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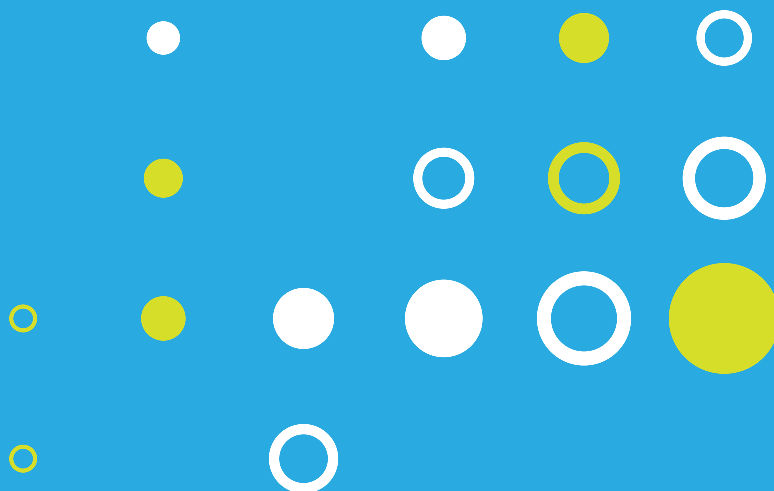

SAIB Sociedad Argentina de Investigaciones en Bioquímica y Biología Molecular

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ARGENTINE SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY
XXXIX ANNUAL MEETING

Sociedad Argentina de Investigación en
Bioquímica y Biología Molecular

BIOPHYSICAL SOCIETY OF ARGENTINA
XXXII ANNUAL MEETING

Sociedad Argentina de Biofísica

BARILOCHE PROTEIN SYMPOSIUM

SAN CARLOS DE BARILOCHE,
ARGENTINA
November 17 - 21, 2003

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"Algunos creen que la ciencia es un lujo
y que los grandes países gastan en ella
porque son ricos. ¡Grave error!
Los países ricos gastan en Ciencia
porque es un gran negocio y porque
de esta forma se enriquecen.
No gastan en Ciencia porque son ricos y prósperos;
son ricos y prósperos porque gastan en Ciencia.
¡Nada da dividendos comparables a los que
proporciona la investigación científica y tecnológica!"

Dr. Bernardo A. Houssay
Premio Nobel de Fisiología y Medicina (1947)

ACKNOWLEDGMENTS

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- Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)
- Agencia Nacional de Promoción Científica y Tecnológica
- Fundación ANTORCHAS
- Instituto Fundación Leloir UBA
- Secretaría de Ciencia y Tecnología – Universidad Nacional de Buenos Aires
- Universidad Nacional de Quilmes
- FONCYT (RC)

OVERVIEW

Monday	17-Nov DAY ONE	Tuesday	18-Nov DAY TWO	Wednesday	19-Nov DAY THREE	Thursday	20-Nov DAY FOUR
		8:30-10:30	Symposium II Lipid-Prot & Prot-Prot Inter	8:30-10:30	Symposium IV Channels & Transporters	8:30-10:30	Symposium V Microbiology
110:00 15:00	**Registration**	10:30-11:00		10:30-11:00		10:30-11:00	
		11:00-12:00	Lecture: Dr. JM Ruysschaert	11:00-12:00	Lecture: Dr. E. Gratton	11:00-12:00	Lecture: Dr. S. Moreno Perez
		12:00-14:00	**Lunch**	12:00-14:00	**Lunch**	12:00-14:00	**Lunch**
		14:00-16:30	Symposium III Protein Folding & Design	14:00-15:00	Lecture: Dr. V. Ruiz	14:00-14:30	Lecture: Dr. U. Hellman
15:00-16:00	Opening Lecture Dr. Dalla-Favera			15:15-16:15	**Lecture:** Dr. E. Sztul	14:30-16:00	Symposium VI Plant Biochemistry
16:00-16:15	Coffee Break	16:30-17:00	Coffee Break	16:15-16:30	Coffee Break	16:00-16:30	Coffee Break
16:15-18:35	Symposium I Glycobiology	17:00-18:00	Lecture: Dr. A. Johnson	16:30-20:00	CO MICROBIOL Sala A CO CELL BIOL Sala B CO STRUCT BIOL Sala C POSTERS: Salón Los Jardines	16:30-17:00	Session *in mem* A Stoppani
18:45-19:45	Lecture: Dr.Gierasch	18:00-20:00	CO LIPIDS Sala A CO CHANNELS Sala B CO PLANTS Sala C POSTERS: Salón Los Jardines	20:00-22:00	**Dinner**	17:00-18:00	Lecture: Dr. J. Johnson
19:45-21:00	Science & Politics	20:00-22:00	**Dinner**	22:00-24:00	Assemblies SAB & SAIB	18:00-20:00	CO SIGNAL TRANSD Sala A CO BIOTECNOL Sala B CO PLANT/MICROB Sala C POSTERS: Salón Los Jardines
21:00	**Dinner**					20:00-24:00	**Dinner**

SAIB
PS
General
SAB

CO: Communications
Sala A: Salon Los Maitenes
Sala B: Salon Los Radales
Sala C: Salon Los Arrayanes

PROGRAM

MONDAY, November 17, 2003.

11:00 - 15:00 (Hall Salón Auditorio) **Registration**

15:00 - 16:00 (Salón Auditorio)

OPENING LECTURE

Dr. Riccardo Dalla-Favera
(Institute for Cancer Genetics, Columbia University, USA)
"Molecular genetics of cancer: lessons from B cell lymphoma"
Chairperson: Dr. Ernesto Podestá - (Facultad de Medicina, Universidad de Buenos Aires) - Argentina

16:00 - 16:15 **Coffee break**

Symposium I (Salón Auditorio)

"Luis Federico Leloir" Symposium: Glycobiology
Chairpersons: Dr. Armando Parodi and Dr. Ricardo Wolosiuk (Instituto Fundación Leloir, Buenos Aires). Argentina.

Speakers:

16:15 - 16:50 **S1** **STRUCTURAL BASIS OF GLYCOGEN SYNTHESIS**
Dr. Pedro Alzari (Institut Pasteur, Paris, France)

16:50 - 17:25 **S2** **THE MULTI-FACETED MANNOSE 6-PHOSPHATE RECEPTORS**
Dr. Nancy Dahms (Medical College of Wisconsin, USA)

17:25 - 18:00 **S3** **THE NUCLEOTIDE SUGAR TRANSPORT/ANTIPORT CYCLE OF THE ENDOPLASMIC RETICULUM AND GOLGI APPARATUS: FROM BASIC SCIENCE TO DISEASE**
Dr. Carlos Hirschberg (Boston University, USA)

18:00 - 18:35 **S4** **DOMAIN ORGANIZATION AND PATTERN RECOGNITION OF UDP-GLC:GLYCOPROTEIN GLUCOSYL TRANSFERASE (GT)**
Dr. Armando Parodi (Instituto Fundación Leloir Buenos Aires, Argentina)

18:45 - 19:45 (Salón Auditorio) **PLENARY LECTURE**

Dr. Lila M. Gierasch
(University of Massachusetts at Amherst, USA)
"Folding of a predominantly beta-sheet protein *in vitro* and *in vivo*"
Chairperson: Dr. Gonzalo de Prat Gay (Instituto Fundación Leloir, Buenos Aires). Argentina.

19:45- 20:30 (Salón Auditorio) **Science and Politics Workshop**

Chairperson: Dra. Norma Sterin de Speziale (Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires). Argentina

Invited Members of Science Administration Organisms

Dr. Lino Barañao. Presidente de la Agencia Nacional de Promoción Científica y Tecnológica.

Dr. Juan Carlos Pugliese. Secretario de Políticas Universitarias.

Dr. Ricardo Farías. Miembro del Directorio del CONICET.

Lic. Mario José Lattuada. Vicepresidente de Asuntos Tecnológicos del CONICET.

20:30 **Dinner**

TUESDAY, November 18, 2003.

Symposium II (Salón Auditorio)

Lipid-Protein and Protein-Protein Interactions

Chairperson: Dr. Silvia Alonso (Universidad Nacional de Quilmes) and Dr. Horacio Garda (Universidad Nacional de La Plata). Argentina.

Speakers

8:30 - 9:00 **S5** **STRUCTURAL STUDIES OF MELANOCORTIN PEPTIDES IN AQUEOUS AND LIPID MEDIA**
Dr. M. Teresa Lamy-Freund (Instituto de Física, Universidade de São Paulo, Brazil)

9:00 - 9:30 **S6** **LIPID-PROTEIN INTERACTIONS AT THE OUTER AND MIDDLE RINGS OF THE ACETYLCHOLINE RECEPTOR TRANSMEMBRANE DOMAINS**
Dr. F.J. Barrantes (INIBIBB, Univ. Nac. del Sur, Bahía Blanca, Argentina)

9:30 - 10:00 **S7** **PROTEIN-PROTEIN AND PROTEIN-LIGAND INTERACTIONS OF THE GTPASE DYNAMIN**
Dr. David Jameson (University of Hawaii, Honolulu, Hawaii,USA)

10:00 - 10:30 **S8** **PROTEIN-LIPID AND PROTEIN-PROTEIN INTERACTIONS: DIRECT VISUALIZATION BY 2-PHOTON MICROSCOPY**
Dr. Susana Sanchez (University of Illinois at Urbana-Champaign, USA)

10:30 - 11:00 **Interval**

11:00 - 12:00 (Salón Auditorio) **PLENARY LECTURE**

Dr. Jean Marie Ruysschaert
(Université Libre de Bruxelles, Belgium)
"Detection of conformational changes in multidrug transporters"
Chairperson: Dra. Silvia. Alonso (Universidad Nacional de Quilmes). Argentina

12:00 - 14:00 **Lunch**

Symposium III (Salón Auditorio)

Protein Folding and Design

Chairpersons: Dr. Jose Maria Delfino (IQUIFIB, Universidad de Buenos Aires) and
Dr. Alejandro J. Vila (Universidad de Rosario). Argentina.

Speakers:

14:00 - 14:35 **S9** **METALLOPROTEIN DESIGN: ENGINEERING METAL-BINDING SITES INTO NATIVE PROTEIN SCAFFOLDS**
Dr. Yi Lu (University of Illinois at Urbana-Champaign, USA)

14:35 - 15:10 **S10** **FROM SEQUENCE TO CONSEQUENCE**
Dr. Dagmar Ringe (Brandeis University, USA)

15:10 - 15:45 **S11** **STRUCTURAL PROPERTIES AND KINETIC ROLE OF EARLY INTERMEDIATES IN PROTEIN FOLDING**
Dr. Heinrich Roder (Fox Chase Cancer Center, Philadelphia, USA)

15:45 - 16:20 **S12** **HOW DO MUTANT COPPER-ZINC SUPEROXIDE DISMUTASE PROTEINS KILL MOTOR NEURONS ?**
Dr. Joan S. Valentine (UCLA, Los Angeles, California, USA)

16:20 - 16:30 **Conclusions Symposium III**

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

17:00 - 18:00 (Salón Auditorio) **PLENARY LECTURE**

Dr. Arthur E. Johnson

(The Texas A&M University System Health Sciences Center, USA)

"Cholesterol-dependent structural transitions initiate oligomerization and beta-barrel pore formation by a bacterial protein toxin"

Chairperson: Dr. Fernando Goldbaum (Instituto Fundación Leloir, Buenos Aires, Argentina)

COMMUNICATIONS

SALA A: **Lipids**

Chairpersons: Dr. Carlos Alberto Marra (INIBIOLP, CONICET-UNLP, Cátedra de Bioquímica, Facultad de Ciencias Médicas, UNLP. La Plata, Argentina)
Dra. María del Carmen Fernández Tomé (IQUIFIB-CONICET, Cátedra de Biología Celular, Facultad de Farmacia y Bioquímica, UBA. Buenos Aires, Argentina).

18:00 - 18:15 **LI-C1** **VANADYL SULFATE, AN INSULIN-MIMETIC, DOES NOT ALTER UNSATURATED FATTY ACID BIOSYNTHESIS IN NORMAL OR STREPTOZOTOCIN RATS**
Brenner, Rodolfo R.; González, María S.; Basabe, Juan C. and Bernasconi, Ana M.

18:15 - 18:30 **LI-C2** **SELECTIVE PROTECTION OF C20:4 n6 AND C22:6 n3 BY MELATONIN DURING NON ENZYMATIC LIPID PEROXIDATION OF RAT LIVER, KIDNEY AND BRAIN MICROSOMES AND MITOCHONDRIA**
Leaden, Patricio and Catalá, Angel

18:30 - 18:45 **LI-C3** **PHOSPHATIDYLCHOLINE SYNTHESIS REGULATION BY PGD_2 IS MEDIATED BY MAPK ACTIVATION**
Fernández-Tome, M.; Favale, N.; Speziale, E. and Sterin-Speziale, Norma

18:45 - 19:00 **LI-C4** **HEAT INDUCED CHANGES IN TESTICULAR LIPIDS CONTAINING VERY LONG CHAIN POLYUNSATURATED FATTY ACIDS (VLCPUFA)**
Furland, N.E.; Maldonado, E.N. and Aveldaño, M.I.

19:00 - 19:15 **LI-C5** **GLUCOSE-INDUCED DECREASE OF PHOSPHATIDYL-ETHANOLAMINE AFFINITY FOR THE TRANS-MEMBRANE SURFACE OF MEMBRANE PROTEINS**
Levi, Valeria; Villamil Giraldo, Ana M.; Castello, Pablo R.; Rossi, Juan P.F.C. and González Flecha, F. Luis.

19:15 - 19:30 **LI-C6** **c-FOS REORGANIZES PHOSPHOLIPIDS AT THE INTERFACE**
Borioli, Graciela; Rosetti, Carla; and Maggio, Bruno

19:30 - 19:45 **LI-C7** **INTERACTION OF AN ASPARTYL PROTEASE WITH LIPID INTERFACES CONTAINING PHOSPHATIDYLGLYCEROL OR PHOSPHATIDYL ETHANOLAMINES**
Martini, M. Florencia; and Disalvo, E. Anibal.

19:45 - 20:00 **LI-C8** **COLLISIONAL TRANSFER OF FATTY ACIDS FROM IFABP TO MEMBRANES: IMPORTANCE OF THE LYSINE RESIDUES IN THE -HELICAL DOMAIN**
Laborde, Lisandro; Falomir, Lisandro; Corsico, Betina; Garda, Horacio A. and Storch, Judith.

SALA B: **Channels and Transporters**

Chairpersons: Dr. Graciela Berberian (INIMEC, Universidad Nacional de Cordoba) and
Dr. Luis Gonzalez Flecha (IQUIFIB, Universidad de Buenos Aires).

Speakers:

18:00 - 18:15 **CA-C1** **MEASUREMENT OF CELL VOLUME CHANGES IN WILD TYPE AND AQUAPORIN TRANSFECTED RENAL CELLS**
Ford, Paula; Rivarola, Valeria; Chara, Osvaldo; Parisi, Mario and Capurro, Claudia.

18:15 - 18:30 **CA-C2** **NICOTINIC RECEPTOR M3 TRANSMEMBRANE DOMAIN: ROLE IN CHANNEL ACTIVATION**
De Rosa M.J. and Bouzat C.

18:30 - 18:45 **CA-C3** **ACTIVATION OF PLANT PLASMA MEMBRANE H^+-ATPase (AHA2) BY 14-3-3 PROTEIN MEDIATED OLIGOMERIZATION**
Yudowski G, Berberian G, Palmgren M, Beaugé L, and Roberts G.

18:45 - 19:00 **CA-C4** **SITES INVOLVED IN THE SPONTANEOUS OCCLUSION OF K^+ IN THE Na,K ATPase**
González-Lebrero RM, Kaufman SB, Garrahan PJ, Rossi RC.

19:00 - 19:15 **CA-C5** **FORSKOLINE ACTIVATED CURRENT IN WILD TYPE AND VINCRISTINE RESISTANT K562 CELL LINE**
Assef, Yanina A; Cavarra, Soledad; Damiano, Alicia; Zotta, Elsa; Ibarra, Cristina; Kotsias, Basilio

19:15 - 19:30 **CA-C6** ***ARABIDOPSIS THALIANA*** **PLANT PLASMA MEMBRANE AQUAPORINS SHUT DOWN AT LOW CYTOPLASMIC pH**
Sutka, Moira; Alleva, Karina; Tournaire-Roux, Colette; Parisi, Mario; Maurel, Christophe; and Amodeo, Gabriela.

19:30 - 19:45 **CA-C7** **LIPID PHASE DISTRIBUTION OF NICOTINIC ACETYLCHOLINE RECEPTOR PROTEIN**
Wenz, Jorge J. and Barrantes, F. J.

19:45 - 20:00 **CA-C8** **A MODEL ACCOUNTING FOR SARCOPLASMIC RETICULUM Ca-ATPase MAXIMAL ACTIVATION AT HIGH ATP AND METAL CONCENTRATIONS**
González Débora A., Ostuni Mariano A. and Alonso Guillermo L.

SALA C **Plant Biochemistry and Physiology**

Chairpersons: Dr. Alberto A. Iglesias (Fac. Bioquímica y Cs. Biol., Univ. Nac. Litoral) and Dr. Lorenzo Lamattina (Universidad Nacional de Mar del Plata). Argentina.

18:00 - 18:15 **PL-C1** **POTASSIUM UPTAKE-KINETICS AND GENE EXPRESSION ALONG BARLEY ROOT AXIS**
Vallejo AJ, Peralta MI, Danna CH and Santa-María GE.

18:15 - 18:30 **PL-C2** **CELL WALL DEGRADING ENZYMES DURING RIPENING OF STRAWBERRY FRUIT**
Rosli HG, Civello PM, Martínez GA.

18:30 - 18:45 **PL-C3** **ENHANCED TOLERANCE TO IRON DEFICIENCY IN TRANSGENIC TOBACCO PLANTS EXPRESSING A BACTERIAL FLAVODOXIN**
Tognetti VB, Zubriggen MD, Valle EM, Carrillo NJ, Morandi E, Fillat M.

18:45 - 19:00 **PL-C4** **INDUCTION OF TWO ENDOPROTEOLYTIC ACTIVITIES IN SECESCENT WHEAT LEAVES**
Roberts IN, Passeron S, Barneix AJ.

19:00 - 19:15 **PL-C5** **CLONING AND CHARACTERIZATION OF FRUCTOSYLTRANSFERASES GENES IN GRAMINEAE**
del Viso F, Heinz R, Puebla AF. **Poster PL-P56 (p. 147) (INTERVAL)**

19:15 - 19:30 **PL-C6** **PECTINOLYTIC ACTIVITIES IN POTATO-FUSARIUM INTERACTION**
Olivieri FP, Machinandiarena MF, Daleo GR.

19:30 - 19:45 **PL-C7** **INVOLVEMENT OF LeCDPK AND PP2A IN RESPONSE TO ABIOTIC STRESS IN TOMATO PLANTS**
Capiati D, Pais SM, Coluccio MP, Téllez-Iñón MT.

19:45 - 20:00 **PL-C8** **CLONING, EXPRESSION AND BIOCHEMICAL CHARACTERIZATION OF THE FRATAXIN HOMOLOG FROM ARABIDOPSIS THALIANA (Athfh)**
IIB-INTECH, Argentina: Busi MV, Burgos JL, Zabaleta, E, Gomez Casati DF.

18:00 - 20:00 (Salón "Los Jardines") **POSTER SESSION I**

Microbiology: MI-P1 to MI-P48
Biotechnology: BT-P1 to BT-P35
Structural Biology: BE-P1 to BE-P38
Cell Biology: BC-P1 to BC-P19

WEDNESDAY, November 19, 2003.

Symposium IV (Salón Auditorio)

Channels and Transporters: Structure and Function

Chairperson: Dr. Cecilia Bouzat (INIBIBB, Bahía Blanca, Argentina) and
Dr. Gabriela Amodeo (Universidad de Buenos Aires, Argentina).

Speakers:

8:30 - 9:00 **S13** **INTRASTERIC REGULATION OF THE CA2+ TRANSPORTER FROM PLASMA MEMBRANES**
Dr. Hugo Adamo (IQUIFIB, Buenos Aires, Argentina)

9:00 - 9:30 **S14** **REGULATION AND FUNCTIONAL ROLE OF AQUAPORIN WATER CHANNELS IN HEPATOCYTES**
Dr. Raúl Marinelli (Universidad Nacional de Rosario, Argentina)

9:30 - 10:00 **S15** **NICOTINIC RECEPTORS OF COCHELAR AND VESTIBULAR SENSORY SYSTEMS: FORM MOLECULAR STRUCTURE TO FUNCTION**
Dr. Belen Elgoyhen (INGEBI Buenos Aires, Argentina)

10:00 - 10:30 **S16** **MOLECULAR BASIS OF CHANNEL GATING OF CYS-LOOP RECEPTORS**
Dr. Cecilia Bouzat (INIBIBB, Bahía Blanca, Argentina)

10:30 - 11:00 **Interval**

11:00 - 12:00 (Salón Auditorio) ***Gregorio Weber*** **PLENARY LECTURE**

Dr. Enrico Gratton
(University of Illinois at Urbana-Champaign, USA)
"3-D Particle Tracking in a Two-photon Microscope"
Chairperson: Dr. David Jameson (University of Hawaii, Honolulu, Hawaii, USA)

12:00 - 14:00 **Lunch**

14:00 - 15:00 (Salón Auditorio) **PLENARY LECTURE**

Dr. Valentina Ruiz Gutierrez
(Instituto de de la Grasa (CSIC). Seville, Spain)
"Influence of virgin olive oil on cardiovascular risk factor"
Chairperson: Dr. Angel Catala (Universidad Nacional de La Plata). Argentina

15:00 - 15:15 **Interval**

15:15 - 16:15 (Salón Auditorio) **PLENARY LECTURE**

Dr. Elizabeth Sztul
(University of Alabama at Birmingham, USA)
"Molecular regulation of membrane traffic between the ER and the Golgi"
Chairperson: Dr. Cecilia Alvarez (Universidad Nacional de Córdoba). Argentina

16:15 - 16:30 **Coffee Break**

COMMUNICATIONS

SALA A: **Microbiology**

Chairpersons: Dra. Cristina Nowicki (Universidad Nacional de Buenos Aires) and
Eleonora García Véscovi (Universidad Nacional de Rosario) Argentina.

16:30 - 16:45 **MI-C1** **CHEMICAL MODIFICATION OF AN α-MANNOSYL-TRANSFERASE FROM *Acetobacter xylium***
Abdian, Patricia L.; Barreras, Máximo; Geremia, Roberto A.; and Ielpi, Luis.

16:45 - 17:00 **MI-C2** ***Rhodobacter capsulatus SOD* MUTANT DISPLAYS INCREASED SPONTANEOUS DNA MUTAGENESIS**
Bortolotti, Ana; Bittel, Cristian; Tabares, Leandro C.; and Cortez, Néstor.

17:00 - 17:15 **MI-C3** **THE RPON GENE OF *BRUCELLA ABORTUS* IS IMPORTANT FOR THE BACTERIAL PERSISTENCE IN MICE**
Iannino F, Ciocchini A, Vidal Russell R, Ugalde RA, and Iñón de Iannino N.

17:15 - 17:30 **MI-C4** **MOLECULAR CLONING, DNA SEQUENCING AND EXPRESSION OF AN UBIQUITIN CONJUGATING ENZYME GENE FROM *Trypanosoma cruzi***
Pravia, Carlos; Búa, Jaqueline; Bontempi, Esteban; Ruiz, Andrés.

17:30 - 17:45 **MI-C5** **EXPRESSION OF OAT ARGININE DECARBOXYLASE (ADC) GENE IN *Trypanosoma cruzi* EPIMASTIGOTES**
Serra MP, Carrillo C, Huber A, González NS, and Algranati ID.

17:45 - 18:00 **MI-C6** **IDENTIFICATION AND ANALYSIS OF TRYPOMASTIGOTE STAGE-SPECIFIC GENES IN *Trypanosoma cruzi***
Tekiel V, Agüero F, and Sánchez D.

18:00 - 18:15 **MI-C7** **CLONING AND EXPRESSION OF A PLAUSIBLE CYTOSOLIC TYPE MALATE DEHYDROGENASE (cMDH) FROM *Leishmania mexicana***
Aranda A, Leroux A, Cazzulo JJ, and Nowicki C.

18:15 - 18:30 **MI-C8** **TRANSCRIPTIONAL REGULATION OF SUCROSE BIOSYNTHESIS IN *Anabaena* sp., A NITROGEN- FIXING CYANOBACTERIUM**
Cumino, Andrea C.; Giarrocco, Laura E.; Salerno, Graciela L.

18:30 - 18:45 **Interval**

18: 45 - 19:00 **MI-C9** **IDENTIFICATION BY MICROARRAYS OF A NOVEL DELETION IN A MEMBER OF *Mycobacterium tuberculosis* COMPLEX: *M.microti***
Caimi K, García-Pelayo C, Bigi F, Romano MI, Gordon S, and Cataldi A.

19:00 - 19:15 **MI-C10** ***Vibrio cholerae*-INDUCED APOPTOSIS OF MAMMALIAN CELLS MEDIATED BY EL TOR HAEMOLYSIN**
Saka Héctor A., Bidinost Carla, Echenique José, Chinen Isabel, Bonacci Gustavo and Bocco José L.

19:15 - 19: 30 **MI-C11** **CHARACTERIZATION OF AN ADAPTIVE ACID-TOLERANCE MECHANISM IN *Streptococcus pneumoniae*: ANALYSIS OF QUORUM-SENSING MUTANTS AND IDENTIFICATION OF ACID-INDUCED PROTEINS**
Cortes, Paulo; Piñas, Germán E. and Echenique, José R.

19:30 - 19:45 **MI-C12** **A SALMONELLA SPECIFIC TRANSCRIPTIONAL REGULATOR THAT REPONDS TO GOLD**
Checa, Susana K.; Botta, Pablo E.; Spinelli, Silvana V. and Soncini, Fernando C.

19:45 - 20:00 **MI-C13** **REGULATION OF SPORULATION AND ENTEROTOXIN PRODUCTION OF THE GAS GANGRENE PRODUCER *Clostridium perfringens* TYPE A FOOD-POISONING**
Philippe Valeria, Orsaria Lelia and Grau Roberto.

20:00 - 20:15 **MI-C14** **EFFECT OF THE MUTATIONS ON THE Cys RESIDUES IN THE *Escherichia coli* NADH-DEHYDROGENASE-2 ACTIVITIES**
Volentini, Sabrina; Solbiati, José; Rapisarda, Viviana; Rodríguez Montelongo, Luisa and Farías, Ricardo.

SALA B: **Cell Biology**

Chairpersons:
Section I (16:30 - 17:45): Dr. María I. Colombo (Universidad Nacional de Cuyo) and Dr. Luis Mayorga (Universidad Nacional de Cuyo)
Section II (18:00 - 19:15): Dr. Cecilia Alvarez (Universidad Nacional de Córdoba) and Dr. José Mordoh (Instituto L. F. Leloir)
Section III (19:30 - 20:30): Dr. Beatriz Caputto (Universidad Nacional de Córdoba) and Dr. Silvia Moreno (Universidad de Buenos Aires).

16:30 - 16:45 **BC-C1** **DYNAMICS PROPERTIES OF THE GTPase RAB1 IN LIVING CELLS**
Monetta Pablo, and Alvarez Cecilia.

16:45 - 17:00 **BC-C2** **GANGLIOSIDE GLYCOSYLTRANSFERASES ORGANIZE IN DISTINCT MULTIENZYME COMPLEXES IN CHO-K1 CELLS**
Giraudo, Claudio G. and Maccioni, Hugo J.F.

17:00 - 17:15 **BC-C3** **AUTOPHAGY AS A NEW TARGET FOR CONTROL OF COXIELLA AND MYCOBACTERIUM REPLICATION**
Gutierrez, M.; Vazquez, C.; Munafó D.; Berón, W.; Master, S.; Deretic, V.; and. Colombo, M.I.

17:15 - 17:30 **BC-C4** **NUCLEOSIDE DIPHOSPHATASE AND GLYCOSYLTRANSFERASE ACTIVITIES CAN LOCALIZE TO DIFFERENT SUBCELLULAR COMPARTMENTS**
D´Alessio, Cecilia; and Parodi, Armando J.

17:30 - 17:45 **BC-C5** **DYNAMICS OF SNARE ASSEMBLY AND DISASSEMBLY DURING HUMAN SPERM EXOCITOSIS**
De Blas, G; Tomes, C; Yunes, R and Mayorga, LS.

17:45 - 18:00 **Interval**

18:00 - 18:15 **BC-C6** **THE CATION-DEPENDENT MANNOSE-6-PHOSPHATE RECEPTOR IS NECESSARY FOR DEVELOPMENT OF RAT LIVER LYSOSOMES**
Romano P, López C, Carvelli L, Sartor T, and Sosa MA.

18:15 - 18:30 **BC-C7** **ASSOCIATION OF TETRASPANIN CD63 WITH INTEGRINS IN HUMAN DENDRITIC CELLS: IMPLICATIONS FOR CELL MIGRATION. A COMPARATIVE STUDY WITH OTHER TETRASPANINS**
Mantegazza, Adriana R.; Barrio, Marcela and Mordoh, José.

