomass and DHA were obtained, confirming that low nitrogen concentration in the production medium is critical to DHA accumulation.

MI-P77

AN INTEGRAL STUDY OF NATIVE-PGPR TO IMPROVE PLANT GROWTH IN SOILS AFFECTED BY ENVIRONMENTAL STRESS

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We have selected strains from saline soils of Cordoba province able to promote the growth of different plants. These isolates belong to *Pseudomonas*, *Ochrobactrum*, and *Bacillus* genus. In order to advance on the characterization of these strains different strategies were applied: Survival of *Pseudomonas* sp. SF 4C in different soils: The viable cell number was determined at 15, 30 and 60 days post-inoculation in the rhizosphere and bulk soil. This strain was able to survive in the wheat rhizosphere in both tested soils along this experiment, while in the bulk soil the survival at 60 days was only observed in the non-fertilized soil. Identification of functions localized on a megaplasmid of *Ochrobactrum* sp. 11a: the megaplasmid was mobilized to *A. tumefasciens* free-plasmid UBAPF2. The selected *A. tumefasciens* UBAPF2 transconjugants showed the same resistance to heavy metal than *Ochrobactrum* sp. 11a and the presence of this plasmid was confirmed by Eckhardt. Partial characterization of metabolites associated to biocontrol of phytopathogen fungy. Supernatans obtained from *Bacillus* sp. cultures showed antifungal activity in vitro against several species of *Fusarium* and *Sclerotinia* genus. The supernatants were analyzed by reversed-phase HPLC. Peaks were fractionated and examined against *Sclerotinia sclerotiorum* on APD. Some of these fractions showed antifungal activity.

MI-P78
SPECIFICITY OF CITOTOXIC ACTIVITY OF RECOMBINANT DOMAIN *St*AP-PSIr

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We have previously reported that Potato Aspartic Proteases (*St*AP1 and *St*AP3) have antimicrobial activity towards plant pathogenic microorganisms and human pathogenic bacteria. Besides, we have cloned and expressed the Plant Specific Insert (PSI), a domain present in the *St*APs sequence. This recombinant peptide (*St*AP-PSIr) has antifungal activity against *F. solani*. In this study, we tested the capacity of *St*AP-PSIr to affect the viability of cysts of *Phytophthora infestans*, an economically important potato pathogen, as well as three bacterial strains which cause human disease (*Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus*). The results showed that *St*AP-PSIr has antimicrobial activity against *P. infestans* (IC_{50}: 0.22 µM) involving plasma membrane permeabilization. *St*AP-PSIr presents cytotoxic activity on bacteria cultures in a dose-dependent manner. The IC_{50} obtained for *B. cereus*, *E. coli* and *S. aureus* were 0.24 µM, 0.3 µM and 2.67 µM respectively. On the other hand, we analysed the specificity of *St*AP-PSIr, using a culture of plant cells and human erythrocytes. When tobacco cells and human erythrocytes were incubated with *St*AP-PSIr, the cell viability was not affected. Therefore, we conclude that *St*AP-PSIr have specific antimicrobial activity against plant and human pathogenic microorganisms.

MI-P79
INITIAL CONIDIA ADHESION OF *ASPERGILLUS NIGER* AND ITS ROLE IN PELLETS FORMATION

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Introduction: Environmental conditions markedly influence the growth pattern of filamentous fungi, which can range from a pelleted to a dispersed filamentous form, influencing in this way both the growth rate and the product formation. Although pellets formation is primarily due to conidia agglomeration, the involved mechanism has not been clearly elucidated. The aims of this work were to quantify the initial conidia adhesion of *A. niger* under different environmental conditions and to establish its role in pellets formation. Methods: Assays of conidia adhesion were done in 96-well polystyrene microtiter plates by using sulforhodamine B staining to quantify the adherent conidia. The adhesion unit was defined as the amount of adherent substance that produces an increase in absorbance at 570 nm of 0.01 per min per ml of culture. The effect of modifications in the environmental conditions was tested by changing either the temperature of incubation or the initial pH of the medium as well as by the addition of either $CaCl_2$ or $FeCl_3$. Results and conclusions: Differential adhesion degree in response to environmental conditions was detected. Under our experimental condition, initial adhesion units lower than 0.4 obtained either at initial pH 2 or in presence of 1 g/l $FeCl_3$ were not compatible with pellets formation. [This work was supported by grants 693/04 CONICET and PICTO 761].

MI-P80

GROWTH AND ENZYME ACTIVITY OF ULOCLADIUM BOTRYTIS ON CARBOXY-METHYLCELLULOSE UNDER A PH-RANGE

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Ulocladium botrytis, a fungus from Scutia buxifolia leaf-litter, was cultured both on a liquid and solid medium with Na-carboxy-methylcellulose (CMC, 0.5 %) as sole C source and different buffer solutions, to evaluate its growth under a pH-range between 4 and 10. Solid-state fermentation (SSF) cultures on S. buxifolia leaves were also carried out for analyzing the ability of the fungus for modifying the substrate and for producing the cellulase complex. The effect of pH and reaction buffer on endoglucanase activity of the water-soluble fraction (WSF) from SSF cultures was also analyzed as well as its stability against the pH. Growth on CMC-agar media was maximum at pH 6 after 7 days. However, highest biomass levels from liquid cultures with CMC were registrated at pH 8 after 7 days. The strain on SSF cultures reduced the mass of the leaves at a 5.8 % and increased the pH of the WSF compared to uninoculated ones as well as produced extracellular activities of β-glucosidase (2.4 U dry-leaf g-1), cellobiohydrolase (3.9 10-3 U dry-leaf g-1) and endoglucanase (2.0 U dry-leaf g-1) after 30 days. This enzyme activity on CMC revealed maximal levels at pH 6 when Mc Ilvaine buffer (2.1-9) was used in the reaction, and higher ones when 150 mM Na-tartrate (pH 5) was used as reaction buffer compared to Na-acetate and Mc Ilvaine buffers. Likewise, it was stable at pH 7 and 25 ºC for 24 h.

MI-P81

AURORA KINASES FAMILY IN *TRYPANOSOMA CRUZI*.

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The chromosomal passenger protein aurora kinases have been implicated in regulating chromosome segregation and cell division. Three chromosome passenger proteins, aurora A, B, and C, were identified in mammals. Consistent with their localizations, aurora A regulates spindle assembly, aurora B controls chromosome segregation and cytokinesis initiation and aurora C was found in testis and certain tumor cell lines and localized to spindle poles during late mitosis. This work describes three aurora kinase homologues (TcAUK1, 2 and 3) in *Trypanosoma cruzi*. The protein products were obtained by expression in *E. coli* and evaluated for enzymatic activity by using H2AS Histone mix from Sigma, and Myelin Basic Protein as substrates. It is know that human aurora-B is phosphorylated at Thr-232 through interaction with the inner centromere protein (INCENP) *in vivo*. The phosphorylation of Thr-232 occurs by means of an autophosphorylation mechanism, which is indispensable for the aurora-B kinase activity. According to this observation we explored the TcAUKs looking for the modification site and interestingly the phosphorylable Thr was present in all of them. To study the effect of the putative phosphorylation we changed Thr x (Asp or Glu) by site-direct mutagenesis and expressed the mutated enzymes in *E. coli*. In addition we expressed TcAUK1 in mammalian cells and kinase activity was measured.

MI-P82

TOXOCARA CANIS EXCRETION-SECRETION ANTIGEN (TES) ACTION ON CELLULAR LINE NEURO-2A

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Toxocara canis, a dog roundworm, causes Visceral Larva Migrans (VLM), Ocular Larva Migrans and Covert Toxocarosis in paratenic host as a man. The commitment of the Central Nervous System (CNS) has been described as a VLM manifestation. In previous studies we detected T. canis larvae in CNS using PCR and brain maceration. The parasite produces the antigen of excretion-secretion (TES), that is, a set of glycoproteins and secreted enzymes like superoxide dismutase. TES is able to induce oxidative stress in hepatocytes and macrophages. The aim of this work was to study the action of TES on Neuro-2a. Nitric oxide (NO) levels were measured by Griess reagent in the supernatant of cell cultures. Reactive oxygen species (ROS) were determined by measuring cell fluorescence with DFCH-DA. Neuron viability was evaluated by MTT method, reading the formed formazan salts at 540 nm. The hightest NO level, 14µM nitrite±0.05/mg of protein was observed in neurons tried with 40 µg/ml of TES. ROS was 12000±584 IF/mg protein in cultures tried with 100 µg/ml respects to the value obtained without TES. It was observed a decrease (40%) in cell viability respect the one observed in the control, $p<0.01$. The cell injuries, based on NO and ROS generation, could in vivo cause protein, DNA and lipids injuries and eventually could induce cell death by necrosis or apoptosis.

MI-P83

FLUBENDAZOLE INDUCE PROTOSCOLICIDAL EFFECT BY A CALCIUM-DEPENDENT MECHANISM ON *ECHINOCOCCUS SP*

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The chemotherapeutic treatment of human echinococcosis remains unsolved. Benzimidazole (BZD) as mebendazole (MBZ) and albendazole (ABZ) are broad-spectrum antihelminthic agents and the only drugs licensed for treatment of hydatid cysts. These drugs bind directly to β-tubulin causing the disruption of microtubule-based processes in helminths although little is known about the molecular bases of their multiple biological activities. Recently has been demonstrated a clear *in vitro* effect of flubendazole (FLBZ), against *E. granulosus* larvae (Elissondo et al., 2006), whereas vitality tests were consistent with a faster appearance of morphological damage than larvae cultured with others BZD. Hence, we examined the biochemical and molecular changes *in vitro* of this halogen-derivate BZD on protoscoleces. We observed that only FLBZ caused a rapid increase in intracellular Ca^{2+} concentration determined by labeling with Fluo-3 AM and imaged by a confocal laser microscope after treatment with ruthenium red and EGTA for distinguishing subcellular signal localization. On the other hand, these results were correlated with significant glycogen depletion and mitochondrial gene expression. Therefore FLBZ, as others halogeno-benzimidazoles (Andrzejewska et al., 2004), may interfere with a wide spectrum of cell regulatory mechanisms. [Supported by Carrillo-Oñativia-2005, FIBA, CONICET and UNMdP].

MI-P84

DEVELOPMENT OF A *SRPK*-NULL MODEL FOR EVALUATING SR NETWORK COMPONENTS IN TRYPANOSOMATIDS

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SR proteins are the main non-snRNP components of spliceosome and together with their specific kinases constitute the "SR Network". Several kinases have been reported to phosphorylate RS domain-containing splicing factors, including SRPK's and Cdc28/Cdc2-like kinase (Clk/Sty). Gene expression is also synchronized with the cell division cycle. Therefore, intricate interplay exists among pre-mRNA splicing, transcription, and cell cycle. SR proteins and SR protein-specific kinases may constitute a protein relay or networks to regulate the coupling of splicing, transcription, and cell cycle in mammalian cells. In trypanosomatids, the SR network is composed by only one SR and its specific kinase protein, TcSR-TcSRPK in *T. cruzi* and TSR1-TbSRPK in *T. brucei*. These proteins were functionally characterized in different *in vitro* and in vivo models, as HeLa cells, yeast and *T. brucei*. The fission yeast *Schizosaccharomyces pombe* presents two SRPKs, Dsk1 (SRPK) and Kic1 (Clk/Sty). Yeast *dsk1*-null *kic1*-null mutant cells grew extremely slowly and formed microcolonies. The current work presents the development of TbSRPK-deleted cell line. The initial characterization shows differences in growth rate compared to 1913 strain (*wild type*), suggesting TbSRPK-deleted cell line must be a useful model to study SR Network components in a homologous model.

MI-P85

POLYADP-RIBOSYLATION IN TRYPANOSMATIDS IN RESPONSE TO DNA DAMAGE

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Poly(ADP-ribose)polymerase (PARP) is a nuclear enzyme present in most eukaryotes. Its activity depends on the presence of DNA strand breaks (DNAsb) and has been involved in many important genomic processes such as DNA replication, DNA repair and gene expression. The poly(ADP-ribose) polymer (PAR) is catabolized by the poly(ADP-ribose)glycohydrolase (PARG). Previously we reported the cloning and expression of *Trypanosoma cruzi* and *Trypanosoma brucei* genes for PARP-2 homologues (TcPARP and TbPARP). The present work, describes the biochemical and functional characterization of all these enzymes. We have also found, cloned and expressed the catabolic counterpart PARG, suggesting an analogous metabolism to the known in higher eukaryotes. In silico analysis of TcPARP and TbPARP showed conserved WGR, regulatory and catalytic domains. TcPARP and TbPARP activities were strongly activated by DNAsb. We also demonstrated the attachment of PAR synthesized by TcPARP, to it-self and to *T. cruzi* histones by using SDS-PAGE and autoradiography. PAR synthesis was confirmed in vivo by indirect immunofluorescence assay with antiPAR antibodies. Under standard growth conditions *T. cruzi* epimastigotes display low signal at the nucleus, which drastically increased when the cells were exposed to DNA damaging agents. These results point out a physiological role for PARP in trypanosomatids DNA repair systems.

MI-P86

CYTOCHROME P450 REDUCTASES IN *TRYPANOSOMA CRUZI*. A POSSIBLE DRUG RESISTANCE MECHANISM

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Cytochrome P450s (CYP) system is involved in the synthesis of endogenous compounds such as steroids, fatty acids and prostaglandins as well as activation and detoxification of foreign compounds including therapeutic drugs. Cytochrome P450 reductase (CPR) is the electron donor necessarily associated to CYP to achieve optimal activity. A NADPH dependent cytochrome c reductase activity has been previously demonstrated in *T.cruzi* by biochemical approaches. We describe here the cloning and characterization of three sequences coding for respective proteins homologous to CPRs, all of them show the FMN, FAD and NADPH domains characteristic of reductases superfamily. Two of them, CPR (A) and CPR (B) were subcloned and expressed in a bacterial system. The kinetic behavior of both enzymes is similar to those of mammalians, and they were active *in vitro* using reconstituted heterologous systems. The expression of both enzymes was demonstrated in soluble subcellular fractions of epimastigotes from the CL Brener Strain, and confirmed by immuno-fluorescence (IFI) microscopy. The overexpression of CPR (B) was achieved in epimastigote cells and confirmed by Southern and Western blot, enzyme specific activity and IFI. Parasites overexpressing CPR (B) showed increased survival capability to the anti-trypanosomal drugs Nifurtimox and Benznidazole.

MI-P87

CLONING AND BIOCHEMICAL CHARACTERIZATION OF BROAD SPECIFICITY AMINOTRANSFERASES FROM TRYPANOSOMES

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The gluconeogenic pathway has been recently demonstrated to be essential for virulence of *Leishmania* sp., aspartate being utilized among other amino acids for de novo synthesis of carbohydrates. These findings fit in well with our recent observation that a cytosolic aspartate aminotransferase with broad substrate specificity is expressed in all the biological cycle stages of *L. mexicana*. In the present work, we report the cloning and functional expression in *E. coli* cultures of cytosolic and mitochondrial aspartate aminotransferases (ASATs) from *T. cruzi* and *T. brucei*. The nucleotide sequences encoding the putative enzymes, identified by Blast searching in the genome data-bases, were amplified by PCR and cloned into pET28 vector. Upon purification to electrophoretic homogeneity, the recombinant *T. cruzi* and *T. brucei* putative cytosolic-type ASATs were able to transaminate actively aspartate in addition to aromatic amino acids and methionine. Leucine was also utilized, although less efficiently. Conversely, the putative mitochondrial-type ASATs specifically recognized the substrate pair aspartate/2-oxoglutarate. As judged from Western blot analyses, the cytosolic-type ASATs are expressed in all the stages of *T. cruzi* and *T. brucei*. These broad specificity enzymes might be essential along the whole life cycle of trypanosomatids, very likely playing a conserved function.

MI-P88

TWO MALIC ISOZYMES OF TRYPANOSOMA BRUCEI ARE EXPRESSED IN ALL THE ADAPTIVE FORMS OF THE PARASITE

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Trypanosoma brucei is the causative agent of sleeping sickness, one of the most neglected diseases. We have previously reported that the cytosolic malate dehydrogenase is the unique MDH isozyme expressed in the bloodstream forms of *T. brucei*. The biological function of this isozyme is believed to provide malate, the substrate required by the NADP-linked malic enzyme (ME) to synthesize NADPH, the essential coenzyme for biosynthetic processes and oxidative stress defense. Two different sequences coding for putative MEs (Tb11.02.3130, METb1; and Tb11.02.3120, METb2) were identified by Blast searching of the *T. brucei* genome data-bases. The nucleotide sequence encoding each of the MEs was amplified by PCR, cloned into an expression vector, pET28, and the functionality of the recombinant enzymes was proved by heterologous expression in *Escherichia coli*. Both MEs showed similar apparent K_m values, when malate (0.17 mM) and NADP (0.057 mM) were assayed. Conversely to *T. cruzi* homologues, the *T. brucei* enzymes neither were inhibited by oxaloacetate nor activated by aspartate. The presence of both MEs was evidenced in the crude extracts of procyclic and bloodstream forms by Western-blotting analyses. Hence, the two functional MEs are expressed in all the stages of *T. brucei* and their kinetic properties differ from those reported for *T. cruzi* enzymes.

MI-P89

ADENYLATE KINASE 1 (TZADK1) IN *TRYPANOSOMA CRUZI*

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Adenylate kinases are phosphotransferases, which catalyze the interconversion of adenosine nucleotides. These enzymes maintain the cellular ATP homeostasis and duplicate ATP´s energetic potential. While eukaryotic cells typically contain three adenylate kinase isoforms, kinetoplastid parasites present at least three additional isoenzymes. The unusual large amount of adenilate kinase variants present in these organisms is explained by their differential intracellular positioning. In this way each variant presents particular structural characteristics which account for their localization. In *Trypanosoma cruzi* TzADK1 is an isoform with a particular long N-terminal domain. This enzyme is homologous to TbADKA from *Trypanosoma brucei*, which has been shown to be targeted to the flagellum. In this work we study the localization of TzAdK1 and the association to cellular structures by means of Western-Blot and the expression of GFP-fusion proteins in *Trypanosoma cruzi*. Other adenylate kinase isoforms were also characterized.

MI-P90
BROMODOMAIN FACTORS (BDF) AND HISTONES INTERACTION IN *TRYPANOSOMA CRUZI*
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Chromatin structure regulation can be accomplished by at least two mechanisms: ATP-dependent chromatin remodeling and covalent histone modifications. Several chromatin remodeling factors and HAT complexes contain at least one bromodomain motif. This motif binds acetyl-lysine and it is present in many transcriptional regulators, participating in chromatin structure remodeling and transcription control. Little is known about chromatin structure and transcriptional regulation in trypanosomatids. Recently, chromatin modification has been described along T. cruzi life cycle and epimastigotes cell cycle. Coding sequences for putative methytransferases, HAT, HDAC and BDF have been revealed after the genome project. So, an epigenetic gene control regulation is suggested. We are studying BDF functions in T. cruzi transcription and chromatin structure regulation. TcBDF1, 2 and 3 were studed in T. cruzi epimastigotes. TcBDF2 was detected by WB in nuclear extracts and its localization was confirmed by IFI. However we were not able to detect TcBDF1 and 3. To find TcBDF2 in vivo interactors, we used TAP-Tag system. Protein complexes were recovered and eluted proteins were evidenced by SDS-PAGE. In parallel they were found to interact with T. cruzi histones by Far WB, using histones purified by acid extraction and recombinant TcBDFs. Different interaction patterns have been shown for each TcBDF.

MI-P91
RNAi ANALYSIS OF THE SCF COMPLEX IN *TRYPANOSOMA BRUCEI*
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Regulated protein degradation has emerged as a key recurring theme in multiple aspects of cell-cycle regulation. The importance of this process appears evident in the organization of the cell division cycle triggered by the destruction of key regulatory proteins. Many proteins are targeted to the proteasome degradation by a family of E3 ubiquitin-ligases, named SCF complexes, which link substrate proteins to an E2 ubiquitin-conjugating enzyme. Three core proteins compose SCFs: Skp1, Cdc53/Cullin1, Rbx1/ROC1. It has been shown that this complex is a key regulator in the transitions between G1/S, G2/M and in mitotic progression. Querying the Trypanosoma brucei genome database (GeneDB) we have identified the homologues to the human components of the SCF complex. The cell cycle of protozoan parasite T. brucei is a highly coordinated process, which ensures that there is correct replication and segregation of its single organelles: the flagellum, nucleus and kinetoplast. We have used RNA interference to study the involvement of the SCF components in T. brucei cell cycle regulation. Procyclic parasites were analyzed for defects in cell cycle progression by assessment of growth and by microscopy to visualise the distribution of organelles and DNA content. In this presentation, the phenotypes observed by knocking down these proteins will be discussed.

MI-P92
ADVANCES ON TRYPANOSOMATID TCPIN1 FUNCTION

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Studies in multiple systems have suggested that PIN-1 is a protein that plays a critical role for mitosis progression in mammalian and yeast cells. PIN1 participates in the phosphorylation-dependent prolyl isomerase that changes the conformation of its substrates controlling cell-cycle progression. The peptidyl-prolyl cis/trans isomerases (PPIases) are a conserved family of proteins that catalyze the cis/trans isomerization of proline preceding peptide bonds. This post-phosphorylation isomerization can lead to conformational changes in the substrate proteins and modulate their functions. In Trypanosoma cruzi we identified TcPIN1 as a homologue of the essential hPin1 parvulin PPIase. Based on functional assays, subcellular localization and preliminary enzymatic PPIase activity, we showed that TcPIN1 is a member of the Pin1-type PPIases, suggesting the existence of an additional conserved level of post-translational control in trypanosomatids. To gain a broader understanding of how TcPIN1 is regulated and to explain its role in trypanosomatids cell biology, a two-hybrid screen was performed using TcPIN1 as bait. Several different open reading frames were identified that codify for putative interacting proteins. Although the outcome of a screening often results in many new hypotheses, they still need to be validated by other techniques. The determination of the actual function of these novel candidates will be discussed.

MI-P93
AN APPROACH FOR TEACHING LACTOSE METABOLISM

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It is a difficult challenge for an instructor to teach students the relation between metabolism, nutrients availability and how a multicellular organism operates. We developed a didactic proposal for teaching the metabolism of carbohydrates. Initially we worked on lactose metabolism; utilizing lactose intolerance as an associated disease. For that aim, we developed a kit which consists of Petri dishes with MEF medium (Milk, Yeast Extract, plant Fertilizer), bakery yeast and yoghurt bacteria dilutions, glass balls and screw lid tubes. When MEF was inoculated with bakery yeast, colonies showed diameters lesser than 1 mm. In media in which Milk had a higher glucose content the diameter increased. Bacteria showed a normal growth in MEF independently of lactose level, comparable to bacteria present in large gut. When MEF medium with bromocresol purple was inoculated with yoghurt bacteria, each colony adjacent presented acidification. Through these assays, students can see that yeast cells could not grow properly, since they can not hydrolyse lactose, like gut´s mucosa cells. Also the acidification produced from lactose metabolisation of bacteria, can be detected easily. The kit is easy to manipulate and its components can be acquired easily, are non toxic and cheap. These allow students to analyse their own results increasing their interest in this topic making easier its learning.

NS-P01
DUAL AGONIST-CHANNEL BLOCKER ACTIVITY OF LAMOTRIGINE ON THE NICOTINIC ACETYLCHOLINE RECEPTOR

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Lamotrigine (LTG) is an antiepileptic drug employed in the treatment of partial epilepsies. We studied its possible interaction with channels other than its known therapeutic target, the voltage gated sodium channel, using the adult muscle nicotinic acetylcholine receptor (AChR) as a model. Patch-clamp recordings showed that LTG (50-400 μM) affected AChR channel function, behaving as an open-channel blocker when co-applied with the natural agonist, acetylcholine (Vallés et al., NeuroReport 2006, in press). Here, single-channel recordings with LTG alone demonstrate that LTG (0.05-100 μM) is able to activate the AChR channel by itself. [^{125}I]-α-bungarotoxin binding studies further indicate that LTG does not bind to the ACh binding site. Moreover, fluorescence experiments using the probe crystal violet, which displays higher affinity for the desensitized (D, in the presence of agonist) than for the resting AChR conformation (R, in the absence of agonist) show that LTG is able to induce the transition from the R-state to the D-state in the presence of α-bungarotoxin, i.e. when the canonical agonist binding site is blocked. We conclude that LTG displays dual agonist /channel blocker activities on the AChR, which operate through different sites.

NS-P02
DIFFERENT CONFORMATIONAL STATES OF AChR INDUCED BY STEROIDS AND FREE FATTY ACIDS

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Steroids and free fatty acids (FFA) are non-competitive inhibitors of the nicotinic acetylcholine receptor (AChR). They are purportedly located at the lipid-AChR interface, and their exact mechanism of action is still unknown. We studied the effect of FFA and steroids on AChR conformational equilibrium in *T. californica* AChR-rich membranes using the probe crystal violet (CrV), which displays higher affinity for the desensitized conformation (D) than for the resting conformation (R). Increasing concentrations of steroids (cortisone, hydrocortisone) shifted the equilibrium towards the D form even in the absence of agonist. In contrast, different FFA stabilized the AChR in a conformation with low affinity for CrV, similar to that of the R state, even in the presence of agonist. Cholesterol-depletion of AChR-rich membranes through cyclodextrin treatment led to an AChR conformation similar to that resulting from FFA treatment. Both FFA and cholesterol depletion increase lipid fluidity, whereas steroids decrease it, as measured by Laurdan generalized polarization using Förster energy transfer conditions. Thus, AChR conformational states disclosed by CrV fluorescence can be correlated to the physical state of the AChR lipid microenvironment.