18:30 - 18:45 **BC-C8** **RESTRUCTURING OF FOCAL CONTACTS BY BRADYKININ IN RAT RENAL PAPILLA**
Marquez, Gabriela; Serrano, Diego; Gagliano, Laura; Sterin-Speziale, Norma.

18:45 - 19:00 **BC-C9** **ACTH-INDUCED CAVEOLIN-1 PHOSPHORYLATION IS RELATED TO PODOSOME ASSEMBLY IN Y1 ADRENAL CELLS**
Colonna C. and Podestá E.

19:00 - 19:15 **BC-C10** **IDENTIFICATION OF TARGETING SEQUENCES WITHIN PROTEIN TYROSINE PHOSPHATASE 1B (PTP1B) TO DIFFERENT CYTOPLASMIC COMPARTMENTS**
Davies Sala, Georgina, and Arregui, Carlos O.

19:15 - 19:30 **Interval**

19:30 - 19:45 **BC-C11** **INFLUENCE OF SPHINGOLIPIDS ON NICOTINIC ACETYLCHOLINE RECEPTOR ASSEMBLY, TRAFFICKING AND CELL-SURFACE TARGETTING**
Baier CJ, and Barrantes FJ.

19:45 - 20:00 **BC-C12** **POSSIBLE MECHANISMS INVOLVED IN c-FOS ACTIVATION OF PHOSPHOLIPID SYNTHESIS**
Portal MM, Gil GA, Renner ML, and Caputto BL.

20:00 - 20:15 **BC-C13** **CYTOPLASMIC c-FOS: A NOVEL TARGET FOR CANCER THERAPY?**
Silvestre D, Gil GA, and Caputto BL.

20:15 - 20:30 **BC-C14** **NUCLEO-CYTOPLASMIC LOCALIZATION OF P8, CELL CYCLE AND SIGNAL TRANSDUCTION PATHWAYS**
Valacco P, Varone C, Iovanna J, and Moreno S.

SALA C: **Structural Biology and Enzymology**

Chairpersons: Dr. Mario Ermácora (Universidad Nacional de Quilmes) and
Fernando Goldbaum (Fundación Instituto Leloir, Universidad de Buenos Aires).

16:30 - 16:45 **BE-C1** **ENERGETIC MAPPING OF A PROTEIN-DNA INTERFACE**
Ferreiro, Diego U.; Dellarole, Mariano; Centeno, Juan M.; Nadra, Alejandro D. and Prat Gay, Gonzalo.

16:45 - 17:00 **BE-C2** **MULTIPLE PARTIALLY FOLDED STATES OF β-LACTAMASE AT EQUILIBRIUM**
Santos, Javier; Risso, Valeria A.; Ferreyra, Raúl G.; Gebhard, Leopoldo G. and Ermácora, Mario R.

17:00 - 17:15 **BE-C3** **UDP-GLC:GLYCOPROTEIN GLUCOSYLTRANSFERASE RECOGNIZES SUBSTRATES WITH MINOR STRUCTURAL PERTURBATIONS**
Caramelo, Julio J.; Meras, Andrea A.; Castro, Olga C. and Parodi, Armando J.

17:15 - 17:30 **BE-C4** **A SIALIDASE MUTANT DISPLAYING TRANS-SIALYLATION ACTIVITY**
Paris, Gastón; Ratier, Laura and Frasch, Alberto C.C.

17:30 - 17:45 **BE-C5** **CHARACTERIZATION OF A TRANSCRIPTION FACTOR-ANTIBODY INTERACTION**
Cerutti, M.Laura; Ferreiro, Diego U.; Prat Gay, Gonzalo; and Goldbaum, Fernando

17:45 - 18:00 **BE-C6** **THE HPV16 E7 ONCOPROTEIN CAN FORM SPHERE-LIKE PARTICLES THAT RESEMBLE RING PROTEINS**
Alonso, Leonardo; García Alai, Maria; Smal, Clara; Iacono, Rubén; Castaño, Eduardo and Prat Gay, Gonzalo.

18:00 - 18:15 **BE-C7** **STUDYING INTERFACES IN PROTEIN-PROTEIN COMPLEXES WITH A PHOTOCHEMICAL PROBE**
Gómez GE, Cauerhff AA, Craig PO, Goldbaum FA, Delfino JM.

18:15 - 18:30 **BE-C8** **ANALIZYNG LOCAL PROTEIN STRUCTURE PERTURBATIONS WITH COLORES**
Lema, Martin A. and Echave, Julian.

18:30 - 18:45 **Interval**

18:45 - 19:00 **BE-C9** **NMR CHARACTERIZATION OF POLYAMINE COMPLEXES WITH α-SYNUCLEIN**
Fernández CO, Hoyer W, Zweckstetter M, Jares-Erijman EA, Subramaniam V, Griesinger C, and Jovin TM.

19:00 - 19:15 **BE-C10** **MOLECULAR DYNAMICS SIMULATIONS OF LIVER BASIC FATTY-ACID BINDING PROTEIN (LB-FABP) IN LIPID MEMBRANES**
Montich, Guillermo G.; Nolan, Veronica; Perduca, Massimiliano; Monaco, Hugo L, and Villarreal, Marcos A.

19:15 - 19:30 **BE-C11** **ANALYSIS OF THE CHOLESTEROL-DEPENDENT INTERACTION OF PERFRINGOLYSIN O WITH MEMBRANES USING FLUORESCENCE SPECTROSCOPY**
Heuck AP, Ramachandran R, Johnson A.

19:30 - 19:45 **BE-C12** **DIRECTED MOLECULAR EVOLUTION OF A METALLO-β-LACTAMASE: DEVELOPING BAD MANNERS**
Tomatis, Pablo E. and Vila, Alejandro J.

19:45 - 20:00 **BE-C13** **PURIFICATION, CRYSTALLIZATION AND PRELLIMINARY X-RAY ANALYSIS OF TRIATOMA VIRUS (TrV) FROM TRIATOMA INFESTANS**
Costabel, Marcelo D.; Rozas-Dennis, Gabriela S.; Guérin, Diego M.A.; Squires, Gaëlle; Lepault, Jean; Navaza, Jorge; and Rey, Félix A.

20:00 - 20:15 **BE-C14** **CHARACTERIZATION OF THE SUBSTRATE BINDING DOMAIN IN BACTERIAL ADP-GLUCOSE PYROPHOSPHORYLASE**
Erben, Esteban; Figueroa, Carlos; Fusari, Corina; Demonte, Ana; Aleanzi, Mabel; Iglesias, Alberto A.

18:00 - 20:00 (Salón Los Jardines) **POSTER SESSION II**

Signal Transduction	TS-P1 to TS-P27
Plants	PL-P1 to PL-P55
Lipids	LI-P1 to LI-P37
Channels and Transporters	CA-P1 to CA-P22

20:15 - 22:00 **Dinner**

22:00 **SAIB Ordinary General Assembly** (Sala A: "Los Maitenes")

SAB Ordinary General Assembly (Sala B: "Los Radales")

THURSDAY, November 20, 2003.

Symposium V (Salón Auditorio)

Microbiology

Chairperson: Dr. Antonio Uttaro (Universidad Nacional de Rosario) and
Dr. Juan Díaz Ricci (Universidad Nacional de Tucumán). Argentina.

Speakers:

9:00 - 9:30 **S17** **MOLECULAR EVIDENCES FOR THE ACQUISITION OF ENDOSIMBIONTS BY THE *Euglenozoa***
Dr. Fred R Opperdoes (Universite Catholique de Louvain, Brussels, Belgium)

9:30 - 10:00 **S18** **THE *Salmonella enterica* MAGNESIUM STIMULON**
Dr. Fernando Soncini, (IBR-CONICET, Facultad de Cs. Bioquimicas y Farmaceuticas, UN Rosario, Argentina)

10:00 - 10:30 **S19** **REGULATION OF *Xanthomonas campestris* VIRULENCE FACTORS AND THEIR ROLE IN THE INTERACTION WITH PLANTS**
Dr. Adrián A. Vojnov (Fundación Instituto Leloir, Buenos Aires, Argentina)

10:30 - 11:00 **Interval**

11:00 - 12:00 (Salón Auditorio) **Alberto Sols PLENARY LECTURE**

Dr. Sergio Moreno Pérez
(Instituto de Biología Molecular y Celular del Cáncer, Spain)
"Molecular mechanisms regulating cell cycle exit"
Chairperson: Dr. José Luis Bocco (Universidad Nacional de Córdoba). Argentina

12:00 - 14:00 **Lunch**

14:00 - 14:30 (Salón Auditorio) **CONFERENCE**

Dr. Ulf Hellman
(Ludwig Institute for Cancer Research, Uppsala Branch, Sweden)
"Peptide Sequencing by PSD MALDI-ToF MS using CAF methodology"
Chairperson: Dr. Juan José Cazzulo (Universidad Nacional San Martín). Argentina

Symposium VI

SAFV-SAIB Symposium: Plant Biochemistry and Physiology
Organized by the Argentinean Plant Physiology Society and the Argentinean Society for Research in Biochemistry and Molecular Biology

Chaipersons: Dr. Horacio Tigier (Universidad Nacional de Rio Cuarto, Córdoba) and
Dr. Alberto A. Iglesias (Universidad Nacional del Litoral, Santa Fe). Argentina

Speakers:

14:45 - 15:15 **S20** **PHYTOREMEDIATION EMPLOYING *IN VITRO* CULTURES**
Dr. Elizabeth Agostini (Universidad Nacional de Río Cuarto, Argentina)

15:15 - 15:45 **S21** **STRUCTURE AND REGULATION OF THE NDH COMPLEX FROM CHLOROPLASTS**
Dr. Hernan Ramiro Lascano (IFFIVE INTA, Cordoba, Argentina)

15:45 - 16:15 **S22** **PA AND NO ARE TWO SECOND MESSENGERS INVOLVED IN PLANT-PATHOGEN INTERACTIONS**
Dr. Ana Laxalt (Instituto de Investigaciones Biológicas, FCEyN, Universidad Nacional de Mar del Plata, Argentina)

16:15 - 16:30 **Coffee Break**

16:30 - 17:00 (Salón Auditorio)
"HOMAGE TO DR. ANDRES STOPPANI - His Personality and Works"
Dr. Rodolfo R. Brenner.
Universidad Nacional de La Plata, Buenos Aires, Argentina

17:00 - 18:00 (Salón Auditorio) **PLENARY LECTURE**

Dr. John E. Johnson
(The Scripps Research Institute, USA)
"RNA and DNA virus capsids as nano platforms and nano machines"
Chairperson: Dr. José María Delfino (IQUIFIB, Argentina)

COMMUNICATIONS

SALA A: **Signal Transduction**

Chairpersons: Dra. Cristina del Valle Paz (Fac. Medicina, Univ. Bs. As.) and
Dra. Ana Russo de Boland (Dpto. Biología, Bioq. y Farmacia, Univ. Nacional del Sur).

18:00 - 18:15 **TS-C1** **ISOLATION OF THE STYLE PARTNER OF POLLEN RECEPTOR KINASES LePRK1 AND LePRK2 FROM TOMATO (*LYCOPERSICON ESCULENTUM*)**
Wengier, Diego; Cabanas, María; Salem, Tamara; Sanchez, Sabrina; de Paz Sierra, Pablo and Muschietti, Jorge.

18:15 - 18:30 **TS-C2** **CDK4/6 INHIBITOR p19INK4d INCREASES THE DNA REPAIR ABILITY IN FIBROBLAST**
Cánepa, Eduardo; Julio, Miguel; Ceruti, Julieta; Carcagno, Abel; Guberman, Alejandra and Scassa, María.

18:30 - 18:45 **TS-C3** **STUDIES ON ACTIVATION AND PHOSPHORYLATION OF PROTEIN KINASE A DURING THE TRANSITION FROM RESPIRATORY TO FERMENTATIVE METABOLISM IN *Saccharomyces cerevisiae***
Portela P, and Moreno S.

18:45 - 19:00 **TS-C4** **TYROSINE PHOSPHATASES ACT ON STEROIDOGENESIS THROUGH THE ACTIVATION OF AA RELEASE**
Cornejo Maciel F, Cano F, Podestá EJ.

19:00 - 19:15 **TS-C5** **EXPRESSION OF PROTEIN PHOSPHATASES FROM THE PP2A FAMILY IN POTATO PLANTS**
Vozza N, Raíces M, Téllez-Iñón MT.

19:15 - 19:30 **TS-C6** **INSULIN MODULATES PHOSPHATIDIC ACID METABOLISM IN CEREBRAL CORTEX SYNAPTOSOMES**
Salvador GA, Pasquaré SJ, Ilincheta de Boschero M, and Giusto NM.

19:30 - 19:45 **TS-C7** **$1\alpha 25(OH)_2D_3$ AND PTH SIGNALING IN RAT INTESTINAL CELLS: ACTIVATION OF CYTOSOLIC PLA2**
Gentili, Claudia; Morelli, Susana; and Russo de Boland, Ana.

19:45 - 20:00 **TS-C8** **NITRIC OXIDE, cGMP, CDPKs AND MAPKs ARE INVOLVED IN THE IAA-INDUCED ADVENTITIOUS ROOT FORMATION IN CUCUMBER**
Lanteri, M. Luciana, Pagnussat, Gabriela C. and Lamattina, Lorenzo.

SALA B: **Biotechnology**

Chairpersons: Dr. Estela Valle (Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Argentina) and
Dr. Carlos Argaraña (CIQUIBIC, Universidad Nacional de Córdoba, Argentina).

18:00 - 18:15 **BT-C1** **SELECTIVE AND COVALENT IMMOBILIZATION OF PROTEINS ONTO POLYMERIC SURFACES. BIOTECHNOLOGYCAL APPLICATIONS**
Carbajal ML, Santos J, Ermacora MR, and Grasselli M.

18:15 - 18:30 **BT-C2** **ERYTHROMYCIN A BIOSYNTHESIS IN HETEROLOGOUS HOST**
Peirú, Salvador; Menzella, Hugo G.; Kurth, Daniel G. and Gramajo, Hugo C.

18:30 - 18:45 **BT-C3** **EVALUATION OF THE EFFECT OF N-ACETYL-GLUCOSAMINE ON THE PRODUCTIVITY AND THE GLYCOSYLATION PATTERN OF rhEPO**
Didier, Caroline; Etcheverrigaray, Marina and Kratje, Ricardo

18:45 - 19:00 **BT-C4** **UNCULTURED γ- PROTEOBACTERIA DOMINATE 16S RDNA CLONE LIBRARIES FROM NONYLPHENOL ETHOXYLATE-ENRICHED ACTIVATED SLUDGE**
Figuerola, Eva; Itria, Raúl; Lozada, Mariana; de Tullio, Luis; Erijman, Leonardo.

19:00 - 19:15 **BT-C5** **PLASMID DESIGN TO PRODUCE MEMBRANE-PERMEANT RECOMBINANT PROTEINS**
Lopez, Cecilia; Magadán, Javier; Mesa, Rosana and Mayorga Luis.

19:15 - 19:30 **BT-C6** **DEVELOPMENT OF DNA VACCINES AGAINST HEMOLYTIC UREMIC SYNDROME (HUS)**
Bentancor, Leticia; Pistone Creydt, V.; Giambartolomei, Guillermo; Meiss, Roberto; Ghiringhelli, Daniel; Palermo, Marina.

19:30 - 19:45 **BT-C7** **SELECTION OF CAMELID ANTI-IDIOTYPIC VHHs BEARING DNA INTERNAL IMAGE BY PHAGE-DISPLAY**
Zarebski LM, Urrutia M, Vila Melo G, Goldbaum FA.

19:45 - 20:00 **BT-C8** **PROTEOMIC ANALYSIS OF A MELANOMA CELL LINE: INSIGHT INTO A MOLECULAR PATHWAY OF TUMORIGENESIS**
Sosa, María S.; López, Juan A.; Camafeita, Emilio; Juárez, Silvia; Albar, Juan P.; Podhajcer, Osvaldo and Llera, Andrea S.

SALA C **Plants - Microbiology**

Chairpersons: Dr. Lorenzo Lamattina (Universidad Nacional de Mar del Plata) and Dr. Mario Lozano (Universidad Nacional de Quilmes), Argentina.

18:00 - 18:15 **PL-C9** **EASTERN BLOTTING. A HIGH-THROUGHPUT FUNCTIONAL ASSAY OF PHOSPHATASE ACTIVITY**
Senn AM, and Wolosiuk RA.

18:15 - 18:30 **PL-C10** **POLYAMINE METABOLISM IN NODULES AND ROOTS OF SOYBEAN PLANTS UNDER CADMIUM STRESS**
Balestrasse KB, Benavides MP and Tomaro ML

18:30 - 18:45 **PL-C11** **FUNCTIONAL ANALYSIS OF THE MAIZE PHOTOSYNTHETIC NADP-MALIC ENZYME BY SITE DIRECTED MUTAGENESIS**
Detarsio E, Andreo CS and Drincovich MF

18:45 - 19:00 **PL-C12** **PURIFICATION AND ANTIFUNGAL ACTIVITY OF A SUNFLOWER LIPID TRANSFER PROTEIN EXPRESSED IN *Escherichia coli* AS A GST-FUSION**
Espinosa Vidal, Esteban; Martín, Mariana; de la Canal, Laura.

19:00 - 19:15 **MI-C15** **THE SPECIFICITY AND ARCHITECTURE OF ACYL-COA CARBOXYLASE β SUBUNIT IN *Streptomyces coelicolor* A3(2)**
Diacovich L, Gago G, Tsai S-C.(S), Khosla C, and Gramajo H.

19:15 - 19:30 **MI-C16** **GENETIC VARIABILITY AND RECOMBINATION IN ARENAVIRUSES**
Goñi S, Posik D, Romanowski V, Ghiringhelli PD, and Lozano ME.

19:30 - 19:45 **MI-C17** **GALACTOSIDES METABOLISM OF *L. plantarum*, GENES AND THEIR FUNCTION: REGULATION IS THE KEY**
Silvestroni A, Connes C, LeBlanc J-G., Piard J-C, Sesma F, Savoy de Giori G..

19:45 - 20:00 **MI-C18** **REGULATION OF EXPRESSION OF THE TWO-COMPONENT SYSTEM CitST IN *Bacillus subtilis***
Sender, Pablo D.; Blancato, Victor S.; Lolkema, Juke; and Magni, Christian.

18:00 - 20:00 (Salón Los Jardines) **POSTER SESSION III**

Microbiology:	MI-P49 to MI-P95
Cell Biology:	BC-P20 to BC-P63
Structural Biology:	BE-P39 to BE-P77
Bioenergetic	BG-P1 to BG-P4
New Technologies	NT-P1 to NT-P3
Plants	PL-P56

L1.

MOLECULAR GENETICS OF CANCER: LESSONS FROM B CELL LYMPHOMA

Riccardo Dalla-Favera (USA)

L2.

FOLDING OF A PREDOMINANTLY β-SHEET PROTEIN *IN VITRO* AND *IN VIVO*

Lila M. Gierasch.
Departments of Biochemistry & Molecular Biology and Chemistry. University of Massachusetts at Amherst. USA. E-mail: gierasch@biochem.umass.edu

This talk will describe ongoing studies of the mechanism by which cellular retinoic acid binding protein I (CRABP I) takes up its native structure, and how local and global sequence information specifies its fold. Additionally, we seek to observe the folding of CRABP I in cells, including assessing its thermodynamic stability, kinetics of folding, the nature of folding intermediates and the energy landscape of folding, and the effects of mutations, and recent progress in this area will be described. CRABP I is a member of the large family of intracellular lipid binding proteins, whose structures are comprised of a short helix-turn-helix and two nearly orthogonal five-strand β-sheets wrapped around a central cavity. Kinetic analysis by stopped-flow fluorescence and CD, hydrogen exchange, and probing of ligand binding has provided a description of the landscape of refolding of CRABP I. In a 250 μs kinetic phase, upon dilution from urea into folding conditions, the ensemble of CRABP I conformers is hydrophobically collapsed and contains significant local secondary structure. Native-like topology, as indicated by ligand binding, develops in a ca. 100 ms kinetic phase. Strikingly, stable hydrogen bonding in the β-sheets forms in a fully cooperative manner in a later (1 s) phase, during which specific packing interactions also develop. The presence of significant secondary structure along with hydrophobic collapse suggests that both global and local forces are acting in the earliest folding events. We observed previously that the formation of the helix-turn-helix sub-domain of CRABP I is dictated by local sequence, and more recently examination of peptides corresponding to the turns in CRABP I reveals that two (turns III and IV) are strongly biased to native structure by local sequence. Hence, local sequence may limit the conformational space available to CRABP I in the early folding phases. Subsequent kinetic phases likely arise as the conformational ensemble forms longer-range contacts that specify native topology (~100 ms) and finally native interstrand hydrogen bonds and tertiary structure (1 s). Analysis of sequences and structures for CRABP I and homologues has identified a network of conserved pairwise hydrophobic interactions that is likely to specify native-like global topology. Recently, CRABP I was mutated to incorporate in a surface-exposed Ω-loop the sequence CCGPCC, which binds specifically to a membrane-permeable, biarsenical fluoroscein dye. Unfolding of labeled 'tetra-Cys CRABP I' is accompanied by enhancement of dye fluorescence, which made it possible to determine the free energy of unfolding by urea titration in cells and to follow in real time the formation of inclusion bodies by the slow-folding, aggregation-prone mutant. Aggregation *in vivo* displayed a concentration-dependent lag time characteristic of protein aggregation in purified *in vitro* model systems. Carrying out studies of folding and aggregation with a protein such as CRABP I, whose folding is well-characterized *in vitro*, will provide insight into the mechanism of folding in cells and the nature of the species that initiate the cellular aggregation process.

L3.

DETECTION OF CONFORMATIONAL CHANGES IN MULTIDRUG TRANSPORTERS

*C. Vigano, L. Manciu and J.-M. Ruysschaert**

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The multidrug resistance is mainly due to the overexpression in tumor cells of proteins that use the energy derived from ATP hydrolysis to transport drugs, out of the cell, against a concentration gradient.These proteins are composed of two homologous halves, each formed by six putative transmembrane helices and one nucleotide-binding domain.The mechanism of coupling ATP hydrolysis, at the cytoplasmic nucleotide binding domain to drug transport involves conformational changes in the protein structure. To gain further insight into the mechanism by which multidrug transporters-mediated drug transport occurs, our group investigates the different transient conformations adopted by the protein in the presence of nucleotide ligands and drugs. Multidrug transport proteins (Pgp, MRP, LmrA) are reconstituted into proteoliposomes in such a way that they conserve their ATPase and drug transport activity. Our studies including infrared spectroscopy, tryptophane quenching, enzymatic proteolysis demonstrate that multidrug transport proteins change their conformation during the catalytic cycle and that several conformational states are involved in the drug transport. Structure changes are transmitted between the cytosolic domains and the membrane domains. This coupling between the drug binding site and the catalytic site plays a crucial role in the transport mechanism. Interestingly, it is differently affected by drugs which accumulate or do not accumulate in resistant cells.

L4.

CHOLESTEROL-DEPENDENT STRUCTURAL TRANSITIONS INITIATE OLIGOMERIZATION AND BETA-BARREL PORE FORMATION BY A BACTERIAL PROTEIN TOXIN

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Perfringolysin O (PFO) is secreted from the Gram-positive bacterium, Clostridium perfringens, as a water-soluble and stable monomeric protein. But upon encountering a mammalian cell membrane that contains cholesterol, PFO binds, oligomerizes, and forms a very large hole in the bilayer with a diameter near 300 Å. Using multiple independent fluorescence techniques, we showed previously that the transition of PFO from a water-soluble monomer to a membrane-inserted oligomer containing about 50 subunits involves the conversion of six short α-helices in each monomer into two transmembrane β-hairpins in the oligomer. We have now found that this major structural transition is initiated by the binding of one end of the PFO molecule to the membrane surface. This association elicits a conformational change at the other end of the molecule that exposes an otherwise-protected region of PFO that forms the interface between adjacent proteins in the oligomer. The cholesterol-dependent structural changes in PFO therefore extend throughout the entire molecule and are required to initiate oligomerization. The oligomerized monomers then act cooperatively to puncture the membrane. The initial binding of PFO to cholesterol at the membrane surface therefore triggers a sophisticated sequence of coupled and cooperative intramolecular and intermolecular conformational changes that ultimately lead to pore formation on the appropriate target membrane.

L5.

3-D PARTICLE TRACKING IN A TWO-PHOTON MICROSCOPE

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Particle tracking in a cell offer the possibility to determine the diffusive or non-diffusive behavior of a particle over large distances. The presence of obstacles, flow and particle interaction with the substrate is recognized by the analysis of the particle trajectory. Conventionally, particle tracking has been performed using cameras and reflecting or fluorescing particles. In addition, interferometric or reflecting techniques have been used to determine the particle position. In this work, we explore the possibility to perform particle tracking in 3-D using the 2-photon excitation microscope. We show that rapid tracking is achievable over large distances. The tracking bandwidth is essentially limited by the number of photons that can be collected during the interval of time used for the feedback of the particle position. For small movements, it is not necessary to center feedback the particle position, but the 3-D position can be determined by a simple algorithm. We present result of particle tracking in the nucleus of cells.

L6.

INFLUENCE OF VIRGIN OLIVE OIL ON CARDIOVASCULAR RISK FACTOR

Valentina Ruiz Gutierrez (Spain)

L7.

MOLECULAR REGULATION OF MEMBRANE TRAFFIC BETWEEN THE ER AND THE GOLGI

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Protein transfer between membrane bound compartments of eukaryotic cells occurs by membrane traffic. Proteins traverse the secretory pathway through progressive steps of vesicle formation, movement, targeting and fusion. Over the last decade, significant insight has been gained into traffic from the Endoplasmic Reticulum (ER) to the Golgi, the first "membrane station" in the secretory pathway. It is now apparent that proteins exit the ER at specialized subdomains called ER exit sites. Vesicles bud from ER exit sites and form new compartments called the vesiculo-tubular clusters (VTCs). VTCs become transport-competent after they are remodeled by the removal of select proteins. VTCs then attach to microtubules and in a motor-mediated process move to the Golgi region where they fuse. One of the key challenges in cell biology is to uncover the spatial and temporal sequence of events that occur at each step. The progress has been on two fronts: the description of the compartments through which proteins move, and the identification and functional characterization of molecules that mediate traffic. Among them are small GTPases, activators and inactivators of those GTPases, COPII and COPI coat components, tethering factors and SNAREs. Despite significant progress, active investigation continues to uncover the relationships between these molecules. This lecture will highlight some of the molecular events regulating ER to Golgi transport.

L8.

MOLECULAR MECHANISMS REGULATING CELL CYCLE EXIT

Sergio Moreno Perez (Spain)

L9.

PEPTIDE SEQUENCING BY PSD MALDI-TOF MS USING CAF METHODOLOGY

Ulf Hellman.
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L10.

RNA AND DNA VIRUS CAPSIDS AS NANO PLATFORMS AND NANO MACHINES

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Viruses are two-edged swords. Many are dangerous pathogens that cause extraordinary human suffering, mortality and economic loss (e.g HIV and SARS), but benign viruses are now recognized as viable reagents for applications in nano technology, chemistry and biology. We used a static, icosahedral, 30nM plant virus as an addressable "nano block" for molecular electronics, protein immobilization, and novel particle patterning on gold surfaces. RNA and DNA virus capsids that exhibit large-scale, pH sensitive, protein reorganization have recently been mechanistically characterized by biophysics and molecular genetics for the harnessing of these properties for nano devices. The characteristics of these remarkable nano machines will be presented and potential applications discussed.

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S1.
STRUCTURAL BASIS OF GLYCOGEN SYNTHESIS

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Glycogen and starch are the major carbon and energy storage compounds in most living organisms. The understanding of glycogen metabolism is an important subject in general biochemistry. Glycogen synthase [EC 2.4.1.21] catalyzes the addition of individual glucosyl subunits to the growing chain of glycogen. It is a key component of the enzymatic machinery involved in glycogen metabolism, together with glycogen phosphorylase and the branching/debranching enzymes. Glycogen synthases from bacteria and higher plants (starch synthases) are α-retaining family 5 glycosyl transferases (for a classification of glycosyl transferases, see http://afmb.cnrs-mrs.fr/CAZY) that use ADP-glucose as sugar donor and have MW around 50 KDa. Mammalian and yeast GSs belong to family 3 glycosyl transferases, are larger enzymes (~80 KDa) and prefer UDP-glucose to ADP-glucose as the sugar donor. We now present the 3D structure of a bacterial GS at 2.3 Å resolution. The recombinant enzyme from *Agrobacterium tumefaciens* was purified to homogeneity and crystallized. The structure was determined by single-wavelength anomalous diffraction methods, revealing a two-domain α/β folding topology. Crystals of GS in complex with specific ligands identified the catalytic center and its architecture strongly suggest that glycogen synthase and glycogen phosphorylase are evolutionary related enzymes, indicating that glycogen is synthesized and degraded by homologous enzymes. The implications of the 3D structure in terms of protein folding, catalytic mechanism and activity regulation will be discussed.

S2.
THE MULTI-FACETED MANNOSE 6-PHOSPHATE RECEPTORS

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The 46kDa cation-dependent mannose 6-phosphate receptor (CD-MPR) and the 300kDa cation-independent mannose 6-phosphate/ insulin-like growth factor II (IGF-II) receptor (CI-MPR) are the sole members of the P-type lectin family and are distinguished from all other lectins by their ability to recognize phosphorylated mannose residues. These receptors play an essential role in the generation of functional lysosomes within the cells of higher eukaryotes by directing newly synthesized lysosomal enzymes bearing the mannose 6-phosphate (M6P) signal from the trans Golgi network to lysosomes. The CI-MPR has been implicated in several other processes, including cell growth, apoptosis, and cell migration, due to its ability to bind a wide range of M6P-containing (e.g., latent transforming growth factor-beta, granzyme B, proliferin) and non-M6P-containing (IGF-II, retinoic acid, urokinase-type plasminogen activator receptor (uPAR), plasminogen) molecules at the cell surface. The ability of the CI-MPR to interact with many different proteins and a lipophilic molecule is facilitated by the receptor's ~2,270-residue extracytoplasmic region comprised of 15 homologous domains in which several binding sites have been localized to individual domains. Our studies have provided a detailed view of the mechanism of high affinity (nM) phosphomonoester recognition by both MPRs. Our recent structural studies have provided insights into the nature of plasminogen and uPAR recognition by the CI-MPR and has allowed us to generate a model of the entire extracytoplasmic region that provides a context with which to envision the numerous binding interactions carried out by this multi-faceted receptor.

S3.
THE NUCLEOTIDE SUGAR TRANSPORT/ANTIPORT CYCLE OF THE ENDOPLASMIC RETICULUM AND GOLGI APPARATUS: FROM BASIC SCIENCE TO DISEASE

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Approximately half of the proteins in eukaryotes are either membrane bound or secreted. Eighty percent undergo posttranslational modifications, such a glycosylation, sulfation and phosphorylation in the lumen of the endoplasmic reticulum and Golgi. Nucleotide sugars, nucleotide sulfate and ATP are substrates for these reactions and must first be transported from the cytosol into the lumen of the above organelles. This transport is coupled to the exchange with the corresponding nucleoside monophosphate, an antiport. We have purified, reconstituted into liposomes and cloned several transporters of the Golgi as well as the enzymes responsible for generating the antiporter nucleoside monophosphate molecules. While transporters are multitransmembrane spanning proteins nucleotide disphosphatases are type II membrane proteins. Proteins of very different amino acid sequences may transport the same substrates. Genetic studies have shown that mutations or deletion of transporter or diphosphatase genes result in defects of glycosylation of proteins and lipids. In *C. elegans* we found that several transporters of dual substrate specificities have very different protein sequences, cell type differential expression and biological functions. One of the nucleotide diphosphatase mRNAs is upregulated by conditions leading to endoplasmic reticulum stress suggesting that it plays a role in quality control of glycoprotein folding. . Finally a disease has been recently described in which the Golgi apparatus GDP-fucose transporters is partially defective leading to severe growth and developmental impairments.

S4.
DOMAIN ORGANIZATION AND PATTERN RECOGNITION OF UDP-GLC:GLYCOPROTEIN GLUCOSYL TRANSFERASE (GT)

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GT is the key element of the quality control of glycoprotein folding occurring in the endoplasmic reticulum (ER). The enzyme only glucosylates glycoproteins not displaying their native structures. Structures created by GT (monoglucosylated *N*-glycans) are specifically recognised by two ER resident lectins (calnexin and calreticulin) thus precluding transit of not properly folded glycoproteins to the Golgi. GT proved to be formed by at least two tightly associated domains, the C-terminal or catalytic domain (20% of the molecule) and the N-terminal, presumably involved in conformation recognition. In spite of showing a poor similarity at the primary sequence level (16.3%), the GT N-terminal domains derived from two different species could be functionally interchanged. Using chemically glycosylated derivatives of chymotrypsin inhibitor 2 displaying several truncations from the N-terminus, and thus different conformations, as GT substrates we determined that GT recognises hydrophobic amino acid patches exposed in molten globule-like conformations. Moreover, the enzyme was able to differentially glucosylate conformers showing minor structural differences.

S5.

STRUCTURAL STUDIES OF MELANOCORTIN PEPTIDES IN AQUEOUS AND LIPID MEDIA

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In most vertebrates the cationic linear tridecapeptide α-melanocyte stimulating hormone (α-MSH) is known as the most relevant physiological hormone regulating skin darkening. It is also involved in a variety of other physiological and neurological processes. The hormone α-MSH, and several of its derivatives will be discussed regarding the peptides biological activities, their partition and interaction with lipid bilayers, and their structures in aqueous and lipid media. For such studies, the peptides natural fluorescent probe Trp^9 was monitored, via steady state and time-resolved fluorescence spectroscopy. Besides Trp, some of the derivatives contain the aromatic fluorescent amino acid β-(2-naphtyl)-D-alanine (D-Nal) and the paramagnetic amino acid probe 2,2,6,6-tetramethylpiperidine-N-oxyl-4-amino-4-carboxylic acid (Toac). Hence, the fluorescence of Trp and D-Nal and the electron paramagnetic resonance (EPR) signal of Toac were used to analyze structural properties of the peptides in different media. To monitor the effect the peptides cause in the lipid bilayer, the ESR signal of spin labels intercalated in the membrane were investigated.

S6.

LIPID-PROTEIN INTERACTIONS AT THE OUTER AND MIDDLE RINGS OF THE ACETYLCHOLINE RECEPTOR TRANSMEMBRANE DOMAINS

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Fluorescence spectroscopy and structural studies are beginning to delineate a topographic map of the nicotinic acetylcholine receptor (AChR) transmembrane (TM) region. Site-directed mutagenesis and single-channel electrophysiology help dissect the involvement of lipid-sensitive residues in channel gating, opening and closing mechanisms. Recent structural data reveal that the TM segments form three concentric layers around the ion channel. An inner ring, shaped by five M2 segments (one from each subunit) excluded from contact with the lipid, forms the walls of the pore proper. The middle ring is formed by M1 and M3 segments, which exhibit contact with lipids, and the M4 TM domains constitute an outer ring, distant from the channel and loosely separated from the middle ring. Although they are not part of the ion conduction pathway, the lipid-exposed middle and outer rings appreciably modulate AChR function. Establishing structural-functional correlations of the lipid-exposed TM rings requires the identification of sites for lipid modulation and more precise knowledge of the TM structure. Examples of current strategies used in our laboratory will be discussed in the presentation.

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S7.

PROTEIN-PROTEIN AND PROTEIN-LIGAND INTERACTIONS OF THE GTPase DYNAMIN

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Dynamins are closely related GTPases of approximately 100,000 MW that are essential for receptor-mediated endocytosis and synaptic vesicle recycling. Two forms of dynamin have been extensively characterized: a neuron-specific form called dynamin I and an ubiquitously expressed form called dynamin II. These two proteins have a similar organization of functional domains which include an N-terminal GTPase domain (residues 1-300), a pleckstrin-homology (PH) domain that interacts with lipids (residues 510-620) a GTPase effector domain (GED) which stimulates dynamin GTPase activity (residues 620-750) and a C-terminal proline/arginine-rich domain (PRD) which targets dynamin to the clathrin-coated pit and binds to a subset of SH3-domain containing proteins, negatively-charged phospholipids, and microtubules. Dynamin's role in endocytosis has been variously attributed to being a force-generating enzyme or a signalling protein. Here we review our studies on the oligomeric state of dynamin at high ionic strength conditions. We also review our work on the elementary processes of the dynamin GTPase at high ionic strength and compare these to the ATPase of the force generating protein myosin and the GTPase of the signalling protein Ras. Data on the interaction of dynamin with a giant unilamellar vesicles, studied using two-photon fluorescence microscopy, will also be discussed.

S8.

PROTEIN-LIPID AND PROTEIN-PROTEIN INTERACTIONS: DIRECT VISUALIZATION BY 2-PHOTON MICROSCOPY

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We have used two-photon excitation fluorescent microscopy to study the interaction of Phospholipase A2 (PLA2) and HDL particles with lipids. The interaction of PLA2 with Giant Unilamellar Vesicles of different lipid composition show morphological and fluidity changes as a manifestation of the enzymatic activity. Our studies on the monomer-dimer equilibrium of PLA2 using micelles and Fluorescence Correlation Spectroscopy indicate a very strong dimer that will not dissociate until 10^{-11} M. A second system we have studied is the interaction between reconstituted particles of HDL with membranes; in this case we have used the microscopy version of Laurdan Generalized Polarization technique to study the capability of these particles to remove cholesterol from Giant Unilamellar Vesicles of different lipid composition.

S9.
METALLOPROTEIN DESIGN: ENGINEERING METAL-BINDING SITES INTO NATIVE PROTEIN SCAFFOLDS

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Metalloproteins catalyze some of the most difficult biological reactions and can fine-tune the reactivity at the highest level. While much progress has been made on the study of native metalloproteins and their variants, little is known about how to design a metalloprotein with desired structure and activity. By using stable, easy-to-produce, and well-characterized proteins as scaffolds, we have successfully designed protein models of Cu_A and Cu_B-heme centers from cytochrome *c* oxidase, a Mn(II)-binding center from manganese peroxidase and a heme-thiolate center from cytochrome P450.[1] Our successes provide an alternative approach to *de novo* protein design. Nature is known to use only a limited number of thermodynamically stable protein scaffolds and yet is able to achieve diverse functions by designing different active sites into the same scaffold. Therefore, as in nature, we can choose stable scaffolds for designing metalloproteins. More importantly, the study of the designed proteins has contributed to the understanding of the structural and functional properties of several complex metalloenzymes and has helped resolve long-standing issues in the field. Recent progress in this endeavor will be presented.

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S10.
FROM SEQUENCE TO CONSEQUENCE

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Genomics, the study of the properties of genes and gene products on a whole-organism scale, is revolutionizing all aspects of biology. The ultimate goals are the determinations of the functions of all gene products of an organism within the context of the organism, at all levels from the molecular to the organism as a whole. Among the fields that has the potential to aid in that process is the field of structural biology. Structural genomics has as its stated goals the filling-in of the catalog of known protein folds and the assignment of function to gene products whose functions are not known by structural similarity to proteins of known function. How realistic are these goals? How robust are the predictive methods that take structure to function? How can computational methods be used to predict function? These questions will be discussed in light of the methods we have developed to address them.

S11.
STRUCTURAL PROPERTIES AND KINETIC ROLE OF EARLY INTERMEDIATES IN PROTEIN FOLDING

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While small globular proteins typically require milliseconds to seconds to complete the process of folding, there is growing evidence that important conformational changes, including secondary structure formation and chain collapse, occur on a much shorter time scale[1,2]. These early events are crucial for understanding how protein folding is initiated and directed along productive channels. A highly efficient capillary mixer[3] coupled with optical probes or NMR-detected H-D exchange has enabled us to follow structure formation during folding over the time range from tens of microseconds to minutes. Our observations of rapid changes in fluorescence, absorbance and amide protection on the sub-millisecond time scale provide insight into the structural characteristics and kinetic role of early intermediates populated during the folding of a diverse set of proteins, including cytochrome c[4,5], protein G[6], Im7[7], β-lactoglobulin[8], staphylococcal nuclease[9] and acyl-CoA binding protein[10].

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S12.
HOW DO MUTANT COPPER-ZINC SUPEROXIDE DISMUTASE PROTEINS KILL MOTOR NEURONS?

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Amyotrophic lateral sclerosis (ALS) is caused by selective death of motor neurons. In a small fraction of the cases (familial ALS or FALS), it is inherited as a autosomal dominant trait associated with mutations in the gene encoding copper-zinc superoxide dismutase (CuZnSOD). These mutations are known to confer new and toxic properties on the protein and thereby cause the disease, although the nature of the toxic property(ies) is still in dispute. We have purified a series of ALS mutant human CuZnSODs and found altered metal binding properties, chemical reactivities, structures, and stabilities in the mutant proteins relative to the wild type proteins. These and related observation may help to explain what is perhaps the most perplexing question in CuZnSOD-associated FALS, how such a diverse set of mutations could result in the same gain of function that causes motor neurons to die.

S13.

INTRASTERIC REGULATION OF THE Ca^{2+} TRANSPORTER FROM PLASMA MEMBRANES

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The term intrasteric regulation was introduced to describe autoinhibition of protein kinases and phosphatases by internal sequences acting directly at the active site. it is now clear that this powerful mechanism of control extends to diverse enzymes including the Ca^{2+} transporter from plasma membranes (PMCA) that is responsible of fine tuning the cytosolic Ca^{2+} concentration in animal cells. The PMCA is a highly regulated ion transport P-ATPase, with a well characterized calmodulin binding-autoinhibitory segment located at the C-terminal end of the molecule. It has been proposed that in the apoPCMA (without calmodulin) the autoinhibitory segment blocks the access of substrates to the catalytic site. Calmodulin binding to a sequence partially overlapping the autoinhibitory segment would result in not yet well defined conformational changes that disengage the inhibitor from the active site. We have been able to detect calmodulin dependent changes in FRET between the blue and green fluorescent proteins fused to both ends of the PMCA which are indicative of important rearrangements of the protein upon activation. however different intermediate states seem possible as suggested by the fact that substitution of aspartate 170 by asparagine activates the enzyme without completely separate the autoinhibitory sequence from the catalytic core.

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S14.

REGULATION AND FUNCTIONAL ROLE OF AQUAPORIN WATER CHANNELS IN HEPATOCYTES

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Hepatocytes express aquaporin water channels, a family of integral proteins that increase cell membrane water permeability, facilitating the osmotically driven movement of water. The water channel aquaporin-8 is located primarily within the interior of hepatocytes in a vesicular compartment (1,2). Glucagon, via the cAMP-dependent protein kinase A signal transduction pathway, stimulates the microtubule-mediated polarized trafficking of aquaporin-8 vesicles to the canalicular plasma membrane domain (i.e., the bile secretory pole of hepatocytes) (3). Water transport studies support the corresponding hormone-induced increase of membrane water permeability (3,4). The hepatocyte water channel aquaporin-9 is present exclusively in sinusoidal membranes not undergoing regulated trafficking (2). Thus, hepatocytes seem to control their canalicular water permeability by changing the number of aquaporin molecules. This mechanism would allow efficient coupling of osmotically active solutes and water transport during canalicular bile formation.

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S15.

NICOTINIC RECEPTORS OF COCHLEAR AND VESTIBULAR SENSORY SYSTEMS: FROM MOLECULAR STRUCTURE TO FUNCTION

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The sensory epithelia of organs responsible for hearing (the cochlea) and balance (vestibular labyrinth) share a unique subset of hair cells which transduce mechanical stimuli into electrical signals. These cells are under the influence of efferent fibers originating in the brain, which modulate the dynamic range of afferent fibers. Acetylcholine is the principal neurotransmitter released by efferent axons. The existent data suggest a central role for an atypical, nicotinic subtype of receptors (nAChRs) located at the synapse between efferent fibers and hair cells. Over the recent years we have cloned two novel nAChR genes involved in hair cell physiology: $\alpha 9$ and $\alpha 10$. We demonstrate the existence of two functional nAChRs: homomeric $\alpha 9$ and heteromeric $\alpha 9 \alpha 10$. While both $\alpha 9$ and $\alpha 9 \alpha 10$ nAChRs exhibit similar pharmacological profiles, the presence of $\alpha 10$ modifies key biophysical characteristics of $\alpha 9$ nAChRs. Both $\alpha 9$ and $\alpha 10$ transcripts are observed in adult cochlear outer hair cells and sensory epithelia of the otolithic organs and the semicircular canals. However, while cochlear inner hair cells express $\alpha 9$ from embryonic through adult stages, $\alpha 10$ transcripts are only observed during early development, before the onset of hearing. Our results indicate that efferent modulation of vestibular and outer hair cell function occurs via heteromeric nAChRs assembled from both $\alpha 9$ and $\alpha 10$ subunits.

S16.

MOLECULAR BASIS OF CHANNEL GATING OF CYS-LOOP RECEPTORS

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Cys-loop receptors of the ligand-gated ion channel superfamily play key roles in synaptic transmission. Their strategic positions in the pathway of information flow makes them molecular targets for drugs and neurological diseases. Neurotransmitters interact with a ligand-binding site triggering a conformational change in the protein that results in the opening of an ion channel. The detailed structural mechanism of this process, which is known as gating, remains a mystery. The cys-loop channels are pentameric proteins composed of homologous subunits. Each subunit contains an amino terminal extracellular domain which carries the signature disulfide loop and the binding sites and four transmembrane domains (M1-M4). Our goal has been to understand the molecular arrengements underlying channel gating. To this end, we have combined site-directed mutagenesis and chimeric subunits with single-channel and macroscopic current recordings. We identified residues in the less studied M1 and M3 domains as well as in the lipid-exposed M4 domain of the nicotinic receptor (AChR) that are involved in channel gating and described their mechanistic contributions. Our results revealed that these domains govern opening and closing rates in a subunit-selective manner. We also clarified the fundamental mechanistic steps that are altered in several myasthenic syndromes associated with mutations in the AChR. We constructed a chimeric subunit linking the soluble ACh binding protein (AChBP) to the channel of the 5-HT_3 receptor. Replacement of domains in AChBP until gating is achieved is our current strategy to identify residues involved in channel activation.

S17.

MOLECULAR EVIDENCES FOR THE ACQUISITION OF ENDOSIMBIONTS BY THE Euglenozoa

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S18.

THE *Salmonella enterica* MAGNESIUM STIMULON

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In the pathogenic bacterium *Salmonella enterica*, magnesium plays a fundamental role during the infection process. *Salmonella* utilizes the level of environmental Mg^{2+} as a signal to control the expression of several essential virulence determinants. We have identified that this sensing process is mediated by a specific signal-transduction system known as PhoP/PhoQ. Extracellular Mg^{2+} controls a specific response-regulator (PhoP) phosphatase activity in the membrane-associated sensor protein PhoQ, inactivating the transcriptional regulator. Phosphorylation of the response regulator promotes its self-association, allowing the protein to recognize and interact with a direct-repeat sequence located in the promoter region of a set of PhoP-activated genes (*pags*). On the other hand, *pags* that do not harbor the PhoP-box at their promoter regions are indirectly controlled by this system. We are currently investigating putative intermediaries in their Mg^{2+}-controlled regulation. Interestingly, most of these indirectly-regulated genes are *Salmonella*-specific and were probably acquired by horizontal transfer. Recently, we have uncovered a novel Mg^{2+} regulatory system that coordinates with PhoP/PhoQ the cellular distribution and homeostasis of Mg^{2+}. The analysis of this system is also relevant to understand how intracellular Mg^{2+} is kept within a precise range to guarantee the proper function of vital intracellular mechanisms.

S19.

REGULATION OF *Xanthomonas campestris* VIRULENCE FACTORS AND THEIR ROLE IN THE INTERACTION WITH PLANTS

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The phytopathogenic bacterium *Xanthomonas campestris* pv. *campestris* (*Xcc*) is the causal agent of black rot disease of cruciferous plant. *Xcc* produces a range of extracellular enzymes and extracellular polysaccharide (EPS) which are collectively essential for pathogenesis. The production of these factors is regulated by a cluster of genes called *rpf* (for *r*egulation of *p*athogenicity *f*actors). Two of the genes, *rpfF* and *rpfB*, have previously been implicated in the synthesis of a diffusible regulatory molecule, DSF. The *gum* gene cluster of *Xcc* comprises 12 genes whose products are involved in the biosynthesis of the polysaccharide xanthan. These genes are expressed as an operon from a promoter upstream of the first gene, *gumB*. A reporter plasmid was constructed in which the promoter region of the *gum* operon was fused to *gusA*. The expression of the *gumgusA* fusion in bacteria recovered from inoculated turnip leaves was maximal at the later phases of growth and was subjected to regulation by *rpfF*. In addition, *Xcc* produces a neutral cyclic glucan containing 16 glucose residues. Mutations in both *rpfF* and *rpfC* lead to reductions in levels of cyclic glucan. â-1,2-glucan were infiltrated as a pre-treatment on *Nicotiana benthamiana* leaves 24h previously to *Xanthomonas* infection, restoring the necrosis production in all mutants. By northern blot experiment, glucans turn out to be a suppressor of PR1, a defense gene marker, and hence of the vegetal immunity system.

S20.

PHYTOREMEDIATION EMPLOYING *IN VITRO* CULTURES

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Phytoremediation consists in the use of plants to remove, destroy or sequester hazardous substances from the environment and offers many potential advantages over traditional remediation technologies, particularly its public acceptance and considerably lower cost.

Recently, the number of results obtained with the help of *in vitro* cultures, such as hairy roots, is rapidly increasing. These cultures could be used for screening plants suitable for the removal of organic and inorganic pollutants and they allow to study the enzymatic catalysis involved in the bioconversion of pollutants to non-toxic metabolites without the interference of other plant organs or the microbiota. We have investigated the use of hairy root cultures as inexpensive enzyme-containing tissues that are easy to apply in the removal of phenolic compounds from aqueous solutions. The optimum conditions (H_2O_2 concentration, pH, time of exposure) to obtain high efficiencies in the process were established. Appropriate controls were included. Peroxidase and laccase activities and the remaining amount of phenols, were determined. The polymerization catalyzed by peroxidases would be the main mechanism involved in the removal process. We also determined the possibility of re-using the roots for various consecutive cycles, with high removal efficiency. These features make this system an interesting alternative for use in continuos detoxification programs.

S21.
STRUCTURE AND REGULATION OF NDH COMPLEX FROM CHLOROPLASTS

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The plastid Ndh complex, analogous to the Complex I of the mitochondrial respiratory chain, catalyses the transfer of electrons from NADH to plastoquinone. The Ndh complex activity together with Mehler reaction and a plastoquinol peroxidase might poise the redox level of the photosynthetic electron carriers under different environmental conditions. Hydrogen peroxide and calcium mediate plastid-encoded NDH polypeptides and Ndh complex activity increases under photooxidative stress. Changes in Ndh complex activity could not only be explained by changes in Ndh protein levels. The *in vivo* phosphorylation at threonine residue(s) of the NDH-F polypeptide was demonstrated in both thylakoids and immunopurified Ndh complex using monoclonal phosphoamino acid antibodies. The Ndh complex phosphorylation level, modulated by H_2O_2 and calcium, closely correlated with its activity. The understanding of the molecular basis of the Ndh complex function regulation requires structural studies. The topologies of Ndh complex and its NDH-F subunit were investigated using bioinformatic tools, proteolitic assays on intact and permeabilised thylakoids and antibodies against specific sequences of different NDH subunits. The Ndh complex structure may be similar to that of respiratory Complex I. NDH-F subunit would have up to 15 transmembrane helices. The 181-Thr of NDH-F seems to be the phosphorylation site of Ndh complex since its highly conserved and is the unique potential Thr phosphorylation site located in a stromal hydrophilic. Bioinformatic predictions and the conserved 349-His in the X transmembrane helix, suggest that NDH-F could be a proton channel.

S22.
PA AND NO ARE TWO SECOND MESSENGERS INVOLVED IN PLANT-PATHOGEN INTERACTIONS

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Nitric oxide (NO) and phosphatidic acid (PA), two emerging molecules in plant stress signaling, are both involved in the induction of plant defense. We are interested in how NO and PA exerts their effect. In the last few years, evidence have been provided that plant cells contain a variety of phospholipid-based signaling pathways. These pathways include phospholipase C (PLC) and D (PLD) activities, which generate the emerging plant second messenger PA. PA accumulates in tomato cell suspensions treated with aspecific elicitors such us xylanase, chitotetraose and flagellin. PA also accumulates in tobacco cells expressing tomato CF-4^{+} resistance gene treated with the specific *C. fulvum* elicitor AVR4. Inhibition of phospholipases responsible for this PA accumulation, blocks responses associated with plant defense like the generation of reactive oxygen species (ROS). Moreover, exogenously applied PA has been shown to generate ROS, which in turn can induce the hypersensitive response (HR), a form of programmed cell death (PCD). In animals, NO and ROS act together triggering PCD. In plants, NO accumulates during plant-pathogen interactions and inhibitors of NO accumulation compromise HR and PCD. The first experimental evidence show that-NO-treated cells accumulate PA, and that xylanase-triggered PA accumulation depends on the NO presence. This opens an exciting new field in signal transduction pathways that could drive plant disease resistance.

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LI-C1.
VANADYL SULFATE, AN INSULIN-MIMETIC, DOES NOT ALTER UNSATURATED FATTY ACID BIOSYNTHESIS IN NORMAL OR STREPTOZOTOCIN RATS

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It is widely accepted that Vanadium, either in V^{+4} (vanadyl) or V^{+5} salts, shows insulinmimetic properties in experimental animals, isolated tissues and cell preparations. In streptozotocin (STZ) rats, Vanadyl normalizes the glycemia and corrects the gene expression for glucokinase, 6-phosphofructokinase, fatty acid synthetase, etc. On the other hand, insulin recovers the Δ9, Δ6 and Δ5 desaturase activities depressed in insulin-dependent diabetes. In consequence to compare effects, we examined the action of vanadyl sulfate added to the drinking water (0.5 g/L for 1 week, and 1g/L for three weeks) on control and STZ rats. Glycemia, insulinemia, Δ6 and Δ5 desaturase activities, and fatty composition of liver microsomes were determined. In diabetic rats vanadyl alleviated the hyperglycemia without modifying the insulinemia. However, neither the liver Δ6 and Δ5 desaturation activities nor the fatty acid composition were altered. Comparing these results to the up-to-now recognized insulin and vanadium downstream biochemical events, we may deduce: Vanadyl controls glucose homeostasis, but undoubtedly it is unable to use the pathways of insulin that lead to the regulation of the desaturases. The activation of insulin receptor and insulin receptor tyrosine phosphorylation mechanism would be essential.

LI-C2.
SELECTIVE PROTECTION OF C20:4 n6 AND C22:6 n3 BY MELATONIN DURING NON ENZYMATIC LIPID PEROXIDATION OF RAT LIVER, KIDNEY AND BRAIN MICROSOMES AND MITOCHONDRIA

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Melatonin (MLT) (N-acetyl-5-methoxytryptamine), the main secretory product of the pineal gland, is a free radical scavenger that has been found to protect against lipid peroxidation in many experimental models. In the present study the effect of MLT on lipid peroxidation of long chain polyunsaturated fatty acids located in rat liver, kidney and brain microsomes and mitochondria was determined. The incubation of rat liver, kidney and brain microsomes or mitochondria in the presence of ascorbate-Fe^{2+} resulted in lipid-peroxidation of membranes as evidenced by light emission and decrease of docosahexaenoic acid 22:6 n-3 and arachidonic acid 20:4 n-6. In the presence of MLT (0.5, 1.0, 1.5 mM), light emission percent inhibition of microsomes was: (liver-3.33, 9.98, 39.40) (kidney-46.79, 61.88, 68.36) and (brain-33.36, 28.89, 43.32), whereas light emission percent inhibition of mitochondria was: (liver-2.74, 19.62, 33.91) (kidney-8.41, 14.66, 44.31) and (brain-4.43, 7.90, 23.55). Not all fatty acids were equally protected after the addition of melatonin to the incubation medium. Our results indicate a selective protection of C20:4 n6 and C22:6 n3 by melatonin during non enzymatic lipid peroxidation of rat liver, kidney and brain microsomes and mitochondria.

LI-C3.

PHOSPHATIDYLCHOLINE SYNTHESIS REGULATION BY PGD_2 IS MEDIATED BY MAPK ACTIVATION

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Phosphatidylcholine (PC) is a main lipid of biomembranes with important structural and functional roles. In previous works, we have demonstrated that renal papillary PC synthesis is regulated by endogenous-synthesised prostaglandin D_2 (PGD_2) and that such regulation occurs on PC biosynthetic enzymes. The purpose of the present work was to evaluate the mechanism by which PGD_2 exerts its modulatory action on PC synthesis. We first studied which receptor could be activated by PGD_2 interaction and found that sulprostone (a PGE_2 receptor, EP3 full-agonist) mimicked the effect of PGD_2 on PC synthesis. Hence, we studied if MAPK activation, which was reported to be involved in EP3 actions, was mediating PGD_2 regulatory action on papillary PC synthesis. Thus, we evaluated renal papillary PC biosynthesis as ^{32}Pi incorporation to PC in the absence or in the presence of PGD_2 with or without the addition of U0126, a selective MEK inhibitor. We found that PGD_2 induced MAPK-phosphorylation which was prevented by U0126. This inhibitor also blocked the PGD_2 effect on PC biosynthesis with the same pattern observed for MAPK activation, thus suggesting the involvement of this pathway in the maintenance of PC synthesis in renal papillary membranes.

LI-C4.