NS-P03
INTERACTIONS BETWEEN NICOTINIC ACETYLCHOLINE RECEPTOR AND LIPID DOMAINS

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Biochemical and fluorescence spectroscopy methods were used to investigate the interactions between the nicotinic acetylcholine receptor (AChR) and purported "raft" lipids in model membranes. Affinity-purified AChR from *T. californica* and a synthetic peptide (γM4) corresponding to the M4 transmembrane domain, which in the whole receptor interacts with neighboring lipid molecules, were reconstituted into liposomes with a "raft" lipid composition (phospholipid:sphingomyelin:cholesterol 1:1:1). Partition of AChR or γM4 between detergent-resistant membranes (DRM) or soluble domains was assessed by Triton X-100 treatment at 4°C, centrifugation, and SDS-PAGE. The efficiency of the Förster resonance energy transfer (*E*) between the intrinsic fluorescence of the protein (or the peptide) and dehydroergosterol (fluorescence cholesterol probe, used as a reporter of DRM) was used as an assay of the protein distribution in the membrane. The fluorescent probe Laurdan was employed as a control acceptor fluorophore since it shows no preferential partition into any domain in particular. Laurdan generalized polarization (GP) was used to learn about the biophysical properties of the membranes. The results indicate that whereas the γM4 peptide alone partitions preferentially into DRM domains, the whole AChR displays no preferential interactions with these domains.

NS-P04
EFFECTS OF NERAMEXANE, AN OPEN CHANNEL BLOCKER OF NMDA RECEPTORS, ON THE α9α10 NICOTINIC RECEPTOR

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The aim of this work was to study the effects of neramexane, an uncompetitive NMDA receptor antagonist, on the α9α10 nicotinic receptor (nAChR). cDNAs coding for the rat α9 and α10 subunits were expressed in *Xenopus laevis* oocytes and agonist-evoked currents were measured under two-electrode voltage-clamp. Electrophysiological recordings in inner hair cells (IHCs) were performed by patch-clamp in acutely excised apical turns of the organ of Corti. The effects of neramexane were compared with those of memantine, a well-studied pore blocker of NMDA receptors. Our results indicate that, in oocytes, both compounds block ACh-evoked responses at micromolar concentrations with similar apparent affinities (neramexane IC50: 0.39 ± 0.03 μM; memantine IC50: 1.2 ± 0.4 μM). Blockage of rat α9α10 by neramexane was voltage-independent while blockage by memantine was slightly voltage-dependent. Neramexane inhibited ACh currents in a mixed competitive and noncompetitive manner. In IHCs, neramexane blocked ACh-evoked responses with an IC50 value (0.3 ± 0.08 μM) similar to that found for recombinant α9α10 receptors. In addition, neramexane blocked responses to synaptically released ACh. Since memantine and neramexane are effective when administered to animal models for various disease states, our findings suggest that the actions of these compounds at nAChRs may be of therapeutic relevance.

NS-P05

NOVEL EXPRESSION IN BRAIN AND OVARY OF FSH-β AND LH-ß SUBUNITS IN THE CICHLID FISH *C. dimerus*

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It is widely known that FSH and LH, which play key roles in vertebrate gametogenesis and steroidogenesis, are synthetized and secreted exclusively in the pituitary gland. While investigating the origin and ontogeny of FSH and LH cells in the cichlid fish *Cichlasoma dimerus* by immunohistochemistry (IHC), we unexpectedly found immunoreactivity (ir-) in neurons of the preoptic area, which send their projections through different brain areas and the neurohypophysis, and in pre-vitellogenic and early-vitellogenic oocytes. Western blot and IHC techniques applied to adult brain and ovary also showed ir-bands for LH-ß (24 kDa) and FSH-ß (18 and 15 kDa) which had the same molecular weight found in pituitary extracts. To further demonstrate the extra-pituitary expression of these hormones, we cloned, sequenced, and confirmed the presence of FSH-ß and LH-ß subunits mRNAs in the pituitary, brain and ovary of *C. dimerus*. The expression of these transcripts in the three organs was consistent with their peptide expression showing a high sequence homology when compared with other phylogenetically related fish (ie: tilapias *Oreochromis mossambicus* and *O. niloticus*). The expression of these hormones outside the pituitary suggest important and novel roles for them, such as hypohysotropic factors / neuromodulators at the brain level and autocrine / paracrine functions at the ovarian level.

NS-P06

CHALCEDONY (INCIPIENT FOSSILIZATION PROCESS) IN HUMAN BRAIN CORTEX AND CEREBELLUM FROM AGED PATIENTS

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Aluminum and silicon were observed in human degenerated brain by X-ray microanalysis using EDS-SEM. Similarly, Al and Si in electric organs were detected by us. On these evidences we decided to explore the presence of silica (silicon mineral) by transmitted light on a polarization microscope. Cortex of brain and cerebellum were collected after autopsy from three patients (mean: 80 years old): a woman who died for septic shock (renal infection) and two men (cancer) who died with a CVA. Thin sections of brain (frontal) and cerebellum staining with hematoxilin-eosin were observed using a Leica DMLP microscope. Shape, size and color were analyzed on transmitted light while birefringence and extinction angle were observed under polarized light. Chalcedony (a silicon mineral) distribution in the three layers of the cerebellar cortex (woman and a man) was identified; it was more important in cerebellum than in the brain. Chalcedony is rounded in shape, 12-20 µm in size, non color, low refraction index; it has first grade gray color of birefringence and radial extinction. It is a typical mineral of fossilization processes in different organic matter along geological record. This was recognized in Psammobatis extenta electric organs by us. This is the first mention of chalcedony in Central Nervous System. May be fossilization process is related to age and it would be independent of cancer.

NS-P07
MOLECULAR DIAGNOSIS OF NEUROFIBROMATOSIS TYPE 2

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The NF2 tumor suppressor gene is involved in the development of multiple tumors of the nervous system, mainly schwannomas and meningiomas. To evaluate the predisposition to these tumors we analyzed the NF2 gene for mutations and at-risk haplotypes in affected families. 7 families were studied by: A. Segregation of 3 microsatellites STR(CA)n, two intragenic and one 3Mb telomeric to NF2; B. Heteroduplex/SSCP/Sequence assay. Segregation analysis was informative in two families: 1.Two siblings and 3 patient´s offsprings were excluded from being NF2-carriers; 2. The at-risk haplotype, defined by LOH in the tumor, could be useful for patient´s offspring. Heteroduplex/SSCP analysis of 16 NF2-exons showed an altered pattern in 5 exons suggesting a mutation in 5 different patients. These data allowed the testing of patients´ relatives: Two asymptomatic children resulted mutation-carriers, they also shared the same haplotype; 6 individuals from 3 families were excluded from risk. Sequence analysis of these exons with forward primers showed a 13-bp deletion in exon 2. The other 4 exons are sequenced with reverse primers to identify the mutation.
The use of two-step strategy, segregation of STRs and heteroduplex/SSCP/sequence analysis allowed us to detect the at-risk haplotype and/or causative mutations in 6 NF2-families.

PL-P01
TOBACCO PLANTS WITH REDUCED LEVELS OF FERREDOXIN USING TWO DIFFERENT TECHNIQUES

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One approach to study the role of a protein in a biological system is reverse genetic. *Antisense* technology and RNAsi allow us to reduce the physiological level of a target protein in vivo. Using these tools, we evaluate the role of Ferredoxin (Fd) in the context of the antioxidant defences of the plants. Plant-type Fds, that have one 2Fe-2S cluster per molecule, act as a soluble electronic transporters between Photosystem I and ferredoxin-NADPH reductase (FNR) during the light phase of photosynthesis, to generate NADPH needed for CO_2 fixation. Plants with reduced levels of this protein were obtained using both techniques. Antisense 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. Moreover, only heterotrophic growth was observed in the plants with the lowest levels of Fd. All these results suggest that a small reduction in the levels of Fd would lead to drastic alterations in the growth and development of plants. The functional sustitution of Fd of plant origin by Fd and flavodoxin (Flx) of *Anabaena*, a filamentous cyanobacteria, was evaluated in double transformants.

PL-P02
TOBACCO PLANTS OVEREXPRESSING FERREDOXIN-NADP(H) REDUCTASES DISPLAY ENHANCED STRESS TOLERANCE

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Ferredoxin-NADP(H) reductase (FNR) catalyses the last step of photosynthetic electron transport in chloroplasts, driving electrons from two molecules of ferredoxin to a single molecule of $NADP^+$. Antisense tobacco plants with reduced amounts of FNR display diminished photosynthetic activity (Hajirezaei *et al* Plant J. (2002) 29:281) and enhanced photo-oxidative damage (Palatnik *et al* Plant J. (2003) 35:332). Furthermore, in response to oxidative stress Escherichia coli induces the expression of the flavoprotein FNR and its substrate flavodoxin (Krapp *et al* J Bacteriol. (2002) 184:1474). In order to investigate if the overexpression of this enzyme improves oxidative stress tolerance in plants we prepared transgenic *Nicotiana tabacum* cv. Petit Havana plants expressing pea or cyanobacterial FNR from *Anabaena* PCC7119. In this work we evaluate the photosynthetic efficiency of the transgenic lines using fluorescence chlorophyll measurements, CO_2 fixation rates and biomass accumulation. We found that FNR overexpression had no effect on growth or photosynthetic parameters. On the other hand, we studied tolerance to oxidative stress using high light treatments or redox-cycling herbicides that propagate reactive oxygen species, such as methyl viologen (MV). Interestingly, the transgenic plants with higher levels of the alien protein exhibited augmented tolerance to photooxidative damage and MV.

PL-P03
ANALYSIS OF AN OXIDATIVE STRESS TOLERANT MUTANT OF *Arabidopsis thaliana*

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The goal of this study is the analysis of a mutant line of *Arabidopsis thaliana* with a phenotype of tolerance to methyl viologen (MV). This mutant was isolated in an assay aimed to identify lines tolerant to oxidative stress, in which 8,850 independent lines that had been mutated by activation tagging (Weigel et al., 2000) were exposed to MV. A line named 44/1 was isolated and the T-DNA insertion site in the Arabidopsis genome was identified using the plasmid rescue technique. Sequencing of the rescued plasmids revealed that the T-DNA had been inserted within the activation range of two genes, one of unknown function (UFG) and one that codes for a 2-Cys peroxiredoxine (2-Cys Prx). The participation of both genes as candidates responsible for the oxidative stress tolerant phenotype was analyzed *in silico*. Although the *2-Cys Prx* gene was the most likely candidate owing to the involvement of this protein in the antioxidant defense, the analysis of the expression of these genes revealed that the UFG could be responsible for the phenotype. This is indicated by the variation of the mRNA levels of UFG, which increased when exposed to oxidative stress, while the mRNA levels of *2-Cys Prx* remained constant.

PL-P04
CATALASE ACTIVITY AND EXPRESSION IN DEVELOPING SUNFLOWER SEEDS AS RELATED TO CADMIUM STRESS

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Sunflower seed development is associated with marked changes in catalase (CAT) activity. In mature seeds, CAT is located mainly in glyoxysomes. Although at least eight isoforms of CAT isoenzymes have been described, results from the association of 55 and 59 kDa subunits, four different genes, *CATA1* to *CATA4*, have been identified in sunflower cotyledons. The objective of the present work was to evaluated CAT activity and expression in developing sunflower seeds as related to oxidative stress induced by cadmium. Sunflower seeds were imbibed in water (control) or concentrations up to 100 Cl_2Cd µM and stored in dark at 24 ^oC for 24 h. Seeds germinability was unaltered by cadmium under the assayed conditions. H_2O_2 production, measured as precipitation of 3,3 diamine bencidine, was observed in root apix with up to 10 µM cadmium concentration. In the seedlings, all *CATAs* transcripts were detected. Although *CATA1* and *CATA3* transcripts level increased with cadmium treatment, *CATA2* and *CATA4* decreased its expression. CAT activity in Cd-treated seedlings remained similar to control. Modification in the expression pattern of catalase subunits in seeds under cadmium treatment and no variation in the activity are indicating that new CAT isoforms synthesis are responsible of a major resistant to the stress condition.

PL-P05
ACCUMULATION OF δ-AMINOLEVULINIC ACID IS RESPONSIBLE OF CADMIUM-INDUCED OXIDATIVE STRESS IN SOYBEAN

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Cadmium toxicity has been extensively studied in plants, however its biochemical mechanism of action has not yet been well established. To fulfill this objective, four-weeks-old soybean nodulated plants were treated with 200 µM Cd^{2+} for 48 h. δ-aminolevulinate dehydratase activity and protein expression, as well as δ-aminolevulinic acid (ALA) and porphobilinogen concentrations were determined in nodules, roots and leaves. In vitro experiments carried out in leaves were performed using leaf discs. Oxidative stress parameters were also determined. Cadmium treatment caused 100% inhibition of δ-aminolevulinate dehydratase activity in roots and leaves, and 72% inhibition in nodules whereas protein expression remained unaltered in the three studied tissues. Plants accumulated ALA in nodules (46%), roots (2.5-fold) and leaves (104%), respect to controls. When leaf discs were exposed to ALA or Cd^{2+}, it was found that TBARS levels were enhanced, while GSH content and SOD and GPOX activities were diminished. The protective role of S-adenosyl-L-methinone against oxidative stress generated by Cd^{2+} and ALA was also demonstrated. This work let us to establish that accumulation of ALA in nodules, roots and leaves of soybean plants due to treatment with cadmium is highly responsible for oxidative stress generation in these tissues.

PL-P06
HEAVY METALS PRODUCED MACROMOLECULES OXIDATION AND MODIFIED PROTEOLYTIC ACTIVITY IN SUNFLOWER LEAVES

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The increase and availability of metal in soil has initiated extensive studies about their tolerance-toxicity in plants. A comparative analysis about the effect of 100 µM of $AlCl_3$, $CoCl_2$, $CuCl_2$, $CrCl_3$, $HgCl_2$, $NiCl_2$, $PbCl_2$ or $ZnCl_2$ on macromolecules oxidation and modified proteolytic activity was performed in sunflower leaves after 4 days of plant treatment. Except Pb, metals caused a decrease in leaf area. Co, Cr, Cu and Hg increased lipid peroxidation. Total proteins shared similar patterns, however, Co, Cu, Hg and Ni produced proteins oxidation, specially with molecular weight lower than 150 kDa. Except Al and Zn that increased acid proteases activity, the rest of metals decreased it. Similar pattern was observed with neutral activity. While Co and Pb decreased basic activity; Cu, Hg and Zn increased it. Metals decreased 20S proteasome hydrolyzing activities. Nor protein fragmentation either modification in the proteasome protein abundance was observed (except Hg that decreased 20S protein). New bands with low molecular weight of ubiquitin conjugated proteins were observed with Al, Cr, Cu, Hg and Ni treatment. In order to understand the different mechanisms of metal toxicity and the plant response certain common features can be established that included decline in the antioxidant defense system, increase of ROS production, or diminished capacity for removal of oxidized proteins.

PL-P07
CADMIUM TOXICITY IN TRANSGENIC ARABIDOPSIS DEFICIENT IN PUTRESCINE BIOSYNTHESIS

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Polyamines (PAs) are aliphatic amines with proposed antioxidant properties. Cadmium (Cd) is a strongly phytotoxic pollutant. The aim of this work was to evaluate Cd toxicity in a mutant deficient in putrescine (Put) biosynthesis to look into the relationship between PAs levels and oxidative stress. Arabidopsis FN (wild type) and transgenic adc2-1 were germinated and then transferred to soil for 20 days. Plants were irrigated with Hoagland solution (Controls) or 0.5 mM CdCl2 for 3 or 6 days. Chlorophyll, TBARS, H2O2 and PAs contents and SOD and CAT activities were measured. Cadmium decreased chlorophyll in both FN and adc2-1-treated plants in a similar manner. A slight increase in TBARS was observed in adc2-1 Cd-treated plants whereas H2O2 increased significantly in adc2-1 exposed to Cd, accompanied by a large inhibition in SOD and CAT activities. However, the basal activities of these enzymes were significantly higher in adc2-1 than in FN control plants. PAs were slightly reduced in adc2-1 plants respect to FN, as expected considering the deficiency in Put biosynthesis of the mutant. However, the three PAs were moderately increased or without changes in FN Cd-treated plants. Constitutive increased SOD and CAT activities in the mutant could be a compensation for a reduced capacity to synthesized PAs in adc2-1 plants, considering that both lines presented similar toxicity symptoms.

PL-P08

CADMIUM-INDUCED ROOT GROWTH INHIBITION IN WHEAT IS RELATED TO INCREASED POLYAMINES AND NITRIC OXIDE

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Nitric oxide (NO) plays a key role as an intra and intercellular messenger in several processes in plants. Recently, it has been demonstrated that polyamines induce NO biosynthesis in plants. Since both molecules are related to developmental and defense processes, as well as in their biosynthetic pathways, the aim of this work was to study whether polyamines and NO were involved in cadmium root growth inhibition of wheat plants. Triticum aestivum seeds were germinated in Petri dishes and after 48 h, they were transferred to hydroponics with Hoagland solution (control) or with 0.1 mM CdCl2, 0.1 mM NO, 0.01 mM NO, 1 mM Spm exogenously added to the same nutrient solution. cPTIO was used as NO trap in control treatments. Plants were harvested after 7 days of growth and root elongation, polyamine content and NO formation were evaluated. All treatments caused an inhibition of root growth compared to the controls. This inhibition was accompanied by an increase in the level of the three main polyamines Put, Spd and Spm and an evident NO formation (detected with the fluorescent probe DAF-FM). When cPTIO was added together with Cd NO or Spm, a reduction of NO release and a partial reversion of root growth inhibition was observed. These results suggest that NO and polyamines could be involved in cadmium toxicity that leads to a significant root growth inhibition in wheat plants.

PL-P09

***Arabidopsis thaliana* FRATAXIN (AtFH) IS INVOLVED IN IRON HOMEOSTASIS AND OXIDATIVE STRESS**

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Iron is an essential cofactor for many reactions in the cell; in particular, it is present in heme and FeS proteins. Moreover, mitochondria contain the machinery for FeS cluster biogenesis and export into the cytosol. However, excess iron can generate via Fenton reaction highly toxic-free radicals generating oxidative damage to the cell. Nitric oxide (NO) can attenuate the Fenton oxidative damage preventing the formation of oxidants by scavenging either iron or superoxide limiting hydroxyl radical formation. Thus, plants employ a sophisticated homeostasis mechanism to control cellular iron. Frataxin is a mitochondrial protein that is conserved throughout evolution. The function of frataxin is still essentially unknown. It has been postulated that this protein is an essential protein in plants, required for full activity of mitochondrial Fe-S proteins and plays a protective role against oxidative damage. In this work we present evidences that frataxin-deficient *Arabidopsis* plants accumulate iron in their mitochondria. We suggest that an excess of free iron could be involved in the production of reactive oxygen species (ROS) and could be responsible for the oxidative damage to Fe-S clusters as a consequence these plants have increased sensitivity to oxidative stress. We discuss if the higher NO content observed has an effect protective or deleterious in frataxin-deficient plants.

PL-P10
ISOLATION OF ANTIOXIDANTS FROM TRIPODANTHUS ACUTIFOLIUS (RUIZ & PAVÓN) VAN THIEGEM LEAVES

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Introduction: Hydroxyl radicals (HO•) are oxygen species generated in reactions with iron or copper ions. Proteins and lipids are target of HO• attack. Within the cell nucleous HO• damages deoxyribose and nitrogen bases of DNA, causing heritable mutations and cancer. Many diseases are atributed to the deleterious effects of HO• attack. Objective: to study the HO• scavenging activity of infusion from T. acutifolius leaves and to isolate and identify the responsible substances. Methods: deoxyribose assay was performed to asses the site specific (SS) and non-site specific (NSS) scavenging activities of T. acutifolius infusion. The isolation of the antioxidant substances was performed by Silica gel column chromatography (CC), Sephadex LH-20 CC, and preparative Silica gel TLC plates. The identification of eluted compounds was achieved by comparison of their UV-visible spectra with standards. Results: T. acutifolius infusion showed HO• scavenging activity by SS and NSS assays (EC50= 55.2 and 213 ppm respectively). The bioactive compounds were identified as glycoflavonoids: rutin, isoquercitrin and hyperoside. Conclusions: Flavonoids have the ability to protect biosystems against HO• attack. Those flavonoids may act as direct HO• scavengers or iron chelating substances. The antiinflammatory effect of T. acutifolius infusion could be partially attributed to the identified flavonoids.

PL-P11
ATP MODULATES PEROXIDASE ACTIVITY AND ELICITS AUTOPHOSPHORYLATION OF RAPESEED 2-CYS PEROXIREDOXIN

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2-Cys peroxiredoxins (2-Cys Prx) are ubiquitous peroxidases devoid of selenium- and the heme-prosthetic group that have been implicated in cell proliferation, differentiation, apoptosis, and photosynthesis. Although the contribution to the cell redox status has been characterized and the chaperone capacity was recently reported, the precise control of functional characteristics remain unknown. We found that ATP impair reversibly the peroxidase activity associated to chloroplast rapeseed 2-Cys Prx. In particular, the noncovalent interaction with ATP not only modifies the catalytic activity but also changes the structural features of the recombinant protein. More importantly, ATP triggers the autophosphorylation of the chloroplast 2-Cys Prx when the protein is successively treated with a reductant and an oxidant. Contrary to the peroxidatic activity, the conserved Cys175 and Trp179 residues are essential for the incorporation of radioactivity from [γ-^{32}P]ATP. Despite significant differences, the process of autophosphorylation is also observed with orthologs from a helminth parasite (Schistosoma mansoni 2-Cys Prx1) and bacteria (Salmonella typhimurium AhpC). These results uncover a previously unknown function of ATP in the regulation of 2-Cys Prx and give a new insight not only to the removal of obnoxious reactive oxygen species but also to the control of signal transduction pathways.

PL-P12
INCREASE IN APOPLASTIC PEROXIDASES ACTIVITIES DURING THE DEVELOPMENT OF SUNFLOWER LEAVES

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Peroxidases are enzymes associated to a wide variety of physiological processes including defense responses and auxin catabolism. They are involved in H2O2 reduction by using auxins, lignin precursors and other biomolecules as substrates. Paradoxically, the extracellular isoforms grouped in the class III subfamily, have been associated to both, the inhibition as well as the promotion of cell growth. In this work we examined apoplastic activities during the development of leaves inserted in even shoot positions of sunflower plants. Seeds of *Helianthus annuus* Ha 89 variety were grown in greenhouse under no limiting conditions. At different times from sowing intercellular washing fluids were obtained by infiltration and centrifugation of the leaves. The peroxidase activity was spectrophotometrically recorded every 10 seconds using tetramethylbenzidine as substrate (3 mg/ml). Results showed that, independently of the position in the shoot, the apoplastic peroxidase activity increased continuously with development of the leaves, reaching its maximun value when they became fully expanded. The contribution of this phenomenon to the regulation of cell development will be discussed. [*Both authors equally contributed to this work].

PL-P13
APPLICATION OF PEROXIDASES IN THE REMOVAL OF PHENOLIC COMPOUNDS: OPTIMIZATION OF REACTION CONDITIONS

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Phenolic compounds, present in the drainage from several industries, are harmful pollutants. Here we describe the use of peroxidase isoenzymes from tomato hairy roots, which were able to oxidise phenol and 2,4-dichlorophenol (2,4-DCP) from polluted solutions. We used different enzyme fractions: Total proteins (TP), ionically bound to cell wall proteins (IBP), basic (BP) and acidic peroxidases (AP). We analyzed the optimum conditions, the effects of Polyethyleneglycol (PEG-3350) on the removal and on the enzyme activities to obtain the maximum efficiency in the process. The optimal H2O2 concentration for 2,4-DCP and phenol removal were 1 and 0.1 mM respectively. The addition of PEG (10-100 mg/l) to solutions containing 2,4-DCP showed no effect either on the removal efficiency or on the enzyme activity. However, PEG (100 mg/l) increased the removal efficiency of phenol by BP and IBP. Also, peroxidase activities from BP and IBP were 3 and 13 times higher, respectively, than those detected for the same fractions in solutions without PEG. Peroxidase activities from AP and TP fractions were no affected by PEG. As IBP and BP fractions contained the isoenzymes mainly involved in removal of phenol, the protective effect of PEG, which depends on the contaminant as well as of the enzyme fraction used, would be important to improve the removal efficiency with these isoenzymes.

PL-P14

MECHANISMS FOR THE REGULATION OF WHEAT NON-PHOSPHORYLATING GLYCERALDEHYDE-3-PHOSPATE DEHYDROGENASE

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Non-phosphorylating, $NADP^+$-dependent glyceraldehyde-3-phosphate dehydrogenase (EC: 1.2.1.9; NP-GaPDHase) is a cytosolic enzyme found photosynthetic eukaryotes. The enzyme is involved in a shuttle system exporting NADPH from chloroplasts to cytosol. In heterotrophic plant cells its function is less clear and it could couple NADPH production to glycolysis. In wheat endosperm (but not in leaves) NP-GaPDHase is post-translationally modified by phosphorylation, after which it interacts with 14 3-3 proteins. We heterologously expressed the gene encoding a NP-GaPDHase from wheat (NCBI; NºAcc:AF521191) and a NP-GaPDHaseS404A mutant form, and we purified both enzymes to a high degree. NP-GaPDHase was phosphorylated by wheat endosperm extracts under reaction conditions specific for SNF1-related protein kinases, but not for another kinases (SOS2, GSK-3, and MAPK). The mutant S404A enzyme exhibited higher specific activity and was recalcitrant to be phosphorylated under the different conditions. These results reinforce the structural model predicting Ser404 as a regulatory phosphorylation site in the enzyme. NP-GaPDHase was also inactivated by the thiol oxidative chemicals DTNB and Diamide. This inactivation was reverted by DTT and reduced thioredoxin, suggesting that NP-GaPDHase could be regulated by redox systems operating in the cytoplasm of plant cells.

PL-P15

REDOX METABOLISM IN *Phaeodactylum tricornutum*

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Diatoms are important components of marine phytoplankton, being particularly relevant for geochemical cycling of minerals (mainly silica), and global carbon fixation. Diatoms are brown unicellular algae, thought to be responsible for around one-fifth of global primary productivity. Being such important players in the global ecosystem, their ecology and physiology have been the focus of research. The cellular redox state is a crucial mediator of multiple metabolic, signalling and transcriptional processes in cells. Also, protein thiols in the form of cysteine residues are key players in redox sensing and regulation. The thioredoxin (TRX) and glutathione (GSH) systems control cellular redox potential, keeping an intracellular state rich in reduced thiols. In this work, we present the molecular cloning of the genes coding for two TRXs, a PMSR (peptide methionine sulfoxide reductase) and a putative PrxQ (peroxiredoxin Q), all enzymes involved in redox metabolism, from *P. tricornutum* total RNA. In addition, we measured glutathione reductase activity in cellular extracts of *P. tricornutum*. Our results support the occurrence of TRX and GSH system in the diatom. Results afford valuable information to the incipient *P. tricornutum* genomic and proteomic, data that are of key relevance for the understanding of the alga biochemistry. [Granted by UNL, CAI+D 2006; ANPCyT, PICT´03 01-14733, PAV´03 137].

PL-P16

DIFFERENTIAL RESPONSES OF *TRITICUM AESTIVUM* SUPEROXIDE DISMUTASES UPON HYDRIC STRESS

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Reactive Oxygen Species (ROS) generation represents an early response associated to biotic and abiotic stresses. ROS may cause cell damage due to their high reactivity with biomolecules while they could also act in defense signaling. We have previously observed changes in the redox status of vascular system of seedlings under water deficit. Here, to further study the effects of this stress on the antioxidant system, we focused on apoplastic Superoxide Dismutases (SODs). Intercellular fluids (IF) were isolated by vaccuum infiltration and centrifugation of control and stressed leaves. After five days of witholding water, a reduction of 73% was observed in the activities of Total Soluble Extracts (TLSE), while no significant change was detected in IF fraction. We also analysed by zymograms the pattern of SOD isoforms using specific SOD inhibitors. Interestingly, both qualitative and quantitative differences were observed in these compartments. No changes in the levels of the four apoplastic isoforms (Cu/Zn-, Fe- and Mn-SODs) were detected; while, the levels of four out of six isoforms present in TLSE decreased due to the treatment. Altogether, our results present evidence of differential responses of components of the antioxidant system in plants underlining the importance of studying each compartment separately. To our knowledge, this is the first report of an apoplastic Fe-SOD.

PL-P17

THE INTRON OF THE COX5C GENE ENHANCES THE DROUGHT TOLERANCE CONFERRED BY HAHB-4 TO TRANSGENIC PLANTS

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Hahb-4 is a member of the sunflower subfamily I of HD-Zip proteins. Transgenic Arabidopsis thaliana plants constitutively expressing this gene exhibit a strong tolerance of water stress in concert with morphological defects and a delay in development. To obtain drought-tolerant plants without morphological associated phenotype, several stress inducible promoters were isolated and cloned directing Hahb-4 expression. These plants showed unchanged morphology and enhanced drought tolerance compared with non transformed plants, but no as high as the one exhibited by the constitutively transformed genotype.
A chimerical construct between the Hahb-4 promoter and the intron of the Arabidopsis Cox5c gene was made either directing gus or Hahb-4 expression. GUS activity increased in transgenic plants after induction, showing the same distribution pattern as in plants transformed with a construction lacking the intron. Transgenic plants, bearing the chimerical construct, are morphologically indistinguishable from wild type plants in normal growth conditions whereas the water stress tolerance achieved was as strong as the one shown by the constitutive genotype. This fact seemed to be due to a combination of an increase in transcription and translation rates. Similar strategies could be applied in the future for the obtaining of suitable promoters responsive to other external agent.

PL-P18

CHARACTERIZATION OF ROOT MEDICAGO TRUNCATULA TRANSCRIPTION FACTORS INVOLVED IN SALT STRESS RESPONSE

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The root architecture of the model legume Medicago truncatula is determined by the action of environmental stresses on specific genes, which adapt root development to this constraint. In this work, we isolated and characterised Transcription Factors (TFs) expressed in Medicago roots in response to salt stress. TFs were identified using genomic approaches and massive quantitative RT-PCR. Among a collection of 39 TFs, we chose 4 from the HD-ZIP, NAC, RR and WRKY families, respectively, to analyse their regulation and expression in detail. Full-length cDNA clones corresponding to these TFs were obtained by retrotranscription followed by PCR and cloned in both an appropriate vector to transform Arabidopsis plants and Medicago roots. Fusions of these cDNAs with GST from Schistosoma japonicum served to express the encoded proteins. Such proteins were purified by affinity chromatography and in vitro analysis, consisting of EMSA and PCR assisted binding site selection (SELEX), were carried out. This analysis enabled us to characterise protein-DNA interactions for these TFs. Our aim is to dissect the regulatory pathways involved in the response to salt stress in legume roots and eventually identify genes able to confer plant tolerance to such adverse environmental conditions.

PL-P19

IDENTIFICATION OF KEY SEQUENCES IN THE HAHB-4 GENE PROMOTER FOR THE REGULATION OF ITS EXPRESSION

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Sunflower Hahb-4 encodes a transcription factor from the HD-Zip family. Its expression is transcriptionally regulated by drought, ABA, ethylene and other abiotic stresses. In this work we describe the functional characterization of the Hahb-4 promoter, with the aim of identifying the cis elements within this regulatory segment of DNA. In order to achieve our aim we obtained several constructs bearing fractions of the promoter fused to the gus reporter gene, occasionally including chimeras and point mutations. These constructs were used to transform Arabidopsis thaliana plants. The expression patterns of the reporter gene were analyzed by histochemistry while transcript expression levels were quantified using Real-Time RT-PCR.
Our results suggest that this promoter present a bipartite structure. The first region, adjacent to the transcription start point would contain functional boxes of response to hormones and also of tissue-specific expression, whereas the further one would include enhancer elements, essential for an efficient expression. Additionally, we could conclude that most of the elements identified in silico are not actually functional in this promoter.

PL-P20
EVALUATION OF RELATIONSHIP BETWEEN PUTRESCINE AND ABA IN Arabidopsis thaliana

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Putrescine (Put), spermidine (Spd), and spermine (Spm) form a class of aliphatics amines that are ubiquitous in living organisms, and have been implicated in a wide range of biological processes, including plant growth and development. However, their mode of action remains a matter of speculation. In recent years, attention has been focused on the involvement of PAs in response to environmental stresses. In plants PAs biosynthesis begins principally with Arg decarboxylation by Arginine decarboxylase (ADC; EC 4.1.1.19). There are general agreement in consider this enzyme as a key enzyme playing a decisive role in the regulation of the PAs levels. On the other hand, abscisic acid (ABA) appears to play an important role in plant response to these stresses. In orther to further analyze PAs involvement in abiotic stresses and their relationship with stress hormone ABA, we obtained transgenic Arabidopsis thaliana plants expressing oat ADC under the stress inducible promoter rd29A. When treated with ABA or acclimated to cold, differences in the expression of genes involved in stress response (analyzed by real-time PCR) were observed. We also evaluated the performance of these plants under freezing and dehydration stresses, suggesting that endogenous Put levels could be involved in the tolerance mechanism and the different gene expression patterns observed in the transgenic plants.

PL-P21
POSTHARVEST UV-B RADIATION IN LEMON FRUITS OF DIFFERENT SEASONS

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UV-B radiation acts as an environmental stressor, leading to multiple responses in primary and secondary metabolisms. In plants, production of flavonoids and other phenols is important for tolerance against UV-B and pathogens. The aim of our work was to analyze the flavonoid and malondialdehyde (MDA) contents in lemons exposed to different UV-B radiation and to check fruits susceptibility to wound infection by *Penicillium digitatum*. Lemons were collected either in summer or winter from CITRUSVIL S.A. packing. Fruits were exposed to UV-B for different periods of time and the flavonoid and MDA were measured in flavedo 24 h after the treatment. Then, the exposed fruits were infected with *P. digitatum* spores. In control lemons, the flavoniod contents were higher in summer than in winter. The UV-B treatment produced a 6-time increase in flavonoids in summer fruits, whereas no changes were observed in winter. The levels of MDA in irradiated lemons were higher in winter than in summer. In general, a decreased in the infection rate was observed upon UV-B treatments. UV-B radiation could induce in lemon flavedo signals associated to pathogen/UV-B protection.

PL-P22
REGULATION OF PLANT DNA MISMATCH REPAIR BY ULTRAVIOLET RADIATION

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Plant DNA is continuously damaged by ultraviolet (UV) irradiation. Plants generally have a higher tolerance for UV than animals due to the absorption of UV by waxy substances on leaf surfaces, cell walls and intracellular chemical compounds such as flavonoids. However, UV radiation that is not absorbed induces DNA damage that should be repaired to maintain genetic stability. Different DNA repair mechanisms exist that are both specific and overlapping. Mismatch repair (MMR) systems repair DNA mismatches and participate in responses to a variety of adducts induced by endogenous and exogenous agents. The MMR system functions through the interactions between several proteins, such as MSH2, MSH6, MLH1 and PMS1. Besides these proteins plants also encode a unique MSH7. To investigate the regulation of plant MMR by UV-B, we evaluated the transcripts levels of MMR proteins by qRT-PCR. Our results demonstrate that MSH2, MSH6 and PMS1 mRNAs are induced by UV-B in *A. thaliana* leaves and flowers, while MLH1 remained unchanged in these tissues. A similar effect was observed for MSH2 and MSH6 from *Z. mays*. Interestingly, MSH7 mRNA levels in controls plants of both species were undetectable, while rapid induction was observed after UV-B irradiation. The correlation with protein expression and the effect of mutations in those genes in Arabidopsis using SALK-lines are under progress.

PL-P23
FUNCTIONAL ANALYSIS OF *Arabidopsis thaliana* MutS AND MutL COMPLEXES IN YEAST

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Highly conserved mismatch repair (MMR) systems promote genomic stability by correcting DNA replication errors, antagonizing homeologous recombination, and responding to various DNA lesions. *Arabidopsis* and other plants encode a suite of MMR protein orthologs: the recognition heterodimers MSH2-MSH6 and MSH2-MSH7, and the recognition-excision coupling component MLH1-PMS1. To analyze the function of these complexes in yeast we constructed vectors for expression of AtMSH2, AtMSH7, AtMLH1 and AtPMS1. The vectors utilize a relaxed 2µ origin, the inducible GAL1-10 promoter to achieve high level of protein expression and different selection markers. The effects of AtMMR protein expression on mutation rates were assessed in strains carrying a sensitive reporter based on a long homonucleotide run of 14 A-T base pairs within the LYS2 locus resulting in a +1 reading frame. Hence, reversion to LYS+ occurs by deletion of a single A-T base pair within the homonucleotide run. Our results suggest that expression of AtMSH2 and/or AtMSH7 has not impact on the mutation rate of the *msh6* mutant strain, and expression of the individual proteins does not increase the low mutation rate of a wild-type strain. However, co-expression of AtMSH2 and AtMSH7 at high levels in wild-type yeast reduced reversion events compared to the empty vector.

PL-P24
CHARACTERIZATION OF PROTEIN PHOSPHATASES TYPE 2A IN SOLANACEAE

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Protein phosphorylation/dephosphorylation plays critical roles in growth and development of plants, being serine/threonine phosphorylation the predominant postranslational modification. Ser/Thr phosphatases include phosphatases type 1, 2A and 2C. PP2A contain a highly conserved catalytic subunit, a structural subunit (A) and a regulatory subunit (B). A potato phosphatase type 2A (StPP2A1c) was described previously in our lab and evidence on the participation of this isoform in tuber development was obtained. In tomato plants, changes in PP2A levels under abiotic stress were observed suggesting the participation of one or more isoforms in the stress response. The aim of this study was to characterize the catalytic subunit of PPase 2A present in potato (Solanum tuberosum) and tomato (Solanum lycopersicum). Sequence homology analysis revealed six isoforms in potato and five in tomato. Presence of functional domains, prediction of subcellular localization and phylogenetic analysis were carried out using bioinformatics analysis. Expression pattern profiles of PP2A isoforms in potato and tomato under different environmental conditions (mainly stimuli related to tuber development for potato and abiotic stress for tomato) were determined. Bioinformatics and expression profile analysis yielded evidence on the physiological roles of different isofoms of PP2A in potato and tomato.

PL-P25
ACTIVATION MECHANISM OF SALICYLIC ACID-RESPONSIVE GENES IN *Arabidopsis thaliana*

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Salicylic acid (SA) plays a crucial role in the activation of defense genes in response to stress. Nevertheless, the mechanism of gene activation by SA is not clearly understood yet. According to their activation kinetics, SA-responsive genes are classified in early and late genes. It has been suggested that differential requirement of the co-activator NPR1 and cis-elements could exist for the activation of these groups of genes. Using transcriptome analysis in response to SA we identified 231 genes whose expression increases after 2,5h of treatment. For a group of selected genes we confirmed the kinetics of induction and the NPR1 dependence by using Northern blot. To determine the mechanism of gene activation, we also evaluated the effect of SA on the mRNA stability, by using transcription inhibitors. *In silico* analysis of the promoter sequences allowed to identify different putative *cis*-elements in NPR1-dependent and independent genes. For a few selected model genes we request and analyzed T-DNA insertional mutants. We also analyzed the subcellular localization of the corresponding proteins, by visualizing the GFP fusion proteins in agroinfiltrated tobacco leaves by confocal microscopy. [This work was supported by research grant 1060494 from Fondecyt-CONICYT, Chile].

PL-P26
CROSS-TALK BETWEEN BIOTIC AND ABIOTIC STRESS IN ARABIDOPSIS THALIANA

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Oxidative stress, arising from an imbalance in the generation and removal of reactive oxygen species (ROS), is a challenge faced by all aerobic organisms. In this study we used the GeneChip technology in order to monitor gene expression of the entire genome of *A. thaliana* under oxidative stress generated by MV. Using MapMan software genes were categorized according to their function in the plant cell. Among the highly induced genes were a number of genes related with receptor kinases, calcium regulation, protein modification and transcription factors. The WRKY family of transcription factors was significantly up regulated during MV treatment. We further studied *WRKY30* which was highly expressed under this MV treatment. Its expression profile was studied *in silico* using Genevestigator database and *in vivo* with the reporter gene GUS fused to the *WRKY30* promoter. *WRKY30* was clearly induced under biotic or abiotic oxidative stress conditions. The convergence of these stresses was also analysed by cross tolerance experiments carried out with Arabidopsis plants. Results showed that there are multiple stress perception and signalling pathways, some of which are specific, but others may cross-talk at various steps. WRKY30 could be a node of convergence for integrating biotic and abiotic signalling events, regulating downstream genes involved in the defense against ROS damage.

PL-P27
MULTIPLE SILENCING OF DIFFERENT TOSPOVIRUSES NUCLEOCAPSID GENES BY A SINGLE hpRNA CONSTRUCT

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Tospovirus is a genus of plant-infecting viruses that causes significant economic losses in a wide range of crops. Today, conventional control methods of these viruses are limited and ineffective. We attempted a multiple virus control based on RNAi mechanism. We chose to target the three tospovirus species present in Argentina: *Tomato spotted wilt virus* (TSWV), *Tomato chlorotic spot virus* (TCSV) and *Groundnut ringspot virus* (GRSV). To generate a hairpin-RNA construct (hpRNAc) we determined and cloned a highly conserved 173bp sequence with 97% identity from the nucleocapsid gene of TSWV, arranged it invertedly flanking an intron and inserted this cassette between the 35S promoter and the NOS terminator. To test this hpRNAc we developed 3 fusion protein constructs between each viral N gene and the GFP coding sequence (N-GFPc). Each N-GFPc alone or combined with the hpRNAc were agroinoculated into *N. benthamiana* leaves. Neither N protein, nor GFP expression was detected by DAS-ELISA test and under UV light when any of the N-GFPc were co-agroinfiltrated with the hpRNAc. These results demonstrate the efficiency of the latter to generate a broad viral gene silencing. The new technology presented here based on sequence homology dependant degradation of multiple genes by a single hpRNAc is an efficient and safe tool for simultaneous molecular control of different species of plant viruses.

PL-P28

COMPARATIVE AND FUNCTIONAL GENOMICS APPLIED TO THE STUDY OF PVX PATHOGENESIS IN SOLANUM TUBEROSUM

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Several resistance genes, including R1 against Phytophthora infestans and Nb against PVX-ROTH1, were previously mapped to a resistance gene cluster on the short arm of potato chromosome V. To explore the functional and evolutionary significance of clustering in the generation of novel disease-resistance genes, a physical map of the R1-Nb region was constructed for each of the three genomes of Solanum demissum. Sequence analysis of the region, predicts the presence of more than 40 resistance genes candidates (RGCs) belonging to distinct resistance-gene families: R1, Prf and Bs4. The S. demissum Bs4 homologue, is a highly structurally conserved Type II gene, and its localization is coincident with the position of Nb. Thus, we hypothesized that the Bs4 homologue present in S. tuberosum cv Pentland Ivory is a candidate Nb gene. To test these, we mapped the RGCs-Bs4 in a recombinant population of Nb. Genetic and molecular analysis show that one of the sequences belongs to Nb. We developed an agroinfiltration system to asses the transient expression of the viral elicitor, the 25 kDa protein. We analyzed the biochemical and molecular changes triggered by defense response such as the level of reactive oxygen intermediates and the expression of PR-1a in resistant and susceptible plants. Our results suggest that this system can be used for the functional complementation analysis of Nb-RGC.

PL-P29

ROLE OF XANTHAN GUM IN THE INTERACTION OF XANTHOMONAS AXONOPODIS PV. CITRI AND CITRUS LIMON

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The phytopathogenic bacterium Xanthomonas axonopodis pv. citri (Xac) is responsible for the canker disease affecting citrus plants throughout the world. Previously, we had shown that Xac biofilm structure was dramatically influenced by xanthan production in vitro. In this work, we demonstrated that xanthan is essential for bacterial attachment, epiphytic growth and survival on lemon leaves. We used confocal laser scanning microscopy to analyze lemon leaves infected with GFP-labeled Xac cells in order to test how closely biofilm formation on abiotic surfaces mimics pathogen-induced canker development in citrus plants. As we observed in the in vitro experiments, the xanthan-deficient mutant (MgumB) grows as single cells and does not induce microcolonies on lemon leaves during the length of experiment, indicating that xanthan affects aggregate formation. Coinoculation of wild-type Xanthomonas campestris pv. campestris (Xcc) and the Xac gum mutant onto lemon leaves reverted the canker phenotype, confirming the role of the EPS in canker development. We analyzed the biochemical and molecular changes triggered by Xac and Xac MgumB in Citrus limon plants, such as deposition of callose and expression of pathogenicity related genes (PR-1a). Our preliminary results suggest that xanthan are able to suppress, in earliest stages of infection, the defense response in lemon leaves.

PL-P30
ANALYSIS OF A SUNFLOWER LIPID TRANSFER PROTEIN BOUND TO PLASMA MEMBRANES

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Lipid transfer proteins (LTPs) are extracellular low-molecular-mass proteins extensively studied in higher plants. Our group has characterized a sunflower LTP named Ha-AP10 that exhibit apoplastic localization and antifungal activity. We have previously anticipated that a fraction of the extracellular Ha-AP10 pool was peripherically bound to microsomal membranes. To determine if Ha-AP10 was bound to plasma membranes we perform plasma membrane isolation by two-phase partitioning in Dextran-Polyethylenglicol. Western blot analysis revealed that Ha-AP10 was present in the plasma membrane enriched fraction. In order to verify the enrichment of the upper phase in plasma membranes, we achieve a western blot analysis of both upper and lower phases using an antibody anti-plasma membrane-V-ATPase. The results demonstrate that the upper phase was effectively enriched in plasma membranes. To bear out the absence of contamination of the plasma membrane fraction with other microsomal membranes we evaluate the activity of enzymatic markers. The outcome of this assay revealed the absence of contamination of the plasma membrane fraction with tonoplast nor mitochondria, so we conclude that Ha-AP10 was effectively bound to plasma membranes. In addition, treatment of sunflower seeds with several plant hormones revealed changes in the accumulation of Ha-AP10. Assays are in progress to determine the biological meaning of this finding.

PL-P31
IDENTIFICATION OF SEPTORIA TRITICI CONIDIOSPORES PROTEINS DEGRADED BY WHEAT LEAF APOPLAST PROTEASES

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We have previously described a wheat leaf intercellular fluid serine protease that blocks *Septoria tritici* conidiospore germination. Thus, it controls the wheat leaf blotch caused by *S. tritici* in resistant cultivars. Based on these results, the identity of *S. tritici* spore proteins degraded by the intercellular fluid serine protease was explored. For it, 2D SDS-PAGE patterns of spore protein extracts incubated with the intercellular fluid at 37 °C for 3 hours were analyzed. Degradation of several spore proteins was detected. The identity of these proteins, as well of other nondegraded, was examined by HPLC - Mass spectrometry followed by alignment of sequences in databases. Results suggest that main protease substrate is 2-oxoglutarate dehydrogenase. It comprises multiple copies of three enzymes which are analogues of those of pyruvate dehydrogenase. Conversely, aldehyde dehydrogenase plus a still nonidentified spot were main stable proteins. Corroboration of these identifications and the posterior analysis of other proteins separated by 2D SDS-PAGE will allow advancing significantly in understanding the defense of wheat against *S. tritici*. [Financed by ANPCyT, CONICET, UNMdP].

PL-P32
ANTIOXIDANT ACTIVITY AND SA ACCUMULATION ASSOCIATED TO THE DEFENSE RESPONSE IN STRAWBERRY

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Plant resistance to hemibiotrophic fungi is a poorly understood process. In a previous study, we found that the isolate M23 of *Colletotrichum* presents an incompatible interaction with *Fragaria ananassa* cv Pájaro. To isolate genes differentially induced in the incompatible interaction we have used mRNA differential display. This technique was performed with total RNA prepared from strawberry leaves 48 hai. The cDNAs of interest were excised from the gel, reamplified, cloned and sequenced. One clone named I(6,5)2B carrying an insert of 288 bp showed 50% identity with GST 18 from *Glycine max* and other plant species. An increase of GSTs expression after pathogen attack was reported in several plant-pathogen interactions. It has also been postulated that antioxidant activity of GSTs plays an important role in limiting the damage caused by ROS. Positive DAB and NBT staining were observed in strawberry leaves 2-4 hai with M23. Leaves extracts were assessed for TBA/MDA reaction showing peaks at 4 and 24 hai and a decrease 48 hai, accompanied by ascorbate peroxidase activity. Results also revealed a peak of free and conjugated salicylic acid (SA) at 48 hai in phloem exudates. Taken together these results suggest the involvement of complex redox regulation in the establishment of the defense response in strawberry.

PL-P33
A XANTHOMONAS AXONOPODIS PV. CITRI PLANT NATRIURETIC PEPTIDE-LIKE PROTEIN INVOLVED IN PATHOGENICITY

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Xanthomonas axonopodis pv. *citri* (Xac) is the casual agent of citrus canker, which affects most commercial citrus cultivars resulting in significant losses worldwide. The Xac genome has been sequenced, although some conserved genes involved in plant-Xac interactions have been characterized, little is known about many Xac hypothetical genes. Xac has an hypothetical protein, only found in this bacteria (XacPNP), with sequence similarity to plant natriuretic peptides (PNPs), a class of peptide hormones implicated in the regulation of cell homeostasis. We observed an increase of XacPNP mRNA levels in a medium that mimics the intercellular space of leaves, suggesting a role for XacPNP in pathogenicity. To evaluate the participation of XacPNP in this process, we constructed a XacPNP deletion mutant. The lesions produced by this mutant were less rough and more necrotic than the lesions observed with Xac. Moreover, when we over-expressed XacPNP in Xac or in *X. campestris* pv. *vesicatoria* the resulting transgenic bacteria caused less necrotic lesions in their host plants than wild type bacteria. All these transgenic bacteria showed the same hypersensitive response in non-host plants. Our results suggest that XacPNP is involved in Xac pathogenicity interfering with plant tissue necrosis allowing a prolonged survival of plant cells and thus, maintaining the interaction with non-dead tissue.