HEAT INDUCED CHANGES IN TESTICULAR LIPIDS CONTAINING VERY LONG CHAIN POLYUNSATURATED FATTY ACIDS (VLCPUFA)

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Hyperthermia is known to result in adverse consequences for the normal adult testis of several mammals, including a reduction of testicular weight and a period of partial or complete infertility, since it selectively damages germ cells. We observed previously that experimental cryptorchidism in rats resulted in a marked reduction of germinal cells, concomitantly with a decreased amount of testicular lipids containing polyunsaturated fatty acids (PUFA) with long and very long chains. In this work we aimed at studying the long-term effects on lipids of a single exposure (15 min) of the testis to moderate heat (43ºC). Four days after the heat shock, there was a significant increase in the amount of cholesterol esters and 1-alkyl-2,3 diacylglycerols. The fatty acid profile of both showed a slight increase in pentaenoic PUFA (22:5n-6) and a concomitant decrease of pentanoic VLCPUFA (especially 28:5n-6). The earliest effect observed (6 hours posttreatment) was a significant, apparently transient, increase in the amount of ceramides (Cer). The increased Cer showed a peculiar fatty acid pattern, with an increase in fatty acids other than VLCPUFA. After four days, the proportions of PUFA and VLCPUFA were significantly reduced, and after 10 days the VLCPUFA were virtually absent from the Cer remaining in the testis.

LI-C5.

GLUCOSE-INDUCED DECREASE OF PHOSPHATIDYL-ETHANOLAMINE AFFINITY FOR THE TRANS-MEMBRANE SURFACE OF MEMBRANE PROTEINS

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Integral membrane proteins span biological membranes defining a lipid-protein interface. The amphiphiles located in contact with the protein surface have a restricted mobility as a consequence of interactions with surface residues of the transmembrane domain. Hence, the transmembrane segments detect modifications in the bilayer and induce changes on protein conformation, which -in some cases- are translated into functional changes. We have recently developed a simple resonance energy transfer method to quantify amphiphile-membrane protein interactions [Levi *et.al., Anal Biochem* 317: 171-179, 2003], which uses a phospholipidic fluorescent probe that competes with the unlabeled amphiphiles for positions on the transmembrane surface of the protein. In this study, the plasma membrane calcium pump was reconstituted in mixed micelles composed of the detergent $C_{12}E_{10}$, phosphatidylcholine and phosphatidylethanolamine (PE) or PE preincubated with glucose. The protein fluorescence spectrum was registered at different mole fractions of the micelle components, and the exchange constants were determined being 1.8 ± 0.2 for the control and 1.0 ± 0.1 for glucose treated lipids. These values indicate that incubation of PE with glucose produce a significant decrease of PE-affinity for the transmembrane domain of the protein. Similar results were obtained using the Na,K-ATPase reconstituted in the same micellar systems.

LI-C6.

c-FOS REORGANIZES PHOSPHOLIPIDS AT THE INTERFACE

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The transcription factor c-Fos has recently been studied from viewpoints unrelated to nuclear transcription. It has been shown that it activates phospholipid biosynthesis *in vivo* and *in vitro* through a citoplasmic activity associated with endoplasmic reticulum. It also is tensioactive, interacts with phospholipids, especially phosphatidyl inositoldiphosphate (PIP_2), and undergoes two reversible molecular reorganizations in response to lateral pressure changes in monolayers. Furthermore, it modulates PLA_2, sphingomyelinase and PLC activity on lipid monolayers in a finely tuned way depending on surface lateral pressure. Aimed at elucidating the mechanism by which c-Fos could play a role in phospholipid metabolism, we analyse the compressional behavior of lipid-protein film mixtures seen by surface lateral pressure and surface potential monitoring, epifluorescence and Brewster angle microscopy. We find that c-Fos affects phospholipid intermolecular organization. Mixed films with either PIP_2 or dilauroylphosphatidylcholine (PC) are respectively condensed or expanded. The contribution to deviation from ideality of each component in the mixture depends on the film's packing and composition. In addition, mixtures with PIP_2 show domain segregation and shifts in both of the protein's interfacial reorganizations, while those with PC are largely homogeneous with protein reorganizations that remain independent of the lipid. The capacity of c-Fos to affect interface structuring according to packing state, lipid nature and the protein's own proportion, further support the idea that it could participate as a signal transducer in membrane function.

LI-C7.
INTERACTION OF AN ASPARTYL PROTEASE WITH LIPID INTERFACES CONTAINING PHOSPHATIDYLGLYCEROL OR PHOSPHATIDYL ETHANOLAMINES

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Aspartyl proteases are proteolytic enzymes with two aspartyl groups in the active site that are involved in various human diseases.
Previous papers of our laboratory have shown that an aspartyl protease has a significant adsorption on lipid interfaces depending on the net charges and the lipid composition.
The penetration of the protein is higher in phosphatidylcholine than in phosphatidylethanolamine monolayers at the same initial surface pressure. The penetration in PC monolayers was correlated with a slight decrease in the enzyme activity at 25°C with respect to its activity in water. In contrast, the activity increased when the lipid interface was composed by phosphatidylethanolamine in which the penetration was much lower. A comparable result was obtained when the activity was measured in the presence of membranes composed by phosphatidylglycerol.
For these reasons, our interest was to explore the changes in the structural properties of both the protein and the membrane when they are composed by phosphatidylethanolamine or phosphatidylglycerol.

LI-C8.
COLLISIONAL TRANSFER OF FATTY ACIDS FROM IFABP TO MEMBRANES: IMPORTANCE OF THE LYSINE RESIDUES IN THE α-HELICAL DOMAIN

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Fatty acid (FA) transfer from intestinal FABP (IFABP) to PL membranes occurs by a collisional mechanism. Electrostatic interactions are crucial in this process, so lysines residues of the IFABP surface could be interacting with acidic groups in membrane. We have shown that the helical domain of IFABP is critical in determining the FA transfer mechanism. To analyze the role of the Lys residues of the α-helical domain on the transfer mechanism, we eliminated and reverted the charge of these residues (K16I, K16E, K20I, K20E, K27I, K27E, K29I and K29E) by site directed mutagenesis. Analysis of the structural integrity of point mutants by CD spectra and quantum yield showed no major differences compared to the wild-type protein. Binding to the native ligand and anthroyloxy-oleate (12AO) were also unaltered.Transfer rates for 12AO to zwiterionic membranes revealed a collisional transfer mechanism for all the mutants except for K27I. Positions 27 and 29 showed the largest decrease in transfer rate. More drastic changes were observed for vesicles containing anionic phospholipids; the highest decrease was shown by K27E and K29E mutants, with a 78% and 90% decrease in transfer rate to cardiolipin-containing vesicles respectively. These results indicate that lysines 27 and 29 have a important role in this process.

CA-C1.
MEASUREMENT OF CELL VOLUME CHANGES IN WILD TYPE AND AQUAPORIN TRANSFECTED RENAL CELLS

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Measurement of water transport across cell membranes, with high temporal resolution and sensitivity, is essential for determining osmotic water permeability and changes in cell volume associated with stimulation or inhibition of ion transport mechanisms under isotonic conditions. Various techniques have been employed to study cellular volume changes. The use of fluorescent dye-dilution, based on an inversely proportional relationship between relative fluorescence intensity and relative cell water volume, provides excellent sensitivity for measuring volume changes in single cells. However the application of this technique in confluent monolayers of epithelial cells results on a directly proportional relationship, opposited to what was expected. These results were interpreted by other authors as a consequence of fluorescence self-quenching. In the present study, we investigated in detail whether this relationship depends on the region of the cell studied. To do this we used a cellular line established from rat cortical collecting duct ($RCCD_1$), which maintains the cellular types and the transport characteristics of the native duct. We found that the relationship between relative fluorescence intensity and relative cell water volume does change with the region of the cell studied. Comparative studies between wild type and water channel (aquaporin-2 transfected) cells were made using this technique.

CA-C2.
NICOTINIC RECEPTOR M3 TRANSMEMBRANE DOMAIN: ROLE IN CHANNEL ACTIVATION

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The nicotinic acetylcholine receptor (AChR) is a pentamer of homologous subunits. The primordial AChR presumably contained only one type of α subunit and evolution led to subunit diversity resulting in a wide spectrum of structurally and functionally different AChRs. AChRs in the adult muscle have a composition of $\alpha_2\beta\varepsilon\delta$. Each subunit contains 4 transmembrane domains (M1-M4). The role of M3 in channel gating is not well understood. To determine its contribution to AChR activation, we constructed muscle α chimeric subunits containing M3 segments of the neuronal α7 and the ε subunit. The chimeric subunits were coexpressed with non-α subunits and the functional changes were evaluated at the single channel and macroscopic current level. Single-channel recordings of AChRs in which αM3 was replaced by α7M3 showed briefer open durations and prolonged closing intervals within clusters. Kinetic analysis revealed a 6-fold decrease in the channel opening rate and a 2-fold increase in the closing rate. Qualitatively different changes were observed in the AChR containing the chimeric αεM3α subunit. These channels showed significatively prolonged durations, indicating a ~ 20-fold decrease in the closing rate, and brief mean closed times within clusters. The open probability is about 1 at ACh concentrations as low as 1 μM. Taken together our results add new experimental evidence that gives support to the involvement of the M3 domain in channel gating and gives new insights into the structural functional relationships of its contribution to channel gating.

CA-C3.
ACTIVATION OF PLANT PLASMA MEMBRANE H^+-ATPase (AHA2) BY 14-3-3 PROTEIN MEDIATED OLIGOMERIZATION

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Plant plasma membrane H^+-ATPase is regulated by an autoinhibitory C-terminal domain that serves as binding site for scaffolding protein 14-3-3. The 14-3-3 protein releases the constraint exerted by this domain. We had established that activation of the catalysis by C-terminal blocking or deletion produces a strong negative cooperativity (or double michaelian) on the substrate kinetic. In this work we have employed fluorescent resonance energy transfer (FRET) between a donor-acceptor fluorescent pair to follow self-association between H^+-ATPase monomers in response to treatments related with activation of the pump. We used recombinant AHA2 y ahaΔ92 (C-terminal truncated form). The results show that loss of cooperativity occurs in parallel with disruption of protein-protein interactions. The activation of H^+-ATPase by 14-3-3 protein changes the substrate kinetics towards a negative cooperativity and produces oligomerization. Also, oligomerization was observed when lipids restore the negative cooperativity (lost after solubilization) in C-terminal truncated enzyme. These results suggest that there are specific supramolecular arrangements involved in the optimal performance of this pump and the role of the C-terminal domain of H^+-ATPase might be related to prevent self-association and further interaction.
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CA-C4.
SITES INVOLVED IN THE SPONTANEOUS OCCLUSION OF K^+ IN THE Na,K-ATPase

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The Na^+/K^+-ATPase is a protein of the cell membrane that exports 3 Na^+ and imports 2 K^+ ions in each transport cycle, during which these ions become temporarily occluded (trapped within the protein). Occluded K^+ can also be formed by mixing the enzyme with the cation in media lacking Na^+, Mg^{2+} and ATP ("direct route"). While K^+ occlusion in physiological conditions occurs through extracellular sites of the enzyme, it is believed that occlusion via the direct route takes place through intracellular sites, but this is still unclear. We here investigated whether the sites of the enzyme implicated in the occlusion of K^+ via the direct route are extracelullar or intracellular. Under these conditions, K^+ occlusion and deocclusion occur through ordered processes with a fast and a slow components of similar sizes. This behaviour resembles that found for K^+ deocclusion in the presence of Mg^{2+} and orthophosphate (MgPi) where the sites involved are extracellular. Using $^{86}Rb^+$ as a K^+ congener, and a double incubation sequence (with $^{86}Rb^+$ or Rb^+) we labelled the slow- or fast-exchange pools. Depending on whether the deocclusion velocity of $^{86}Rb^+$ in the presence of MgPi was slow or fast after the double incubation sequence, we could infer if the occlusion via the direct route occurred through extracelullar or intracellular sites of the enzyme. Results suggest that the release of Rb^+ in media with MgPi take place through the same sites as those from which the cation entered the enzyme, i.e. the external ones.
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CA-C5.
FORSKOLINE ACTIVATED CURRENT IN WILD TYPE AND VINCRISTINE RESISTANT K562 CELL LINE

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The cell line K562 obtained from a chronic myeloid leukemia express integral membrane proteins such as CFTR (cystic fibrosis transmembrane regulator) and MDR1 (multidrug resistance). We studied with patch clamp and RT-PCR techniques, the relationship between ion currents and the pattern of expression of these proteins, in wild type (K562WT) and vincristine resistant (K562vinc) cell lines. The amplified products in K562WT showed two bands of 300 bp and 170 bp corresponding to CFTR and MDR1, respectively. These two bands were of smaller and greater intensity in K562vinc cells. In the whole cell configuration, forskoline (20 ì M), an activator of adenylate cyclase, added to the extracelular side turned on an outward current in both cell lines, 8 out of 10 experiments in K562WT and 5 out of 9 in K562vinc. The cAMP-activated current was blocked by diphenylamine-2-carboxylate (DPC, 0.5 mM) added to the bath. Normalized current (I/Imax) at 80 mV were: 3.92 ± 0.95 and 2.69 ± 0.56 for forskoline and DPC in K562WT, respectively. In K562vinc the currents were: 9.62 ± 4.81 and 4.56 ± 2.14 for forskoline and DPC, respectively. The presence of an outward cAMP-activated current blocked by DPC in both cell lines suggest that these currents are not altered in spite of the different pattern of expression of CFTR and MDR1.

CA-C6.
***ARABIDOPSIS THALIANA* PLANT PLASMA MEMBRANE AQUAPORINS SHUT DOWN AT LOW CYTOPLASMIC pH**

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Mechanisms that regulate water permeability (Pf) in plants are basic to understand plant responses under limiting factors like abiotic stress. One of the proposed candidates involved in aquaporin modulation is pH (J. Memb. Biol., 2002 187:175-184; Plant. J., 2002 30:71-81). In order to understand its action we performed the following approaches: 1) At the cell level, isolated leaf protoplasts showed very low Pf values (10-20 $\mu m.s^{-1}$) in the presence of an acidic bath medium (pH 5.5) when employing a developed micropipette technique combined with video microscopy. 2) At the membrane level, when plasma membrane vesicles were purified and studied using stopped flow spectrophotometry, we found that Pf was blocked by protons when medium pH was lowered on both sides of the membrane. In these experiments, when vesicles were equilibrated at different pH (final concentration 10 mM of buffer) Pf showed a half-inhibition at pH values of 7.2-7.5. 3) At the molecular level, PIPs were expressed in *Xenopus* oocytes. In osmotic swelling assays, Pf was markedly sensitive (85%) to a 10 min pre-treatment by sodium acetate (pH 6.0) which induced a marked cytosolic acidification.
These findings clearly demonstrate not only that pH allows short-term regulation of aquaporin activity but also that these proteins can be directly blocked by an intracellular acidification.

CA-C7.

LIPID PHASE DISTRIBUTION OF NICOTINIC ACETYLCHOLINE RECEPTOR PROTEIN

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Fluorescence quenching, fluorescence polarization, and Förster resonance energy transfer (FRET) techniques in combination with a detergent insolubility assay were used to investigate the ability of purified nicotinic acetylcholine receptor (AChR) to partition in lipid environments having defined phase characteristics. Fluorescence data indicate that in reconstituted bilayers composed of DPPC, DOPC, the fluorescent probe DPH, and the quenching lipid 12-SLPC, which form a distinct two-phase system, the AChR favours the saturated lipid (DPPC)-enriched phase. This segregation becomes more evident in the presence of cholesterol. In addition, the presence of AChR or cholesterol enhances Triton X-100 insolubility of the phospholipid systems, supporting the hypothesis that the protein is preferentially distributed in the saturated DPPC-rich, Triton-insoluble, ordered lipid phase.

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CA-C8.

A MODEL ACCOUNTING FOR SARCOPLASMIC RETICULUM Ca-ATPase MAXIMAL ACTIVATION AT HIGH ATP AND METAL CONCENTRATIONS

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The sarcoplasmic reticulum Ca-ATPase uses Mg^{2+} as ATP cosubstrate. Results already presented strongly suggest the participation of 2 Me^{2+} and 2 ATP for maximal enzyme activation. Previously, we showed that 2 Mn^{2+} (Mg^{2+} analogue) are involved in maximal phosphorylation with Pi. To evaluate the stoichiometry for the binding of Me^{2+} and ATP, we studied, in the absence of enough Ca^{2+} to activate the enzyme, the ATP effect on micromolar (^{54}Mn)Mn binding and the Mn^{2+} effect on the intrinsic fluorescence changes induced by ATP binding. We also measured ATPase activity in an extended range of [Mg^{2+}] and [Mn^{2+}], in the presence of different [ATP]. The explanation of all these results required the participation of 2 Me^{2+} and 2 ATP. Here we present a model for the ATPase cycle that includes species with 2 Me^{2+} and 2 ATP simultaneously bound. We assigned the kinetic constants values and the initial concentrations of the species in the model to obtain, by numerical simulation, the steady state concentrations of the species at a given time. Pi production is the index of the ATPase activity. The simulated curves for activity as a function of [Mn^{2+}] satisfactorily reproduce the experimental results.

PL-C1.

POTASSIUM UPTAKE -KINETICS AND GENE EXPRESSION ALONG BARLEY ROOT AXIS

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Potassium acquisition by higher plants requires the involvement of outer root cell layers. It has been postulated that the AKT1 inward rectifier K^+ channel and HAK1 transporters play a major role in this process. While evidence supporting this assertion has been already provided for AKT1 in *Arabidopsis thaliana*, it remains scant for HAK1. In situ studies performed with barley roots shown that *HvHAK1* is preferentially expressed at the root epidermis. A detailed examination of the longitudinal *AKT1* and *HAK1* expression patterns showed that both genes are expressed at lower levels in the first centimeter from the apex relative to mature zones. A similar expression pattern was observed for the putative K^+ transporters *HvHAK1b*, *HvHAK2* and *HvHAK3*. If the contribution of different transport entities differs along the root, and if these entities differ in their kinetic properties, a change in Rb^+ transport characteristics along the root could be expected. We observed that all root zones exhibit a saturable high-affinity component and a linear "non-saturable" one, being the Vmax minimal in the growing region. No differences were observed for the Rb^+ Km and the K^+ Ki among root segments. Cs^+ and NH_4^+ inhibited high-affinity rubidium uptake in all the segments, while Ba^{2+} and TEA did not. However, 10 mM Na^+ exerted a differential inhibition along the root that matches the HvHAK1b/HvHAK1 expression quotient. These results indicate that high-affinity K^+ transport in all root zones is mediated by several HAK1-like transporters, which share most, but not all, kinetic attributes.

PL-C2.

CELL WALL DEGRADING ENZYMES DURING RIPENING OF STRAWBERRY FRUIT

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The primary cell wall of fruits undergoes structural changes during ripening which leads to softening. Cell wall modification is mediated by enzymes like polygalacturonase (PG), pectin methyl esterase (PME), endoglucanase (Egase), ß-xylosidase (ß-Xyl) and ß-galactosidase (ß-Gal). We studied the activity of these enzymes during ripening of three strawberry cultivars with different softening extent (Camarosa was the firmest, Toyonaka the softest and Pajaro intermediate). Fruits were classified in large green (LG), white (W), 50% red (50%R) and 100% red (100%R). PG activity was detected from W in Toyonaka and Pajaro, being the first higher than the second. Toyonaka maintained this level throughout ripening and Pajaro increased reaching values similar to those of Toyonaka in 100%R. PG activity started from 50%R in Camarosa and reached higher values than the other varieties in 100%R. PME activity increased during ripening until 50%R, without significant differences among varieties. From 50%R to 100%R, only Pajaro increased its Egase activity, having Toyonaka the highest values, with almost no differences between Camarosa and Pajaro. ß-Gal activity increased in the three cultivars. Toyonaka presented the highest values, Camarosa the lowest and Pajaro intermediate. ß-Xil presented two peaks of activity, one at W and other at 100%R. For all stages Toyonaka showed higher activities, Camarosa and Pajaro had similar levels except in 100%R. We conclude that the higher activity levels presented by the softer cultivar might be one of the reasons for differences in firmness.

PL-C3.

ENHANCED TOLERANCE TO IRON DEFICIENCY IN TRANSGENIC TOBACCO PLANTS EXPRESSING A BACTERIAL FLAVODOXIN

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One of the most severe limitations for agricultural development is the availability of iron, particularly pronounced in plants grown in alkaline, calcareous soils, which cover more than one third of earth's surface and represent a major deterrent for agriculture. Soil-based organisms have evolved various adaptive mechanisms to ameliorate the consequences of iron deficit. For instance, cyanobacteria and phytoplankton induce the expression of flavodoxin (Fld) to replace ferredoxins (Fd), whose iron-sulphur clusters cannot be assembled in iron-restricted media. Fld are small hydrophillic proteins containing FMN as prosthetic group that collaborate with and/or substitute Fd in many different processes, including photosynthesis. They are found in prokaryotes and some algae, but not in vascular plants. The reported recruitment of bacterial Fld under iron deficit encouraged us to assess the behaviour of tobacco plants expressing Fld in chloroplasts (*pfld*) or cytosol (*cfld*) when exposed to low iron concentrations. We report here that transgenic tobacco lines expressing *Anabaena* PCC7119 Fld in chloroplasts displayed enhanced resistance relative to wild-type or *cfld* plants to low iron availability. Tolerance results from functional replacement of Fd. Modulation of Fld levels in plants might therefore provide entirely novel tools to improve the field tolerance of agronomically important crops.

PL-C4.

INDUCTION OF TWO ENDOPROTEOLYTIC ACTIVITIES IN SENESCENT WHEAT LEAVES

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Senescence is the ultimate stage of every plant organ. One of its most characteristic features is the high rate of protein degradation. Endoproteolytic activity measured as azocasein hydrolysis is strongly increased in dark-induced senescent wheat leaves. Analysis of extracts from senescent leaves on DEAE-Sepharose allowed separation of the azocaseinolytic activity in two major peaks. One appeared in the flow-through (P_1) and the other one (P_2) retained in the column was eluted by ionic strength. Previous isolation and purification of P_1 activity allowed its identification as a subtilisin-like serine endoprotease. An antiserum prepared against purified P_1 was used for western blot analysis. It showed that this enzyme appeared in senescent leaves induced by incubation in darkness but was not detectable when induction was achieved by nitrogen deprivation. A band of 84 kDa in SDS-PAGE correlating with P_2 activity was also revealed through several steps of purification. This protein, already present in non-senescent leaves increased its amount in extracts from senescent leaves induced by darkness or by nitrogen starvation. The fact that P_2 reacted with the antibody against the subtilisin-like endoprotease P_1, indicates that these two enzymes are structurally related. As previously demonstrated for P_1, P_2 activity was inhibited by the protease inhibitors chymostatin and PMSF and was able to hydrolyse Suc-AAPFpNA suggesting that P_2 also belongs to the chymotrypsin-like proteases.

PL-C6.

PECTINOLYTIC ACTIVITIES IN POTATO-*FUSARIUM* INTERACTION

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To colonize potato tissue, Fusarium solani f.sp. eumartii degrades plant cell wall. The aim of this work is to relate the pectinolytic activities (Polygalacturonases, PG; Pectin Methyl esterases, PME) with the different susceptibility to Fusarium of two potato cultivars: Spunta, moderately resistant and Pampeana, susceptible. In addition, we compared two F. solani isolates: F. solani f.sp. eumartii isolate 3122, pathogenic and F. solani isolate 1402, no pathogenic. Both isolates grown in liquid culture containing pectin as carbon source, produced similar amount of PG activity. Conversely, in potato intercellular washing fluids (IWF), this activity was higher for the susceptible cultivar inoculated with pathogenic isolate than for the no pathogenic one. Also the PG isoforms detected by zymograms were different. The amount of total PG activity in the IWF changed in each cultivar when Ca++ was present in the reaction mixture. When 0.1 mM CaCl2 was added, PG activity of infected Spunta IWF was higher than in absence of CaCl2. For Pampeana IWF, the effect of Ca++ was less evident. On the other hand, we compared the PME activity between Spunta and Pampeana cultivars. Different isoforms were visualized by zymograms. We suggest that the different PME activity between both cultivars could modify the PG activity produced by the pathogenic fungus. In addition, preliminary evidences indicate that the major level of Polygalacturonase-inhibiting proteins accumulated in the more resistant cultivar could be related with its low level of PG activity.

PL-C7.

INVOLVEMENT OF LeCDPK AND PP2A IN RESPONSE TO ABIOTIC STRESS IN TOMATO PLANTS

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Plants have developed different mechanisms to avoid damage cused by exposure to different stress conditions as biotic (virus, fungus, hervibores) and abiotic (cold, drought, salinity) factors. The defensive response involves the activation of signal transduction pathways that lead to the modulation of gene expression. A calcium-dependent protein kinase, CDPK, induced local and systemically by mechanical damage in tomato (Lycopersicon esculentum) plants, was described previously in our lab. To obtain more information about the involvement of this kinase in plant defense response, LeCDPK expression was determined in tomato plants exposed to different abiotic stresses. The kinase was induced by high salinity and cold in different parts of the plant. Protein phosphatase PP2A expression was also determined suggesting its particiaption in the response to abiotic stress. To determine if the systemic induction of LeCDPK is mediated by systemin, the expression of the kinase was determined in response to systemin. The results obtained indicate that LeCDPK is induced in response to high salinity and cold, being the systemical induction mediated by systemin, and also suggest the involvement of the phosphatase PP2A in the response to abiotic stress.

PL-C8.

CLONING, EXPRESSION AND BIOCHEMICAL CHARACTERIZATION OF THE FRATAXIN HOMOLOG FROM ARABIDOPSIS THALIANA (*Athfh*)

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Frataxin is a nuclear encoded mitochondrial protein required for maintenance of normal mitochondrial iron levels and respiration. This protein is highly conserved from bacteria to mammals. It has been suggested that frataxin could play the same role in all these organisms, but its precise function remains unclear. It has been also predicted the role of this protein in iron-sulfur cluster protein assembly. Thus, plant frataxin homologue might be involved in the assembly of the respiratory chain complexes at the inner membrane in plant mitochondria. Our general working hypotesis is that frataxim null mutants are defective in Fe-S cluster assembly and result in abnormal iron trafficking and Fe-S protein deficiency within plant cells. Recently, we have identified a putative plant frataxin homologue (Athfh). The expression pattern of Athfh shows an induction in Arabidopsis flowers. In u-atp9 Arabidopsis transgenic plants (showing a mitochondrial dysfunction) this protein is further induced. Purified recombinant Athfh from Escherichia coli shows a ferroxidase activity as reported to occur with the yeast homologue. These results, combined with physiological studies using transgenic u-atp9 Arabidopsis lines support a direct role of this protein in plant iron metabolism.

PL-C9.

EASTERN BLOTTING. A HIGH-THROUGHPUT FUNCTIONAL ASSAY OF PHOSPHATASE ACTIVITY

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Phosphatases (EC 3.1.3.1) hydrolyze phosphate esters of primary, secondary and tertiary alcohols. Most of current high-throughput procedures to assay this ubiquitous activity rely upon artificial substrates that yield a signal upon phosphate release; e.g. the appearance of p-nitrophenol after the hydrolysis of p-nitrophenylphosphate. We adapted a well-known assay for the estimation of phosphate [*Anal.Chem*. 28: 1756-1758 (1956)] to solid surfaces. As a consequence, the method became specific and general for phosphatase reactions.
The current protocol, which does not require *the lysis of bacterial cells*, was used to reveal the presence of active forms of two acid and two alkaline phosphatases cloned in different plasmids; [acid glucose phosphatase (*agp*), acid phosphatase (*appA*)] and [chloroplast fructose-1,6-bisphosphatase, alkaline phosphatase (*phoA*)], respectively. Moreover, it can be multiplexed to detect these enzymes not only at a range of concentrations close to those used *in vitro* but also in the presence of specific inhibitors.
Taken together these data indicate that the novel method profiles active and inactive phosphatases, thereby can be used to screen efficiently these enzymes in expression libraries prepared by directed evolution or other procedures.

PL-C10.

POLYAMINE METABOLISM IN NODULES AND ROOTS OF SOYBEAN PLANTS UNDER CADMIUM STRESS

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Polyamines (Pas) have been reported to be involved in several kinds of abiotic stresses in plants. However, there is no information regarding putrescine (Put), spermidine (Spd) and spermine (Spm) metabolism in soybean plants under cadmium stress. Both cadmium treatments, 50 µM and 200 µM, modified Pas metabolism in soybean, by increasing Put and Spm in nodules, and Put and Spd in roots of treated plants. In roots and nodules, Put formation could be attributed to the activity of both biosynthetic enzymes, arginine decarboxylase (ADC), which was 7 times higher than ornithine decarboxylase (ODC) in controls and 50 µM Cd-treated nodules. ODC was responsible for Put formation in nodules under 200 µM cadmium, where ADC activity was almost undetectable. A clear increase in Spm and Spd content were observed in nodules and roots respectively, after day 6 under the metal treatments and this increase was concurrent with a drop in ethylene formation. In nodules under cadmium stress, Spm was higher than controls, and this effect was attributed to S-adenosylmethionine decarboxylase (SAMDC) activity. However, the highest Spm level was observed after SAMDC began to decline. Our results demonstrate that the increase in Pas and the decrease in ethylene in soybean nodules and roots did not avoid the senescence process.

PL-C11.