PL-P34
BIOINFORMATIC ANALYSIS OF A POTATO DEFENSOME

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One important disease of the potato tubers 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. In this work we report the identification and analysis of 500 cDNAs detected during the screening. Approximately 95% of the cDNA sequences showed similarity to previously described genes. They were classified according to Arabidopsis thaliana functional categories. Genes involved in basic cellular processes: metabolism and protein synthesis represented 28%. Genes involved in cell regulation processes: folding and protein degradation (8%) transcription and RNA processing (6%) and signal transduction (4%), were also represented. Genes with functions related to stress and cell defense comprised 19% of the cDNAs. Transport, energy and cellular organization and biogenesis were the least represented categories. 24% of the cDNAs comprised putative proteins with unknown functions. Taken together these results give a preliminary and global picture of the mechanism that activate in potato tuber during Fusarium eumartii attack. In addition, this collection constitutes a rich source of defense/stress-related and regulatory genes worth to be further studied. [Supported by UNMdP, CONICET and ANPCyT].

PL-P35
POTENTIAL EFFECTS OF PROLINE ACCUMULATION IN PLANT-PATHOGEN INTERACTIONS

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The Hypersensitive Response (HR) is a defense program stimulated upon recognition of avirulent pathogens that triggers the apoptosis of infected cells. We have recently found that tissues developing HR accumulate high levels of L-proline, although we do not know if this response contributes to activate defenses. We here evaluate two possible consequences of proline increase in HR developing tissues: a) avoidance of pathogen proliferation by rise of extracellular proline levels and consequent reduction of apoplastic water potential; b) enhancement of proline degradation and accumulation of pro-apoptotic derivatives. Both effects were analyzed in *Arabidopsis thaliana* leaf tissues infiltrated with virulent/ avirulent races of *Pseudomonas syringae*. We found that the levels of apoplastic proline accumulated in HR were not sufficient to inhibit bacterial growth *in vitro*. On the other hand, we detected transcriptional alterations of genes controlling proline degradation that suggest accumulation of pro-apoptotic compounds at initial steps of HR. In addition, treatment with salicylic acid (SA), an inducer of defenses, stimulates identical behaviour of these catabolic genes. These results suggest that proline degradation may contribute to signal cell death in HR involving SA-dependent responses.

PL-P36
TOMATO EXPRESS A DIR1-LIKE PROTEIN, AN ORTHOLOG OF A GENE INVOLVED IN SYSTEMIC ACQUIRED RESISTANCE

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A novel gene called dir1 has been involved in the establishment of the systemic acquired resistance (SAR) in the model plant *Arabidopsis thaliana*. This gene displays the signature of Lipid Transfer Proteins (LTPs) and is responsible for the production and/or transport of the SAR mobile signal through the plant. However, the mechanism of action of DIR1 is still unknown. The aim of this work was to detect and characterize a putative dir1 ortholog from tomato (*Lycopersicon esculentum*). Data mining allowed the detection of four tomato unigenes similar to *Arabidospsis* dir1 (AF342726). A detailed analysis in silico was undertaken in order to characterize each unigene and identify the putative ortholog. Hence, an EST (TC 159463), associated to one of the genes, was selected taking into account the properties of the putative protein that include the molecular mass (7 kDa), the presence of the LTP signature, the hydrophobic profile, and the acidic pI (3.8). The sequence of this dir1-like cDNA let us to design an antigenic peptide that was further used to develop polyclonal antibodies. Western blot analyses revealed a 7 kDa peptide in leaf apoplastic fluids, thus confirming the existence of a dir1-like protein in tomato. Studies are in progress to analyze the pattern of expression and mode of action of DIR1.

PL-P37
TOWARD DECIPHERING NITRIC OXIDE FUNCTION IN AUXIN SIGNALING PATHWAY

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Nitric oxide (NO) is an important signaling molecule involved in a diverse array of physiological processes. However, the mechanism by which NO could be acting in the auxin induced responses remains unknown. It has been already reported that auxins regulate plant developmental processes through its ability to specifically bind to TIR1 (F-box protein of the ubiquitin ligase complex, SCF^{TIR}) promoting Aux/IAA-TIR1 interaction. Previously, we demonstrated that exogenous indole-acetic acid (IAA) increased NO levels in Arabidopsis seedling roots. In addition, the treatment of the transgenic Arabidopsis line BA::GUS with IAA and NO donors overinduced the promoter actvity of BA3 auxin-response element in a dose response maneer. GST pull down assays indicated that NO donors promotes Aux/IAA-TIR1 interaction. In this way, our challenging was to understand the action of NO on Aux/IAA-TIR1 interaction. Thinking that NO regulates this process via direct reaction (S-nitrosylation) with cysteines residues of the TIR1 protein, we tested the effects of cisteine-modifying agents (DTT and NEM) on IAA responses. These compounds blocked the IAA-mediated induction of GUS expresion as well as different physiological IAA regulated responses. The precisely role of NO in the auxin signaling is now revealing. [Supported by UNMdP, CONICET and ANPCyT].

PL-P38
EXTRACELLULAR ATP INDUCES NO PRODUCTION IN TOBACCO CULTURED BY2 CELLS

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ATP is a vital molecule used by living organisms as a universal source of energy. In animals, extracellular ATP (eATP) is a well documented regultatory signal involved in neurotransmissions, muscle contraction, regulation of blood preasure and immune responses. On the other hand, nitric oxide (NO) is a bioactive molecule that exerts a number of diverse signal functions in phylogenetically distant species. Studies in animal systems have revealed that guanylil cyclase is an ATP sensor coupling NO signaling to cell metabolism. It is also known that ATP and NO modulate intracellular calcium in isolated cells. Recent findings revelead that intact plant tissues release ATP to the extracellular matrix and this eATP functions as an endogenous external metabolite regulating plant cell viability. In this work, we report for the first time that eATP treatments (ranging from 0.5µM till 10 mM) induces a burst of NO within minutes (starting at 15 min till 2 h) in BY2 cells. These observations allowed us to hypothesize that both eATP and NO might have common signaling pathways in plants. Preliminary results supporting this work will be presented and discussed. [Supported by ANPCyT, CONICET, UNMDP].

PL-P39
IRON DEFICIENCY-INDUCED RESPONSES IN *ARABIDOPSIS THALIANA* ROOTS: ROLE OF ETHYLENE

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A low iron availability in soil generates a series of plant root responses that lead to the increase of iron uptake. Fe^{+3}-reductase (FRO2) under the control of the transcription factor (FIT1) and morphologic changes in roots have been described to be induced by iron deficiency. Since different reports have involved nitric oxide (NO) and ethylene in plant iron metabolism, we decided to study the role of ethylene and its crosstalk with NO in plant root responses to iron deficiency. Plants of *Arabidopsis thaliana* grown in presence or absence of iron were treated with ethephon (ethylene donor) or $CoCl_2$ (ethylene synthesis inhibitor). The morphologic analysis of the roots showed that depletion of endogenous ethylene completely inhibited roots hairs development. NO production in roots was increased by ethylene treatment and decreased when adding $CoCl_2$. Beside, FRO2 and FIT1 expression were evaluated by semiquantitative RT-PCR. Results indicated that, while $CoCl_2$ did not affect the FRO2 and FIT1 mRNA levels in presence or absence of iron, the ethylene addition decreased those levels. In conclusion, ethylene would play a role in root hairs development and NO production and, at less extent, would also influence FRO2 and FIT1 expression as well as FRO2 activity. [*These authors have contributed equally to this work].

PL-P40

NITRIC OXIDE (NO) PROTECTIVE EFFECT IN UV-B IRRADIATED MAIZE SEEDLING

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To determine the role of NO in the protection of plants against UV-B, maize seedlings were maintained for 10 days in normal conditions (H), or were pre-treated with Sodium Nitropruside (SNP), a NO donor (N). After that, H and N seedlings were irradiated 8 h with 3 W/m2 of UV-B (Hi and Ni respectively). Twenty four hours after the UV-B irradiation, Hi and Ni seedlings displayed the same macroscopic aspect than non-irradiated ones. However, DAB staining showed a high concentration of reactive oxygen species (ROS) in Hi seedlings. Ni seedlings contained a greater concentration of NO detected with the fluorescent probe DAF-FM. and a strong reduction in the ROS amount compared to Hi. Five days after the UV irradiation, Hi seedlings displayed an important reduction of the leaves length, an increase of the cellular permeability and a high ROS content. Those effects were considerably attenuated in Ni. Five days after the UV irradiation, flavonoids in organic solvent extracts increase 25% in Hi and 50% in Ni, and anthocyanins increase 41% in Hi and 82% in Ni respect to the non-irradiated controls. Seedlings with alterations in flavonoids and anthocyanins synthesis did not respond to SNP. Our results indicate that NO reduces the oxidative stress generated by UV-B, scavenging the ROS and increasing the concentration of other ROS sacavengers like flavonoids and anthocyanins.

PL-P41

NITRIC OXIDE MODULATES THE INDUCTION OF FRO2 AND FIT1 IN IRON-DEFICIENT *ARABIDOPSIS THALIANA*

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Iron (Fe) is an essential mineral for plants and its deficiency is associated with the presence of insoluble forms in soil. To solve this, plants develope strategies to improve the Fe uptake. The molecular responses under Fe deficiency in *Arabidopsis thaliana* involve the induction of Fe^{+3}-reductase (FRO2), ion metal transporter (IRT1) and the transcription factor FIT1, that regulates FRO2. Nitric oxide (NO), synthesized by the nitrate reductase (NR) and other enzymes, has emerged as a signaling molecule in Fe-deficiency responses in tomato. The aim of this work was to know the role of NO in regulating the expression of FRO2 and FIT1 in roots of Fe-deficient *A. thaliana*. Plants were treated with the NO donor nitrosoglutathione (GSNO) or inhibitor of NR, tungstate (Tg) and the expression of FRO2 and FIT1 was analyzed by RT-PCR. The induction of FRO2 was enhanced by GSNO only in Fe-deficient plants and was not inhibited by Tg. However, the activity of Fe-reductases was increased by GSNO and reduced by Tg. Microscopic observation revealed an increase in NO content in Fe-deficient roots. Tg-treated roots diminished NO levels suggesting that NR activity is the main source of NO in *A. thaliana* during Fe-deficiency. These results support a role for NO in regulating the molecular response of *A. thaliana* to Fe-deficiency. [*These authors have equally contributed to the work].

PL-P42
AUXIN AND NITRIC OXIDE IN PLANTS RESPONSES TO IRON DEFICIENCY

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Iron (Fe) is an essential micronutrient for plants. *Arabidopsis thaliana* responds whit the type I strategy in Fe-deficient conditions. Strategy I response involves an induction of H^+-ATPase (AHA2), ferric-chelate reductase (FRO2) and an iron transporter (IRT1). It is known that many features of root physiology are controlled by hormones. Among them, auxin effects have been extensively studied in different root systems. The aim of this work was to study the role of auxin in physiological, morphological and molecular responses induced by Fe-deficiency in *Arabidpsis thaliana* roots. Thereby, *Arabidposis* seedlings were treated with the auxin naftil acetic acid (NAA) and the auxin transport inhibitor N-1-naphthylphthalamic acid (NPA). Seedlings were grown under Fe-deficient or –sufficient conditions. Treatments with NPA indicate that increase of both nitric oxide (NO) levels and root hair density in response to Fe-deficiency, are under endogenous auxin control. In addition, results obtained by analyzing FRO2 activity and expression by semi-quantitative RT-PCR, indicate that both parameters are also under endogenous auxin control. It is concluded that endogenous auxin might be involved in *A. thaliana* root responses during Fe-deficiency. [1 contributed equally to this work].

PL-P43
AtMBF1 GENES PARTICIPATE DURING THE GERMINTAION PROCESS MEDIATED BY ABA

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Multiprotein bridging factor 1 (MBF1) is known to be a transcriptional co-activator that mediates transcriptional activation by bridging between an activator and TATA-box binding protein (TBP). MBF1 genes from *Arabidopsis thaliana* (AtMBF1a, AtMBF1b, and AtMBF1c) show distinct tissue-specific expression patterns and responses to phytohormones. In this study, we carried out the analysis of different *A. thaliana* MBF1 mutant lines: T-DNA insertion mutant (Syngenta collection) and transgenic over-expressing lines for the three AtMBF1 genes (a, b or c). AtMBF1abc triple mutant seeds, were treated with different phytohormones (ABA, SA, Ethephon). After ABA treatments triple mutant seeds showed a delay in the germination rate compared to the wild type ones. However, the over-expressing lines (35S::AtMBF1 a, b and c) did not show differences on germination compared to the wild type under the doses tested (0 till 2.5 uM ABA). On the other hand, SA or ethephon treatments did not alter the germination responses. All these findings support a potential function of AtMBF1 genes on the control of germination mediated by ABA. Supported by ANPCyT, CONICET, UNMDP.

PL-P44
ENHANCED VITAMIN C PRODUCTION IN TOMATO HAIRY ROOTS BY PRECURSOR FEEDING

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We have studied the effects of sugar precursors such us D-galactose (Gal) and D-galacturonic acid (GalUA) on ascorbic acid (AsA) production in tomato hairy roots (HR). A wild type HR clone and a transgenic UR4 HR clone were elicitated. UR4 overexpresses *GalUR* gene which encodes a GalUA reductase that is involved in an alternative pathway of AsA biosynthesis described in fruits. WT and UR4 were treated with 30 mM Gal and 30 mM GalUA on exponential growth phase. A detrimental effect on biomass growth and darkening of the roots was observed in both treatments 96 h post-elicitation. UR4 clone elicited with GalUA or Gal showed the highest AsA content at 168 h post-elicitation. In UR4, GalUA induced an increase of about 100% respect to UR4 controls treated with distilled sterile water, 200% respect to GalUA elicited WT HR and of 260% respect to WT HR control. On the other hand Gal induced a similar effect in WT HR, an AsA pool increase since 16 h, reaching a maximal value at 96 h post-elicitation (about 100% higher than WT HR controls treated with distilled sterile water). Our results evidence that *in situ* and *de novo* AsA biosynthesis in HR occurs via uronic acid pathways; that sugar precursors availability may be a limiting factor in AsA production in HR and that it can be enhanced (about 3-fold) by overexpression of *GalUR* combined with precursor feeding.

PL-P45
HYOSCYAMINE-6-β-HYDROXYLASE GENE: OBTENTION OF RECOMBINANT HAIRY ROOTS OF *Brugmansia candida*

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Current experimental work is being performed in order to obtain hairy roots of *Brugmansia candida* that over-express h6h cDNA and the enzyme H6H. The cDNA encoding H6H was isolated and cloned from anthers of *B. candida* flowering plants. The gene was cloned in the vector pJIT with the double promoter CAMV35S and nos terminator. The size of the insert was 1.1 Kb, encoding a sequence of 344 amino acids that was highly homologous to the same enzyme derived from other Solanaceae. *Agrobacterium rhizogenes* LBA 9402 was transformed with this constructs. The colonies were screened by subculture in YMB medium with rifampycin (5 mg/L), obtaining the strain LBA-H6H. Plants of *B. candida* maintained in vitro were used to infect them with *A. rhizogenes* strains LBA 9402 and LBA-H6H. Subsequently, *A. rhizogenes* strains LBA9402-PR and 15834-PR (gift of Dr. Rocha) and LBA 9402-Hn (gift of Dr. Oksman-Caldentey) were used in order to obtain HR lines over-expressing cDNA for H6H from others Solanacea. The induction of hairy roots from B. candida plants was successful (80%). The hairy roots that appeared at the infection sites were excised and cultured individually on B5/2 liquid medium supplemented with sucrose 15 g/l, ampicillin 2 g/l, rifampycine 5 mg/L, kanamycin 30 mg/L and agar 8 g/l. We are now in the process of choosing the clones based on their growth index and alkaloid production.

PL-P46

SEARCHING FOR A PFT1 ROLE IN THE REGULATION OF FLOWERING TIME

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Our aim is to understand the biochemical role of PFT1 (PHYTOCHROME AND FLOWERING TIME 1), a gene that plays a central role in the regulation of flowering time by light quality. PFT1 might have a transcriptional role. It is a nuclear protein that has a C-terminal glutamine rich region, activates transcription in yeast when fused to a DNA binding domain and regulates FT mRNA levels. PFT1 has also homology with a component of the Human Mediator Complex, a high molecular weight complex known to mediate between specific transcription factors and the basal transcriptional machinery. PFT1 has no obvious DNA binding domain, so it might require other proteins to bind DNA. We followed two approaches to understand the PFT1 role; i) we screened for PFT1 interacting proteins using the two hybrid technology and ii) generated transgenic plants with PFT1 fusions to a variety of tags, TAP tag, HA and the GR (glucocorticoid receptor) with the aim of purifying PFT1 from plant tissues. We isolated two RING-finger proteins that interact with PFT1 and generated double knockout plants that seem to flower as late as the pft-1 mutant. The tagged versions of PFT1 are functional "in vivo". We determined by size exclusion chromatography that PFT1 could be part of a high molecular weight protein complex. We are planning to characterize this complex and to study its targets using the inducible GR-PFT1 fusions.

PL-P47

Lic1, A NEW MUTANT AFFECTING LIGHT SIGNALLING AND CLOCK FUNCTION IN ARABIDOPSIS

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Circadian clocks allow plants to adjust multiple physiological and developmental processes in anticipation of daily and seasonal changes in the environment. A feedback loop composed of cycling gene products that control their own synthesis has been suggested as the central oscillatory mechanism. The clock is synchronized on a daily basis by light cycles perceived by phytochromes and cryptochromes, but the signalling pathway connecting these photoreceptors to the clock is largely ignored. We have conducted a high-throughput screening using *Arabidopsis thaliana* as a model system to identify novel components of the circadian signalling network. Here we report the characterization of a novel mutant, *lic1* (Light Input to the Clock 1), in which the circadian period of the rhythm of leaf movement is lengthened compared to the wild type. The *lic1* mutant is also defective in light dependent responses across multiple wavelengths, suggesting that it may encode a signalling component that integrates light perceived through multiple photoreceptors into the clock. The regulation of flowering time is also disrupted in the *lic1*mutant, which flowers later than the wild-type. The pattern of expression of clock associated and flowering time genes in lic1 will be presented. Molecular mechanisms underlying the circadian and flowering time phenotypes will be discussed.

PL-P48

ECTOPIC EXPRESSION OF GAMMA CARBONIC ANHYDRASES AFFECTS RESPIRATION RATES IN PLANTS

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Arabidopsis thaliana γ-Carbonic Anhydrase 2 (AtγCA2) is a nuclear encoded member of a small protein family present in mitochondria. The submitochondrial localization of these proteins has been established as structural subunits of complex I (NADH:ubiquinone oxidoreductase) forming a plant-specific extra domain. Transgenic plants containing gammaCA2 under the control of 35S promoter express this protein ectopically and show dramatic phenotypic changes causing male sterility by indehiscence of anthers. Western blotting analyses on mitochondrial fractions show increased levels of this protein imported into the mitochondria in derived cell cultures. To analyse this phenotype in more detail, physiological parameters were measured. While CO2 assimilation was unchanged, respiration rate was affected. Reactive Oxygen Species (ROS) production was strongly affected both, in leaves as well as in flowers of transgenic plants. This result is consistent with the variation in respiration rates measured in transgenic leaves as O2 consumption. Transcript coding for proteins involved in redox status and alternative respiration were as well monitored. These data demonstrate the relevance of complex I gammaCAs in mitochondrial respiration.

PL-P49

ANALYSIS OF Arabidopsis thaliana GAMMA CARBONIC ANHIDRASE 2 PROMOTER USING REPORTER GENES

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Arabidopsis thaliana γ-Carbonic Anhydrase 2 (AtγCA2) is a nuclear encoded member of a small protein family present in mitochondria. The submitochondrial localization of these proteins has been established as structural subunits of complex I (NADH:ubiquinone oxidoreductase) forming a plant-specific extra domain. Previous studies by in situ hybridization, and RT-PCR showed that gammaCA2 is expressed mainly in flowers, specifically in the tapetum of anthers. In this work, three AtγCA2 promoter deletions were fused to the reporter GUS gene. AtγCA2 promoter 2000 bp-, 1500 bp-, 500 bp- and 300 bp-GUS constructs were used to transform Arabidopsis by floral dip methods. Whole transgenic seedlings, adult leaves, roots and flowers were analysed for in situ GUS expression. Results showed that transcriptional activation of gammaCA2 promoter is affected in the different tissues and deletions analyzed, suggesting the presence of several tissue-specific regulatory elements.

PL-P50
INDUCTION OF GENES INVOLVED IN ANAPLEROTIC PATHWAYS IN MALE STERILE A. THALIANA

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Mitochondrial retrograde signaling is a pathway of communication from mitochondria to nucleus under normal and abnormal conditions. It involves multiple factors that sense and transmit mitochondrial signals to make changes in nuclear gene expression. These changes lead to a reconfiguration of the metabolism to accommodate cells to defects in mitochondria. To study the effect of a mitochondrial dysfunction induced by the expression of the unedited form of the subunit 9 of ATP synthase gene (u-ATP9) in Arabidopsis, we constructed transgenic plants expressing u-ATP9 under the control of two different floral promoters: Apetala3 and A9. Both plants expressing u-ATP9 exhibited a male sterile phenotype. Using microarrays, we analyzed the transcriptome changes in Arabidopsis AP3- and A9-u-ATP9 vs. wt plants. We found altered levels of several genes codifying for enzymes involved in glycolysis and Krebs cycle, such as two up-regulated malate dehydrogenases (At5g43330 and At1g04410), and down-regulation of a pyruvate decarboxylase (At5g54960). In agreement with the microarray data, we confirmed these results by RT-PCR and also correlate with changes in their enzymatic activity. This differential activity seems to enhance the production of energy under reduced pO2 as a hallmark of the hypoxic response.

PL-P51
A SIMPLY METHOD FOR THE ADDITION OF ROTENONE IN ARABIDOPSIS LEAVES

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The NADH:ubiquinone oxidoreductase (complex I), a component of the respiratory chain, is a large multi-subunit complex and has been well characterized in bacteria, fungi, mammals and plants. Mechanistic studies showed that rotenone block the electron transport between a Fe-S cluster and ubiquinone. It has been described that several genes participating in protein import to mitochondria, molecular chaperones and genes involved in respiratory chain assembly are highly expressed after treatment of Arabidopsis cell cultures with rotenone. However, some methodological problems remain to be solved when used rotenone directly on Arabidopsis leaves instead of cell cultures. In the present work, we report a simple procedure for the treatment of Arabidopsis leaves with rotenone. The usefulness of the procedure was determined by the analysis of the expression two genes: COX19, involved in respiratory chain assembly and known to be induced in Arabidopsis cell cultures after rotenone treatment, and AOX, showing no alteration in the presence of rotenone. Results show that treated leaves have an increased expression of COX19 and no effects on AOX transcription. Moreover, rotenone/Triton X-100 incubated leaves presented an inhibition of oxygen uptake. The simplicity of the procedure shows this methodology useful for studying the effect of the addition of rotenone to a photosynthetic tissue in situ.

PL-P52
ROLE OR SITE II MOTIFS IN MITOCHONDRIAL CYTOCHROME C OXIDASE GENE PROMOTERS

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Cytochrome c oxidase (COX) is composed of several subunits, 3 of them encoded by the mitochondrial genome and the rest encoded by the nuclear genome. In this work, we study the structure of the single COX6a and the three COX6b gene promoters from Arabidopsis thaliana. All of these promoters contain copies of a motif named site II (TGGGCC/T) involved in expression in meristematic cells in other genes. We obtained transgenic plants that express the gus -glucuronidase) gene under the control of COX6a and COX6b promoter sequencesβ(and analyzed GUS enzymatic activity by histochemical assays. Transgenic plants that included COX6a promoter sequences revealed GUS activity only in pollen grains. The COX6b1/3 lines showed similar expression patterns in roots, cotyledon veins, leaf veins, shoot apical meristem and pollen. Deletions of the respective promoters down to approximately -200 (a fragment that included the site II motifs) produced plants with the same expression patterns as those observed with larger fragments. In transgenic plants that contained COX6a and COX6b promoters with mutations in the site II motifs the expression disappeared or was extremely low. The results suggest that site II motifs are essential for expression of all Arabidopsis COX6a and COX6b genes. These motifs may participate in the coordinate regulation of the expression of these genes.