FUNCTIONAL ANALYSIS OF THE MAIZE PHOTOSYNTHETIC NADP-MALIC ENZYME BY SITE DIRECTED MUTAGENESIS

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NADP-malic enzyme (NADP-ME) catalyses the oxidative decarboxylation of L-malate to yield pyruvate, CO_2 and NADPH. In C_4 plants, a plastidic isoform is located in bundle sheath chloroplasts, which provides the CO_2 to be fixed by RuBisCO. Using the expression system we have previously developed for the maize photosynthetic NADP-ME in *E.Coli*, four mutants were constructed to analyse the specific function of the mutated aminoacids. The mutants R237L and K255I produced drastic kcat reduction, which suggests that both aminoacids can directly participate in the catalytic mechanism. The double mutant K425/6L showed no substantial alteration in the kcat and the Km of malate, although the Km for NADP increased 9-fold, indicating that one or both of these aminoacids´ positive charge are important for the proper interaction of the enzyme with NADP. Curiously, the Km of NAD was 4 times lower than that of the wild type, which indicates that this mutation favours the interaction with the alternate cofactor NAD. The mutant Y184F showed modification only in the Km of malate, suggesting that the hydroxyl group eliminated with this mutation is implicated in malate-binding. Fluorescence emission and circular dichroism spectra showed no differences between these mutants and the wild type. The mutations introduced in the present work are also discussed in the context of a constructed three-dimensional model of the enzyme.

PL-C12.

PURIFICATION AND ANTIFUNGAL ACTIVITY OF A SUNFLOWER LIPID TRANSFER PROTEIN EXPRESSED IN *Escherichia coli* AS A GST-FUSION

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Lipid transfer proteins (LTPs) are low-molecular-mass, basic, antifungal proteins extensively studied in higher plants. A wide array of limitations have been found to express antifungal protein *in vitro,* but in order to produce enough protein to develop structural and functional studies we have tempted the expression of an antifungal protein in *Escherichia coli.* We have previously isolated, characterized and cloned Ha-AP10, a potent sunflower antifungal protein homologous to members of the LTP family. This work describes the cloning of the PCR-amplified ADN encoding Ha-AP10 into an *Escherichia coli* expression vector, and its expression as a fusion with gluthathion-S-transferase (GST). The optimal condition of the expression recombinant protein was determined and a protein with the expected molecular mass was detected in the soluble fraction. In addition, the specific antibody against sunflower-extracted Ha-AP10 recognized the fusion protein purified by affinity chromatografy. A preliminary characterization of the antifungal activity of GST-Ha-AP10 showed it was unable to inhibit the germination of *Fusarium solani* spores, which can be a consequence of structural changes in the protein bound to GST. When the fusion protein was cleaved with thrombin, small amounts of Ha-AP10 were produced to enable a detailed characterization. Although, low yields are obtained, the system proved to be feasible. Scale-up process will allow the production of the recombinant protein to perform structural and functional studies.

MI-C1.

CHEMICAL MODIFICATION OF AN α-MANNOSYL-TRANSFERASE FROM *Acetobacter xylinum*

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AceA is a non-processive α-1,3 mannosyltransferase from *Acetobacter xylinum*, which transfers mannose from GDP-mannose to polyprenyl-pyrophosphate linked cellobiose (Glc_2-PP-Lip) during the assembly of the repeat unit of the exopolysaccharide acetan. AceA belongs to CaZY family 4 of retaining glycosyltransferases. We have previously identified a series of conserved residues, performed site-directed mutagenesis and found that acidic aminoacids may be involved in sugar donor binding and catalysis. We have now confirmed our findings by chemical modification using the carboxyl specific reagent 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (EDAC), resulting in a time- and concentration-dependent inactivation of AceA. The semi-logarithmic plots of residual enzyme activity vs time at different EDAC concentrations resulted in a linear relationship, indicating pseudo-first order rate kinetics. A log plot of inactivation rate constants vs EDAC concentration indicated that at least one acidic aminoacid is important for AceA activity. The effect of substrates was investigated showing that a synthetic analog of the acceptor Glc_2-PP-Lip partially protected the enzyme from inactivation by EDAC.

MI-C2.

***RHODOBACTER CAPSULATUS SOD* MUTANT DISPLAYS INCREASED SPONTANEOUS DNA MUTAGENESIS**

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The purple bacterium *Rhodobacter capsulatus* can live under a broad range of enviromental conditions. When illuminated in anaerobiosis this microorganism synthesizes ATP through a cyclic electron transport around a single photosystem, whereas in the presence of air it shifts to a respiratory metabolism. In this condition, reactive oxygen derivatives such as superoxide, peroxides and the hydroxyl radical are produced by reduced intermediates of the electron transport chain.

Superoxide dismutases (SOD) are the first line of antioxidant defense in most aerobic organisms.

We have cloned the promoter region of the single SOD present in *Rhodobacter capsulatus* and determined the transcription start site by primer extension. Besides canonical –35 and –10 elements, one putative binding site for the redox regulator RegA was identified. A *sod* insertional mutant was studied to evaluate the fisiological role of SOD. Lucigenin luminesence was determined as measurement of intracellular superoxide level, indicating a two-fold raise in the mutant strain.

The oxidative damage on Fe/S centers was evaluated by measuring aconitase activity. An increased rate of spontaneous mutagenesis suggests that superoxide itself or Fe released from Fe/S clusters promoted DNA oxidative injury.

MI-C3.

THE RPON GENE OF BRUCELLA ABORTUS IS IMPORTANT FOR THE BACTERIAL PERSISTENCE IN MICE

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rpoN encodes the alternative sigma factor σ54, which is required for transcription of a wide range of genes involved in diverse physiological functions such as nitrogen metabolism, dicarboxylic acid transport and xylene degradation. σ54 is also involved in the regulation of virulence-related factors in both plant and animal pathogens, including synthesis of flagellar and pilus components. A gene with high homology to *Agrobacterium tumefaciens* σ54 was identified in *Brucella abortus*. Given the facts that RpoN activates the expression of a wild variety of environmentally regulated genes and is required for virulence in a variety of pathogens we hypothesized that RpoN would play a central role in *B. abortus* virulence. In this study we describe the results of experiments that involved the construction of a *B. abortus rpoN* mutant in order to study the role of σ54 in *B. abortus* pathogenesis in HeLa cells, murine macrophage J774 cell line and in Balb/c mice. We concluded that *B. abortus rpoN* is not essential for infection and multiplication in nonphagocytic or phagocitic cells, however *rpoN* may be important for the bacterial persistence in Balb/c mice.

MI-C4.

MOLECULAR CLONING, DNA SEQUENCING AND EXPRESSION OF AN UBIQUITIN CONJUGATING ENZYME GENE FROM *TRYPANOSOMA CRUZI*

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The covalent conjugation of ubiquitin to other intracellular proteins fullfils essential functions in the eukaryotic cells. The ubiquitination requires the sequential actions of three enzymes. The first one is an activating enzyme (E1) wich activates ubiquitin. Following activation one of several ubiquitin conjugating enzymes (E2 enzymes) transfers ubiquitin from E1 to a member of the ubiquitin protein ligase family, E3, that catalyzes the covalent attachment of ubiquitin to the substrate. We describe here the identification and cloning of one *Trypanosoma cruzi* ubiquitin conjugating enzyme, E2Tc. A cDNA clone 27o4 derived from CL Brener cDNA library encodes a protein of 151 aminoacids with a calculated MW of 17,5 kDa. This DNA sequence showed 81% similarity and 67% identities *Drosophila melanogaster* ubiquitin conjugating enzyme E2-17Kd, "bendless protein". The 27o4 clone hibridized with two chromosomal bands of approximately 1,350 and 760 Kbp. The RT-PCR from epimastigotes mRNA amplified one main band of around 550 bp. In order to obtain the recombinant E2Tc protein, the putative coding region was cloned into pMALp2 vector. A fusion protein, with a molecular mass close to the calculated value was obtained. The enzymatic and immunological characterization are currently being performed.

MI-C5.

EXPRESSION OF OAT ARGININE DECARBOXYLASE (ADC) GENE IN *Trypanosoma cruzi* EPIMASTIGOTES

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Trypanosoma cruzi epimastigotes cultivated in a semisynthetic medium can normally proliferate only when putrescine or spermidine is added to the medium. This auxotrophy for polyamines is caused by parasite inability to synthesize putrescine. Previous work from our laboratory has shown that wild-type strains of *T. cruzi* do not contain ornithine decarboxilase (ODC) enzymatic activity due to the absence of the corresponding gene in the parasite genome. Recent experiments carried out to search for the putative conversion of arginine into agmatine, which would allow putrescine biosynthesis through the only other possible metabolic pathway leading to polyamines, also gave negative results in all the strains of *T. cruzi* assayed. We were able to demonstrate that the absence of ADC enzymatic activity in wild-type *T. cruzi* is not caused by inhibition of the enzyme expression inside the parasite. In fact after transfections of *T. cruzi* with a recombinant plasmid bearing the coding region of oat ADC inserted in an appropriate vector, we have obtained transgenic *T. cruzi* showing an appreciable level of transient expression of ADC enzymatic activity. The heterologous ADC has been characterized by identification of its products, agmatine and CO_2, as well as by the stoichiometry of the catalysed reaction and the specific inhibition by difluoromethylarginine (DFMA).

MI-C6.

IDENTIFICATION AND ANALYSIS OF TRYPOMASTIGOTE STAGE-SPECIFIC GENES IN *Trypanosoma cruzi*

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T. cruzi, the aetiological agent of Chagas disease, affects 10% of the Latin American population. The non-replicative trypomastigote is one of the mammalian parasite-stages, and is involved in cell invasion and tissue dissemination. We constructed a cDNA epimastigote-subtracted library for trypomastigotes by suppressive subtractive hibridization (library T-E). A total of 403 good-quality sequences were obtained and a trypomastigote-specific database constructed. More than 45% of the clones represent new genes as no matches against any database available were found and 29 putative new *T. cruzi* genes were identified. Trypomastigote-specific mRNA expression was confirmed by reverse Northern blot, Northern-blot and semi-quantitative RT-PCR. Based on sequence similarity, the T-E library was grouped into 23 clusters, each containing several contigs. A conserved ~220 bp region, present in about 25% of the clones, was identified and defined as "T element" (*Te*). Searches against *T. cruzi* genome database at TIGR, revealed the presence of the *Te* in the 3' UTR of a wide range of putative proteins. Almost all ORFs flanking the T element in the TIGR's WGSs, were found in our T-E library. Differential expression in trypomastigotes of some ORFs as well as the *Te* were confirmed. The presence of the *Te* in the 3' untranslated region of mature transcripts was also determined. Results indicate that the use of subtractive libraries is a useful tool for the identification of novel and stage-specfic genes and stage-specific regulatory elements.

MI-C7.

CLONING AND EXPRESSION OF A PLAUSIBLE CYTOSOLIC TYPE MALATE DEHYDROGENASE (cMDH) FROM *LEISHMANIA MEXICANA*

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L. mexicana, similarly to *T. cruzi*, possesses a broad specificity aromatic aminotransferase but contrary to *T. cruzi*, this parasite neither produces aromatic lactate derivatives as end products nor possesses an enzymatic activity equivalent to the *T. cruzi* aromatic L-α-hydroxy acid dehydrogenase (AHADH). The latter, a cytosolic enzyme, is a member of the MDH family, unable, however, to reduce oxaloacetate. Preliminary purification protocols allowed us to isolate from the crude extracts of *L. mexicana* three different chromatographic fractions with MDH activity. In the present work we report the cloning and functional expression in bacteria of two of these three isoforms. Amino acid sequence alignments and the protein targeting sequences suggest that one isoform may be located in the mitochondria, and the other does not possess a typical glycosome targeting sequence suggesting a possible cytosolic localisation. Both recombinant MDHs were purified by Ni^{2+} affinity chromatography with specific activities of 23 and 389 U/mg for the putative mitochondrial and cytosolic type MDHs, respectively. Glycosomal membranes are impermeable for molecules such as NAD^+/NADH; thus, trypanosomatids probably need a NADH dependant dehydrogenase to regenerate the cytosolic NAD^+. This role might be played by AHADH in *T. cruzi* and by the cytosolic type MDHs in *T. brucei* and *L. mexicana.*

MI-C8.

TRANSCRIPTIONAL REGULATION OF SUCROSE BIOSYNTHESIS IN *Anabaena* sp., A NITROGEN- FIXING CYANOBACTERIUM

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In *Anabaena* sp. we have previously identified the biosynthesis of sucrose through the sequential action of sucrose-phosphate synthase (SPS) and sucrose-phosphate phosphatase (SPP), and have reported the presence of two SPS genes (*sps*A, *sps*B) and one SPP gene (*spp*A). In this work we show that the expression of those genes are subjected to transcriptional regulation in response to changes in the nitrogen source, to salt stress and to the physiological cell stage. RT-PCR and primer extension experiments demonstrated that *sps*A is highly transcribed in osmotic stress, stationary phase and nitrogen-starved cells. In contrast, *sps*B expression is only increased under nitrogen deficiency. *Anabaena* sp. PCC 7120 sucrose biosynthesis genes showed multiple transcripts with different 5' ends analyzed by primer extension, suggesting multiple transcription start sites. Primer extension experiments also revealed an upstream sequence in the *sps*B putative promoter related to a nitrogen transcriptional regulator binding site. Thus, sucrose synthesis is regulated through a high transcriptional control at the first step of the pathway.
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MI-C9.

IDENTIFICATION BY MICROARRAYS OF A NOVEL DELETION IN A MEMBER OF *Mycobacterium tuberculosis* COMPLEX: *M.microti*

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The *Mycobacterium tuberculosis* complex consists of slow-growing, pathogenic mycobacteria. The group includes *M. tuberculosis*, the agent of human tuberculosis; *M. bovis*, which causes both bovine and human tuberculosis; *M. africanum*, which causes human tuberculosis on the African continent; and *M. microti*, which is pathogenic for the vole, a wild rodent. *M. microti* has been used as a live vaccine against tuberculosis in man and cattle. However, recent reports suggest that some strains can cause disease in humans. Deletions were clearly a driving force in the genome evolution of the tubercle bacilli. Using comparative genomic approaches, 16 regions of difference (RD1–16), ranging in size from ~2 to 12.7 kb, have been described in *M. bovis* and BCG strains relative to *M. tuberculosis* H37Rv, with 5 regions deleted from *M. tuberculosis* H37Rv. To explore the *M. microti* genome we used a *M. tuberculosis* H37Rv genomic DNA microarray to detect gene deletions among *M. microti* isolates. A number of deletions were identified that correlated with those described previously by Brodin *et al.* A novel *M. microti* deletion was also found (MiD4) which removes 5 genes that code for ESAT-6 family antigens and PE-PPE proteins. Southern blot experiments showed that this region was also deleted from *M. pinnipedii*, a mycobacterium isolated from seals that is closely related to *M. microti*. Genes encoding ESAT-6 antigens and PE-PPE proteins appear to be frequently deleted from *M. microti*, and the implications of this are discussed.

MI-C10.

VIBRIO CHOLERAE-INDUCED APOPTOSIS OF MAMMALIAN CELLS MEDIATED BY EL TOR HAEMOLYSIN

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El Tor Haemolysin (ETH) is a pore-forming toxin encoded by the *hlyA* gene of *V. cholerae* O1 biotype El Tor and most of *V. cholerae* non-O1/ non-O139 (VCN) isolates. Previous studies demonstrated that ETH is able to induce cytolytic, enterotoxic and vacuolating activity, suggesting that this toxin may contribute to the pathogenesis of gastroenteritis caused by *V. cholerae* strains lacking the cholera toxin (CT). In order to explore further mechanisms of cell damage, the potential involvement of apoptosis triggered by ETH was investigated. To this end, COS-7 cell monolayers were exposed to sterile culture supernatant from a clinical VCN strain, lacking CT, or from its isogenic *hlyA* null mutant. At different times post-incubation the apoptosis phenomenon was analyzed by DNA fragmentation, flow cytometry and TUNEL. The parental strain but not its isogenic *hlyA* null mutant induced internucleosomal DNA fragmentation, hypodiploidia and TdT-mediated incorporation of fluorescent d-UTP, demonstrating the apoptotic state of treated cells. Apoptosis was also observed after exposure to 75 ng/mL of purified ETH. Furthermore, apoptotic activity of both parental strain and purified ETH was completely abolished after pre-incubation with an anti-ETH antibody. These results demonstrate that ETH is capable to induce apoptosis of mammalian COS-7 cells and points to previously unknown interactions between *V. cholerae* and its host.

MI-C11.

CHARACTERIZATION OF AN ADAPTIVE ACID-TOLERANCE MECHANISM IN *Streptococcus pneumoniae*: ANALYSIS OF QUORUM-SENSING MUTANTS AND IDENTIFICATION OF ACID-INDUCED PROTEINS

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During the invasion process, *S. pneumoniae* has to overcome different stress conditions due to host defence mechanisms, like pH decrease produced by phagosomes. The aim of this work was to study a putative acid tolerance response (ATR) in *pneumococcus*. We found that log- phase cells, grown previously at neutral pH, survived 2 hours at pH 4.4. In contrast, stationary-phase cells required a pre-incubation in an acetate-bufferized medium at pH 5.6 during 1 h at 37°C to tolerate this pH condition. With the purpose to study the cellular density effect on ATR induction, we analysed *comE* mutants. The *comE* gene encodes a response regulator that belongs to the quorum-sensing system ComCDE, involved in competence regulation. The null mutant, *comE::km*, showed no significant effect on ATR, however, the hyperactive mutant *comE38KE* repressed either acidic or log-phase ATR induction. These results demonstrate that ATR induction is controlled by the ComE pathway. In addition, we searched acid-induced proteins in different proteic fractions obtained by acetone precipitation and urea solubilization. The proteic band patterns were compared by PAGE-SDS, and we found four proteins clearly induced by pH 5.6 (30, 70, 80 and 120 kDa). At present, we identified by peptide sequencing and pneumococcal genome analysis two proteins: an ATP-dependent protease and a fructose aldolase. In the future, we will determine the impact of these genes on ATR induction by mutagenesis assays.

MI-C12.
A SALMONELLA SPECIFIC TRANSCRIPTIONAL REGULATOR THAT REPONDS TO GOLD
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The MerR family is a group of transcriptional regulators with similar N-terminal helix-turn-helix DNA binding regions and C-terminal effectors binding regions that respond specifically to certain environmental stimuli, such as heavy metals, oxidative stress or antibiotics. We searched for new transcriptional regulators in *Salmonella enterica* serovar Typhimurium and identified a gene with high homology in sequence to members of the *merR* family of metalloregulatory proteins. This gene, *hmrR*, was located within a *Salmonella* specific region of the genome. *hmrR* forms a two-gene operon with a gene coding for a putative metal-transporting P-type ATPase (*hmrT*), and is located upstream to a gene showing high similarity to a family of metal binding proteins (*hmrB*).
We showed here that HmrR regulates the expression of the two neighbouring genes, and probably its own synthesis. By measuring β-galactosidase activity, we found that expression of these genes is strongly regulated by gold, but responds poorly to copper or silver ions, as well as other heavy metals. We mapped hmrT and hmrB promoters by primer extension analysis and noticed that their promoter/operator regions have signature elements that distinguish promoters controlled by transcriptional regulators of the MerR family: a suboptimal spacing between -10 and -35 elements and sequences of dyad symmetry. To our knowledge, this is the first example of a metalloregulatory protein that responds specifically to gold.

MI-C13.
REGULATION OF SPORULATION AND ENTEROTOXIN PRODUCTION OF THE GAS GANGRENE PRODUCER *Clostridium perfringens* TYPE A FOOD-POISONING
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The spore forming, anaerobic bacterium *Clostridium perfringens* is responsible for the human gangrene apart from being an important cause of gastrointestinal and histotoxic infections. The enterotoxigenic pathology is due to the production of a potent toxin (CPE) that induces cell death and the symptoms of enterotoxemia, intestinal cramping, and diarrhea. Expression of *cpe* is temporally and genetically linked to sporulation. Here we examined the molecular mechanism of spore formation and its role in CPE production. An isogenic *spo0A* knockout mutant of *C. perfringens* was constructed by allelic exchange. The *spo0A* knockout mutant failed to produce Spo0A and it was completely deficient in sporulation and CPE production. By complementation of the *spo0A* knock out mutant with a recombinant plasmid carrying the intact *spo0A* gene it was possible to restore full efficiency in sporulation and CPE formation. By the expression of a clostridial DNA library in a *Bacillus subtilis* strain deficient in the expression of the sensor sporulation kinases KinA and KinB it was possible to identify a clostridial clone that restored *Bacillus* sporulation. DNA analysis indicated the presence of a putative sensor histidine kinase coding gene (*cp-kinA*) involved in inorganic metabolism. A *cp-kinA* knock out mutant strain of *C. perfringens cpe*$^+$ was severely affected in sporulation and CPE production. The overall results are relevant for the development of antidotes to prevent gastrointestinal diseases and the gas gangrene.

MI-C14.
EFFECT OF THE MUTATIONS ON THE Cys RESIDUES IN THE *Escherichia coli* NADH-DEHYDROGENASE-2 ACTIVITIES
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NADH dehydrogenase-2 (NDH-2) of *Escherichia coli* is a membrane-bound flavoprotein linked to the respiratory chain. Our previous studies showed that purified NDH-2 has a Cu(I)-bound and exhibits Cu(II)-reductase activity supported by NADH. Moreover, bioinformatic analysis indicated that this protein has a cysteine-rich domain (domain III) conserved in primary and secondary structure with the HMA (Heavy Metal Associated) domains that could be the site of copper binding. Domain III presents the $Cys_{315}XXCys_{318}$ and the $MetXXCys_{339}$ motifs. In order to study the participation of all the Cys present in domain III on the NDH-2 activities, we prepared a set of plasmids bearing *ndh* gene mutagenized in the three Cys and then transformed into strain IY12 that lacks the NADH- dehydrogenase activities (*nuo* and *ndh*). We found that the mutations affected the NADH-dehydrogenase and the NADH-oxidase activities of the membranes, compared with one preparation containing the wild-type protein. We saw that both activities decreased about 80% in the C318S mutant, and almost disappeared in the double mutant (C315S-C318S). On other hand, the activities increased about 50% in C339S mutants. These results indicating that this mutations affected some of the NDH-2 activities, suggest that $Cys_{315}XXCys_{318}$ and $MetXXCys_{339}$ motifs are involved on the functionality of the enzyme directly or due to the Cu (I) putatively bound there.

MI-C15.
THE SPECIFICITY AND ARCHITECTURE OF ACYL-COA CARBOXYLASE β SUBUNIT IN STREPTOMYCES COELICOLOR A3(2)
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Two acyl-CoA carboxylase complexes, acetyl-CoA carboxylase (ACC) and propionyl-CoA carboxylase (PCC) have been characterized in *Streptomyces coelicolor* whose main physiological role is to provide malonyl- and methylmalonyl-CoA for both fatty acid and polyketide biosynthesis. Both complexes share the same biotinylated α subunit, AccA2 (biotin carboxyl carrier protein, BCCP), that also contains a biotin carboxylase domain. The β and the ε subunits are specific for each of the complexes (AccB-AccE and PccB-PccE to ACC y PCC respectively). The β subunit has transcaboxylase activity and the ε subunit forms a subcomplex with the β subunit that increases drastically the activity of the enzymatic complex. ACC and PCC in *S. coelicolor* are 1 MDa multienzyme complexes containing at least 18 polypeptide chains. The β subunit, PccB and AccB are 360 kDa homo-hexamers. Apo and substrate-bound crystal structures of PccB hexamer were resolved to 2.0 – 2.4 Å. Overall, the hexamer assembly of the core 360 kD β subunit forms a large ring-shaped complex as two stacks of trimers related by two-fold symmetry. The structural studies shed light on the molecular basis of substrate recognition and the nature of the assembly.

MI-C16.
GENETIC VARIABILITY AND RECOMBINATION IN ARENAVIRUSES

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Arenaviruses possess a bipartite single stranded RNA genome. Both molecules (RNAs S and L) present an ambisense coding strategy, with two genes in each one, separated by an intergenic region constituted by self-complementary sequences. Sources of genetic variation can have diverse origins including the error rate of the RNA replicases and, in multipartite viruses, the genomic rearrangement during progeny generation. In addition, recent evidences suggest that recombination among RNA molecules is a quite frequent event, that can have an important role as genetic variation source. In this work, we present alternative models of RNA recombination based on the bioinformatic analysis of complete S RNA sequences from arenaviruses. The main molecular mechanisms that would enable the formation of recombinants include copy-choice, primer-alignment-and-extension and trans-esterification. Whitewater Arroyo virus, Bear Canyon virus (both isolated in USA) and Rio Carcarañá virus (isolated in Argentina) provide evidences of natural recombination among arenaviruses detected by our theoretical analysis. All these possible genetic sources of variation, added to ecological and human activity changes, suggest that the number of arenaviruses would be increased in the next years. Molecular-epidemiological surveillance programs that contemplate these possibilities would be able to help to minimize the risks of the emergency or re-emergency of potential pathogens.

MI-C17.
GALACTOSIDES METABOLISM OF *l. plantarum*, GENES AND THEIR FUNCTION: REGULATION IS THE KEY

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In this study we present evidence that *Lactobacillus plantarum* has sugar metabolism functional genes but fail to metabolize these carbohydrates probably because they lack a crucial step of their metabolic pathway. In *L. plantarum* ATCC8014, a 7.5 Kb gene cluster involved in galactosides metabolism was identified and sequenced. Their relevant genes, an á–galactosidase (*melA*) and a galactosides permease (*rafP*) were cloned and expressed in heterologous hosts showing enzymatic activity and lactose transport, not present in the native strain. Using the cloned genes, the á-Gal activity was evidenced in different *E. coli* strains and *Lactococcus lactis*, while functional lactose transport was demonstrated in *E. coli*. The metabolic capacity of two *L. plantarum* strains were compared as was the complete genome region encoding the *melA* and *rafP* genes at the nucleotide and amino-acid level. Only one of these strains was able to grow on melibiose and lactose. However, sequence comparison showed no relevant differences, suggesting that regulatory genes could be responsible for these divergent results. This comparison provides another good example that genes "are live and are functional" only in a specific genetic environment.

MI-C18.
REGULATION OF EXPRESSION OF THE TWO-COMPONENT SYSTEM *CitST* IN *Bacillus subtilis*

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The two-component signal transduction system is a ubiquitous mechanism for sensing and responding to several environmental stimuli in bacteria. In *Bacillus subtilis*, citS and citT encode a two-component system involved at the regulation of the citrate transporter CitM. Induction of citM is mediated by CitS (sensor kinase), which recognizes external citrate, and CitT (response regulator) that work as a transcriptional activator by binding to the promoter region of citM. Due to the expression of citM depends on the expression of the two-component citS and citT, in this period we start the molecular characterization of the gene expression of this two-component system to understand more about the exquisite regulation in the citrate-Mg^{+2} transport in *B. subtilis*. Our experiments shown that an expression of the two-component system depends on the constitutive promoter located upstream region of citS. The control of the citS expression is under the CcpA protein, which binds to the cre site present in the internal region of this gene. The expression of citST is not repressed by arginine in rich medium suggesting that this repression is operated in the citM region.

BE-C1.
ENERGETIC MAPPING OF A PROTEIN-DNA INTERFACE

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Protein-DNA interactions play a central role in cell physiology. Within these, site-specific recognition is a primary topic that underprints the expression profile of whole genomes. Although many biological aspects of these non-covalent interactions are becoming clearer, detailed biophysical understanding of site specific recognition is lacking. Using the papillomavirus E2c transcription factor as a model system we aim a physicochemical dissection of its DNA binding properties. We set-up an spectroscopic binding essay, readily done in solution, which allowed us to quantify DNA binding parameters in energetic terms, detect conformational changes exerted on both macromolecules upon binding and relate them to the high resolution structures available. An extensive site-directed mutagenesis analysis over the entire DNA binding region led us to assign the energetic contributions of individual sidechains to the overall binding energy. The small individual contribution of sidechains (< 1.0 kcal/mol) correlates with a highly dynamic interface. This, together with the additive free energy contributions by aminoacid-base interactions, is indicative of absence of "hot-spots" at the interface. Furthermore, the global energetics can be mapped into separate patches that may be related to the interface solvent accessibility. With the integration of structural, biophysical and computational techniques, the molecular basis of this complex macromolecular interactions are beginning to picture into thermodynamic models that may help us grasp how these specific interactions are achieved.

BE-C2.
MULTIPLE PARTIALLY-FOLDED STATES OF β-LACTAMASE AT EQUILIBRIUM

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Site-directed mutagenesis was used to introduce single cysteine residues into β-lactamase (ES-βL), an α+β protein that naturally does not contains any cysteine residue. Two mutants, S126C and S265C, in which a single cysteine residue substitutes the isosteric residue serine, were prepared. The new thiol moieties were used as a chemical probes to map the two domains of ES-βL in urea-induced unfolding experiments. Fluorescence and CD were also measured. In terms of protein stability, the S126C substitution is well tolerated, but S265C ES-βL is significantly less stable than wild-type ES-βL. The single Cys of the two variants is unreactive in the native state. In the urea-unfolded state Cys reacts (100%), with the same rate as free β-mercaptoethanol. Under partially denaturat conditions, the stability, thiol accessibility, and secondary structure suggests multiple partially folded states at equilibrium. In order to compare the stability of the two subdomains in a globally isoenergetic unfolding process, we built a double mutant, S126C, S265C. Our results suggests that folding of proteins with complex architecture involves a hierarchical.