PL-P53
ANALYSIS OF THE COX5B-1 PROMOTER FROM ARABIDOPSIS THALIANA

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The promoter of the Arabidopsis thaliana nuclear gene COX5b-1 (At3g15640), encoding subunit 5b of mitochondrial cytochrome c oxidase, was analyzed using plants transformed with different promoter fragments fused to the -glucuronidase reporter gene. In a previous work, histochemical stainingβ indicated that the COX5b-1 promoter directs tissue-specific expression (meristems, anthers, pollen and vascular tissues of cotyledons, roots and hypocotyls). In this work, the analysis of progressive upstream deletions showed that a fragment located between nucleotides -328 and -259 from the translation start site is required for expression in vegetative tissues. Mutagenic analysis of this fragment revealed that the segment located between nucleotides –298 and –288 was absolutely required for expression in vegetative tissues and the presence of several elements required for maximal expression. Site-directed mutagenesis of a G-box (CACGTG) element present at -228 completely abolished expression of a reporter gene in all organs/tissues. Electrophoretic mobility shift assays showed that proteins present in cauliflower nuclear extracts were able to specifically bind to the G-box element. These observations suggest that the G-box element is essential for COX5b-1 expression and that additional cis elements determine the expression patterns of this gene in vegetative tissues.

PL-P54

THE PROMOTERS OF GENES ENCODING THE ARABIDOPSIS COPPER CHAPERONE ATCOX17 ARE INDUCED BY STRESS

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AtCOX17 genes encode Arabidopsis thaliana homologues of the yeast metallochaperone Cox17p, involved in the delivery of copper for cytochrome c oxidase assembly. Two different AtCOX17 genes, located in chromosomes 1 and 3, are present in the Arabidopsis genome. Sequences upstream from the translation start sites of AtCOX17 genes, which include an intron located in the 5' leader region, were introduced into plants in front of the gus gene. For both genes, expression was localized preferentially in young roots, anthers and siliques, but almost 10-fold higher GUS activity levels were observed in plants transformed with AtCOX17-1 upstream regions. Both promoters were induced to different extents by wounding, treatment of leaves with the bacterial pathogen Pseudomonas syringae, incubation with agents that produce oxidative stress and metals. AtCOX17-2 showed similar responses to these factors, while AtCOX17-1 was more strongly induced by relatively low (10-100 µM) copper. The results indicate that both AtCOX17 genes have similar, though not identical, expression characteritics and suggest the existence in their promoters of elements involved in tissue-specific expression and in response to factors that may produce mitochondrial damage.

PL-P55

CLONING, EXPRESSION AND CHARACTERIZATION OF SORBITOL-6-P DEHYDROGENASE FROM APPLE AND PEACH LEAVES

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In addition to sucrose and starch, sorbitol is a major photosynthetic end-product in many economically important fruit-bearing tree species, including apple and peach. Despite this relevance, relatively few studies have been performed on the regulation of sorbitol synthesis. Recently, two enzymes from mature apple leaves were purified and characterized, demonstrating that sorbitol formation is catalysed by the sequential action of sorbitol-6-P dehydrogenase (S6PDHase; Glc6P + NADPH ‹› Sor6P + $NADP^+$), and sorbitol-6-P phosphatase (Sor6P + H2O › sorbitol + Pi). The sugar-alcohol is translocated from mature leaves to growing tissues, such as fruits and young leaves, where it may serve as osmoprotectant, as it occurs with mannitol in celery. In our aim to elucidate the structure to function relationships of S6PDHase, we have cloned the genes encoding for this enzyme from apple and peach leaves. The recombinant proteins were expressed in *Escherichia coli* cells, purified and characterized. The results obtained are in good agreement with the previously reported data. The recombinant enzymes were specific to reversibly reduce Glc6P utilizing NADPH. Also, a 3D model of S6PDHase was built with the SWISS-MODEL server, using the human aldose reductase as template. To the best of our knowledge, this is the first time that S6PDHase is successfully expressed in a prokaryotic system.

PL-P56
CHARACTERIZATION OF STARCH SYNTHASE III FROM A. THALIANA

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Glycogen and starch constitute the main form of storage of energy and carbon compounds in living organisms, including mammals, yeast, bacteria and plants. In plants, starch synthase (SS) catalyzes the elongation of α-(1,4) glucans by adding glucose units from the sugar-nucleotide to the non-reducing end of the growing chain. Five classes of SS were described in higher plants: granule-bound SS (GBSS), and soluble SSs (I, II, III and IV/V). All the SS members share high sequence similarity in the C-terminal catalytic domain which belongs to the glycosyltransferases 5 family (GT5). However, they differ significantly in their N-terminal domain to the extent that no sequence similarity could be found between each other. In particular, SSIII from Arabidopsis thaliana contains an N-terminal transit peptide followed by a non-catalytic SSIII-specific domainand the common C-terminal domain common to all the SS isoforms. In this work, we report the cloning, expression and purification of full length and truncated forms of SSIII from Arabidopsis thaliana. All the proteins were purified to near homogeneity in a one-step procedure. Our results suggest that the N-terminal non-catalytic domain of SSIII play an important role in the modulation of the enzyme activity.

PL-P57
BINDING OF ARABIDOPSIS THALIANA SSIII-SBD TO GLYCOGEN, STARCH AND ITS COMPONENTS

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The starch-synthase III (SSIII, At1g11720), with a total of 1025 residues, is one of the enzymes involved in plant starch synthesis. SSIII from Arabidopsis thaliana contains a putative N-terminal transit peptide followed by a 557-amino acid SSIII specific domain with three internal repeats and a C-terminal catalytic domain of 450 amino acids. Using computational characterization techniques, we showed that each of the three internal repeats encodes a starch-binding domain (SBD). To address whether the different SSIII-SBDs are able to bind to polysaccharides, we cloned and expressed in E. coli cells His-tagged proteins with three (SBD-full), two (SBD-int) or one (SBD-small) SBD domains. The three proteins were purified to near homogeneity by one-step purification using a Ni++ column. SDS-PAGE and western blot analysis revealed the presence of three protein bands of 66, 34 and 15 kDa for each SBD in agreement with the predicted molecular mass. We tested the ability of each SBD to bind to glycogen, amylose and amylopectin using a co-sedimentation assay. Our results showed that each SBD could bind to each polymer with different affinites.

PL-P58
NICOTIANA TABACUM NADP-MALIC ENZYME ISOFORMS: CLONING AND BIOLOGICAL ROLE ANALYSIS

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NADP-Malic Enzyme (NADP-ME, EC 1.1.1.40) catalyzes the oxidative decarboxylation of L-malate producing pyruvate, CO2 and NADPH. In plants, the most studied is the isoform involved in carbon fixation in bundle sheath chloroplasts of some C4 plants and in cytosol of some Crassulacean Acid Metabolism plants (CAM). Although some NADP-MEs have been found in cytosol and plastids of different tissues of C3, C4 and CAM plants, the biological role remains elusive. The presence of a NADP-ME isoform in stems of vascular bundles and petioles of Nicotiana tabacum was proposed to be related to the occurrence of a C4-like cycle. To test this hypothesis and to characterize N. tabacum NADP-ME isoforms, cDNAs encoding two NADP-MEs were isolated from roots, stems, leaves and flowers. The complete cDNAs isolated from leaves (Ntnadp-me 1-2, GenBank DQ923119, DQ923118) were cloned and expressed in E. coli and the proteins are being biochemically characterized. The response of each isoform against different biotic and abiotic stresses in different tissues is being evaluated through Real Time PCR and activity assays. Computational sorting prediction programs indicate that NtNADP-ME1 contains a putative plastidic peptide transit directing the protein to the plastids whereas NtNADP-ME2 do not possess any predicted organellar targeting sequence, being cytosolic.

PL-P59
CLONING OF NADP DEPENDENT MALIC ENZYME FROM C4, C3 AND C3-C4 FLAVERIA SPECIES

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C4 plants have evolved independently from C3 species many times during the evolution. NADP-malic enzyme (NADP-ME) is a widely distributed enzyme involved in different metabolic pathways. The photosynthetic isoform of this enzyme has evolved from non-photosynthetic isoforms. In order to analyze the origin of the NADP-ME C4-specific isoform, cDNAs encoding this enzyme were isolated in the genus Flaveria. This genus is well suited for studying the evolution of photosynthesis because it contains a more or less continuous range of species between C3 and C4, including C3-C4 and C4-like species. In the present work, five cDNAs codifying NADP-ME isoforms were isolated by RT-PCR. Four of these cDNAs correspond to plastidic isoforms from C3 F. pringlei, C3-C4 F. floridana and C4 F. bidentis and F. trinervia species. The remainig cDNA correspond to a cytosolic isoform from F. pringlei. The amplified products were cloned into pGEM-T vector, sequenced and the complete codifying sequences corresponding to mature proteins subcloned into the pET 32 expression vector. These recombinant proteins will be use for kinetic and structural characterizations. In addition, small portions to 3' ends to cDNAs encoding NADP-ME from intermediate species F. anomala, F. palmeri and F. sonorensis were amplified by RT-PCR. These sequences (previously unknown) will be used to obtain completes cDNAs with RACE method.

PL-P60

THE NAD-DEPENDENT MALIC ENZYME OF ARABIDOPSIS THALIANA: TWO PROTEINS WITH DIFFERENT PROPERTIES

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The mitochondrial NAD-dependent malic enzyme of plants (NAD-ME) has been suggested to be a heterodimer of α and β subunits. At present, several isoforms have been purified and characterized, but none of them in a recombinant manner. Alignment analysis based on Solanum tuberosum NAD-ME subunits cDNA sequence showed that Arabidopsis thaliana genome contains two putative nad-me genes. The predicted aminoacid sequences of α and β subunits showed 62% identity. A multiple sequence alignment among plant NAD-ME showed that α and β subunits are clustered in different groups. In this work, cDNA encoding the α and β subunits of the A. thaliana NAD-ME were cloned, expressed in a prokaryotic system and the proteins obtained structurally and biochemically characterized. Contrary to that previously reported, the separated subunits showed NAD-ME activity. The α subunit showed sensitivity to the activator CoA and a dimeric native aggregation state. The β subunit was not activated by CoA and presented a tetrameric native oligomeric state. In order to evaluate the α-β interactions in-vitro, both subunits were also co-expressed. The results indicate the possibility of the existence of two separate proteins with NAD-ME activity within Arabidopsis thaliana mitochondrias.

PL-P61

MECHANISMS OF microRNA PROCESSING IN PLANTS

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MicroRNAs (miRNAs) are non-conding RNAs that regulate gene expression widely distributed in plants and animals. They are small RNAs of ~21 nt that recognize partially complementary sites in target mRNAs and guide them to cleavage or translational arrest. MiRNAs are transcribed as larger precursors that form fold-back structures and are recognized by RNAse III enzymes, which cleave the precursors to release the mature miRNA. The precursor supposedly contains spatial determinants that indicate the position of the miRNA along its sequence. In plants, the biogenesis of miRNAs occurs in the nucleus and the identification of processing intermediates has proven a difficult task. Therefore the mechanism of miRNA processing remains still largely unknown in plants. Currently, we are characterizing the biogenesis of the Arabidopsis thaliana miRNAs miR319, miR172 and miR156 which regulate TCP, AP2 and SPL transcription factors respectively. We have analyzed the miRNA processing in wild type plants, transgenics overexpressing the miRNAs and mutant plants deficient in miRNA biogenesis. We have developed a novel PCR based technique that allows us to map miRNA processing intermediates in vivo. A comparison between miRNA processing in plants and animals will be presented.

PL-P62
SPECIFICITY DETERMINANTS OF microRNA-TARGET INTERACTION IN PLANTS

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MicroRNAs (miRNAs) have been recognized as a novel mechanism to regulate gene expression in multicellular organisms. They are small RNAs of ~21 nucleotides in length that recognize partially complementary sequences in target mRNAs and guide them to cleavage or translational arrest. They have been implicated in many key processes such as development and stress responses. miRNAs are generally encoded by small gene families of identical or similar sequence. It is currently unknown if all members of a certain miRNA family regulate their putative targets with the same efficiency or if there is miRNA sub-functionalization. Although plant miRNAs are largely conserved during evolution we have observed that specific miRNA variants are found only in certain species. We performed a bioinformatic analysis of miRNAs from different plants in order to systematically classify this natural variation. To study the role of these changes during evolution we are modifying Arabidopsis miRNAs in order to express these new variants. These transgenic miRNAs are then expressed ectopically in Arabidopsis and their effects are compared to the wild type versions. We developed a novel technique to detect miRNA cleavage that allows us to differentiate between the activities of some closely related miRNAs in vivo. The effects of small sequence changes on miRNA activity will be discussed.

PL-P63
EXPRESSION AND FUNCTION OF MICRORNA CONTROLLED TCP TRANSCRIPTION FACTORS

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TCPs are a plant specific transcription factor family with 24 members in Arabidopsis thaliana, sharing a TCP domain involved in DNA binding. We have previously reported that five TCP genes are regulated by miRNAs. The jaw-D mutant overexpresses miR-319a, which causes degradation of this group of TCPs, leading to crinkly leaves. We show here that KOs for TCP2, 4 and 10 have larger leaves than wild type. The triple knock out resembles closely the jaw-D phenotype. The TCP2 promoter is active in leaves and floral organs, while the TCP4 promoter is active in the vasculature of cotyledons and young leaves. Expression of the reporters is increased in jaw-D, suggesting that promoter regulation involves a miRNA-dependent feed back loop. To understand the biological role of the TCPs, miRNA resistant TCPs (rTCPs) were generated. Expression of rTCP4 is mostly lethal. rTCP4:GFP plants survive, with smaller and rounder leaves, which is roughly the opposite of jaw-D. We analyzed the genes regulated by the TCPs using a combination of microarray data from different tissues and transgenics. The results indicate the participation of TCPs in JA biosynthesis, a phytohormone involved in wounding, senescence and pathogen response. Moreover, we found that jaw-D leaves have a delay senescence. The results suggest a role of TCPs in many leaf processes.

PL-P64

PHYSICOCHEMICAL PROPERTIES OF AMARANTH GLOBULINS AND THEIR SENSITIVITY TO PROTEASES

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Amaranth storage proteins are mainly hexameric globulins of 300 kDa. They are composed of polypeptides of 30 and 20 kDa joined by disulfide bridges. As other storage globulins, they came from a multigene family and exhibit molecular heterogeneity. Amaranth globulins comprised 11S-globulin and globulin-P which showed different physicochemical properties. On the contrary of 11S-globulin, globulin-p presented a great tendency to polymerization. In this work we analyzed the relationship among the physicochemical properties of the two globulins with their sensitivity to proteases and their biological function. Globulin-P and 11S-globulin from germinated and non-germinated seeds were analysed by chromatography, electrophoresis and fluorescence spectroscopy. Their sensitivity to the action of papain, urea and 2-mercaptoethanol was tested. Analyses showed that urea affected in a similar way both globulin structures. On the other hand globulin-p aggregates were more sensitive than 11S-globulin to protease and 2-mercaptoethanol. This results correlated with those from germination analyses which showed that globulin-p suffer higher structural modifications than 11S-globulin during the early mobilization. This might be explained by the presence on the globulin-p surface of unprocessed polypeptides more sensitive to protease action.

PL-P65

DEGRADATION OF PHOTOSYNTHETIC PROTEINS IN SENESCENCE-ASSOCIATED VACUOLES OF TOBACCO LEAVES

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Chloroplast degradation is the hallmark of leaf senescence. However, the mechanism underlying the breakdown of chloroplast proteins is poorly understood. Several observations suggest that a novel class of “senescence-associated vacuoles” (SAVs) might be involved in the degradation of photosynthetic proteins. The abundance of SAVs correlates with rates of chloroplast degradation in tobacco, i.e., the number of SAVs decreases in cells where senescence is delayed by treatment with the plant hormone cytokinin, whereas acceleration of senescence with ethylene increases the number of SAVs. Immunolocalization experiments show that the chloroplast-encoded large subunit of Rubisco (RbcL) is re-located to SAVs in senescing leaves. Autodigestion experiments with isolated SAVs incubated at pH 5.5 (the estimated lumenal pH of SAVs) show that RbcL is degraded by SAV proteases. These data strongly support the idea that chloroplast proteins are transferred to SAVs and degraded there during senescence of leaves.

PL-P66
CHLOROPLAST PROTEASES FROM SENESCENT AND NON-SENESCENT WHEAT LEAVES

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Higher plant chloroplasts contain large amounts of proteins, mainly involved in photosynthesis. Stromal proteins (including Rubisco) are the major source of nitrogen in leaves and the preferred point of attack for proteases at senescence. At present, there is a general consensus that at least the first steps in chloroplast protein degradation occur inside the organelle but the identity of the proteases involved remains unknown. The aim of this work was to search for chloroplast proteases with increased activity at senescence, as potential candidates for stromal protein degradation. Chloroplasts were isolated from non-senescent and senescent (72 h of incubation in darkness) wheat leaves by Percoll gradient, lysed by hypotonic shock and the stromal fraction recovered in the supernatant after centrifugation. In-gel protease assays were performed in polyacrylamide gels copolymerised with gelatin. Only one band of gelatin hydrolysis was visualised in the stromal fraction from non-senescent leaves, which was also observed in dark-induced senescent leaves but showing a weaker signal. The addition of different protease inhibitors to the incubation media indicated that it is a serine protease. Two other bands, detected only in dark-induced senescent leaves, were inhibited by o-phenantroline and EDTA indicating that they are metalloproteases. [Supported by grants from ANPCyT and CONICET].

PL-P67
CLONING AND EXPRESSION OF ClpD, A CHLOROPLASTIC CHAPERONE FROM *Arabidopsis thaliana*

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Chaperones of the Hsp100 family (ClpC1/C2/D) have been implicated in protein folding and in the translocation of polypeptides in chloroplasts, events of great importance for normal plant growth. ClpD is the only one expressed under light and cold stress; therefore, could be the main chaperone participating in the protein quality control machinery of chloroplasts and/or the translocation of chloroplastic proteins under these adverse conditions. The goal of this work is to gain further insight into the role of ClpD in the aforementioned processes. The cDNA of the gene *clpd* was obtained from a *Arabidopsis thaliana* cDNA bank. A 2650 bp product was amplified by PCR and cloned in plasmid pET28a, in order to tag the protein with histidines. The construct was transformed into *Escherichia coli* cells; then, the expression of ClpD was assayed. After cellular lysis, the soluble and insoluble fractions were analyzed by SDS-PAGE and Western blot using anti-His antibodies. Under usual expression conditions, the protein was located in inclusion bodies. Thus, the expression was optimized by varying IPTG concentration, induction temperature and induction length. Under all conditions, a band of 98 kDa was observed. Under very mild expression conditions, a 60% of soluble protein was recovered and, by the use of affinity columns, purified. Its folding state and activity were also analyzed.

PL-P68
BIOCHEMISTRY OF POST HARVEST CHILLING TOLERANCE IN TOMATO (*Solanum lycopersicum*)

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Cold storage of fruits can alter the normal ripening process causing chilling injury and economic losses. Oxidative stress has been associated with the appearance of this fisiological disorder and the mitochondria has been assigned as a major source of reactive oxygen species. The objective of this study was to identify biochemical factors involved in chilling injury tolerance. Micro-Tom and minitomato cultivars were evaluated. Fruits were harvested at the mature green stage, stored at 4ºC during four weeks and transferred to 25ºC. The respiratory capacity of the fruits stored at 4ºC increased dramatically immediately after they were transferred to 25ºC. Micro-Tom fruits ripened normally while only 10% of minitomato fruits became red. Catalase (CAT) and glutathione reductase (GR) activities increased 2 and 1.5 fold, respectively, when the Micro-Tom fruits were transferred to non-chilling temperatures. These activity increments were not observed in minitomato fruits. Heat shock proteins (HSPs) Western blot analyses showed the induction of a small HSP (sHSP) in Micro-Tom but not in minitomato. These results suggest that the induction of CAT, GR and sHSP observed could be responsible of the chilling tolerance observed in Micro-Tom. The knowledge of the basis of this process may lead to better post-harvest management of tomato fruit quality.

PL-P69
DIFFERENT PEPTIDASE ACTIVITIES DO NOT CONTRIBUTE TO GLUTAMATE POOL OF RIPE TOMATO FRUIT

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Glutamate is the most abundant free amino acid in ripe fruit of several *Solanum lycopersicum*, particularly in those cultivars producing palatable small fruits. The glutamate sources in non-photosynthetic organs such us ripe fruit are still a matter of discussion. Among them, we explored the *in-situ* enzymatic production of glutamate in the pericarp of mature fruit γ-glutamyl(during the ripening transition. We observed a decrease in transpeptidase activity that may contribute to the glutamate pool more in green fruits than in the red ones. Different endopeptidases are present in mature fruits, being the substrate specificity characteristic of each ripening phase: azocasein was the preferred for ripe fruits and endogenous proteins for green fruits. Free glutamate content did not increase after peptidase activity in green or red fruit endogenous proteins. All these results indicate that several peptidases are present in ripe pericarp fruit, although these activities may not contribute to increase the glutamate content of tomato fruit during the ripening transition.

PL-P70

DXS EXPRESSION IN MORINDA CITRIFOLIA CELL SUSPENSION CULTURES FOR INCREASING ANTRHAQUINONES PRODUCTION

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The isoprenoid moiety constituting of anthraquinones (AQs) in the plant family Rubiaceae is formed from the methylerythritol 4-phosphate (MEP) pathway. 1-deoxy-D-Xylulose-5-phosphate synthase (DXS), the first enzyme in the (MEP) pathway catalyzes the transketolase reaction converting pyruvate and glyceraldehyde 3-phosphate into 1-deoxy-D-Xylulose-5-phosphate. In this study we constructed an expression vector with DXS to investigate its overexpression in Morinda Citrofolia cell suspension cultures. The dxs cDNA obtained from Catharantus Roseus cloned in a pGemTeasy vector, was digested and ligated between the Cauliflower mosaic virus 35S-promoter (CaMV-35S) and the potato proteinase inhibitor terminator (PIt) of pMOG843 (MOGEN, Leiden, The Netherlands). Subsequently, the cassette expression containing dxs was inserted into the binary vector pMOG22-GUS, which contains the hygromycin resistance gene, the GUS reporter gene and the left and right T-DNA borders from Agrobacterium tumefaciens. M. citrifolia cell cultures were transformed using A. tumefaciens strain LBA4404 containing the compatible plasmid pBRIMCS carrying a constitutive virG gene. Transformation was confirmed by analysis of the GUS reporter gene. Overexpression of dxs gene in transformed cell lines is under study.

PL-P71

GLUTAMATE EFFECTS ON SECONDARY METABOLIC PATHWAYS IN RUBIA TINCTORUM CELL SUSPENSIONS

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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. Proline biosynthesis in plants stimulates the pentose phosphate pathway (PPP) which results in an increasing carbon flux through the shikimate pathway. Proline is synthesized from glutamate by a series of reduction reactions. These enzymatic steps generate NADP+, which is the cofactor required in the PPP. We tested the effect of glutamate addition on plant suspension cultures of R. tinctorum in order to study its effect on secondary metabolic pathways. Suspension cultures treated with glutamate 5 mM showed higher levels of AQs at 4 (30%) and 8 (24%) days of culture. Total phenol contents also increased at 2 (11%) and 4 (11%) days of culture. The stimulation of these metabolic pathways could be correlated with the stimulation of the pentose phosphate pathway.

PL-P72
CHARACTERIZATION OF A MILK- CLOTTING PEPTIDASE ISOLATED FROM SALPICHROA ORIGANIFOLIA FRUITS

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Aspartic peptidases (EC 3.4.22) are widely distributed among plant species and have been purified from a variety of tissues. They have been found in seeds, flowers, roots, tubers and leaves; nevertheless APs from fruits have rarely been reported. In this work, a proteolytic enzyme with milk-clotting activity, named "salpichroin", was purified by anion-exchange chromatography from Salpichroa origanifolia fruits. The enzyme hydrolyzes natural substrates (casein, haemoglobin and gelatine) with high specific activity, exhibits an optimum pH around 3.5 to 4.5, it is activated by calcium and inhibited by pepstatin indicating an aspartic peptidase behaviour. Homogeneity of the protein was confirmed by SDS-PAGE, zymograms and isoelectrofocusing. Salpichroin is a heterodimeric enzyme with a pI of 5.2 and a molecular mass of aproximately 29 kDa, containing subunits of 17 and 9 kDa. The amino terminal sequence (20 aminoacids) of the 17 kDa subunit presented a great deal of sequence homology, 95 and 75% respectively, to an APs from Cucurbita pepo and from Brassica oleracea. In addition, a milk-clotting activity was observed for mature fruits when it was assayed on bovine skim milk, yielding characteristic patterns at pH 6.0. These results indicate that the extracts of Salpichroa origanifolia fruits could be useful in the dairy industry as an alternative for cheese making.