BE-C3.
UDP-GLC:GLYCOPROTEIN GLUCOSYLTRANSFERASE RECOGNIZES SUBSTRATES WITH MINOR STRUCTURAL PERTURBATIONS

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Protein folding in the cell involves the action of different molecular chaperones and folding-facilitating enzymes. In the endoplasmic reticulum (ER), the folding status of glycoproteins is stringently controlled by a glucosyltranferase enzyme (GT) that creates monoglucosylated structures recognized by ER resident lectins (calnexin/calreticulin, CNX/CRT). GT serves as a folding sensor because it only glucosylates misfolded or partly folded glycoproteins. Nevertheless, the molecular mechanism behind this recognition process remains largely unknown. We addressed this issue by using a family of chemically glycosylated proteins derived from chymotrypsin inhibitor-2. By progressive truncation from the c-terminus we generated a family of 7 neoglycoproteins, ranging from 53 to 64 residues long, aimed to simulate the last stages of the folding process. Structural characterization of species showing higher glucose acceptor capacity suggests that GT recognizes solvent accessible hydrophobic patches in molten globule-like conformers, mimicking intermediate and advanced folding stages of nascent glycoproteins. Surprisingly, deletion of only one residue from the full-length molecule (the C-terminal one, that was required for attaining the native conformation) triggered GT recognition, pointing to the high sensitivity of GT as a folding sensor.

BE-C4.
A SIALIDASE MUTANT DISPLAYING TRANS-SIALYLATION ACTIVITY

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Trypanosoma cruzi, the agent of Chagas disease, unable to synthe-size sialic acid, requires this monosaccharide on surface molecules for biological processes like protection and cell infection. To in-corporate sialic acid, the parasite expresses a surface trans-sialida-se (TS) that transfers sialic acid from host glycoconjugantes to ter-minal ß-galactoses present in parasite mucins. Another American trypanosmatid, *T.rangeli*, expresses a homologous protein that has sialidase (TrSA) activity but is devoid of transfer activity. Recently, the 3D structure of TcTS and TrSA proteins had been determined. The comparison between both structures reveals that few amino acids close to the active center might explain the trans-glycosylation activity of TcTS. We constructed TrSA exchange mutant proteins with the aim of obtaining TS activity from a sialidase scaffold. TrSA containing only five point mutations at M96V, A98P, S120Y, G249Y and G283P, displayed about 1% of the trans-sialidase activity present in the recombinant TcTS. Inclusion of another mutation at position I37L or G342A in the above framework increased the transfer activity up to 10% of TcTS. 2,3-didehydro-2-deoxy-N-acetylneuraminic acid (DANA), a sialidase inhibitor that has to be used ten times more concentrated to inhibit TS, was used as a probe of the structure of the active site of mutant enzymes. TrSA mutants displaying TS activity loose their sensitivity to DANA. The results show that few amino acid posi-tions in the active site cleft are the ones essential to achieve trans-sialylation activity from a sialidase scaffold.

BE-C5.
CHARACTERIZATION OF A TRANSCRIPTION FACTOR-ANTIBODY INTERACTION

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We have previously generated and characterized a set of monoclonal antibodies (mAbs) against the C-terminal domain of the E2 transcriptional activator (E2C) of the human papillomavirus. Functional epitope mapping analysis revealed that two separate antibodies populations were obtained: those able to form a stable ternary complex with E2C and DNA, and those which recognize the DNA-binding surface of the transcription factor, interfering with its binding to DNA. In order to characterize in more detail the interaction involved in the different antibody:E2C complexes, the affinity constant and kinetic parameters of the reactions were determined. In addition, the effect of the ionic strength on these parameters was analyzed. Experiments were carried out with mAb ED15 (directed towards an epitope in the DNA-binding surface) and mAb ED23 (recognizing an epitope in the opposite surface of the protein). Results show that both antibodies recognize the E2C antigen with high affinity (nanomolar range) and that their association reactions are highly sensitive to ionic strength, being the interactions completely abrogated at NaCl concentrations close to 0.3 M. Furthermore, both mAbs present a high proportion of acidic residues in their variable region sequences, pointing out that these interactions are essentially mediated by electrostatic forces. We present a new system model useful to understand the role of electrostatic interactions in antibody-protein and protein-protein recognition.

BE-C6.
THE HPV16 E7 ONCOPROTEIN CAN FORM SPHERE-LIKE PARTICLES THAT RESEMBLE RING PROTEINS

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High risk HPV16 E7 oncoprotein is an extended dimer capable of undergoing pH dependent conformational transitions that expose hydrophobic surfaces to the solvent. The dimer shares some properties with intrinsically disordered proteins but it is clearly structured. We found that it is capable of forming highly ordered soluble aggregates that elute in the void volume of a gel filtration column. Dynamic light scattering showed monodispersity and molecular weight of 790 kDa, with a radius of 26 nm assuming a globular architecture. Indeed, electron microscopy and atomic force microscopy showed regular and apparently homogeneous sphere-like particles of 40 to 50 nm diameter. The protein undergoes a conformational transition upon formation of these particles, with a substantial increase in β-sheet content as judged by circular dichroism. We introduced a tryptophan residue at position 98, present in the HPV18 E7 protein, and used it as probe for fluorescence studies that indicate an increase in tertiary (and quaternary) structure, also supported by near-UV CD spectra. These structures bind and shift congo red spectra and bind thioflavin T, the standard probes for amyloid structures. However, no insoluble material is formed under any condition tested. The assembly is very slow (t1/2 20 min), which will eventually allow the dissection of the mechanism.

BE-C7.
STUDYING INTERFACES IN PROTEIN-PROTEIN COMPLEXES WITH A PHOTOCHEMICAL PROBE

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The importance of interactions between proteins in biology has made the molecular recognition process an area of considerable interest. A consequence of complex formation is a reduction in the solvent accessible surface area (SASA). Our approach is based on a general photochemical modification of the polypeptide chain, useful for addressing changes in SASA occurring upon protein interaction. ^{3}H-diazirine (^{3}H-DZN) is a photoreactive gas similar in size to water. By irradiation at λ>300nm ^{3}H-DZN generates methylene carbene ([^{3}H]:CH_2), which reacts with its immediate molecular cage, inserting even into C-H bonds. As a model system we used this reagent to probe the complex formed by HEL (hen egg white lysozyme) and the monoclonal antibody IgG_1D1.3. HEL was labeled free or complexed with IgG_1D1.3 yielding 2.76 and 2.32 mmol CH_2/ mol protein at 1mM DZN, respectively. This agrees with the observed decrement in SASA occurring because of complex formation. Tryptic digests derived from labeled HEL - free or complexed - were separated by size exclusion chromatography and RP-HPLC. The measurement of radioactivity and the identification of each isolated peptide showed that those implicated in the area of interaction had the highest differential labeling (H_{15}GLDNYR_{21}; G_{117}TDVQAWIR_{125}; G_{22}YSLGNWVCAAK_{33}). Thus, 'protein footprinting' with DZN emerges as a feasible methodology useful for mapping contact regions of protein domains involved in macromolecular assemblies

BE-C8.
ANALIZYNG LOCAL PROTEIN STRUCTURE PERTURBATIONS WITH COLORES

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COLORES (Comparison of LOcal Residue Environment Structures) is a computer program for performing analysis of local differences among similar protein structures. It is suitable for comparison of different protein mutants or alternate structural predictions for a given protein sequence. Here we introduce the basic algorithms behind the program, we detail its multiple options and we give examples of use. The program is available from the authors for academic purposes for Unix and Windows platforms

BE-C9.
NMR CHARACTERIZATION OF POLYAMINE COMPLEXES WITH α-SYNUCLEIN

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The aggregation (fibrillation) of α-synuclein is characteristic of Parkinson's disease (PD) and other neurodegenerative synucleinopathies. The 140 amino-acid protein is natively unstructured; thus ligands binding to the monomeric form are of potential therapeutic interest. Biogenic polyamines promote the aggregation of α-synuclein and may constitute naturally occurring agents modulating the pathogenesis of PD. We characterized the complexes of natural and synthetic polyamines with α-synuclein by NMR at 15°C.[1] The perturbations of the ^{13}C/^{15}N backbone chemical shifts in the region aa109-140 identified the binding site in the C-terminal domain and yielded dissociation constants for the different polyamines. Greater molecular charge (+2 → +5) correlates with greater affinity and enhancement of fibrillation at 37°C, for which we propose a simple kinetic mechanism involving a dimeric nucleation center. From the derived parameters, the polyamines increase the extent of nucleation by 10^3 and the rate of monomer addition ~40-fold. Significant secondary structure is not induced at 15°C. Instead, NMR reveals changes in a region (aa22-93) far removed from the polyamine binding motif and presumed to adopt the β-sheet conformation characteristic of fibrillar α-synuclein.

[1]Fernández CO, Hoyer W, Zweckstetter M, Jares-Erijman EA, Subramaniam V, Griesinger C, Jovin TM. Submitted (2003).

BE-C10.

MOLECULAR DYNAMICS SIMULATIONS OF LIVER BASIC FATTY-ACID BINDING PROTEIN (LB-FABP) IN LIPID MEMBRANES

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We have performed several molecular dynamics simulations of Lb-FABP in the vicinity of lipid membranes. The different simulations were started with the protein located in the water phase, with none or a few contacts with the lipids. When the membrane was made of the anionic lipid DLPS it was observed that Lb-FABP approaches the membrane and establishes contacts with the phospholpid head groups. At the same time, a conformational change was observed: a beta-hairpin which is distal from the domain that interact with the membrane undergo a partial unfolding with loss of secondary structure. When the membrane was composed of the zwitterionic lipid DPPC, Lb-FABP did not establish contacts with the membrane and no conformational changes were observed as compared with the protein in solution. The partial unfolding induced by the interaction with anionic membranes was in agreement with previous experimental observations [Nolan V, Perduca M, Monaco HL, Maggio B and Montich GG. (2003). *Biochim. Biophys. Acta,* 1611 98-106].

BE-C11.

ANALYSIS OF THE CHOLESTEROL-DEPENDENT INTERACTION OF PERFRINGOLYSIN O WITH MEMBRANES USING FLUORESCENCE SPECTROSCOPY

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Perfringolysin O (PFO) is a β-barrel pore-forming toxin secreted by the pathogen *Clostridium perfringens.* Upon encountering a cholesterol-containing membrane, the toxin oligomerizes and spontaneously inserts to form a large pore. In recent years, we have used fluorescence spectroscopy to analyze the structure of the membrane-inserted oligomer of PFO (*Nat. Struc. Biol.* 2002, 9, 823), and also to examine the mechanism of pore formation (*J. Biol. Chem.* 2003, 278, 31218).
Despite significant progress made on the structural aspects of these bacterial toxins, how cholesterol mediates PFO binding remains one of the main unsolved issues in the field. Which cytolytic step requires cholesterol? Which region of PFO is involved in cholesterol recognition?
Fluorescence resonance energy transfer studies reveal that binding of PFO to the membrane is the critical step that requires cholesterol. We have prepared different derivatives of PFO that have been site-specifically mutated in the membrane-exposed loops of domain 4 of the molecule. The cholesterol-dependent binding properties of these mutants show that the region located close to the unique cysteine residue of the toxin substantially alters the interaction of PFO with the bilayer. In contrast, modification of membrane-exposed residues that are located further away from this conserved loop does not affect PFO binding to the bilayer. These results suggest that the cholesterol-sensing region of PFO is located near the unique cysteine residue.

BE-C12.

DIRECTED MOLECULAR EVOLUTION OF A METALLO-β-LACTAMASE: DEVELOPING BAD MANNERS

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Metallo-β-lactamases (MBLs) are zinc-dependent enzymes able to hydrolyze β-lactam antibiotics. Their active sites are largely conserved in enzymes from various sources, even if they display different metal binding affinities. BcII from *Bacillus cereus* (a non-pathogenic soil bacterium) is a MBL with low specific activity against different substrates. We have proposed that BcII could be an evolutionary precursor of more efficient β-lactamases from pathogenic bacteria.
In order to test this hypothesis, we have employed the iterative method of *in vitro* molecular evolution called DNA shuffling to try to find BcII variants with increased activities. In particular, we decided to improve the enzymatic activity towards cephalexin (a poor substrate of BcII). Cephalexin also displays a low MIC towards *E. coli* cells expressing BcII in the periplasm. After 4 rounds of DNA shuffling and clonal selection of variants being able to confer higher resistance levels to cephalexin, we isolated several clones displaying MIC 64-fold increased. When we analized the sequence of 18 clones, we found that 4 residues showed a high frequency of mutation. Interestingly, one of these is naturally present in some MBLs. The BcIIm5 mutant, which harbours these 4 mutations, was purified and its hydrolytic activities towards different substrates were determined. We found a selective increase in the specific activity towards cephalexin.

BE-C13.

PURIFICATION, CRYSTALLIZATION AND PRELLIMINARY X-RAY ANALYSIS OF TRIATOMA VIRUS (TrV) FROM *TRIATOMA INFESTANS*

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Objective*:* TrV is a viral pathogen of *T. infestans* and *T. Patagonica*, and after TrV genome came out this virus was considered as a possible member of *Cricket paralysis-like viruses* genus.
Methods: Live insects of *T. infestans* experimentally infected with TrV were used to purify TrV and viral particles observed under TEM. Hanging-drop vapour-diffusion technique was used to obtain virus crystals and X-ray diffraction data collected at a synchrotron beamline under cryogenic conditions.
Results: Here we report the purification, crystallization and data collection of TrV crystals. Hexagonal crystals of about 200ìm long were obtained after 5 days. The unit-cell parameters are a=b=306.6Å, c=788.4Å, á=â= 90°, ã=120° and their diffraction reached 3.2Å resolution.
Conclusions: Chagas disease is caused in humans by the infection of the protozoan *Trypanosoma Cruzi* and *T. infestans* is the main vector of this parasite. TrV was proposed as a potential control agent against triatomines and to belong to the *Cricket paralysis-like virus* genus can reinforce this hypothesis. TrV capside is being determined and the structure can give substantial information for the virus classification.

BE-C14.
CHARACTERIZATION OF THE SUBSTRATE BINDING DOMAIN IN BACTERIAL ADP-GLUCOSE PYROPHOSPHORYLASE

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ADPGlcPPase is the key regulatory enzyme for the synthesis of glycogen and starch in bacteria and plants, respectively. The enzyme is allosterically regulated, with specificity for the regulator depending on the source. Accumulated evidence strongly suggests that ADPGlcPPases have a common folding pattern despite different quaternary structure and specificity for activator. Domains for substrate binding have been proposed in the predicted secondary structure of the enzyme. To analyze the validity of the prediction we characterized different mutants of bacterial ADPGlcPPase randomly generated by the linker-scanning mutagenesis. Results reinforce the structure model and agree with domains proposed for binding of ATP and Glc1P. Data also suggest the occurrence of conformational changes in the enzyme upon binding of the activator. The latter was further analyzed by characterization of site-directed mutants. It is suggested the occurrence of distinctive changes induced by ADPGlcPPase specific activators.
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BC-C2.
GANGLIOSIDE GLYCOSYLTRANSFERASES ORGANIZE IN DISTINCT MULTIENZYME COMPLEXES IN CHO-K1 CELLS

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Ganglioside synthesis is compartmentalized in the Golgi complex. In most cells, glycosylation of LacCer, GM3 and GD3 to form higher order species (GA2, GM2, GD2, GM1, GD1b) occurs in distal aspects of the Golgi and the trans Golgi network, where the involved transferases (GalNAcT and GalT2) form physical and functional associations, while glycosylation of the simple species LacCer, GM3 and GD3 occurs in more proximal Golgi compartments. Herein we investigate if the involved transferases (GalT1, SialT1 and SialT2) share the property of forming physical associations. Co-immunoprecipitation experiments from CHO-K1 cell membranes expressing epitope tagged versions of these enzymes indicate that they associate physically in a SialT1-dependent manner, and that their N-terminal domains participate in these interactions. Fluorescence microscopy in living cells confirmed the interactions, showed Golgi localization of the complexes and mapped their formation to the ER. No interactions between either GalT2 or GalNAcT and GalT1 or SialT1 or SialT2 were detected. These results, and triple color imaging of Golgi microvesicles reemerging the ER in Nocodazole treated cells suggest that ganglioside synthesis is organized in distinct units each formed by associations of particular glycosyltransferases, that concentrate in different sub-Golgi compartments.

BC-C1.
DYNAMICS PROPERTIES OF THE GTPase RAB1 IN LIVING CELLS

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Protein transport from the endoplasmic reticulum (ER) to the Golgi requires the action of the GTPase Rab1. Rab1 cycles between a GDP and GTP-bound form and its activity is mediated by the interaction of Rab1-GTP with different proteins. However the relationship between Rab1 dynamics and protein-protein interaction is unclear. To study the dynamics of Rab1 we have used time lapse microscopy of living cells (HeLa) transfected with GFP-Rab1. The GFP-Rab1 localized predominantly to the Golgi and to peripheral punctate structures. The majority of the GFP-Rab1 labeled punctate structures underwent movements within a limited area (less than 2 µm in diameter) during time periods of 5 minutes. Only occasionally longer range, randomly directed movements (aprox. 10 µm) were observed. The binding and release kinetic of Rab1b to and from the Golgi membranes was analyzed by fluorescence recovery after photobleaching (FRAP) in GFP-Rab1b expressing cells. GFP-Rab1b bleached in the Golgi shows rapidly recovery with a half time ($t_{1/2}$) of 80 seconds. Taken together our data suggest that: -Rab1 recruited in to peripheral structures does not move towards the Golgi. -Rapid FRAP occurs as a results of the exchange of Rab1 between Golgi membranes and the cytosol and -Rab1 interacts simultaneously with diferent proteins localized at the donor and acceptor compartments to control protein trafficking at the ER-to-Golgi level.

BC-C3.
AUTOPHAGY AS A NEW TARGET FOR CONTROL OF COXIELLA AND MYCOBACTERIUM REPLICATION

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Many bacteria have evolved mechanisms to invade host cells and survive in the intracellular environment. *Coxiella burnetii* replicates in a compartment with lysosomal characteristics. We have previously observed that *C. burnetii* localizes in a compartment labeled by LC3, a protein that specifically localizes in autophagic vacuoles. Interestingly, our results indicate that autophagy induced an increase in the number of cells infected by *Coxiella*. Furthermore, in cells overexpressing GFP-LC3 a marked increase in the *Coxiella*-infection index was observed suggesting that overexpression of proteins involved in the autophagic pathway favors the generation of the *Coxiella* replicative niche.
Mycobacterium tuberculosis is known to interfere with the normal maturation of the phagosome, preventing acidification. We have observed that under autophagy conditions *Mycobacterium*-containing phagosomes becomes more acidic and mature, hampering the bacterial survival.
Our results suggest that autophagy can play opposing (beneficial or detrimental to the pathogen) roles depending upon the organism. Whereas *Coxiella* transits through autophagosomes as a strategy for survival, induction of autophagy results in a less permissive environment for *Mycobacterium* replication. These observations highlight the significance of the autophagic pathway as a new target for intervention in infectious diseases.

BC-C4.

NUCLEOSIDE DIPHOSPHATASE AND GLYCOSYLTRANSFERASE ACTIVITIES CAN LOCALIZE TO DIFFERENT SUBCELLULAR COMPARTMENTS

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Nucleoside diphosphates generated by glycosyltransferases in cell secretory pathways are converted into monophosphates to provide substrates for antiport transport systems by which entrance of nucleotide sugars from the cytosol is coupled to exit of nucleoside monophosphates. The yeast *Schizosaccharomyces pombe* genome encodes two enzymes with potential nucleoside diphosphatase activity: Spgda1p and Spynd1p. Characterization of the overexpressed enzymes showed that Spgda1p is a GDPase/UDPase whereas Spynd1p is an apyrase. Individual disruption of their encoding genes did not affect cell viability but disruption of both genes was synthetically lethal. Disruption of $Spgda1^+$ did not affect Golgi *N*- or *O*-glycosylation whereas disruption of $Spynd1^+$ affected Golgi *N*-mannosylation. Subcellular fractionation showed that both activities localize to the Golgi. Although no nucleoside diphosphatase activity was detected in the ER, no ER accumulation of misfolded glycoproteins occurred. N-glucosylation mediated by the UDP-Glc:glycoprotein glucosyltransferase (GT) showed a partial (35-50%) decrease in *Spgda1* but was not affected in *Spynd1* mutants. Results show that contrary to what has been assumed for eukaryotic cells, in *S. pombe* nucleoside diphosphatase and glycosyltransferase activities can localize to different subcellular compartments. To test the hypothesis that ER-Golgi vesicle transport might be involved in nucleoside diphosphate hydrolysis, *S. pombe* conditional mutants in ER-Golgi transport bearing also the *Spgda1* or *Spynd1* genotype were constructed. The effect of double mutations on GT-dependent ER glucosylation is currently being assayed.

BC-C5.

DYNAMICS OF SNARE ASSEMBLY AND DISASSEMBLY DURING HUMAN SPERM EXOCITOSIS

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The acrosome reaction (AR) of spermatozoa is a complex, calcium-dependent, regulated exocytosis. Fusion at multiple sites between the outer acrosomal membrane and the cell membrane causes the release of the acrosomal contents and the loss of the membranes surrounding the acrosome. Acrosomal exocytosis is controlled by a protein machinery that is conserved in organisms ranging from yeast to human. SNARE proteins are essential components of this fusion machinery. SNARE proteins exist in different conformations. When packed in tight ternary complexes SNARE proteins are resistant to cleavage by neurotoxins. In contrast, monomeric or loosely assembled SNAREs are sensitive to proteolysis. Here, we report a direct role for the SNARE complex assembly and disassembly in the AR. First, we show that the AR is botulinum (BoNT) and tetanus (TeTx) toxin-resistant when the system is unstimulated. Second, Rab3A promotes disassembly of SNAREs that then become toxin sensitive. Third, SNAREs re-assembly into BoNT/E and BoNT/B sensitive and TeTx-resistant complexes before the efflux of acrosomal calcium. We conclude that SNAREs are not accessible to toxins before sperm activation, suggesting a cis conformation under resting conditions. After stimulation -but before acrosomal calcium release- SNAREs are re-assembled in a loose trans configuration.

BC-C6.

THE CATION-DEPENDENT MANNOSE-6-PHOSPHATE RECEPTOR IS NECESSARY FOR DEVELOPMENT OF RAT LIVER LYSOSOMES

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Mannose-6-phosphate receptors (MPRs) play a role in selective transport of acid hydrolases to lysosomes. Two types of MPR have been described to date; the cation-dependent (CD-MPR) and the cation-independent (CI-MPR) receptor, according to their requirements for bivalent ions. In rodents, MPR expression varies during perinatal development. In a previous study we demonstrated a significant decrease of CI-MPR active binding sites (B_{max}) in rat liver at 10th and 20th day of birth. In contrast, the B_{max} for CD-MPR increased significantly from the 10th day and manteined up to adulthood. From these findings we wondered if CD-MPR can compensate the low activity of CI-MPR at these ages. We measured some acid hydrolases at each age and their interaction with membranes and observed that N-acetyl-â-D-glucosaminidase (NAG), â-glucuronidase (GLU), and â-galactosidase (GAL) activities (per mg of tissue) are higher from the day 10 after birth, whereas the activity of á-mannosidase (MAN) remains unchanged. By immunoblot we confirmed the tendency for MAN and NAG, using specific antibodies. Moreover, the activities of NAG, GAL and GLU bound to membranes correlated with those activities. We also demonstrated that 60% of NAG bearing mannose-6-phosphate was bound to CD-MPR in the livers at 10th day, whilst only 30% was bound to this receptor in adults. We concluded that variations in lysosomal enzyme expression is mostly related to CD-MPR during perinatal development, and this receptor may substitute the CI-MPR at certain ages.

BC-C7.

ASSOCIATION OF TETRASPANIN CD63 WITH INTEGRINS IN HUMAN DENDRITIC CELLS: IMPLICATIONS FOR CELL MIGRATION. A COMPARATIVE STUDY WITH OTHER TETRASPANINS

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Tetraspanins comprise a large group of ubiquitously expressed membrane proteins implicated in many cellular functions. They have the ability to associate with each other and with other surface molecules, including integrins, MHC class I and II molecules and intracellular signaling enzymes. Previously, we have demonstrated that tetraspanins CD9, CD63, CD81, CD82 and CD151 are expressed at the surface of human dendritic cells (DC), the most potent antigen presenting cells. We have also shown that CD63 internalizes and follows the endocytic-MIICs route after extracellular stimuli. We have further characterized the complexes formed by CD63 on DC surface by FACS analysis and immunoprecipitation techniques. We have found that after CD63 internalization upon binding of FC-5.01 monoclonal antibody (Mab), the expression of tetraspanins CD9, CD81, CD82 and CD151 as well as integrins CD29, CD11b, CD18 and α_5, diminished compared to control DC. Moreover, performing chemotaxis assays on immature DC, we have observed that the preincubation with Mabs directed against tetraspanins CD9, CD63, CD81 and CD82 enhanced cell migration induced by MIP-1α and MIP-5 chemoattractants. We propose that the disruption of the complexes between tetraspanins and integrins could be responsible for the observed enhancement on DC migration. Besides, we have observed a differential integrin expression between immature and mature DC, which are known to differ in their migratory ability.

BC-C8.

RESTRUCTURING OF FOCAL CONTACTS BY BRADYKININ IN RAT RENAL PAPILLA

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Focal contacts (FC) are structures of cells attachement to extracellular matrix. We previously found that FC proteins vinculin (V), talin (T) and paxilin (P) are associated with DRMs -detergent-resistant membrane domains; and that bradykinin (BK) modulates the assembly of FC. We have investigated: a) the interaction between T and P with V in DMRs, by immunoprecipitation and immunoblotting, and the mobilisation of V after treatment with BK during 1, 5 and 10 minutes, and b) the "*in vivo*" BK effect in collecting duct cell culture, by immunocytochemistry. Results: a)T and P co-immunoprecipitate with V in DMRs but not in soluble fraction and BK induces a decrease in their association after 10 minutes. b)Intense V staining was localized to FC in control cells, with a diffuse V staining in perinuclear zone. After 1 minutes with BK, V staining was removed from FC and concentrated in perinuclear region, although cells remained attached, which correlates with a dissipation of V from DRM to soluble fraction detected by inmunoblotting. After 5 minutes, V staining dissipated from perinuclear region to FC, and at 15 minutes the pattern resembles that observed in control cells. Since T and P remain associated to V, and V remain localized to FC after BK treatment, we suggest that BK induces a restructuration of FC rather than a dissipation.

BC-C9.

ACTH-INDUCED CAVEOLIN-1 PHOSPHORYLATION IS RELATED TO PODOSOME ASSEMBLY IN Y1 ADRENAL CELLS

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Y1 adrenocortical cells respond to ACTH via protein kinase A with an increase in steroid secretion correlated with a change in cell morphology (rounding-up), which reflects a reorganization of the actin cytoskeleton and focal adhesion disassembly that are critical for transport of cholesterol to the inner mitochondrial membrane. Caveolin is involved in cholesterol transport and cell signaling. We previously showed that ACTH induced caveolin-1 phosphorylation on tyrosine via cAMP. In the present work we investigated phosphocaveolin-1 subcellular distribution and its association with the cytoskeleton. Phosphocaveolin-1 was enriched at focal adhesions in basal conditions, which became rounded after ACTH stimulation with a concomitant increase on the phosphotyrosine content. These structures resembled ring-like arrays (caveolae- rosette), which are associated with filamentous actin. Co-localization with phalloidin showed that when cells are flat, stress fibers are distributed along the cell cortex and phosphocaveolin is present at the edge of actin filaments; after rounding-up stress fibers vanishes and F-actin aggregates at the cell periphery and are now surrounded by phosphocaveolin-1. This effect was blocked in the presence of genistein. These observations along with electron microscopy studies revealed these structures as podosomes, which have a electron dense core. These results show that ACTH induces podosome assembly in Y1 cells, indicating that the morphological and functional responses to PKA activation in steroidogenic cells are related to the cytoskeleton dynamics involving phosphocaveolin-1 localization.

BC-C10.

IDENTIFICATION OF TARGETING SEQUENCES WITHIN PROTEIN TYROSINE PHOSPHATASE 1B (PTP1B) TO DIFFERENT CYTOPLASMIC COMPARTMENTS

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We recently discovered that PTP1B associates with β1-integrin complexes and regulates cell-substrate adhesion. Other authors described PTP1B associated to the endoplasmic reticulum (ER). Here we report that PTP1B is also in mitochondria. Our aim is to unravel the molecular determinants implicated in PTP1B targeting. To this end we constructed different mutants of PTP1B fused to GFP and used them to transfect fibroblasts derived from PTP1B knockout mice. First, we examined two different substrate traps; one in which the Asp 181 was replaced by Ala (DAPTP1B), and another that contains the additional mutation Gln262Ala (DAQAPTP1B). Confocal microscopy showed that both mutants co-localize with proteins of focal adhesions such as vinculin and paxillin. These results suggest the existence of PTP1B substrates in adhesion complexes. A mutant PTP1B in which a SH3 binding motif was disrupted by substituting Pro 309 and Pro 310 by Ala (PAPTP1B) does not co-localize with vinculin and paxillin in these sites, suggesting that this polyproline region is essential. In addition, cells co-expressing DAPTP1B and PAPTP1B co-localize in the ER and mitochondria; however, only DAPTP1B accumulates in adhesion sites. Immunoprecipitation studies show that DAPTP1B associates with p130Cas, a docking protein that localizes in focal adhesions and contains an SH3 domain. We presume that p130Cas may be a candidate protein that recruits PTP1B to cell-substrate adhesion sites.