PL-P73
APOMIXIS AND SEXUAL REPRODUCTION IN CENCHRUS CILIARE (PANICEAE, POACEAE): A MOLECULAR APPROACH

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Understanding reproduction in grasses, the most economically important plant family, is relevant from a biological, evolutionary and biotechnological point of view. Apomixis, asexual reproduction through seeds, could be potentially exploited to indefinitely propagate specific genotypes in crops. To study sexual and apomictic reproduction in the aposporic grass *Cenchrus ciliare*, we performed SDS-PAGE and silver staining of ovary proteins of different developmental stages, verified by cytological investigations. With this approach, differences in protein expression patterns between leaves and ovaries, soluble and membrane ovary proteins and early and late developmental stages were found. We developed polyclonal antibodies that specifically recognize soluble ovary proteins in western blots and constitute useful tools for posterior studies. To look for transcription factors involved in reproduction we performed MADS box genes PCR and RT-PCR studies. We identified two MADS box genes expressed in sexual and apomictic spikelets and ovaries: one similar to class D/C maize ZMM1 and ZmOV23, and the other similar to class E LHS1, which have a demonstrated role in the production of reproductive organs. Cladistic analyses with their grass orthologs contributed to reconstruct gene evolutionary history in the family. These studies will provide new clues regarding reproduction in *Cenchrus ciliare*.

PL-P74

6-BENCILADENINE AFFECTS CHLOROPHYLLS AND POLYPHENOLS IN *Physalis ixocarpa* BROT VAR PURPLE DE MILPA

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Physalis ixocarpa Brot var Purple de Milpa is native of Mexico and Central America. Their violet fruits are used in sauces or candies. Total polyphenols (PTT) (flavonoids and antocianins, ATT) have great antistress oxidative power. There is a positive correlation between this activity, PTT and ATT. This specie contains in its fruits antocianins, responsible for its colour. The objective of this work was evaluate chlorophyll content (CHR), PTT and ATT from leaves and fruits from crops supplemented with 0, 1 or 2 doses of Fertilizer (F) and/or 6-bencyladenine (BA, 5mg/l). CHRs were quantified with the Minolta SPAD502 Meter. Dates were validated against the regression curve. Samples were smashed with Metanol-HCl and centrifuged to 10,000 xg, 20'. PTT were determinate with saturated solution of CO_3Na_2 and Folin-Ciocalteu reagent and measured at 755 nm. ATT were evaluated using the differential pH method, in buffers for pH 1.0 and 4.5, quantifying absorbance at 510 and 700 nm. Treatments with once or twice BAF increased CHLR (15 and 36%, respectively) and delayed leaves senescence. There were not PTT or ATT in green fruits. ATT and PTT increased with maturation and number of applications of BA (BAF: PTT=157,5; ATT= 87,7; 2BAF: PTT=218,6; ATT= 154,1 mg of /100 gr/fresh fruit). In treatments without BA the PTT and ATT concentration lowered 37 and 20%, respectively.

PL-P75

EFFECT OF CYTOKININS ON *P. GRANATUM* CUTTINGS INOCULATED WITH *G. INTRARADICES* ON DROUGHT STRESS

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Arbuscular mycorrhizas are mutualistic fungi that colonize roots of mostly plants. In general, this association produces a nutritional improvement (P_2, N_2 and H_2O from microagregates where roots can't take them) of the host and they exchange photosintates. Cytokinins are vegetal hormones that characteristically retard senescence. Our objective was to evaluate the effect of mycorrhiza in combination with cytokinins and drought stress. Cuttings of *Punica granatum* were inoculated with *Glomus intraradices;* half of them received 3 applications from aspersion with a solution of cytokinins (5 mg/ml), each cutting received 0,5 ml per application. A half was well watered whereas the other was watered stressed for 10 days. We measured root colonization, chlorophylls "a", "b" and totals, total poliphenols, soluble proteins, catalase, ascorbate peroxidase and superoxide dismutase activities. We observed an improvement on the phenolic content, on the chlorophylls "a" and "b" and protein contents in presence of drought stress (independently of the presence or absence of cytokinins) between inoculated and non inoculated cuttings. Inoculated cuttings presented low levels of catalase, superoxide dismutase and ascorbate peroxidase levels on drought stress, whereas the non inoculated ones didn't. In conclusion, mycorrhizas are more important protectors of drought stress than cytokinins.

PL-P76
POSTHARVEST QUALITY OF CUT FOLIAGE OF *Asparagus cetaceus* cv NANUS AFFECT BY 6-BENCYLADENINE

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Asparagus cetaceus cv Nanus has been used as cut foliage. Life in vase is the most important variable that restrains the election for this purpose. It implies the time that shoots maintain a fresh aspect, without senescence signals. Cytokinins control different functions within the plant, but their most important effect is on senescence. It is defined as a process that finalizes with functional activity of cells, tissues or organs. Shoots were joined together in bunches and placed in jars. Basal stems were immersed in water. Half of them were supplemented weekly (five times) with 6-benciladenine (BA) (5mg/ l). Jars were incubated 7 weeks, in a Growth Chamber, at 23±2ºC and 16 hours of light and 8 of dark. Weekly, the consumed water was compensated. Underneath each jar a tray allowed to gather the fallen foliage. Chlorophylls (CHL) were extracted with N-N,Dimetilformamide as described by Inskeep and Bloom. Proteins (Pr) were determined according to Bradford. Total polyphenols (PTT) were extracted in Metanol-HCl and supplemented with CO_3Na_2 and Folin-Ciocalteu reagent; absorbance was measured at 755 nm. Differences in water consumption only were of significance and higher in the first week for shoots without BA. Initial CHL, Pr and PTT were 2,3; 11,6 and 1,8 mg/g of leaves, respectively. BA delayed senescence decreasing loss of CHL (5 vs 26%), Pr (19 vs 37%) and lessening leaf abscission (140 vs 709 mg). Declined in PTT was lesser (3 vs 15%).

SB-P01
EPITOPE REPETITIVENESS INFLUENCES IMMUNOGENICITY: THE BRUCELLA LUMAZINE SYNTHASE (BLS) MODEL

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BLS is highly immunogenic and very stable decameric 180 KDa protein. We used BLS as a carrier by replacement of the amino terminus of each monomer by different peptides giving rise to chimeric proteins. Incubation of BLS in GuHCl 2M generates intermediate folded pentamers and increased concentrations, unfolded monomers. Using these different steps of the unfolding process and mixing two chimeras in different proportion we obtained different kind of mixed chimeras (MC). To obtain the MC BLSW22F:BLS-OMP31, we used a BLSW22F mutant (the unique Trp22 replaced by Phe) and the chimera BLS-OMP31, where OMP31 is a 27-mer exposed loop of the Brucella abortus membrane protein OMP31. We analyzed the MC decameric structures with variable copies of OMP31 peptides through intrinsic tryptophan fluorescence. We immunized BALB/c mice with MC BLSW22F:BLS-OMP31 9:1 and 5:5 in presence or absence of adjuvant. After 3rd and 4th immunizations a differential humoral response was observed in the sera as measured by ELISA. Groups immunized with MC 9:1 did not raise humoral response. The group immunized with MC 5:5 in absence of adjuvant developed a low response but with adjuvant this group raised a strong response similar to that of the group immunized with BLS-OMP31, although at longer times. Overall, these results demonstrate that the repetitiveness of the exposed peptide influences its immunogenicity.

SB-P02
STABILITY AND PLASTICITY ALSO MODULE AFFINITY MATURATION OF ANTI-PROTEIN ANTIBODIES

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Affinity maturation of antibodies against protein antigens is carried out accompanying the switch process from IgM to IgG isotype. The study of the molecular basis of affinity maturation mechanism is important to understand aspects of the humoral immune response and also for biotechnological and biomedical applications. We study two anti-lisozyme antibodies derived from the same germ lines genes recognizing the same epitope on HEL surface. The first antibody, D44.1 was obtained after a short immunization plan, and the second, mAb F10.6.6 was obtained after a long term immunization plan. F10.6.6 has a ~10^3 increase in affinity for antigen over D44.1. Several spectroscopic and calorimetric studies were performed in order to characterize both binding and stability. We found that Fv F10.6.6 has an increased thermal and chemical stability than D44.1 and that contacts made by V_H domain contribute to the association rates while the V_L domain contacts module the dissociation rates. We also tested ANS binding to Fvs molecules, finding a correlation between stability and ANS binding: the more stable the protein, the lesser the light emitted by the probe. Thus, an improvement of the variable domain stability that increases the plasticity of the V_H-V_L interaction results in the improvement of the binding properties of the antibody towards the antigen.

SB-P03
STRUCTURAL BIOLOGY OF POTENTIAL PROTEIN DRUG DESIGN TARGETS FROM *TRYPANOSOMA CRUZI*

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Introduction: *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 main objective of the Chagas´ Disease Research Network is the search for protein targets in metabolic routes absent in the human host and common in trypanosomatids Materials and Methods: The *T. cruzi* proteins studied at the present are: TcAK (arginine kinase), TcNDPK (nucleoside diphosphate kinase), TcALArac (alanine racemace), TcTAF9 (tata binding factor 9), TcFIP1-like (factor interacting with Pap1) and TcCPSF30 (cleavaje polyadenylation specificity factor). They are expressed in *E. coli* as a fusion protein with N-terminal His-tag. We have overexpressed and purified these proteins for crystallization and other structural assays. We have carried out activity assays. Results and Discussion: His-TcNDPK was overexpressed, purified and crystallized in differents conditions. It has shown biological activity. We have overexpressed and purified TcTAF9, His-TcFIP1 and TcCPSF30. His-TcAlarac are overexpressed.

SB-P04

NOVEL PROTEIN INTERACTIONS AND FUNCTIONAL ANALYSIS OF THE SPLICEOSOME BASAL COMPLEX IN TRYPANOSOMES

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Trypanosomes undergo trans-splicing as a major RNA processing event. In this reaction, selection of the 3´splice site AG is not restricted by distance from the branch point-PY. Several analyses revealed distances of AG location from branch point of up to 75 nt. This situation is clearly different from the metazoan cis-splicing in which the PY tract is in its close proximity. These events correlate with the properties of the U2AF heterodimers found in mammals and trypanosomes. While mammalian U2AF heterodimer (U2AF65/U2AF35) forms a strong stable complex that binds the PY-AG together, the trypanosome U2AF complex appeared to be disconnected. In fact, it lost the conserved eukaryote “tongue in groove” interaction and the two subunits only interacted weakly with each other. Moreover, there is no trypanosome consensus for branch-point and it is embedded in the PY tract. This situation correlates with the strong interaction that we observed between U2AF65 (PY recognition) and SF1 (Branch recognition). RNAi knock-down assays showed that trypanosome U2AF35 is an essential protein while U2AF65 and SF1 are conditional essential proteins. Here, we present a model that summarizes the similarities and differences between metazoan and trypanosome organization of the factors involved in early spliceosome assembly.

SB-P05

DNA-BINDING PROPERTIES OF HAT3.1, A PHD-FINGER HOMEODOMAIN PROTEIN FROM *ARABIDOPSIS*

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HAT3.1 is a member of the PHD-finger homeodomain protein family found only in plants. The HAT3.1 homeodomain (HD) shows considerable sequence divergence, even at positions that are highly conserved among HDs. In this study, SELEX, footprinting and yeast one-hybrid assays indicated that HAT3.1 shows a preference for the sequence T(A/G)(A/C)ACCA, different from those bound by other HDs. Binding was dependent on HD residues located at positions 50, 51 and 54, that is the same positions that usually participate in DNA binding in most HDs. The study of the interaction of mutants at these positions with DNA with nucleotide changes at specific sites suggested that His51 and Lys50 most likely interact with nucleotides 2 to 4 and 5 to 6, respectively, while Trp54 would establish contacts with position 4. In addition, the DNA binding activity is increased at low pH. Since replacement of His51 with Asn produced a protein non-responsive to pH changes, it can be speculated that the protonation state of His51 modulates the DNA binding efficiency of HAT3.1. Finally, a model in which the sequence T(A/G)(A/C)ACC can be assimilated to TAATCC bound by other HDs is postulated. The presence of His51 and Trp54 represents an innovation among HD structures. The fact that the HAT3.1 HD is able to interact with specific DNA sequences is an evidence of the inherent plasticity of the HD as a DNA binding unit.

SB-P06
CATALYTIC POCKET OF *P. aeruginosa* PHOSPHORYLCHOLINE PHOSPHATASE FOR Mg2+, Zn2+ AND Cu2+

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PA5292 from *P. aeruginosa* PAO1 is the gene responsible for the synthesis of phosphorylcholine phosphatase (PChP). It belongs to the Mg2+ dependent family of phosphomutases and phosphatases containing motifs I, II and III. In PChP these motifs are the aminoacyl residues ^{31}DMDNT35, ^{166}SAA^{168} and K^{242}/^{261}GDTPDSD^{267}, respectively. D33, D262 and D267 are involved in the binding pocket for Mg^{2+}. Since Cu^{2+} and Zn^{2+} were more efficient activators than Mg^{2+} either from the native or the recombinant PChP, the aim of this work was to know the interaction of divalent cations from Cu and Zn in the catalytic pocket for Mg. The substitution of Asp by Glu or Ala in motif I abolished the enzyme activity. The substitution S167T or D267E resulted in enzymes better activated by Mg or Zn than for Cu. Changes in the catalytic efficiency were also observed in D262E and D267E. D262E decreases the affinity for Mg but conserves the same affinity by Cu than the WT enzyme. In D267E, Mg is more efficient than Cu and Zn to activate PChP. These results indicated that the catalytic pocket for Mg is also involved for Cu and Zn and may be explained considering the 3D model, where it predicts a retraction of the carboxilic group of 5 Å in D262E or 2.4 Å in D267E. Since the ionic radius of these cations is similar (0.86-0.88 Å) it is possible that the coordination index is responsible for the described differences.

SB-P07
KINETIC & PHYSICOCHEMICAL PROPERTIES OF *P. aeruginosa* PHOSPHORYLCHOLINE PHOSPHATASE VARIANTS

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P. aeruginosa phosphorylcholine phosphatase (PChP) is secreted to the periplasmic space after the cleavage of the signal peptide (sp). The aim of this work was to known in which manner sp could be able to alter kinetic and physicochemical properties of PChP. Enzymes with ($PChP_{349}$) and without sp ($PChP_{327}$) were expressed in *E. coli* as N-terminal fusion to intein. They were also expressed without intein and purified from inclusion bodies by anion exchange chromatography. The great difference in the catalytic efficiency between $PChP_{327}$ and $PChP_{349}$ was produced by an increased k_{cat} but not by a change of affinity for both substrates phosphorylcholine and *p*-NPP in the first binding site. The sp notably decreased the affinity of the enzyme for the second PCh binding site, increasing Km_2. Mass spectrometry showed that $PChP_{349}$ is completely processed to a 327 protein and $PChP_{327}$ had also the expected MW for the mature protein. N-terminal sequencing data confirmed these findings. CD spectra of $PChP_{349}$ and $PChP_{327}$ indicated typical α-helix predominance and a well-preserved tertiary structure, indicating that despite sp affects the catalytic properties of PChP, it does not affect its structure. These findings indicate that *E. coli* is capable to process PChP exactly as *P. aeruginosa*, and reveals the importance of the secretion pathway to produce an enzyme with high catalytic efficiency.

SB-P08

INSIGHTS INTO THE DESIGN OF A HYBRID ELECTRON TRANSFER SYSTEM COMPOSED BY PROTEINS FROM *ANABAENA*

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The availability of the three-dimensional structures of many redox proteins, together with knowledge of the basis responsible for efficient interaction for electron transfer, opens the possibility of the redesign of already existing enzymatic systems in order to produce new ones able to carry out different functions from that reserved by nature. In this work we analyzed the possibility to obtain a hybrid electron transfer chain by using proteins from two structurally and functionally similar electron transfer chains: one from vertebrates involved in the mitochondrial steroid-hydroxylation system and, the other one, from photosynthetic cyanobacterium *Anabaena* PCC7119 which produce NADPH. To perform and characterize the possible hybrid systems we have used different techniques, such as site-directed mutagenesis, HPLC and stopped flow. Our data indicate that Flavodoxin and, especially, Ferredoxin, are able to efficiently transfer electrons to P450. Moreover, by studying the behavior of different Flavodoxin mutants we have improved the knowledge on how changes in redox properties of electron transfer proteins can influence the overall efficiency of the process.

SB-P09

SPECTROSCOPIC ANALYSIS OF THE INTERACTION BETWEEN NADP(H) AND A BACTERIAL FERREDOXIN-NADP REDUCTASE

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Ferredoxin-NADP+ reductases are enzymes harbouring one molecule of noncovalently bound FAD. These flavoproteins (FNR/FPR) catalyse reversible reactions between obligatory one-electron carriers and a two-electron donors/acceptors. Even though members of the FNR/FPR superfamily (plant-type) exhibit a conserved structure, sequence analysis reveals two clusters, each one with a typical FAD conformation. This raises the distinction between FNRs in a plastidic class, characterised by extended FAD conformation and high catalytic efficiency, and a bacterial FPR class displaying a folded FAD molecule and low turnover rates. The interaction between *Rhodobacter capsulatus* FPR and NADP+ was studied by differential spectroscopy. Modification of the flavin absorption spectrum results from the binding of the nucleotide to the oxidised enzyme. These outcomes bear a resemblance to those reported for plastidic FNRs, suggesting that the interaction between FAD molecule and the nucleotidic partner is similar for both types of flavoproteins, irrespective of FAD conformation. Stopped-flow kinetics under anaerobic conditions was used to study the reaction between oxidised FPR and NADPH. The decrease in flavin absorption is related to its reduction by NADPH, in agreement with results previously obtained for the plastidic enzyme. We also detected two charge-transfer intermediates of the whole reaction.

SB-P10
TYR36PHE MUTATION IN MN(II) SUPEROXIDE DISMUTASES AVOID THE INTERACTION WITH SUBSTRATE ANALOGUES

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Superoxide dismutases are proteins involved in the defense against oxidative stress conditions. They have a mononuclear site with a pentacoordinated metal ion (Mn or Fe). Using high-field electron paramagnetic resonance (HFEPR) spectroscopy we have investigated the Mn(II) site of the *R. capsulatus* protein and its dependence with temperature and addition of azide and fluoride.
Azide was found to directly coordinate the Mn(II). The formation of this complex was characterized by a reduction in the zero-field splitting. Fluoride dramatically affected the HFEPR spectrum but no evidence for six coordinated centers was detected. After incubation of the protein at low temperatures temperature a distinct six-line component was detectable. The newly six-line signal was assigned to the movement of a water molecule which normally lies into the substrate access channel hydrogen bonded to the Tyr36. Tyr36 is part of the substrate access channel and when it was replaced by a Phe there was no detectable interactions with azide or fluoride. By contrast, the low-temperature equilibrium was displaced towards the hexacoordinated form. Based on these results we propose a two-states binding model for these interaction in which Tyr36 acts as bridge between both possible binding sites. This model could represent the mode in which superoxide interacts with the active site during the reductive half-reaction.

SB-P11
THE GlcNAc-BINDING SITE OF *AGARICUS BISPORUS* LECTIN IS FUNCTIONALLY ACTIVE

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Agaricus bisporus lectin (ABL), obtained from commonly known champignon, is a protein with potent antiproliferative effects without any apparent cytotoxicity. The conformation of ABL was determined by x-ray diffraction. Co-crystallization of ABL with several sugars showed two different carbohydrate-binding sites. One recognizes Core 1 (Galbeta1-3GalNAcalpha-O-) of mucin-type O-glycans and the other binds GlcNAc. At present, the last sugar recognition was only demonstrated by crystallography. Using ELISA assays, the ABL interaction with terminal GlcNAc ligands was studied, and affinity constants were measured. The ABL interaction with ovalbumin was selected to demonstrate that binding is mediated by GlcNAc. This sugar and its derivative monosacharides (such as pNPalphaGlcNAc) reveal important inhibitory capacity, showing that GlcNAc-binding site of ABL is actively involved in interaction. ABL binds glycoproteins of nucleus matrix as observed by western blot and immunofluorescence. The GlcNAc inhibition is indicator of ABL recognition to GlcNAcbeta-Ser/Thr O-glycan from nucleus. The C. elegans growth is clearly reduced by the expression of recombinant ABL in E. coli. Purified ABL affects the mobility of C. elegans that is recovered in the presence of glycoconjugates. Through sugar recognition, ABL could affect cellular functions such as gene transcription, cell cycle and proliferation.

SB-P12
MUTATIONAL ANALYSIS AT ASN-73 IN *AGARICUS BISPORUS* LECTIN, A RESIDUE INVOLVED IN T-ANTIGEN

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The lectin from the common edible mushroom *Agaricus bisporus* (ABL), has potent antiproliferative effects on human epithelial cancer cells without any apparent cytotoxicity. The three-dimensional structure of the lectin has been recently resolved by X-ray crystallography (Carrizo, M.E. et al., J. Biol. Chem., 280, 10614-23, 2005). ABL is a tetramer, each monomer presenting a novel fold with two β sheets connected by a helix-loop-helix motif. These studies showed that Galβ1-3GalNAc, the disaccharide moiety of Thomsen-Friedenreich antigen (T-antigen), binds at a shallow depression on the surface of the molecule. On the contrary, GlcNAc binds at a different site on the opposite side of the helix-loop-helix motif. Among the contacts involved in providing the specificity toward T-antigen is the interaction between the side chain of Asn-73 and the acetamido group of GalNAc. Site-directed mutational changes were introduced at this residue with the objective of probing the role of this residue in T-antigen binding and possibly engineering an altered species with increased specificity for T-antigen. The binding activity was analyzed by competitive-enzyme-lectin assays (CELA). The mutants N73E and N73Q showed similar binding affinity for T-antigen, while the last one is less potent for recognition of GalNAc.

SB-P13
SUGAR RECOGNITION OF LECTIN DOMAIN FROM ppGalNAc TRANSFERASES INVOLVED IN MUCIN-TYPE O-GLYCOSYLATION

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Mucin-type O-glycosylation is initiated by a large homologous family of polypeptide GalNAc-transferases (ppGalNAc-T). The glycosylation dependent activity for some ppGalNAc-T isoforms appears to be directed by a lectin domain (ld) found in the C-terminal of most ppGalNAc-Ts. In the present work we initiate the study of carbohydrate-binding specificity from ppGalNAc-T lds. The ld of ppGalNAc-T2 and T4 recognize GalNAc-MUC1, and this interaction showed inhibition in presence of GalNAc derivatives. The sugar recognition of ppGalNAc-T lds reveal changes if protein is cold, labelled with biotin, horseradish peroxidase or acetylated showing an effect of posttranslational modification in carbohydrate-binding specificity. The recognition of biotin ppGalNAc-T4 ld to GalNAc-MUC1 is reduced by presence of cold T11 ld, no affected by cold T4 ld and enhanced by cold T2 ld, showing a cooperation or competition in the interaction depending of each construct. In addition, western blot was used to study binding of soluble ppGalNAc-T2 against nucleus matrix of cell lines. Some bands of interaction are inhibited in presence of GlcNAc. Soluble ppGalNAc-T2 shows ability in the recognition of YSPT(GlcNAc)SPS glycopeptide that is commonly present in tandem repeats of C-terminal of RNA pol II polymerase. The sugar interaction of ppGalNAc-T lds could be involved in gene transcription and cell proliferation.

SB-P14
VISUALIZATION OF 14-3-3 AANAT COMPLEX BY BIMOLECULAR FLUORESCENCE COMPLEMENTATION

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Serine and threonine kinases constitute ~92% of all kinases in humans. 14-3-3 proteins are novel members of this process. We report the implementation of bimolecular fluorescence complementation for the visualization of the 14-3-3 and serotonin N-acetyl transferase (AANAT) complex. In the later, E50 and E87 were defined as anchor residues, whereas R53 and R89 are hot spots by several in silico techniques. We compared 37 AANAT sequences to study conservation of these residues. Both anchor residues were strictly conserved in mammals, while only E50 was invariable in non-mammalian species. The hot spot R53 was strictly conserved in mammals, but R53 and R89 were variable in other species. Data support that anchors and hot spots are important in the 14-3-3-AANAT complex, which is only present in mammals. The interacting proteins were fused to sequences encoding Yellow Fluorescence Protein (YFP) fragments truncated at the residue 155, and cloned within mammalian expression vectors. Standard 17 aa linker residues were used, and the peptides Flag and HA, were maintained. After co-transfection with these constructs, HeLa cells were incubated 24 to 48 h and the stable association was visualized by the YFP fluorescence. We observed fluorescence in the cytoplasm only, due to the presence of a NES sequence in 14-3-3. Anchor and hot spot mutants complemented the analysis by this technique.

SB-P15
CARBOHYDRATE CHARACTERIZATION OF AN EGG CAROTENOPROTEIN FROM THE SNAIL *POMACEA SCALARIS*

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Invertebrate carotenoproteins are a diverse group of proteins still poorly characterized. In molluscs, ovorubin (OR) from Pomacea canaliculata is the only carotenoprotein well studied, showing no similarities with any known invertebrate carotenoprotein. The recently isolated carotenoprotein from P. scalaris eggs, named scalarin (SC) seems to be structurally and functionally related to OR. The aim of this work was to further characterize SC to understand carotenoprotein variability in this group. In particular, the carbohydrate moiety and its influence on particle size and shape were studied. SC was isolated from egg homogenates by ultracentrifugation and size exclusion chromatography (SEC). Carbohydrate characterization was performed by determining the total content, composition by GLC and oligosaccharide moieties by lectin analysis onto dot blotted proteins. We determined the molecular weight of the glycoprotein by PAGE, SEC and small angle X ray scattering (SAXS). SC contains mannose as the major carbohydrate. Five lectins, including ConA, WGA and JAC bound SC oligosaccharides, indicating the presence of N-linked oligosaccharides with high mannose and hybrid type cores, as well as O-linked moieties with a core containing substituted T antigens. MW estimation differed from 300-500 KD, depending on the analytical technique employed, probably due to the 20% carbohydrate content.