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BC-C11.

INFLUENCE OF SPHINGOLIPIDS ON NICOTINIC ACETYLCHOLINE RECEPTOR ASSEMBLY, TRAFFICKING AND CELL-SURFACE TARGETTING

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The effect of sphingolipid (SL) modification on nicotinic acetylcholine receptor (AChR) targeting to the cell surface was studied in living cells using fluorescence microscopy in combination with inhibition of SL biosynthesis at various steps. A significant diminution of fluorescent α-bungarotoxin (αBTX) labelling (60-70%) of the AChR at the plasma membrane was observed when the mutant cell line CHO-SPB1/SPH$^-$, defective in serine-palmytoyl transferase activity, was grown at the non-permissive temperatures (39°C). Myriocin (ISP-1), Fumonisin B-1 and the glucosylceramide synthase inhibitor d,l-PDMP reduced by ~30-40% Alexa^{488}-αBTX AChR staining in control, CHO-K1/A5 cells. Cells treated with FB-1, PDMP, or ISP-1 increased (~50%) their intracellular AChR, which colocalized with ER markers like calnexin and ER-tracker. Non-assembled AChR increased ~30-40% in SL-impaired cells. The results suggest that assembly, trafficking and cell-surface expression of AChR is affected by intracellular SL levels.

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BC-C12.
POSSIBLE MECHANISMS INVOLVED IN c-FOS ACTIVATION OF PHOSPHOLIPID SYNTHESIS

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Tumorigenesis is a multi-step process, reflecting complex genome changes that progressively drive transformation of normal cells into malignant derivatives. Nuclear c-Fos participates, as a component of AP-1 transcription factors, in events of cell growth and differentiation. It´s expression is subjected to tight control checkpoints, and over-expression can lead to cellular transformation. We examined the involvement of c-Fos in tumorigenesis in T98G (glioblastoma multiforme) cell line. We found that c-Fos associates to the ER, activating phospholipid synthesis, main constituents of biological membranes. Blocking c-Fos expression using antisense strategies, inhibits c-Fos dependent phospholipid synthesis activation, and also diminishes cell proliferation. In quiescent cells, the small amount of c-Fos present is phosphorylated in tyrosine residues. Upon stimulation with trophic factors, this state is rapidly lost and de-phosphorylated c-Fos is now able to associate to the ER, thus activating phospholipid synthesis. If this process is impaired by addition to the culture media of an inhibitor of tyrosine phosphatases, c-Fos dependent phospholipid synthesis activation drops down and also does so the proliferation velocity. Interestingly, we found high levels of c-Fos present for at least 18 hours post-stimulation indicating that the stability of c-Fos protein or that of its mRNA could account for its accumulation in these cells. We postulate that this cytoplasmic activity of c-Fos is a key event in cancer progression.
Supported by FONCyT, SeCyT-UNC, Agencia Cordoba Ciencias and JS McDonnell Foundation.

BC-C13.
CYTOPLASMIC c-FOS: A NOVEL TARGET FOR CANCER THERAPY?

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Given our results in PC12 and brain tumor cells, we propose that AP-1-c-Fos is not the critical player in the tumor growth process. If our hypothesis is correct, cytoplasmic c-Fos should be found in actively growing cancer cells. A screening for c-Fos expression in malignant human tumor specimens was performed. Specimens included: testicular (n=7), lung (n=7) breast(n=10) and liver (n=9) tumors. Cytoplasmic c-Fos immunoreactivity was observed in 100% of the tumors and all tumors examined showed ER/c-Fos co-localization. c-Fos expression, phospholipid synthesis activation and proliferation were examined in the breast tumor cells MCF7 and T47D. Proliferating cells showed c-Fos-dependent activated phospholipid synthesis whereas cells fed a c-Fos antisense oligonucleotide showed no phospholipid synthesis activation and no proliferation.
BalB c/c nude mice were inoculated intracerebrally with T98G cells to promote tumor growth. At day 7 animals were separated into 3 groups and were injected at inoculation site, with c-Fos mRNA antisense oligonucleotide or sense oligonucleotide or vehicle every 5 days during 20 days. Brain tissue samples were examined at day 40 for tumor presence. Tumors developed in 6 out of 7 vehicle-, in 3 out of 4 sense-, and in none out of 5 antisense oligonucleotide- injected animals. These results indicate a relevant role of cytoplasmic c-Fos in the growth process of tumor cells.
Supported by: FONCyT, SeCyT-UNC, Agencia Cordoba Ciencias, CONICET, JS McDonnell Foundation.

BC-C14.
NUCLEO-CYTOPLASMIC LOCALIZATION OF P8, CELL CYCLE AND SIGNAL TRANSDUCTION PATHWAYS

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p8, an 8 kDa protein was first identified by its induction during the acute phase of pancreatitis. Its mRNA is expressed in cell lines in response to stress and mitogenic factors. With immuno-cytochemistry, we found that p8 presents nuclear localization in sub-confluent cells, but it localizes in the cytoplasm of confluent cells. Further experiments have shown that nuclear import is energy dependent and that the export of p8 does not involve the CRM1 transporter. Different experiments with cycloheximide suggest that the protein found in the cytoplasm is the result of a de novo protein synthesis, and not of the export of nuclear p8. The objective of this work is to study the sub-cellular localization of the protein along the stages of the cell cycle with immunocytochemistry. Different strategies, such as serum deprivation, hydroxiurea, nocodazole and etoposide were used to arrest cells in the different stages, and it was observed that in sub-confluent cells, arrested in Go, the protein localized in the cytoplasm, and when the cell cycle was restored, p8 migrated back to the nucleus. Since previous results show that inhibitors of MAPK pathways alter the level of p8 expression, their effect on p8 localization was assayed, but none of the drugs had any effect on the protein's localization. In the presence of a histone deacetylase inhibitor, Trichostatin A, sub-confluent cells showed cytoplasmic localization of p8.

TS-C1.
ISOLATION OF THE STYLE PARTNER OF POLLEN RECEPTOR KINASES LePRK1 AND LePRK2 FROM TOMATO (LYCOPERSICON ESCULENTUM)

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After pollen grains germinate on the stigma, pollen tubes traverse the extracellular matrix of the style on their way to the ovules. We characterized two pollen-specific receptor kinases, LePRK1 and LePRK2, that according to their structure, immunolocalization pattern and style extract-mediated specific LePRK2-dephosphorylation, they might interact with signaling molecules within the style extracellular matrix. Recently, we demonstrated that in pollen membranes, LePRK1 and LePRK2 belong to a high molecular complex of ~400 kDa (Wengier et al, PNAS, Vol 100, no. 11, pp. 6860-6865, 2003). This complex is disrupted when pollen is germinated *in vitro* in the presence of style extracts. In yeast, the addition of style extract also disrupts the interaction between LePRK1 and LePRK2. Crude fractionation of the style extract shows that this activity is enriched in the 3- to 10 kDa. fraction. From FPLC fractionation, we obtained a broad peak of ~6 kDa. specifically associated with LePRK2 dephosphorylation. The style component that dephosphorylates LePRK2 is likely to be a heat-stable peptide that is present in the style exudate. Further characterization will be necessary to identify the structure of LePRK ligand.

TS-C2.

CDK4/6 INHIBITOR p19INK4d INCREASES THE DNA REPAIR ABILITY IN FIBROBLAST

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The four proteins of the INK4 family posses a similar structure and bind to CDK4/6 with similar affinity driving to a G1 cell cycle arrest, but they are reported to have different biological roles. The aim of this work was to evaluate if $p19^{INK4d}$ induces a second photoprotective response after DNA damage. Northern blot analysis show that p19 is up-regulated after UV irradiation in BHK cells. Levels of $p19^{INK4d}$ mRNA peaked 12 h after UV treatment. This induction resulted dose dependent and started at 5 mJ/cm^2. The apparent p19 mRNA half life was 3 h in irradiated and non irradiated cells. The same behavior was observed when the protein half-life was estimated in 2,5 h by pull-chase assays with ^{35}S-met. Nuclear run-on experiments revealed an appreciable increase in the rate of p19 mRNA synthesis in UV treated cells in comparison with untreated ones. We measured DNA repair capacity by host cell reactivation assay. The overexpression of p19 resulted in a 80% increased repair of UV-induced DNA damage while the antisense version drove a diminished expression of DNA tested. Nevertheless, mimosine (an inhibitor of G1/S phase progression) or overexpression of $p16^{INK4a}$ displayed a minor effect on the rate of repair as assessed by HCR, suggesting that $p19^{INK4d}$ exerts its action independently from cell cycle arrest. Regardless of $p19^{INK4d}$ precise mechanism the present data demonstrate that this protein is not only associated with cell cycle arrest but also with enhancement of DNA repair.

TS-C3.

STUDIES ON ACTIVATION AND PHOSPHORYLATION OF PROTEIN KINASE A DURING THE TRANSITION FROM RESPIRATORY TO FERMENTATIVE METABOLISM IN *SACCHAROMYCES CEREVISIAE*

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Protein kinase A (PKA) activity was measured *in vivo* after addition of glucose to cells growing on a non-fermentable carbon source, a physiological condition known to produce a transient peak of cAMP. PKA activity followed in time the peak of cAMP. The PKA activity measured in presence of 10 μM of cAMP, also showed a peak of activity. The level of cAMP intracellularly bound to regulatory subunit followed the course of PKA activation. Western blots for regulatory and catalytic subunits and their corresponding activities, assayed in partially purified samples, showed constant levels of both subunits. These results suggest a different state of activability of PKA within the cells, as a consequence of binding of cAMP along the transient rise of cAMP triggered by glucose addition. The relative proportion of phosphorylated species of the catalytic subunit (TPK1), changed during the transition from non-fermentable to fermentable carbon source, in a peak of cAMP and PKA activity-dependent manner. The phosphorylation state of TPK1 was found carbon source dependent: glycerol-grown cells have less phosphorylated isoforms than glucose-grown cells. Preliminary kinetic analysis of crude extracts, carryng phosphorylated isoforms before and after phosphatase treatment showed that the phosphorylation of TPK1 decreases the Km for the kemptide.

TS-C4.

TYROSINE PHOSPHATASES ACT ON STEROIDOGENESIS THROUGH THE ACTIVATION OF AA RELEASE

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We have previously demonstrated a hormone-dependent activation of protein tyrosine phosphatases (PTPs) in adrenocortical and Leydig cells, involved in the cAMP/PKA dependent induction of StAR protein (Steroidogenic Acute Regulatory protein) and activation of steroidogenesis. We also probed that arachidonic acid (AA) release is another key step in the action of StAR protein. Therefore, we questioned whether PTPs and AA release are linked in the regulation of the whole process. Given that AA levels are regulated by the concerted action of an acyl-CoA synthetase (ACS4) and an acyl-CoA thioesterase (ARTISt), we tested the effect of PTP inhibitors on ACS4 expression. We used two steroidogenic cell lines, MA-10 and Y1, stimulated with 8Br-cAMP in the presence and absence of benzylphosphonic acid (BPA, a PTP inhibitor). ACS4 expression, increased after cAMP treatment, was abolished by PTP inhibition. The involvement of PTPs in AA release was also tested using exogenous AA to bypass PTP inhibition, evaluating the effect on progesterone (P4) biosynthesis by MA-10 Leydig cells. P4 production (ng P4/ml) stimulated by 1 mM 8Br-cAMP was inhibited by 0.2 mM BPA (7.6 ± 0.8 vs 2.3 ± 0.4), as already described. AA itself (0.3 mM) has a stimulatory effect (5.3 ± 0.6) and reverses BPA inhibition ($6.5 \pm 0,7$). These results, together with the fact that PTP inhibitors block hormone induction of ACS4, strongly suggest that the stimulation of steroidogenesis involves AA release downstream of PKA and PTPs activation.

TS-C5.

EXPRESSION OF PROTEIN PHOSPHATASES FROM THE PP2A FAMILY IN POTATO PLANTS

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Reversible phosphorylation of proteins is known to be important in the control of plant metabolism, but relatively few proteins whose activities are controlled by phosphorylation have so far been identified. In a previews work, two cDNAs encoding putative catalytic subunits of serine-threonine protein phosphatases from the protein phosphatase 2A family (StPP2A1 and StPP2A2) were identified in *Solanum tuberosum* using a cDNA library from potato tuberizing stolons. Both deduced aminoacid sequences share 80% identity and a high degree of similarity with other plant PP2As. In this work, the expression of StPP2A1 and StPP2A2 was analyzed using gene specific semiquantitative PCRs. StPP2A1 was expressed in all the tissues analyzed but presented higher levels of expression in induced stolons. On the other hand, StPP2A2 was not expressed in leaves or early stolons, it was present in flowers and tubers and it was highly expressed in stolons induced to tuberize.

Potato tuberization is a complex process that results in the differentiation of a stolon into a tuber. The fact that both StPP2A1 and StPP2A2 are upregulated during tuber morphogenesis suggests that these genes could play a role during this developmental process.

TS-C6.

INSULIN MODULATES PHOSPHATIDIC ACID METABOLISM IN CEREBRAL CORTEX SYNAPTOSOMES

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Many subcellular signalling events are initiated with the generation of the biologically active lipid, phosphatidic acid (PA). By excluding the *de novo* biosynthesis, phosphatidic acid can be generated either by phosphatidylcholine (PC) hydrolysis through phospholipase D (PLD) or by diacylglycerol (DAG) phosphorylation through diaglycerol kinase activity (DAGK). In addition, PA can be further hydrolyzed by phosphatidate phosphohydrolase (PAP) yielding DAG. We have previously described that PLD/PAP/DAGK pathway is present in cerebral cortex synaptosomes from adult (4 month-old) and aged (28 month-old) rats. Our purpose was to study the effect of Insulin and of receptor-independent tyrosine phosphorylation on PLD/PAP/DAGK pathway. The effect of Insulin and orthovanadate on this pathway was evaluated as a time function. At 5 min incubation, and in the presence of ATP and orthovanadate, Insulin stimulates both PLD and DAGK activities (120% and 140%, respectively) whereas PAP2 is inhibited (20%). At 30 min exposition to hormone and orthovanadate a 10% and 60% increase was found in PLD and DAGK respectively. Under this condition, in sinaptosomes from aged rats, PLD activity remained unchanged and DAGK activity only showed a 20% increase. These results demonstrate the modulation of PA metabolism through Insulin and tyrosine kinases and its alteration during the aging process.

TS-C7.

1α25(OH)$_2$D$_3$ AND PTH SIGNALING IN RAT INTESTINAL CELLS: ACTIVATION OF CYTOSOLIC PLA2

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In the current study, we have probed the role of cytosolic phospholipase A2 (cPLA2) activity in the cellular response to the calciotropic hormones, 1α25,dihydroxy-vitamin D$_3$ [1α25(OH)$_2$D$_3$] and PTH. Stimulation of rat enterocytes with either hormone, increased release of arachidonic acid [^{3}H-AA] one-two fold in a concentration and time-dependent manner. The effect of either hormone on enterocytes was totally reduced by preincubation with the intracellular Ca^{2+} chelator BAPTA-AM (5 μM), suggesting that the release of AA following cell exposure to the calciotropic hormones occurs mainly through a Ca^{2+}-dependent mechanism involving activation of Ca^{2+}-dependent cPLA2. Calciotropic homone stimulation of rat intestinal cells increases cPLA2 phosphorylation (3-4 fold). This effect was decreased by PD 98059 (20 μM), a MAP kinase inhibitor, indicating that this action is, in part, mediated through activation of the MAP kinases ERK 1 and ERK2. Enterocytes exposure to 1α25(OH)$_2$D$_3$ (1 nM) or PTH (10 nM) also resulted in P-cPLA2 translocation from cytosol to nuclei and membrane fractions, where phospholipase subtrates reside. Collectively, these data suggest that PTH and 1α25(OH)$_2$D$_3$ activate in duodenal cells, a Ca^{2+}-dependent cytosolic PLA2 and attendant arachidonic acid release and that this activation requieres prior stimulation of intracellular ERK1/2. 1α,25(OH)$_2$D$_3$ and PTH modulation of cPLA2 activity may change membrane fluidity and permeability and thereby affecting intestinal cell membrane function.

TS-C8.

NITRIC OXIDE, cGMP, CDPKs AND MAPKs ARE INVOLVED IN THE IAA-INDUCED ADVENTITIOUS ROOT FORMATION IN CUCUMBER

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Adventitious root formation (ARF) is a key step in vegetative propagation. We have previously found that nitric oxide (NO) and cGMP act downstream auxins in the signal transduction pathway leading to ARF in cucumber (Pagnussat *et al*., Plant Physiol. 2002, 129:954-956 and Plant Physiol. 2003, 132:1241-1248). Since ARF is partially dependent on the entrance of calcium from the extracellular space and on the release from intracellular stores, we further tested how auxins and NO affect the calcium dependent protein kinase (CDPK) activity when ARF is induced. *In vitro* CDPK activity increased in explants treated either with the auxin indole-3-acetic acid (IAA) or with the NO-donor sodium-nitroprusside, and was partially prevented by the specific NO-scavenger cPTIO. The activation of a CDPK of 45 kDa was detected by in-gel assays after 1 day of IAA- or NO-treatments. We also investigated the involvement of a mitogen-activated protein kinase (MAPK) signaling cascade during ARF. Our results indicated that MAPK activity is induced by IAA in a NO-mediated pathway. Collectively, these results are strong evidence indicating that ARF is regulated by a complex and intricate set of cellular messengers involving auxins, NO, cGMP, calcium, CDPKs and MAPKs. Further investigations will be directed to establish what type of relationship exists between them.

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BT-C1.

SELECTIVE AND COVALENT IMMOBILIZATION OF PROTEINS ONTO POLYMERIC SURFACES. BIOTECHNOLOGYCAL APPLICATIONS

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Covalent chemical immobilization is an important field of biochemistry and physic chemistry. Also, it is a useful tool in modern biotechnology to develop heterogeneous systems for diagnostic kits. Carbodiimide coupling reaction is one of the most used as a consequence of its very well known mechanism and reaction conditions. However, the high abundance of carboxylic acids and amino groups in protein structures renders this reaction unspecific. In order to study less explored chemical reactions for protein immobilization, sulphur and arsenic containing compounds were reinvestigated. The chemistry of these compounds has convenient features: they form covalent bonds that are reversible under mild conditions and involve short reaction times. In this work, reactions of aminophenyl arsine oxide (APA3), aminophenyl arsine chloride (APA3Cl2), and aminoarsalinic acid (APA5) with biological sulfhidryl compounds were studied in solution. Reaction products were analyzed by UV, HPLC and MALDI-MS. As expected, APA3 reacted readily with mercaptoethanol and dithiotreitol. Interestingly, APA3Cl2 showed higher solubility than APA3 and similar rates of reaction with the thiol compounds. Natural or mutant proteins showing Cys-X-X-Cys motive in their primary structure could be immobilized onto polymeric surfaces containing APA3. Additionally it was possible to selectively immobilize these proteins from a crude extract without previous purification.

BT-C2.
ERYTHROMYCIN A BIOSYNTHESIS IN HETEROLOGOUS HOST

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The increasing knowledge about the chemistry performed by polyketide synthetases (PKSs) has lead to the construction of polyketide libraries, generated by genetic modification of macrolide PKSs. Since these libraries were constructed in heterologous hosts lacking glycosylation pathways, only the corresponding aglycones were produced. We wished to expand the capabilities of the combinatorial biosynthesis strategies to incorporate post-PKS "tailoring" steps, in particular the adition of deoxysugar components. We decided to construct a strain capable to perform the tailoring steps that lead to the formation of Erythromycin A from the aglycon 6-dEB, wich is synthesized by the modular PKS 6-dEB synthetase (DEBS). These reactions include the attachment of two deoxysugar moieties, L-mycarose and D-desosamine, two hydroxilations and a methylation of the mycarose residue. One of the most widely used heterologous host for genetic manipulation of actinomycetes PKSs is *Streptomyces coelicolor*. We decided to genetically engineer the *S coelicolor* A3(2) strain transferring the metabolic pathways involved in the conversion of 6-dEB to Erythromycin A from *Micromonospora megalomicea*. This implied the construction of two operons including the genes involved in the biosynthetic pathways of the deoxysugars TDP-L-mycarose and TDP-D-desosamine and their corresponding glycosyltransferases, two hydroxylases and a methyltransferase**.** This strain could perform the bioconversion of the macrolide 6-dEB to the different erythromycin intermediates in batch cultures fed with this polyketide.

BT-C3.
EVALUATION OF THE EFFECT OF N-ACETYL-GLUCOSAMINE ON THE PRODUCTIVITY AND THE GLYCOSYLATION PATTERN OF RHEPO

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Recombinant human erythropoietin (rhEPO) is a therapeutic protein that promotes red-blood cells maturation. Its biological activity strongly depends on its antennarity and sialylation, being N-acetyl-glucosamine (GlcNAc) an aminosugar contained in carbohydrate chains of rhEPO. In order to optimise the production process, a rhEPO producing clone of CHO cells was adapted to grow in protein free medium (PFM), achieving a 50% increment in cell concentration compared to the standard medium (supplemented with 0.2% FCS), and a similar rhEPO specific production rate. Different culture conditions were then assayed to evaluate their effect on productivity and glycosylation pattern. In particular, four cultures of this clone were initiated in PFM supplemented either without or with 0.2 , 2 and 20 mM GlcNAc. All cultures showed the same specific growth rate, but a decrease in the maximum cell concentration was achieved while increasing the aminosugar concentration (1.9 10^6 and 4.4 10^5 cell/ml for 0 and 20 mM, respectively). Increasing GlcNAc caused increments in specific glucose-consumption rate, and specific lactate-, ammonium- and rhEPO-production rates. Analysis of the molecule performed by isoelectric focusing - Western blot and band densitometry indicated that the addition of GlcNAc caused the disappearance of the most acidic isoform and a decrease in the proportion of the next most sialylated isoform. This effect was probably caused by ammonium accumulation.

BT-C4.
UNCULTURED γ- PROTEOBACTERIA DOMINATE 16S RDNA CLONE LIBRARIES FROM NONYLPHENOL ETHOXYLATE-ENRICHED ACTIVATED SLUDGE

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Nonylphenol ethoxylates (NPEO) are widely used surfactants that enter the environment primarily through sewage and wastewater treatment plants, causing adverse effects on aquatic organisms. We investigated the link between bacterial population and NPEO biodegradation in engineered environments, using cultured-independent methods. Four clone libraries were constructed with amplified full-length insert 16S rDNA from reactors fed with synthetic effluent, two of which received additionally NPEO. A total of 187 clones were digested with restriction enzymes RsaI and HhaI. Changes of relative diversities under different treatment regimens, i.e. presence or absence of NPEO in the feeding solution, within otherwise identical environments, were taken as representative of qualitative changes in the microbial community. NPEO had a significant effect in the increase in relative abundance of a few RFLP patterns compared to control libraries. The sequences of several clones corresponding to the dominant phylotype in libraries from reactors treated with NPEO (ca. 35% of total clones in each replicate reactor) were assigned to uncultured gamma-proteobacteria subclass, suggesting the involvement of this group in NPEO degradation. Two new specific rRNA-targeted oligonucleotide probes were designed from our five 16S rDNA sequences and four other sequences retrieved from public databases of closely related environmental clones. Real time PCR using the specific probes demonstrated their applicability to monitoring the abundance of these so far uncultured species in natural and engineered environments.

BT-C5.
PLASMID DESIGN TO PRODUCE MEMBRANE-PERMEANT RECOMBINANT PROTEINS

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Arginine-rich peptides can permeate inside cells carrying covalently bond proteins. Therefore, these peptides are useful tools to study protein function in cells that cannot be transfected. In order to produce membrane-permeant recombinant proteins the plasmid pGEX-2T-R was designed. The plasmid pGEX-2T was cut on BamHI and EcoRI sites and an oligonucleotide encoding the amino-acid sequence RRRQRRRKRRRQ was ligated. Then *E. coli* BL21(DE3) pLysS cells were transformed and were induced to produce GST-R (arginine-rich peptide covalently bond to GST). Then permeability assays were performed in CHO cells and human spermatozoa. The cells were incubated either with GST-R or GST for three hours and washed. The presence of the protein was determined by indirect immuno-fluorescence and visualised by fluorescence microscopy. Further experiments were carried out in human spermatozoa using either rhodamine-labeled GST-R or GST and visualised by fluorescence microscopy and confocal microscopy. GST-R permeated into both CHO cells and human spermatozoa while GST was excluded. These results suggest that pGEX-2T-R can be used to produce membrane-permeant recombinant proteins.

BT-C6.

DEVELOPMENT OF DNA VACCINES AGAINST HEMOLYTIC UREMIC SYNDROME (HUS)

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We have reported that murine immunization with pCDNA expressing the B subunit gene plus the first codons of the A subunit of Shiga toxin-2 (pStx2ΔA) triggered a Th1-immune response which induced a partial protection from Stx2 toxicity in the mouse model of HUS. Here we studied acute and chronic toxicity of this plasmid and its protein, and the improvement of this vaccine through a novel pStx2ΔA construction and immunization protocols. We purified Stx2ΔA protein from JM109 lysates FPLC. Serial fractions were eluted and analyzed for specificity by dot blot revealed with anti-Stx2 antibody. Mice were injected with pStx2ΔA (50µg), purified Stx2ΔA protein, or with a combined protocol of both. Animals received one injection of 500ng or three injections of 2-500 ng of the protein for acute or chronic toxicity studies. Mice were monthly bled and after 2, 4 and 6 months from the first injection, lungs, livers and kidneys were excised for hystopatological studies. We found low neutralizing activity in sera from protein-immunized mice and different levels of renal damage in mice injected with pStx2ΔA, highest concentrations of protein, or with combined protocol. Finally, we obtained and sequenced a new pStx2ΔA construction using Stu I and Ava I enzymes, carrying the B subunit, and the last 31 aa of the A subunit of Stx2.

BT-C7.

SELECTION OF CAMELID ANTI-IDIOTYPIC VHHs BEARING DNA INTERNAL IMAGE BY PHAGE-DISPLAY

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In camelids, a subset of the immunoglobulins consists of heavy chain homodimers devoid of light chains, and are thus called heavy chain IgGs (hcIgGs). Their variable region (VHH) is the smallest antigen-binding fragment possible, and being just one polypeptide chain it is especially suitable for engineering. From llamas immunized with an anti-DNA mouse IgG, we sought to obtain anti-idiotypic VHHs able to back-elicit anti-DNA antibodies by molecular mimicry. Upon immunization of llamas with ED84, a monoclonal anti-DNA antibody with sequence specificity to a ds18-mer (Site35), high titers (>30000) towards ED84 Fab fragment were obtained. Total IgGs were purified and hcIgGs and conventional IgGs were isolated. By ELISA, both fractions showed significant binding to ED84. On the other hand, the VHHs were amplified from cDNA of mononuclear cells and were subsequently used to construct a phage display library of 10^8 individual clones. After two rounds of solid phase panning with specific elution (i.e., with Site35), a 58% of anti-idiotypic VHHs was obtained. Those monoclonal VHHs, and the idiotypic fraction of polyclonal hcIgGs and conventional IgGs were injected in mice. Preliminary results show that it is possible to obtain anti-DNA antibodies from the response to anti-idiotypic antibodies, confirming the hypothesis of molecular mimicry being attained from an "internal image" of the antigen.

BT-C8.

PROTEOMIC ANALYSIS OF A MELANOMA CELL LINE: INSIGHT INTO A MOLECULAR PATHWAY OF TUMORIGENESIS

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SPARC is a matricellular glycoprotein that elicits changes in cell shape and proliferation. While its normal expression is restricted to tissues under remodeling, SPARC was found to be overexpressed in different tumors, in association with tumor progression and metastasis. Our previous results showed that stable antisense SPARC expression abolished tumorigenicity in an *in vivo* melanoma murine model. Traditional approaches to define a molecular pathway for SPARC-mediated tumor progression have failed to render meaningful results. Thus, we started a proteomic analysis of proteins expressed by melanoma cells with modulated SPARC expression, in order to identify new SPARC-interacting proteins. We studied conditioned culture medium from human MEL-LES clone 1-D (L-1D, showing an 80% decrease of SPARC mRNA and protein expression), as compared with the control MEL-LES clone CMV (L-CMV). Proteomic analysis was done by two-dimensional electrophoresis followed by tryptic digestion of spots and MALDI-TOF peptide fingerprinting analysis. Up to date we have identified 94 proteins, among them those related with SPARC function, as well as extracellular matrix structural proteins and oncoproteins. We are currently focusing in establishing which proteins are differentially expressed in the non-tumorigenic clone L-1D, as opposed to the tumorigenic clone L-CMV, aiming at identifying proteins that may be involved in SPARC molecular pathway.