SB-P16

OVARIAN DEVELOPMENT, HEMOLYMPH AND EGG LIPOPROTEIN LEVELS IN THE CRUSTACEAN *MACROBRACHIUM BORELLII*

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During crustacean reproduction, the HDL lipoprotein Vitellogenins (VTG, female-associated) are transported from the hemolymph into the oocytes by endocytosis, as a precursor for the synthesis of the egg lipovitellin (VT). VT nourishes the embryos and is essential for larval survival. We have characterized the 3 HDL lipoproteins present in adults and eggs of the crustacean M. borellii: VTG, VT and the hemolymph, non-sex associated HDL-1. We have developed an anti-lipovitellin ELISA for the assessment of reproduction and embryogenesis in this prawn and analyzed the relationship among hemolymph, ovary, and eggs of different developing stages, VT and VTG levels by immunological techniques, as well as the onset of HDL-1 in embryos. Typical VTG values for females carrying stage-4 embryos were: hemolymph VTG 235 µg/ml; 350 µg VT/ovary and 344 µg VT/egg clutch. These levels are within the range observed in other crustaceans and indicate that the majority of ovarian VTG would eventually be transferred as VT into eggs. Two HDL were detected in isolated embryos. The most abundant has immunological identity with vitellus VT, incorporated during embryo development. The other one has many characteristics in common with adult HDL-1 and did not cross-react with anti-VT polyclonal antibodies. This would indicate that embryos are capable of synthesizing hemolymph lipoproteins in *M. borellii*.

SB-P17

DISINTEGRIN-LIKE AND CYSTEIN-RICH DOMAINS ISOLATION FROM A *Bothrops alternatus* HEMORRHAGIN

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Snake Venom Metalloproteinases (SVMPs) are multiple domain enzymes whose principal toxic effects are due to disruption of the hemostatic system. That from *Bothrops alternatus* herein studied belongs to the PIII class (SAIB 2005). These enzymes can undergo autolysis to yield a biologically active product, comprising the disintegrin-like and the cystein-rich domains (DC domain).

In this work we optimized the hemorrhagin isolation procedure by means of a combination of gel filtration and ion-exchange chromatography. The purified protein was submitted to autolysis “in vitro” and SDS-PAGE showed fragments in the 28-45 kDa range. The 28 kDa band was electroblotted onto a PVDF membrane and - when submitted to Edman degradation - proved to be the metalloproteinase DC domain on the basis of the comparison of its twenty residue N-terminal sequence with those of Bothrops disintegrins included in data bases. On the other hand, the mixture from the autolytic process was resolved through a Superdex G75 column and fractions analyzed by ESI HPLC-MS.

Different experimental conditions - such as EDTA incubation - were utilized for the purpose of stabilizing the enzyme in order to perform structural studies.

SB-P18
STABILITY AND SPECTROSCOPIC ANALYSES OF A TRYPSIN INHIBITOR ISOLATED FROM *Calliandra selloi seeds*

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A novel trypsin inhibitor was purified from *Calliandra selloi* seeds (CSTI). SDS-PAGE under non reducing conditions showed a single band of approximately 20,000 Da while in reducing conditions two bands of 16,000 and 6,000 Da were observed, indicating that CSTI consists of two polypeptide chains. Molecular masses of 20,078 and 20,279 were obtained by mass spectrometry, although only one pI corresponding to 4.0 was observed. Amino-terminal sequence showed homology to Kunitz-type inhibitors. CSTI was able to inhibit trypsin (Ki: $2.21x10^{-7}$ M), chymotrypsin (Ki: $4.95x10^{-7}$ M) and kallikrein (Ki: $4.2x10^{-7}$ M) but had no inhibitory activity towards elastase. Trypsin inhibitory activity was stable over a wide range of pH and temperature. CSTI was particularly susceptible to DTT treatment, followed by addition of iodoacetamide. Far-UV circular dichroism measurements revealed that CSTI is a β-II protein. Thermal unfolding showed a two-state transition with a midpoint at 68°C. Far-UV CD spectra of CSTI at the pH values tested showed little changes, while more pronounced differences in near-UV CD spectra were detected. Remarkably, treatment with 1 mM DTT caused slight changes in the far-UV CD spectrum, and only after carbamidomethylation there was a marked loss of secondary structure.

SB-P19
CYCLODEXTRIN GLUCANOTRANSFERASE FROM *B. circulans* DF9R: STRUCTURE-FUNCTION RELATIONSHIP

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Cyclodextrin glucanotransferase (CGTase; EC 2.4.1.19) catalyses the conversion of starch and related glucans alpha (1→4) into cyclodextrins (CD) through intramolecular transglycosylation as well as starch hydrolysis and transglycosylation intermolecular reactions. We have modified His residues with diethyl pyrocarbonate and shown that they play an important role in hydrolytic and beta-CD formation activity (SAIB 2005); residues involved belong to two kinetically different families.
In this work we have modified the enzyme with ^{14}C diethyl pyrocarbonate; the ethoxyformylated protein was then digested with trypsin and the peptide mixture submitted to RP-HPLC. Radioactivity was determined in all the fractions and those ^{14}C-labelled were microsequenced. Results indicated that the most reactive His residue is that located at position 233. On the other hand, HPLC-purified fractions from the mixture obtained by trypsin digestion were submitted to ESI mass spectrometry analysis in order to assess sample complexity, those containing only one molecular species were sequenced. Results obtained plus peptide localization in the sequence by homology with other members of the protein family allowed us to confirm that the enzyme has all the domains previously described for CGTases as well as to detect relevant enzyme structural characteristics.

SB-P20

ANOMALOUS FOLDING OF HUMAN APOLIPOPROTEIN A-I INDUCES AMYLOIDOSIS

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Amyloidosis are characterized by extra cellular deposits of anomalous fibrilar proteins. Human apolipoprotein A-I (apoA-I) is not normally involved within these pathologies. However, one case of severe amyloidosis associated with atherosclerosis was observed when apoA-I shows a deletion of a lysine residue in a central region of the protein (apoA-I Lys107-0). In order to detect the possible factors that induce this anomalous aggregation, we analyzed the folding of the mentioned mutant protein, as compared with wild type apoA-I (wt). Analysis of chemical denaturation and by using hydrostatic pressure show that apoA-I Lys107-0 is more unstable and has a stronger tendency to form β sheet structure as incubation time increases, specially at acidic pH. Under these conditions, mutant denaturation is less cooperative, suggesting intermediate states folding. Also, this mutant incubated at low pH shows increase in turbidity and binds more tyoflavine T than wt, showing higher yield of fibers as observed by electron microscopy. These results suggest that the anomalous aggregation of apoA-I Lys 107-0, is mediated by intermediate folded states and β sheet conformation, induced by a pH decrease. This situation could be favored by acidosis associated to cardiovascular disease.

SB-P21

GOB IS A NEW MONONUCLEAR ZINC β-LACTAMASE WITH A NOVEL ACTIVE SITE

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Metallo-β-lactamases (MβL) are Zn(II) dependent enzymes that share a common αβ/βα scaffold with highly conserved metal binding amino acid residues. We describe here the biochemical and biophysical characterization of GOB, an MβL with particular amino acid residue substitutions in the metal binding site. The *gob* gene was amplified from a clinical isolate of the Gram-negative pathogen *Elizabethkingia meningoseptica*, cloned in appropriate expression vectors, and expressed in *E. coli*. Expression of the complete *gob* coding sequence in this host resulted in a native enzyme exported to the periplasm via the Sec machinery, conferring resistance to different β-lactams. Size exclusion chromatography showed that recombinant GOB is a monomer. Kinetic studies indicated that GOB is a broad-substrate spectrum enzyme displaying maximum activity with only one equivalent of Zn(II) per molecule. This contrasts all other known broad spectrum MβLs which are maximally active in their di-nuclear forms. Spectroscopic and kinetic data obtained from the wt enzyme and mutated variants demonstrated that the canonical site 2 is essential for both metal binding and activity. The overall results thus indicate that GOB harbors one Zn(II) ion coordinated by residues D120, H121, H263 and a water molecule in the active site, therefore revealing a novel mononuclear Zn(II) site different from other related MβLs.

ST-P01

TCRPDEA1, AN UNUSUAL cAMP-SPECIFIC PHOSPHODIESTERASE FROM *TRYPANOSOMA CRUZI*

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Cyclic nucleotide phosphodiesterases (PDEs) catalyze the degradation of cAMP and cGMP, and by controlling the levels of these second messengers regulate numerous cellular processes. In particular, the complexity of the life cycle of *Trypanosoma cruzi* requires that this parasite must sense, and rapidly adapt to different environments in its mammalian host and the insect vector. Cyclic nucleotide signal could be an important modulator of such changes, thus cAMP has been shown to downregulate cell proliferation and differentiation in *T. cruzi*. In the present work, we report the identification of TcrPDEA1, a novel phosphodiesterase from *T. cruzi*. This enzyme shows homology with PDE1 subfamily members, is coded by a single copy gene and its sequence contains no regulatory domains outside of the catalytic region. TcrPDEA1 was able to complement a yeast mutant strain deficient in PDEs genes and displayed an unusual high Km value of about 200 µM for cAMP. The enzyme activity decrease according to the incubation time in the presence of EDTA and in a dose' dependent form. Once the enzyme was inactivated, the addition of Mg^{2+} did not restore its activity. It is noteworthy that TcrPDEA1 was not inhibited by several PDE inhibitors even at high concentrations. Moreover, TcrPDEA1 activity was not stimulated by cGMP, indicating that this enzyme is specific for cAMP.

ST-P02

NEW CELL SIGNALING PATHWAYS INVOLVED IN *XENOPUS LAEVIS* NEURAL CREST DEVELOPMENT

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The neural crest (NC) develops at the border between neural plate and the prospective epidermis in all vertebrate embryos. NC cells are highly migratory and generate many cell derivatives (neurons, pigment cells, cartilage, glial cells, etc). It has been demonstrated that the several signals (BMP4, Wnt, FGF, etc.) are involved in the induction of this tissue. However, the participation of other cell signals in NC specification has not been established. The analysis of expression patterns of the components of *Banded hedgehog/Gli3* (*Bhh/Gli3*) and *Endothelin1/Endothelin Receptor A* (*Edn1/Ednra*) cell signaling pathways in *Xenopus* embryos revealed that both pathways are expressed in the NC territory. In order to study the role of *Bhh/Gli3* and *Edn1/Ednra* pathways in the NC development we have carried out conditional gain- and loss-of function approaches. The microinjection of *Bhh* and *Ednra* mRNAs produced an increase in the expression of NC marker genes (*FoxD3, Slug* and *Snail*). On the other hand, the use of specific inhibitors of these signals, the microinjection of dominant negative constructs and morpholino antisense oligonucleotides against *Bhh/Gli3* and *Ednra* caused a decrease in the expression of NC markers. Our results suggest that *Bhh/Gli3* and *Edn1/Ednra* signaling pathways are required for the initial induction, maintenance of specification and migration of NC cells in *Xenopus embryos*.

ST-P03
FUNCTIONAL ANALYSIS OF *HAIRY* GENES DURING *XENOPUS* NEURAL CREST DEVELOPMENT

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It has been demonstrated that the yuxtacrine cell signaling pathway *Notch/Delta* is involved in the control of different developmental processes. The genes of the *Hairy* family encode transcription factors containing a bHLH DNA binding domain that are direct targets of *Notch/Delta* signaling in vertebrates and invertebrates. We have previously shown that *Xenopus Hairy* genes (*Hairy1, Hairy2a*, and *Hairy2b*) are expressed in the neural fold, the prospective neural crest (NC) region, from late gastrula stage onward. The expression patterns of *Hairy* genes suggest that they have a role in neural crest induction and migration. In order to analyze the role of *Hairy* genes during NC development by conditional gain- and loss-of-function, we have prepared chimeric inducible proteins by fusing the coding sequences to the ligand binding domain of glucocorticoid receptor. The overexpression of antisense oligonucleotides or chimeric inducible proteins and their dominant negatives by microinjection of in vitro transcribed mRNAs into developing embryos demonstrated that *Hairy* genes are required for the induction and migration of NC cells. The overexpression of *Hairy2b* mRNA in naïve ectodermal tissue showed that this gene is able to induce neural crest markers. Our results indicate that *Hairy* genes are involved in neural crest development and that they are functionally equivalent.

ST-P04
TOR AND THE cAMP-PKA SIGNALING CONVEY SEPARATE INPUTS TO RIBOSOMAL PROTEIN GENE PROMOTERS

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In S. cerevisiae the TOR and cAMP-PKA signaling cascades respond to nutrients and regulate coordinately expression of genes required for cell growth, including ribosomal protein (RP) and stress responsive (STRE) genes. Several studies have demonstrated that inhibition of Tor signaling by rapamycin results in repression of RP genes and induction of the STRE genes. We and others have reported that mutations that activate the PKA pathway confer resistance to rapamycin, and prevent the RP gene repression induced by this drug. Conversely, mutations that partially inactivate PKA signaling result in rapamycin hypersensitivity and have little impact on the rapamycin induced repression of RP genes. In strains that completely lack PKA activity RP gene expression is impaired and STRE gene expression is enhanced. Remarkably, in the PKA null mutants this altered transcriptional pattern is still sensitive to rapamycin and thus, responsive to Tor signaling. PKA null mutant strains display higher levels of glycogen than WT strains; however these levels are increased after exposure to rapamycin, indicating that Tor regulates glycogen homeostasis via a PKA-independent mechanism. Taken together, these findings support a model in which Tor and cAMP-PKA function as two parallel pathways that respond to nutrient signals to regulate gene expression required for cell growth and stress response.

ST-P05
DIFFERENTIAL REGULATION OF THE TRANSCRIPTION FACTOR KLF6 BY JNK1 AND JNK2 ACTIVITIES

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The transcription factor KLF6 is a potential tumor suppressor gene product whose expression level is responsive to external cell stimulation mediated by growth factors, tumor promoters and DNA-damage agents. We demonstrated that this factor induced the degradation of the c-Jun proto-oncoprotein leading to inhibition of cell proliferation. Interestingly, consensus amino acid sequences which are targets for JNK-mediated phosphorylation in c-Jun are conserved in KLF6. However, the biochemical pathway involved in the KLF6 response to external signals and its interaction with c-Jun is still unresolved. We observed that in *jnk+/+* cells KLF6 protein level was reduced in the cytoplasm upon ectopic expression of both, JNK1 and JNK2. However, JNK2 expression, but not JNK1, enhanced KLF6 detection in the nucleus. In the context of *jnk -/-* cells, the protein level of KLF6 obtained was severely impaired by JNK1 activity and in a lesser extent by JNK2, which was essential for translocation of KLF6 from cytoplasm to the nucleus. In conclusion, these results identified the JNK pathway involved in the regulation of KLF6 stability and cell distribution. Maximal activity of JNK1 correlates with reduced KLF6 protein level whereas c-Jun is stabilized. Conversely, JNK2 substantially increased KLF6 in the nucleus becoming available to regulate its target genes while c-Jun-sustained proliferation is reduced.

ST-P06
17β-ESTRADIOL INHIBITS APOPTOSIS IN C2C12 MUSCLE CELLS ACTING ON THE PI3K/Akt/BAD PATHWAY AND ERβ

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We previously demonstrated antiapoptotic effects of 17β-estradiol in skeletal muscle cells (C2C12 myoblasts). Here we analyzed the participation of the PI3K/Akt/BAD pathway and estrogen receptor (ER) isoforms in the hormone protective action. Immunocytochemistry studies by confocal microscopy revealed activation of Akt by 17β-estradiol. Ly294002 abolished the ability of the estrogen to activate Akt suggesting the participation of PI3K. Western blot assays showed an inhibitory effect of the hormone on H2O2-promoted apoptotic cleavage of PARP, which was abolished by Ly294002 or Wortmanin. Moreover, 17β-estradiol induced Wortmanin-sensitive phosphorylation of BAD, an event known to inactivate its proapoptotic function. In addition, Ly294002 abrogated estrogen inhibition of cytochrome c release elicited by H2O2. The effects of 17β-estradiol on cytochrome c liberation were suppressed by ER β siRNA or in presence of ER antagonist ICI 182780. Thus, our studies indicate that the PI3K/Akt/BAD pathway and ER β are involved in the antiapoptotic action of 17β-estradiol on C2C12 myoblastic cells. This mechanism may be involved in estrogen regulation of skeletal muscle growth, as apoptosis plays an important role controlling the number of myoblasts which undergo differentiation into mature myotubes.

ST-P07

PKC/Src ACTIVATION MEDIATE 1,25(OH)2D3 AND 17β-ESTRADIOL REGULATION OF MAPK PATHWAYS IN MUSCLE CELLS

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The mitogen activated protein kinases (MAPKs) are a superfamily, among which ERK 1/2 and p38 MAPK have been extensively studied. 1,25-dihydroxy-vitamin D3 [1,25(OH)2D3] and 17β-estradiol promote biological responses through MAPK cascades in various cell types. We have previously shown that these steroid hormones stimulate the phosphorylation of transcription factors Elk-1 and CREB via the ERK 1/2 and p38 MAPK pathways in the skeletal muscle cell line C2C12. Now, we have studied upstream steps involved in modulation of MAPK cascades in these cells. Our results demonstrate that 1,25(OH)2D3 and 17β-estradiol activate the non-receptor protein tyrosine kinase c-Src at 60 and 15 minutes, respectively. Moreover, specific inhibition with compound PP1 or knock-down with antisense oligonucleotides revealed the participation of c-Src in ERK 1/2 and p38 MAPK phosphorylation induced by the two hormones. We observed that 1,25(OH)2D3 and 17β-estradiol modulate Src activation in a PKC-dependent manner, possibly through a protein tyrosine phosphatase (PTP). We conclude that 1,25(OH)2D3 and 17β-estradiol act upstream on mitogenic cascades sequentially activating PKC, c-Src via a PTP, and finally ERK1/2 and p38 MAPK. This mechanism is congruent with the anabolic action of both hormones in muscle tissue.

ST-P08

α2M-LRP-1 INDUCES ERK1/2 PHOSPHORYLATION BY INTRACELLULAR CALCIUM AND PKC ACTIVATION IN J774 CELLS

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LRP-1 is a LDL receptor gene family member synthesized and processed into 515-kDa extracelullar α chain and 85-kDa trans-membrane and intracelullar β chain. LRP-1 α chain contains multiple ligand recognition sites and β chain harbors motifs for endocytosis and signaling. In addition to α2-macroglobulin-protease complexes (α2M*), LRP-1 also recognizes proteases and lactoferrin. The receptor-associated protein (RAP) inhibits the binding of LRP-1 ligands. Previously, we have demonstrated that α2M* promotes cell proliferation and intracelullar calcium in J774 cells by LRP-1, but the signaling mechanisms are unknown yet. Herein we evaluate the signaling effects of α2M* and other LRP-1 ligands. By Western blot we observed that α2M* 60 nM promoted MAPK-ERK1/2 phosphorilation, whereas RAP and lactoferrin did not induce it. The α2M*-induced ERK1-2-MAPK phosphorilation was inhibited by MEK-1 PD98059 inhibitor and RAP. When the J774 cells were cultured with Ca2+ antagonist BAPTA and the PKC Calphostin C inhibitor, the α2M*-induced ERK1-2-MAPK phosphorilation was blocked. In conclusion, α2M* induces ERK1-2-MAPK activation by intracellular calcium rises and PKC activation mediated by LRP-1 in J774 cells. Other LRP-1 ligands did not induce intracellular signal. Thus, the ligand recognition in the LRP-1 α chain might regulate and activate different downstream signaling pathways.

ST-P09
INSULIN SELECTIVELY INDUCE IRS-4 TYROSINE PHOSPHORYLATION IN HEPG2

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IRS-4 is the most recently characterized member of IRS family, cloned from human embryonic kidney (HEK) 293 cells. The physiologic role of IRS-4 is still poorly understood. In comparison with other IRSs, IRS-4 exhibits a more limited tissue expression. Besides HEK293 cells, this protein has only been detected in heart and skeletal muscle cells where IRS-4 is not phosphorylated in response to Insulin and IGF-1. IRS-4 interacting proteins have been identified, including PI3-K, Src homology phosphatase, protein kinase Cξ, PP4 and IRAS. We studied the effect of Ins on IRS-4 phosphorylation in HepG2 cell line. HepG2 were stimulated with Ins (10^{-7}M) for different times, in the presence or absence of several tyrosine kinase and phosphatase inhibitors (Wortmanin, Genistein and LY294002, Tyrphostins). Following stimulation, cells were lysated, proteins separated and tyr-phosphorylation was detected by W.B. Ins induce a time dependent tyrosine phosphorylation of IRS-4. This event is blocked by Tyrphostine AG1024, an specific inhibitor of insulin receptor, but not for the AG1478, a specific EGFR inhibitor. The effect was also inhibited by Genistein, while Wortmanin and LY294002 (PI3K inhibitors) increase IRS-4 phosphorylation. We demonstrate, for the first time, insulin-induced tyrosine phosphorylation of IRS-4 in HepG2 cells, an effect which appears to be mediated by PI3 kinase.

ST-P10
ELEMENTS IN THE REGULATORY REGION OF YEAST UGA4 GENE INVOLVED IN THE RESPONSE TO AMINO ACIDS

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The Saccharomyces cerevisiae UGA4 gene encodes the δ-aminolevulic acid (ALA) and γ-aminobutyric acid (GABA) permease. The expression of this gene is highly regulated and depends on GABA induction and Nitrogen Catabolite Repression.UGA4 promoter contains two activating sequences, UASGATA and UASGABA, involved in this regulation. Recently, it has been described that UGA4 expression may also be regulated by the availability of extracellular amino acids through the amino acid sensor complex SPS (SSY1, PTR3, SSY5). The aim of this work was to study the role of SPS sensor in the response to extracellular amino acids and to determine which regulatory sequences are involved in this regulation. For this, B-galactosidase activity was measured on strains carrying YEP-UGA4::lacZ grown in different conditions. Here we report that the SPS sensor is involved in the UGA4 response to extracellular amino acids. Strains lacking sensor components, but not wild type strain, showed UGA4 induction even in the presence of extracellular amino acids. To determine which regulatory sequences are involved in this response, B-galactosidase expression driven by different promoter fragments was analyzed. We found that UASGATA sequences are not the target of the signal triggered by amino acids, while preliminary results suggest that UASGABA sequence would be involved in the external amino acid regulation of UGA4.

ST-P11

EVIDENCE OF NEW ELEMENTS INVOLVED IN *SACCHAROMYCES CEREVISIAE* UGA4 REGULATION

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Uga4p, the delta-aminolevulic acid (ALA) and gamma-aminobutyric (GABA) specific permease, is highly regulated by the TOR kinase signaling pathway in response to carbon and nitrogen availability. GATA transcription factors and UASGABA trans-acting elements are known to be involved in the nutrient responsive permease regulation but the way they interact still remains unclear. Fluorescence microscopy, β-galactosidase activity assays and western blots were performed to study the UGA4 gene regulation under rich or poor carbon and nitrogen sources. Our results complete those reported for expression levels and localization profiles of the positive GATA factor Gln3 in cells grown with acetate as the carbon source, helping to elucidate unclear events of UGA4 regulation. Furthermore, β-galactosidase activity experiments, including those made with two partial deletions of the UGA4 promoter, lead us to the thought that basal expression levels in acetate depends on UASGATA. Nitrogen Catabolite Represion (NCR) is still observed even in the absence of UASGATA and UASGABA. Electrophoretic mobility in SDS-PAGE for different nutrient sources was analyzed to establish Gln3 phosphorylation levels. All this results taken together allowed us to establish correlations between UGA4 expression and localization, phosphorylation and expression levels of Gln3p.

ST-P12

MUSCARINIC RECEPTOR 1 REGULATE THE C-FOS PROMOTER AND C-FOS PROTEIN USING ALTERNATIVE SIGNALING PATHWAY

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M1 muscarinic receptor, is a member of the G-protein-coupled receptors family (GPCRs), and its activation by carbachol (Ch) promotes Immediate Early Gene (IEG) expression and ultimately cell division in NIH 3T3 cells that stably express M1 (1.2 cells). c-fos is an IEG activated rapidly and transiently in response to extracellular signals. c-Fos protein is a transcription factor modified post-translationally by phosphorylation, which regulates its transcriptional activity. The MAPKs constitute key elements in the regulation of gene expression by environmental signals Using 1.2 cells we previously studied the regulation of c-Jun, a protein partner to c-Fos in AP-1 and coded by another IEG, by Ch/M1 induced MAPK cascades. We focused now on M1 induced c-Fos regulation. Our results show that while Ch activates a wide variety of MAPKs in 1.2 cells only ERK1/2 induce c-fos mRNA expression and the activity of some p38 MAPK isoforms counteracts this effect. Some MAPKs have been shown to phosphorylate and transactivate c-Fos under different stimuli, our studies show that the inhibition of any single MAPK cannot abolish c-Fos phosphorylation or transactivation suggesting the involvement of various MAPKs acting together. We conclude that, as is the case for c-Jun, M1 uses analogous but alternative signaling pathways to regulate the c-fos promoter and c-Fos protein.

ST-P13
NEW INSIGHTS INTO THE DES PATHWAY: DESR ACTIVATION MECHANISM AND DISSECTION OF THE DES PROMOTER

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The Des pathway of Bacillus subtilis regulates the synthesis of the Δ5-fatty acid desaturase, encoded by the des gene. Upon a decrease in the membrane lipid fluidity, the histidine kinase DesK phosphorylates DesR. Phosphorylated DesR binds to the des promoter, recruiting the RNA polymerase. The molecular mechanism by which DesR is activated by phosphorylation, as well as the dynamics of its interaction with DNA, remain unknown. To unveil the mechanism by which phosphorylation activates DesR, we constructed a truncated DesR protein, consisting only on its DNA-binding C-terminal domain (DesRC). Electromobility shift assays have shown that DesRC is able to bind to the des promoter regardless of phosphorylation, but incapable of activating des transcription. These results show that DesR activation implies the remotion of inhibitory effects of N-terminal domain over the DNA-binding capacity of the C-terminal domain. In order to comprehend the dynamics of the interaction of DesR-P with Pdes, the two sites, RA and RB, where dissociated through mutagenesis. Mutant versions of Pdes promoter, with different deletions of RB site, have been constructed. EMSA as well as in vivo transcriptional fusion experiments indicate that the RA site, in face with -35 and -10 elements, is enough to allow des transcription. A possible explanation for the existence of the low affinity RB site is proposed.

ST-P14
GENE EXPRESSION LEVELS AND REVERSIBLE DIMORPHIM OF *CANDIDA ALBICANS* PKA MUTANT STRAINS

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Candida albicans cAMP pathway co-ordinates basic cellular processes such as dimorphic shift, carbon metabolism and the cell cycle. Morphogenesis of an achieved set of mutant strains devoid of one or two alleles of PKA catalytic subunits (TPK1 or TPK2) and/or the regulatory subunit (BCY1) was assessed in solid and liquid media. Under conditions in which wild-type and a number of strains exhibited wrinkled colonies composed of filamentous forms (pseudohyphae and true hyphae); and reverted to the yeast morphology when growing in a minimal medium, the homozygous bcy1 in a tpk2 null background was unable to switch to this phenotype. Interestingly the mutant strain tpk2 carrying single alleles of TPK1 and BCY1 gave a non germinative phenotype. It is known that some PKA mutants in the glucose-deprived state are unable to store carbohydrates like glycogen and/or trehalose. Glycogen accumulation was tested in the PKA mutants. It was found that some of them, particularly those devoid of one or both TPK1 alleles were defective in entry into stationary phase an in glycogen accumulation as assessed by iodine/iodide staining, and total glycogen measurement. Also, through RT-PCR we studied the expression of PKA coding genes and those involved in glycogen metabolism. [Supported by grants from University of Saarland (Germany) and CONICET].

ST-P15
PROTEIN SUBSTRATES OF PKA IN *SACCHAROMYCES CEREVISAE*

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The PKA is the most well characterized member of the Ser/Thr protein kinase family. It was widely accepted that cAMP activates PKA however, recent experiments suggest that the substrate would play an important rol in the activation of the holenzyme.The effectiveness of protein phosphorylation by PKA is belived to depend on the primary structure of the protein around the phosphorylation site.Several PKA substrates have been described but is needed to demonstrate that the candidates proteins identified are indeed PKA substrates. Among all the yeast ORF which have a consensus RRXS sequence of PKA phosphorylation,we chose 10 and probed their phosphorylation *in vitro* by yeast PKA. Only three of them were effectively phosphorylated: Pyk1, Pyk2 and Nth1.Synthetic peptides including the consensus phosphorylation sequences from these proteins were phosphorylated *in vitro* and compared with kemptide. Although all of them present the canonical RRXS,only three of them were substrates.Small differences in peptide sequences resulted in significative Kesp difference.The precense of basic residues in -5 position seems to be important for the phosphorylation.The specificity determinants that contribute to the recognition of substrates by PKA described until now is R at the p-6, p-3 and p-2 position. The phosphorylation behaviour of the proteins were compared with the peptides.

ST-P16
***MUCOR CIRCINELLOIDES* PKA, INTERACTION BETWEEN THE R AND C SUBUNITS**

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PKA is a tetramer of regulatory subunits(R) and catalytic subunits(C).The interaction between R and C in *M.circinelloides* PKA is higher than in PKA from *S. cereviciae* and higher eukaryotic organisms.In the range where the PKA activity was no longer proportional to enzyme concentration,NaCl could increse the activity of the holoenzyme, indicating that complete holoenzyme disociation in vitro is not achived upon incubation with cAMP without NaCl. In a cAMP-agarose purification of C subunit it was necessary to use ClNa to obtain this subunit.Our hypothesis is that the N-terminal region of RM,which has acidic residues,is responsible for the difference in the interaction.Wt R and R1 laking the N-terminal and R2 lacking a region of acidic residues were cloned.Interaction between these Rs with C subunits was evaluated by in vitro inhibition or dissociation assays.The IC50 for wtR was lower than those for mutant Rs.Dissociation of reconstituted holoenzyme showed that higher cAMP concentration were required to dissociate the wtR-C than to dissociate the mtR-C. The behaivour of CM or Cb was different.The results indicates that the acidic residues region may be important in the high affinity interaction R-C found *M. circinelloides* PKA.

ST-P17
DISTINCT PHOSPHORYLATION EVENTS OF THE CATALYTIC SUBUNIT OF PROTEIN KINASE A IN *S. CEREVISIAE*

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In AGC family of serine/threonine kinases, the activities of catalytic subunits are regulated by multiple phosphorylations both in the activation loop and at allosteric sites. In S. cerevisiae, the PKA has three TPK1, TPK2 and TPK3 genes encoding the catalytic subunit. Genetic and kinetic experiments indicate an intramolecular phosphorylation mechanism for the Tpk1 isoform during glucose stimulus. We used MALDI-TOF/TOF to identify which aminoacids are phosphorylated in Tpk1 post glucose stimulus. We have identified Ser 32, 43, 179 and 322 as in vivo phosphorylation sites. We constructed strains carrying an inactive version of Tpk1 (tpk1K116R) or a mutant tpk1S179A. In situ and in vitro kinase assays indicate that the phosphorylation in Ser179 contributes to catalytic activity. Immunological analysis showed that inactive Tpk1 is phosphorylated on Ser and Thr241, suggesting an additional intermolecular mechanism of phosphorylation by a heterologous kinase. Additionally, using pull-down assays and mass spectrometry we found that the holoenzyme can consist of different isoforms of catalytic subunit bound to a regulatory subunit dimer in vivo. These results collectively suggest that two phosphorylation pathways can operate on TPK1: i)Tpk1 itself through autophosphorylation in response to glucose stimulus and ii)Tpk1 through Ser/Thr241 phosphorylation by heterologous protein kinase.

ST-P18
EVIDENCES OF BETA 1-3 GLUCANS IN MUCOR ROUXII

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We have cloned and characterized Rho 1 protein from Mucor rouxii. The small GTP binding protein have been shown to be the regulatory protein of the β 1,3 glucan synthase complex, in fungi in which β 1,3 glucans are major components of the cell wall. Even though glucans have not been detected in zygomycetes, caspofungin, an echinochandin known to be an inhibitor of β 1,3 glucan synthase complex, is shown here to have a negative effect on growth and to produce an alteration on morphology when added to M. rouxii growth culture medium. The minimum effective concentration (MEC) was 10 μ g/ml and the fungus produced short, stubby and highly branched hyphae. Localization in germlings was visualized by immunofluorescence, the protein shows to be localized in patches in the mother cell surface and excluded from the germ tubes. Similar results are obtained by using a specific monoclonal antibody anti-β 1,3 glucans. The sresults suggest that Muicor rouxii have β 1,3 glucans in the cell wall.

ST-P19
MICROARRAY ANALYSIS OF IN VIVO GLUCOCORTICOID ANTIAPOPTOTIC ACTION ON MAMMARY GLAND INVOLUTION

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In vivo administration of glucocorticoids (GCs) prevents the apoptosis of epithelial cells of post lactating involuting mammary gland. The aim of this work was to identify GC-regulated genes during this process by performing a genome-wide screen using oligonucleotide microarrays. The involution was induced in BALB/c mice by removing the pups. A subcutaneous injection of Dexamethasone 0.5 mg/100g body weight or vehicle, was applied at 0, 24 and 48 hr after weaning. After additional 24 hours, mammary gland #4 were excised and total RNA prepared. Affymetrix oligonucleotide GeneChip Mouse Genome 430 2.0 array was used and RMA expression values were calculated by the AMARGE web tool. According to the limma package of Bioconductor analysis, it was found that from 34000 genes tested, 492 were differentially expressed between the two experimental groups, being 137 induced (fold changes from 1.49 to 32.8) and 355 repressed (fold changes from 0.68 to 0.12) by the GCs treatment. Hierarchical clustering and Principal components analysis were performed using the dCHIP and TIGR software. According to the EASE analysis of over-represented gene ontology terms, the most relevant category was “Tumor suppressor”, comprised 5 genes all down-regulated by GCs. Thus, these genes could be potential candidates as mediators of the GC antiapoptotic action observed in the mammary gland.

ST-P20
GLUCOCORTICOID-DEPENDENT BCL-X GENE EXPRESSION DURING INVOLUTION OF NORMAL MAMMARY GLAND

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bcl-X gene is highly related to bcl-2 and generates at least five different isoforms which are products of alternative splicing of an unique gene. The 5’-region of the mouse bcl-X gene contains at least five different promoters which are activated in a tissue-specific pattern of promoter usage. The steroid hormone dexamethasone (dex) has demonstrated to be able to control bcl-X expression and to influence the ratio between the isoforms bcl-XL (antiapoptotic) and bcl-XS (proapoptotic) in different tissues. This effect occurs mainly through the control of bcl-XL expression. According to previous reports, there is a decrease in the ratio bcl-XL/bcl-XS of normal mammary gland 48 h after weaning. In the present work we explore the potential regulation of bcl-X gene by glucocorticoids during 72 h of post-lactational involution. RT-PCR assays show that bcl-XL mRNA levels are increased during involution upon daily dex treatment (0,15 mg/ animal) as compared with placebo-treated animals. Moreover, transcripts generated from promoters P1 and P4 are drastically decreased in those animals. On the other hand, we found that LIF expression and p-STAT3 did not change with treatment. This observation correlates with an increase in glucocorticoid receptor analysed by western blot, suggesting that a download of GR during involution may be responsible for the decreased of the antiapoptotic isoform.

ST-P21
STUDY OF GLUCOCORTICOID ACTIVITY OF 21-HEMISUCCINATE-6,19-OP, AN OXYGEN BRIDGE ANALOG

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According to the overall conformation as the three-dimensional structure and spatial distribution of charges, glucocorticoid molecules exhibit a bent skeleton as a consequence of the degree of curvature at the A/B junction of the respective structure. In our approach rigidly bent analogue with some specific anti-glucocorticoid activities was obtained by a 6,19 oxygen bridge introduced in a pregnane structure(21-OH-6,19-OP). This compound devoids affinity at receptoral level for both mineralocorticoid and progesterone receptor. In order to enhance polarity and water solubility we synthesized the esther derivative 21-hemisuccinate-6,19-OP. This new compound has demonstrated to be a highly potent antiapoptotic agent, according to DNA fragmentation and FICT-Annexin V assays when it is co-administrated in combination to dexamethasone in thymocyte primary cultures. However, this compound not only is not able to block dexamethasone induced MMTV-luciferase reporter gene but also it significantly potenciates at its lowest concentration the glucocorticoid action of dexamethasone.

ST-P22
1,25(OH)2-VITAMIN D3 AND PTH PROMOTE APOPTOSIS IN THE HUMAN COLONIC Caco-2 CELLS

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Apoptosis plays a critical role in maintaining homeostasis of the intestinal epithelium. The objective of this study was to delineate the role of PTH and 1,25(OH)2-vitamin D3 [1,25(OH)2D3] in intestinal apoptosis using the human colonic Caco-2 cells. We first demonstrated the presence of the PTH and 1,25(OH)2D3 receptors (R) in Caco-2 cells by immunoblot analysis and immunocitochemistry. Following hormone interaction with its R, PTH (10 nM) rapidly (3 min) and transiently stimulates the serine 473 phosphorylation of AKT and of the pro-apoptotic protein Bad in Ser 136, while 1,25(OH)2D3 (100 nM) causes transient phosphorylation of Bad (1-5 min) and has no effects on AKT. In addition, the steroid hormone opposite to PTH, activates the extracellular signal-regulated kinase ERK 1/2 (+2 fold). Analysis by DAPI staining and, evaluation of the cell survival, reveal that PTH and 1,25(OH)2D3 treatment for longer time-intervals (24 h) increase the number of apoptotic nuclei (+190 % and +260%, respectively) and diminishes the number of cells (-58% and -52%, respectively). Both hormones also induce the expression of cleaved forms of Caspase-3. This apoptosis is associated with reduced levels of phosphorylation of AKT. Taken together, our results indicate that both hormones promote the apoptosis of Caco-2 intestinal cells and may be potentially useful in the therapy of human colon cancer.

ST-P23

NON CLASSICAL VITAMIN D RECEPTOR SUB-CELLULAR DISTRIBUTION IN RAT INTESTINAL CELLS

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1a,25(OH)2-vitamin D3 [1a,25(OH)2D3] stimulates signal transduction pathways in intestinal cells. In the last years increasing evidence for non-genomic hormone effects in different cells types has been accumulated, and led to hypothesize the existence of a cell-surface receptor triggering membrane events. To gain insight into the relative importance of the vitamin D receptor (VDR), in the rapid hormone responses, the localization and binding properties of the VDR was evaluated in subcellular fractions of rat intestinal cells. Confocal microscopy studies revealed that VDR immunoreactive proteins have membrane, mitochondrial, cytosolic and perinuclear localization. Western blot analysis using specific antibodies detected the 60 and 50 kDa immunoreactive bands expected for the VDR in the cytosol and microsomes and, to a less extent, in the nucleus and mitochondria. Low molecular weight immunoreactive proteins were also found in all the subcellular fractions. Since changes in hormone receptor levels appear to constitute a common manifestation of the aging process, we also analyzed 1a,25(OH)2D3 binding properties and VDR levels in subcellular fractions from young (3 months) and aged (24 months) rats. In competition binding assays we detected specific binding sites for [3H]-1a,25(OH)2D3 in all subcellular fractions, with maximum binding in mitochondria and nucleus. VDR protein levels and 1a,25(OH)2D3 binding affinities were diminished with aging. Our results suggest that non classical localization of VDR and related proteins may account for 1a,25(OH)2D3 stimulation of signal transduction pathways in rat intestinal cells previously reported.

ST-P24

PROTEIN PHOSPHATASES REQUIRED IN CX43-MEDIATED ANTIAPOPTOTIC EFFECT OF ALENDRONATE IN OSTEOBLASTS

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Bisphosphonates (BPs) exert antiapoptic effects in osteoblasts through connexin 43 (Cx43) hemichannel opening and activation of ERKs. The events elicited by BPs upstream of hemichannel opening remain unknown. Whole cell binding assays using 3H-alendronate (ALE) showed the presence of saturable, specific and high affinity binding sites in both ROS 17/2.8 osteoblastic cells and rat calvaria-derived osteoblasts, which express Cx43. 3H-ALE bindingf to ROS 17/2.8 cells was displaced by unlabeled ALE, as well as by two other BPs, olpadronate and etidronate, with Kds of 0.65 ± 0.2; 0.6 ± 0.3; and 1 ± 0.15 mM, respectively. Unexpectedly, 3H-ALE also bound specifically to HeLa cells that do not express Cx43, and to ROS 17/2.8 cells pretreated with agents that disassemble Cx channels. Moreover, ALE does not prevent apoptosis of cells in that condition. These results indicate that BPs bind to an entity different from Cx43 and that BP binding is not sufficient to induce survival. We found that 3H-ALE bound to ROS 17/2.8 cells was displaced by phosphatase substrates and also inhibited BP-induced anti-apoptosis of HeLa cells transfected with Cx43, demonstrating that indeed ALE binding is required for anti-apoptosis. These findings indicate that, although required for triggering intracellular signaling by BPs, Cx43 is dispensable for BP binding and that BPs bind to a Cx43-interacting phosphatase.

ST-P25
INSULIN REGULATES A DIACYLGLYCEROL KINASE THAT PREFERENTIALLY UTILIZES 18:0-20:4 DAG IN RAT BRAIN

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We have recently reported that insulin increased phosphatidic acid (PA) synthesis from diacylglycerol kinase activity (DAGK) in rat cerebral cortex (CC) synaptosomes. This effect is mediated by the activation of PC-PLD/PAP2 and PIP_2-PLC pathways to provide DAG, and a direct DAGK activation that seems to be associated to the utilization of a 18:0-20:4 DAG. Synaptosomal DAGK activity was evaluated using either 18:0-20:4 DAG or di-16:0 DAG and [γ-^{32}P]ATP as substrates in an octylglucoside micellar assay. At 1 mM ATP, DAGK from CC synaptosomes preferentially synthesized 18:0-20:4 PA. DAGK affinity for 18:0-20:4 DAG was one order magnitude higher than di-16:0 DAG. Insulin increased PA synthesis from 18:0-20:4 DAG by 100 % when vanadate, was used as Tyr protein phosphatase (PP) inhibitor. Genistein (a TK inhibitor) inhibited the insulin effect. Addition of NaF, a Ser-Threo PP inhibitor increased PA synthesis by 340 %. In this condition PIP_2 phosphorylation was strongly reduced. PA synthesis induced by insulin plus vanadate was not modified when okadaic acid (a PP1 and PP2A inhibitor) or EGTA (a PP2B inhibitor) were present. It is known that DAGK ε selectively phosphorylates 18:0-20:4 DAG, whereas other isoforms show no apparent selectivity. DAGK ε has been reported to be negatively modulated by PIP_2. Our results suggest that DAGK ε participates in insulin signalling in CC synaptosomes.

ST-P26
FREE IRON STIMULATES PI3K/AKT PATHWAY IN RAT CEREBRAL CORTEX SYNAPTIC ENDINGS

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The presence of phosphoinositide-3-kinase (PI3K)/Akt pathway in cerebral cortex synaptic endings has been previously reported. The aim of this work was to determine the involvement of PI3K in oxidative damage triggered by free iron. Purified synaptosomes were exposed to FeSO4 (50 μM) for different periods of time. Fe2+ effects on synaptosomes were evaluated by measuring lactate dehydrogenase (LDH) leakage, protein tyrosine phosphorylation and mitochondrial integrity by MTT assay. The most striking effects on glutamate uptake, LDH release and tyrosine phosphorylation were observed after 1 h of incubation in the presence of the metal ion. For the determination of PI3K activity, synaptosomes preincubated with Fe2+ for 5 min were immunoprecipitated with anti-p85 antibody. PI3K activity from synaptosomes incubated with Fe2+ was 2-fold higher than that under control conditions. Akt activation was only evidenced after 5 min of incubation in the presence of Fe2+. Our results demonstrate an early activation of PI3K/Akt pathway in synaptosomes even though the most deleterious effects of oxidative stress were evidenced at longer times of incubation (60 and 120 min).

ST-P27
GENOMIC AND IMMUNOLOGIC CHARACTERIZATION OF STCDPK1 AND STCDPK2

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During tuberization, dormancy and sprouting of potato tubers, calcium-dependent protein kinase isoforms, StCDPK1 and StCDPK2, are differentially expressed. The genomic characterization of both isoforms was undertaken. The complete genomic sequence of StCDPK1 was obtained and SSCP analysis was used to map the gene. Comparison of StCDPK1 with CDPK genes from Arabidopsis and rice genomes showed that it shares the 8-exons/7-introns structure of the CDPKs from group IIa. StCDPK1 maps in the distal end of chromosome 12, close to the TC69927 marker. A PCR approach was designed to amplify StCDPK2 gene assuming that both kinases share a similar gene structure. We were able to amplify the genomic sequence corresponding to the regulatory domain of StCDPK2. The results obtained show that intron´s length and positon are identical in both kinases. Experiments are being performed to obtain the complete genomic sequence and to map StCDPK2 gene in the potato genome. Western analysis and immunolocalization of StCDPK1 and StCDPK2 were performed. StCDPK1 was detected in the particulate fraction of stolons and histochemical analysis show that it is associated to the outer epidermal layer of cells while StCDPK2 was detected in soluble extracts. A recombinant StCDPK2-6xHis fusion protein was purified and enzymatic assays are being performed to characterize StCDPK2 kinetic parameters.

ST-P28
PTP1B CONTROLS THE ROLE OF NSF DURING THE ACROSOMAL REACTION

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The acrosome reaction (AR) in mammalian sperm is a Ca^{2+}-dependent exocytosis that consists of several steps: tethering of the acrosome to the plasma membrane promoted by Rab3A, priming of the fusion machinery by N-ethylmaleimide-sensitive factor (NSF) and α-SNAP, docking of the vesicle by SNAREs, efflux of calcium from the acrosome and, bilayer mixing. Priming includes the disassembly of unproductive SNARE complexes present in resting sperm. These proteins re-assemble to form molecular bridges between the membranes that are going to fuse (docking). Tyrosine phosphorylation displays a key role on sperm function. PTP1B is a tyrosine phosphatase implicated in numerous signaling processes. We propose that NSF is tyrosine phosphorylated in human sperm and that dephosphorylation is required for its function. We suggest that the relevant PTP is PTP1B. First, we found that a phosphomimetic NSF mutant (NSF-Y83E) but not the wild-type protein blocks Ca^{2+}-induced exocytosis. Second, both an anti-PTP1B antibody and a catalytically inactive PTP1B mutant (PTP1B D181A) prevent the AR. Addition of recombinant NSF relieves the block. Interestingly, when the protein is phosphorylated in vitro, it loses the ability to rescue exocytosis. We conclude that PTP1B acts before priming, releasing NSF from its inactive - tyrosine phosphorylated state - allowing it to achieve its positive role in the AR.

ST-P29

AP-1 REGULATION BY EXPOSURE TO SUBLETHAL DOSES OF CHLORPYRIFOS IN *Bufo arenarum* EMBRYOS

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There is a concern about the effects elicited by some organophosphates (OP) like chlorpyrifos (Cpf) at doses below the recognized anticholinesterase effects. We have been studying the adverse effects of several OP at sublethal doses causing oxidative stress in *B. arenarum* embryos. Here we show the effects of sublethal exposure to Cpf on the expression and function of c-Fos and c-Jun. Western blot analyses of c-Fos showed a decrease when embryos were exposed to 2 mg/L Cpf for 24 h and 48 h, while it increased at 96 h. On the other hand, C-jun showed an increase after 96 h of exposure at the same concentration. Cpf also reduced AP-1 binding (EMSA), at 48 h of exposure. Acetylcholinesterase activity was not affected by 2 mg/L Cpf. Elevation of c-Fos through a non-cholinergic mechanism by Cpf exposure has been reported in other systems, followed by apoptosis. However we could not see any sign of DNA fragmentation by agarose gel electrophoresis in *B. arenarum* embryos treated with 2 mg/L Cpf. Cpf tended to lower catalase and GSH-S-transferase and increase GSH-reductase activities suggesting a response to oxidative stress. Thus, AP-1 downregulation may be reflecting other mechanisms shared by c-Jun, such as Antioxidant Response Element binding.

ST-P30

PROTEIN KINASE ACTIVITIES IN *Bufo arenarum* EMBRYOS EXPOSED TO AZINPHOSMETHYL AND CARBARYL

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Most relevant efforts to understand pesticide actions are presently directed to cellular signaling and gene response. We evaluate here the effects of azinphosmethyl (AM, 9mg/L) and carbaryl (CB, 20mg/L) on basal protein kinase, PKC and PKA activities in *Bufo arenarum* larvae after continuous exposure from fertilization. The activity of the oxidative stress- responding enzyme GST was also evaluated. Kinase assay (^{32}PγATP; Histone) conditions were fitted using adult toad brain. Control larvae showed a significant basal kinase (30.2 dpm/µg protein) and PKA (cAMP-activated phosphorylation; 12.7 dpm/µg protein) activity, but PKC (DAG/PC-activated phosphorylation) was absent at this stage. Exposure to AM caused a significant increase of basal kinase (199%) and PKA (192%), and activated PKC (22.6 dpm/µg protein). Exposure to CB only produced a significant increase in basal kinase activity (124%). Larvae showed an induction of GST activity due to AM exposure (71%) and to a lesser extent to CB (33%) ($p<0.01$). The induction of PKC and PKA observed with AM could be related to the degree of oxidative stress generated by AM compared to CB as suggested by GST induction levels. The appearance of PKC activity in treated embryos may be associated with cell ability to induce detoxifying enzymes such as GST via Nrf2-ARE in response to electrophilic contaminants.