MI-P1.

ARGININE KINASE OVEREXPRESSION IMPROVES *TRYPANOSOMA CRUZI* SURVIVAL CAPABILITY

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Arginine kinase catalyzes the reversible transphosphorylation between N-phospho-L-arginine and ADP. Phosphoarginine and phosphocreatine, generally called phosphagens, play an essential role as energy reserve because the high-energy phosphate can be transferred to ADP when ATP is needed. The molecular and biochemical characterization of arginine kinases in *Trypanosoma cruzi* and *Trypanosoma brucei*, the etiological agents of Chagas´ disease and human sleeping sickness respectively, have been reported by this laboratory. It was established that a single-copy gene encodes for a functional arginine kinase in *Trypanosoma cruzi*. Here we demonstrate that the homologous overexpression of the *Trypanosoma cruzi* arginine kinase improves the transfected cells' ability to grow and resist under nutritional and pH stress conditions. The stable-transfected parasites showed an increased cell density from the day 10 of culture, when the carbon sources became scarce, which resulted in a 2.5-fold respect to the control group on day 28. Additional stress conditions were also tested. We propose that arginine kinase is involved in the parasites' adaptation to environmental changes. Moreover, here we report a successful overexpression model of a *T. cruzi* endogenous enzyme, and the characterization of its biological effects.

MI-P2.

EXPRESSED SEQUENCE TAGS FROM *Trypanosoma cruzi* TRYPOMASTIGOTE AND AMASTIGOTE-LIKE FULL-LENGTH cDNA LIBRARIES

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We have generated 2,796 expressed sequence tags (ESTs) from two cDNA libraries of *Trypanosoma cruzi* CL-Brener. The libraries were constructed from trypomastigote and amastigote-like forms, using a spliced leader primer (miniexon) to synthesize the cDNA second strand, thus selecting for full-length cDNAs. Since the libraries were not normalized nor pre-screened, we compared the representation of transcripts between the two and identify a subset of transcripts that show apparent differential representation. A non-redundant set of 1,626 reconstructed transcripts was generated by sequence clustering. This dataset was used to perform similarity searches against protein and nucleotide databases. 432 ESTs could be assigned a putative identity based on these searches. These are the first ESTs reported for the life cycle stages of *T. cruzi* that occur in the vertebrate host. 481 of these ESTs (29.6%) are novel sequences, not represented in the ESTs obtained from the epimastigote normalized library from which the great majority of the *T. cruzi* ESTs were derived.

MI-P3.

INTERACTION BETWEEN THE *Salmonella* PATHOGENICITY ISLAND 1 AND THE PhoP/PhoQ SYSTEM

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Salmonella infections are initiated when organisms penetrate the epithelial cells within the small intestine. Proteins encoded by a cluster of genes on the chromosome known as "*Salmonella* Pathogenicity Island 1" (SPI-1) play a critical role in the invasion process. The expression of most of these genes requires HilA, a transcription factor encoded on SPI-1. Expression of *hilA* is complex, and it has been demonstrated to be repressed in a *Salmonella* strain harboring a *phoQ* mutant allele, *pho24*. This mutant codes for a sensor protein that is more active at Mg^{2+}-repressing conditions than the wild-type. However, neither the link that connects PhoP/PhoQ with *hilA* regulation nor its Mg^{2+} dependence has yet been elucidated.
Using microarrays, we have detected a PhoP activated gene encoded within SPI1. By creating a *lacZ* transcriptional fusion to this gene, we confirmed its dependence on PhoP/PhoQ, and Mg^{2+} concentration. Interestingly, a deletion in this locus increased *hilA* expression. We propose this gene to be the connection between the *Salmonella* Pathogenicity Island 1 and the PhoP/PhoQ system.

MI-P4.

BEHAVIOR OF *RHODOCOCUS OPACUS* DURING DESICCATION IN THE PRESENCE OF HEXADECANE VAPORS

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Desiccation plays a determinative role in the survival of microorganisms in the environment, principally in desert regions such as in the semiarid Patagonia. The aim of this study was to investigate the behavior of the soil isolate *Rhodococcus opacus* PD630 subjected to desiccation during exposure of hexadecane vapors. Similar situation is found in natural arid environments contaminated with hydrocarbons during non-rainy periods. Exposure of desiccated cells to hexadecane vapors produced the following effects: 1) hexadecane likely exerted a toxic action at the cellular envelope level resulting in cell deformation and a decrease of survival; 2) removal of an extracellular polymer accumulated at colony surfaces during desiccation; 3) production of microcolonies principally in the inner sector of colonies; 4) mobilization of storage lipids (triacylglycerols) presumably for producing the energy necessary for the re-adaptation of cellular physiology; 5) turnover of fatty acids and mycolic acids. Results of this study suggested that *R. opacus* colonies contain cells with different physiological status, some of them being not able to survive under these conditions, whereas others respond actively to the presence of hexadecane vapors during desiccation.

MI-P5.

REDOX-DEPENDANT STRUCTURAL MODIFICATIONS OF tHE *SCHISTOSOMA MANSONI* GLUTATHIONE *S*-TRANSFERASE OMEGA

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The gluthatione *S*-transferases (GSTs) play a key role in phase II of enzymic detoxification of a wide variety of both endogenous and exogenous electrophilic compounds. GSTs are considered the most prominent detoxifying enzymes in helminths. We have cloned an Omega class GST gene from *Schistosoma mansoni* (SmGSTO). The recombinant enzyme shown glutathione-dependent dehydroascorbate reductase, and thiol transferase activities; but low activity towards the classical substrate, CDNB. Spectroscopic experiments showed a structural modification of the enzyme related to its redox state. Reduced form of the enzyme is monomeric. In contrast, in presence of GSSG the enzyme partially dimmerized. The dimmer can be monomerized by DTT or GSH, suggesting the existence of a disulfide bond. The enzyme was able to bind to S-hexyl glutathione agarose, only in its reduced state. Western Blot experiments performed with total protein extracts of adult parasites, cultured under normal or stress conditions (cumene hydroperoxide and hydrogen peroxide), shown that SmGSTO is in its monomer oxidized state. We also observed a greater expression of SmGSTO in male than in female. Immunohistochemistry assays showed that tissues with mayor expression are, intestinal parenchyma, and tegument (including spines and tubercles) exposed to the media. We could not detect SmGSTO neither in ovary nor in testis.

MI-P6.

NEUTRAL LIPIDS ACCUMULATION IN *STREPTOMYCES COELICOLOR* A3(2) AND ITS RELATIONSHIP WITH ACTINORHODIN BIOSYNTHESIS

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The capability for biosynthesis and storage of neutral lipids is widely distributed among eukariotic organisms suchs as yeast, fungi, plants and animals, but the occurrence of TAG in bacteria has only rarely been described. A few examples of substantial TAG accumulation have been reported for species mainly belonging to the actinomycetes. The main function of bacterial TAG seems to be as a reserve compoud, but for S*treptomyces* it has been suggested that neutral lipids might act as the carbon source for those polyketide antibiotics sinthesized from acetyl- and malonyl-CoA precursors. *S coelicolor* produces a wide variety of secondary metabolites, including the blue-pigmented polyketide actinorhodin antibiotic. In our laboratory we are investigating the relationship between the neutral lipids accumulation and the actinorhodin production as a funtion of the external carbon source. We used different culture conditions to analyse and compare the pattern of TAG formation. Cuantification of TAG and actinorhodin production along the growth curve showed that the TAG levels continue increasing in conditions where no significant amounts of pigment was detected. We could perform for the first time a preparative isolation of lipid inclusion from the *S coelicolor* M145 strain under different growth condition. We could analyze their composition by TLC and by GC-mass spectrometry.
In other experiment, we cloned the SCO0958 gene, wich showed a high homology with a recently described bifunctional enzyme from *Acinetobacter calcoaceticus* ADP1 exhibiting acyl-CoA:diacylglycerol acyltransferase activity. Further studies will allow to determine the potencial rol of this gene product in the biosynthesis of TAG.

MI-P7.

EARLY IMMUNE RESPONSES IN BOVINES EXPERIMENTALLY INFECTED WITH THE HEMOPARASITE *BABESIA BOVIS*

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We have studied the early stages of the immunological responses developed in *Babesia bovis*-experimentally infected bovines against several antigens that have been postulated as vaccine and diagnostic candidates. To this end, bovines were inoculated with a vaccinial (R1A) or a pathogenic (S2P) strain of *B. bovis* and serum samples were collected at different time points up to 66 days post infection. Recombinant forms of the following antigens of *B. bovis* R1A strain were produced in a prokaryotic expression system and purified by affinity chromatography: the merozoite surface antigens (MSA) 1, $2a_1$, 2b and 2c and the rhoptry associated protein-1. An indirect ELISA test was developed for each of these antigens. The titers obtained so far show that these tests are able to detect the appearance of specific antibodies against each of the antigens between days 15 and 42 post infection. In addition, increased and decreases of antibody titers were observed along the time period investigated. Further studies are needed to reveal if these could be due to antigenic variations in the parasites during infection. Importantly, our ELISA tests were able to detect antibodies both in infections with the homologous R1A and the heterologous S2P strains, demonstrating the presence of conserved B-cell epitopes in all studied antigens between these two strains.
Supported by ANPCyT PICT 98-083838 and Fundacion Antorchas.

MI-P8.

***IN VITRO* SYNTHESIS OF XANTHAN REPETITIVE SUBUNIT USING RECOMBINANT GLYCOSYLTRANSFERASES**

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Glycosyltransferases (GTs) are enzymes involved in the synthesis of polysaccharides. The bacterial mannosyltransferase GumH, glucuronosyltransferase GumK and mannosyltransferase GumI are involved in sequential steps in the synthesis of the pentasaccharidic subunit of xanthan, an exopolysaccharide produced by *Xanthomonas campestris*. Recombinant His-Tagged GumH, GumK and GumI were cloned, overexpressed and purified to homogeneity. By incubating these recombinant enzymes with its substrates, we achieved xanthan repetitive subunit production in a cell-free assay for the first time. Incubations were carried out in the presence of affinity-resin immobilized or soluble GTs, in the presence of all or a combination of them, and using different glycolipidic acceptors involved in different xanthan biosynthetic steps. Immunoprecipitations using polyclonal or commercial antibodies were carried out in order to ascertain whether these enzymes physically interact or rather act as isolated biosynthetic units. This is a first approach towards understanding the functioning of these enzymes as a multienzymatic complex, and how interaction between these GTs is affecting its function.

MI-P9.

MICROBIAL COMMUNITY STRUCTURE IN POLLUTED MARINE SEDIMENTS DETERMINED BY CULTURE-INDEPENDENT METHODS

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Knowledge about the microbial community structure in polluted marine sediments is required to develop tools for predicting and monitoring natural attenuation. We have conducted culture-independent profiling of microbial communities present in heavily polluted sediments in the Mar del Plata harbor area. Surface sediment samples at depths of 10 to 20 m were collected with a dredge in five sampling stations, representative of different kind of pollution. A procedure based on dipersion, separation and filtration, combined with DAPI staining, was used for enumeration of total cells in all sampled sediments. The number of DAPI-detected bacterium-like particles ranged from $4{,}7 \times 10^8$ to 6.9×10^{10} per g of sediment. Bead mill homogenization in the presence of a phosphate-buffered containing SDS, followed by agarose gel electrophoresis in the presence of polyvinylpyrrolydone (PVP) was used for DNA recovery and removal of PCR inhibitors from crude extracts. Numerical comparison of 16S ribosomal DNA (rDNA)-based denaturing gradient gel electrophoresis (DGGE) profiles of *Bacteria* and *Archaea* was used to analyse the microbial community structures. Sequencing of cloned bands complemented DGGE data. Comparison of 16S rDNA profiles from polluted and nonpolluted sediments points to the relationship between microbial diversity and the degree and kind of pollution.

MI-P10.

EXOPROTEASE ACTIVITY OF PSYCHROTROPHIC MARINE *PSEUDOALTEROMONAS* SP. STRAIN 41, UNDER DIFFERENT CONDITIONS

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Extracellular protease activity has been detected in the marine bacterium *Pseudoalteromonas* sp, during cultivation under different conditions. The strain was isolated from intestinal tract of hake, collected from the San Jorge Gulf (Patagonia, Argentina). To determine whether *de novo* protein biosynthesis was required for exoprotease activity, 5h starved cells were washed and subdivided into three samples. The samples were suspended (i) in mineral base medium (MBM) containing chloramphenicol (250µg/ml), (ii) without addition of antibiotic and (iii) the other sample was cultivated with casein as sole protein source, at 20°C during 24 h. In the absence of substrate, extracellular activity was detected at low basal levels, which increased significantly after addition of casein suggesting the occurrence of an inducible process. Low basal rates of exoprotease activity were found in the chloramphenicol-treated cells during starvation. These observations indicated that *de novo* protein synthesis during starvation was not required and the presence of protease activity in the supernatant fluid, from antibiotic–treated cells, could be a result of secretion of protease preformed and accumulated intracellularly. In gelatin zymograms, extracellular activity showed two proteolityc bands with apparent molecular masses of approximately 31,6 and 62 kDa.

MI-P11.

VARIABILITY OF NUCLEORPROTEIN AND HEMAGGLUTININ GENES OF MEASLES VIRUS

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Measles virus (MV), which is a member of the Morbillivirus genus of the Paramixoviridae family, is an enveloped virus containing a negative sense RNA genome. Although MV is considered to be monotypic, genetic variability has been observed and numerous genotypes have been defined. Molecular epidemiology is based on analysis of nucleoprotein (N) and hemagglutinin (H) genes, which contain the most variable regions of the genome. The aim of this study was to characterize the genetic variability of wild type strains collected during the 1998 Argentinean outbreak.
The 3' end of N gene (605 bp) was amplified by RT-PCR. As this region is known to have the highest variability within the genome, the restriction digested 605-bp fragments were analyzed by Single Strand Conformation Polymorphism (SSCP) to characterize these strains. The nucleotide sequence analysis was only applied to those samples with different SSCP electrophoresis profiles. Besides, the hemagglutinin gene was amplified by RT-PCR in two fragments of 1100 and 1500 bp, with an overlapping region of 400 bp. The amplified fragments were sequence analyzed.
The sequences obtained were compared to previously published sequences belonging to different genotypes. Although some nucleotide differences were found among different isolates, both in N and H genes, all samples showed to belong to the genotype D6.

MI-P12.

CITRATE METABOLISM BY *ENTEROCOCCUS FAECALIS* ATCC29212

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Enterococci compose the microbial association of a variety of fermented foods such as cheese, fermented sausages and fermented vegetables. However members of the genus *Enterococcus* have distinguished themselves from other lactic acid bacteria by their role in human infection, harboring a number of identified virulence factors, and for their acquired resistance to antibiotics. The purpose of the present report was to study the Citrate metabolism in *E. faecalis*. Our results shown that it could grow in MRS glucose, MRS citrate but it grew better when both compounds are present (MRSGC), indicating that citrate was co metabolized. PCR and Southern blot experiments indicate that the cit cluster present in the strain ATCC29212 is similar to that found in the sequenced genome of *E. faecalis* V583. The cluster include the structural genes coding for the citrate lyase subunits (*citD, citE, citF*) and the accessory genes required for the synthesis of an active citrate lyase complex (*citC, citX* and *citG*), the *citM*, a putative oxaloacetate decarboxylase, and two genes encoding the oxaloacetate decarboxylase complex biotin dependent (*aodA, oadB*), *citN* the transporter and a putative regulator protein. Dot Blot and RT-PCR experiments suggest that the expression of the citrate lyase complex in *E. faecalis* is activated by citrate.

MI-P13.

IDENTIFICATION OF TzCYC2 A CYCLIN- LIKE PROTEIN OF *Trypanosoma cruzi*

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Several genes from the family of cdc2-related protein kinases, CRKs, have been cloned in trypanosomatids. The participation of these proteins in the control of the parasites' cell division cycle is under study. Two CRK genes, CRK1 and CRK3, had been cloned in *Trypanosoma cruzi*. Using the yeast two-hybrid system we identified three novel cyclins-like proteins able to associate to TzCRK1, named TzCYC2, 4 and 5. TzCYC2 belongs to the PHO family of proteins which phosphorylate transcription factors controlling phosphate metabolism. Its deduced amino acid sequence (230 aa.) indicates that the identity is restricted to their cyclin box domains (Gómez *et al*., 2001). Southern blot analysis reveals that TzCYC2 is codified by a single copy gene. Northern blot assays show that the mRNA is expressed in all life cycle stages of the parasite, with higher levels of expression in the amastigote form. The TzCYC2 was cloned in the bacteria expression vector pET22(b)+, with a Histidine tag at the C-terminal. The expressed cyclin was purified with a Ni-agarose matrix. The recombinant protein was used to immunize rabbits to obtain a specific antibody. In a functional complementation assay the full-length of TzCYC2 was able to rescue Saccharomyces cerevisiae deficient for G1 cyclins. These results suggest that TzCYC2 could be involved in cell cycle progression of the parasite.
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MI-P14.

IDENTIFICATION OF NOVEL PhoP-REGULATED GENES IN *Salmonella enterica*

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Detection of magnesium plays a fundamental role in *Salmonella* pathogenesis because it triggers the activation of the PhoP/PhoQ major virulence signal-transduction pathway. Mg^{2+} is the signal sensed by the membrane-protein PhoQ that results in changes of the phosphorylation level of the response regulator PhoP. This in turn determines the expression of the PhoP-activated genes (*pags*). We took advantage of the microarray technology to analyze the PhoP-PhoQ regulon in *Salmonella enterica* serovar Typhimurium. We analyzed the transcriptional profile of the wild-type strain compared to Δ*phoPQ* or to Δ*phoPQ*/pPhoP grown in inducing or repressing concentrations of Mg^{2+}. In this way we identified new putative PhoP regulated genes. In order to contrast this approach, we made cromosomal *lacZ* transcriptional fusions to these genes and confirmed that the genes under study were under PhoP control. Interestingly, we determined that four independent loci displayed homology with genes coding for transcription factors. Among them we unravel that the activity of a novel two-component system RstA/RstB depends upon PhoP/PhoQ. However, the tested genes appeared to be neither co-regulators nor intermediates in the PhoP signal pathway to activate the already known indirectly regulated *pags*, indicating the presence of yet unidentified genes belonging to the Mg^{2+} stimulon.

MI-P15.

LicA, A PROTEIN INVOLVED IN THE VIRULENCE OF *Brucella spp*

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Brucella spp. are facultatively intracellular bacteria that persist and multiply in the macrophages of their mammalian hosts, producing brucellosis, a zoonosis distributed worldwide. The genome of three species of *Brucella* has been sequenced, and their analysis has shown the absence of functional sequences for most of the classical virulence factor. We are interested in identify virulence factors in *Brucella* through the inactivation of uncharacterised genes and evaluating their virulence in animal models.

We report the identification of the ORF BMEI0489 as a gene that contributes to the bacterial virulence. A null mutant of this ORF in the virulent *B. abortus* S2308 showed a severely attenuate phenotype in the BALB/c mice model, and it recovered the full virulence when was complemented in *trans* whit the wild type ORF. This ORF was named *iivA* (involved in virulence gene), and encodes for a protein of 11 kDa which presents a high content of alfa-helix, as deduced by prediction of the secondary structure using different algorithms and confirmed by circular dichroism of the recombinant protein. Northern blot analysis suggested that *iiv*A is monocystronic and BLAST homology searches indicated that IivA have uncharacterised homologues in others α-proteobacteria.

Using chemical crosslinking and a bacterial two-hybrid system we showed that Iiva can assembly in homomultimers. The molecular bases of Iiva function could contribute to understand the intimate mechanisms of the *Brucella* virulence.

MI-P16.

ASCOMYCETOUS YEASTS ASSOCIATED WITH *PHOEBE POPHYRIA* AT THE PARK "SIERRA DE SAN JAVIER", TUCUMÁN, ARGENTINA. PHYSIOLOGICAL AND MOLECULAR CHARACTERIZATION

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"Yungas Andinas" is the name of the mountain forests in northwest Argentina. "Sierra de San Javier" Park belongs to the Uiversidad Nacional de Tucumán and is located in this area.

We studied specific yeast communities associated with "Laurel del Monte" (*Phoebe pophyria*). The objetive was to isolate yeast strains and analyze their metabolic and genetic diversity. Yeasts was isolated from alive and dead parts of plants. A preliminary identification of isolated yeasts was made according to conventional techniques. These studies showed some limitations for identification of new isolates, since there was not a 100% matching with reference strains profiles. This work was followed by using molecular techniques based on amplification, restriction fragment length polymorphism analysis (RFLP) and sequence analysis of rDNA. Phylogenetic studies based on 26S sequences showed that the isolated yeast belongs to the ascomycetous yeasts. *Hanseniaspora opuntiae*, *Kodamaea ohmerii*, *Candida haemulonii* and *Pichia rabaulensis* strains isolated show some physiological or molecular differences with type strains.

MI-P17.

UPTAKE OF COPPER BY YEASTS ISOLATED FROM COPPER POLLUTED AREA OF ARGENTINA

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Copper, is utilized as an essential cofactor in critical biological processes. However, excess copper is highly toxic to most organisms. Microorganisms may be used to remediate wastewaters or soils contaminated with cooper. This capacity can be used to concentrate, remove and recover copper from different contaminated sites. The aim of this study was to analyze the growth and uptake copper capacity of two isolated yeasts from copper polluted area in Northwest Argentina. Isolates designated as RCL-3 and RCL-11 were obtained from a sediment sample of a Argentina mine containing high copper level. Yeasts were cultured in minimal liquid medium, yeast nitrogen-base containing Cu^{2+} as $CuSO_4$, at 0.1, 0.2, 0.5 and 1 mM. As positive control, cultures without this heavy metal were performed. Cultures were incubated at 30°C, 250 rpm and pH 5. The growth of yeast was followed by turbidity at 620 nm, and measured by counting cells number. Total copper concentration in the supernatants of the culture media and intracellular were determined by atomic absorption spectrophotometer. The growth rates decreased with the increase copper concentration, but the uptake of copper percentage incresed when the copper concentrations were smaller. The uptake of copper by yeast is important for understanding how these microorganisms develop mechanisms for surviving in presence of toxic copper concentration.

MI-P18.
THE H BOX-HARBORING DOMAIN IS KEY TO THE FUNCTION OF THE *Salmonella enterica* PhoQ Mg^{2+}-SENSOR IN THE RECOGNITION OF ITS PARTNER PHOP

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In two-component signaling systems the transduction strategy relies on a conserved His-Asp phosphoryl exchange between the sensor-histidine kinase and its cognate response-regulator. Structural and functional consensus motifs are found when comparing either histidine kinases or response regulators present in a single cell. Therefore, specific recognition between partners is essential to generate the appropriate adaptive response. We dissected the Mg^{2+}-sensor PhoQ in different sub-domains and examined its interaction with the associated response regulator PhoP. This signal transduction system allows *Salmonella* to withstand environmental Mg^{2+}-limitation by triggering gene expression that is vital throughout the infective cycle. Using resonant mirror biosensor technology and fluorescence anisotropy we calculated the kinetic and equilibrium binding constants and determined that the His-phosphotransfer domain is essential for the PhoQ specific recognition and interaction with PhoP. We also assessed the influence of PhoP phosphorylation in the partners interaction which supports the physiological significance of PhoQ phosphatase activity. Moreover, we showed the role of the His-phosphotransfer domain in the bimolecular transphosphorylation and provide evidence that this region undergoes dimerization.

MI-P19.
IDENTIFICATION OF ESSENTIAL AMINO ACID RESIDUES IN THE BRUCELLA ABORTUS CYCLIC GLUCAN SYNTHASE CGS

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Cgs is an integral inner membrane protein of 316 kDa involved in the synthesis of cyclic β-(1,2)-glucan by a novel mechanism in which the enzyme acts itself as protein intermediate. Cgs uses UDP-glucose as donor-sugar and has the three-enzymatic activities required for the synthesis of the polysaccharide: initiation, elongation and cyclization. The first one may catalyze the transfer of the first glucose from UDP-Glc to an unknown amino acid of the protein intermediate. The second enzymatic activity [UDP-Glc:β-(1,2) oligosaccharide glucosyltransferase] may be responsible for chain elongation. Finally, the third activity may catalyze glucan cyclization and release from the protein**.** By comparing the sequence of Cgs to those of the members of glycosyltransferase family 2 (GTF2), we identified the D_1,D_2,D_3,(Q/R)XXRW motif that is highly conserved in Cgs and in the putative active site of numerous processive β-glycosyltransferases. By site-directed mutagenesis we found that replacement of Asp-636 (D2) by asparagine abolished the enzyme activity *in vitro* and *in vivo*. Single substitutions of each of the amino acids of the RXXRW motif at positions 782, 785 and 786 with alanine resulted in the loss of enzyme activity *in vitro*. *In vivo* production of cyclic glucan was affected in the R785A and W786A mutants but not in the R782A mutant. These results indicate that Asp-636, Arg-785 and Trp-786 are essential for Cgs activity and may be implicated in its elongation activity.

MI-P20.
THE OXIDANT-RESPONSIVE DIAPHORASE OF *RHODOBACTER CAPSULATUS* IS A FERREDOXIN (FLAVODOXIN)-NADP(H) REDUCTASE

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Challenge of *Rhodobacter capsulatus* cells with the superoxide propagator methyl viologen results in the induction of a diaphorase activity identified as a member of the ferredoxin (flavodoxin)-NADP(H) reductase family (FPR) by N-terminal sequencing. The enzyme was purified 400-fold to apparent homogeneity using ion exchange chromatography as a monomer of 30 kDa. The *fpr* gene was cloned and expressed in *Escherichia coli*, rendering a product with essentially the same properties as that isolated from the photosynthetic bacterium. Both native and recombinant forms were able to bind and reduce *Rhodobacter* flavodoxin (NifF) and to mediate typical FPR activities such as the NADPH-driven diaphorase and cytochrome *c* reductase reactions.
In *E. coli*, and presumably also in *Salmonella* and related enterobacteria, FPR provides reduced Fd or Fld for a number of reactions involved in amino acid and nucleotide metabolism, biotin synthesis and iron-sulphur cluster assembly. In nitrogen-fixing bacteria such as *Azotobacter* and *Rhodobacter* species, ferredoxins and flavodoxins are the immediate electron donors to the nitrogenase. Steady-state and stopped-flow kinetic measurements on the recombinant *Rhodobacter* FPR rendered slower reaction rates than the plastidic enzyme, although compatible with electron currents through nitrogenase.

MI-P21.
IN VIVO ERYTHROCYTE FUSION IN MICE WITH EXPERIMENTAL CHAGAS DISEASE

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We study molecular aspects of *Trypanosoma cruzi* (Chagas disease agent) mammalian host cell interaction .We first described that parasite induced normal erythrocyte fusion *in vitro*. The lipidic profile, the increase in parasite phospholipid turnover, the inhibition with parasites cultured in ganglioside supplemented BHI medium and anti-PLA2 antibodies recognition of parasite proteins suggested a parasite PLA2 involvement in the interaction with host cells. Decreased hydrolysis of fluorescent and radioactive substrate (25%) and *in vitro* fusion (50%) when gangliosides were incorporated to substrate or gangliosides or anti-PLA2 antibodies were incorporated at the beginning of the assay to the incubation medium, points to a parasite PLA2 structurally homologous to mammalian PLA2 with extracellular location. From these results erythrocyte fusion seemed to be a good model to evidence PLA2 activity. Now we report that fusion of red blood cells occurs *in vivo*. Fusion bodies were observed in all blood samples of *T.cruzi* infected mice immediately after extraction with heparine at 21ºC, no matter of parasite strain an origin, mouse blood or media supernatant of nurse cells with or without freezing.

MI-P22.
IMMUNE SYSTEM RESPONSE, REPRODUCTION, AND COMPETITION DURING CHAGAS INFECTION

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The biology and pathogenesis of the Chagas disease are not yet completely understood. Biochemical and anatomopathological observations in experimental Chagas and in the human disease indicate a strong involvement of both humoral and cellular immunities. Recently (*J. Theor. Biol.* 208, 1-13, 2001), we presented a model for the interaction between *T. cruzi* and its specific antibodies, which includes parasite reproduction, parasite-generated decoys and immune system learning. The parasite-antibodies interaction can lead to either the coexistence between species (chronicity) or to the exclusion of one of them (healing or death). We have also remarked upon the analogies between parasite-immune system interactions and virus-immune system interactions (*Comm. Theor. Biol.* 8, 1-21, 2003). We now use these analogies to model the simultaneous action of the humoral and cellular responses during the Chagas infection. With this extended model we find the conditions that determine whether one of the responses becomes dominant or whether a competitive coexistence develops between the responses. On the other hand, modeling intracellular amastigotes and invasive tripomastigotes as separate populations allows us to substantially improve our fittings to the available data.

MI-P23.
EVALUATION OF THE RECOMBINANT E2 GLYCOPROTEIN OF BOVINE VIRAL DIARRHOEA VIRUS (BVDV) AS IMMUNOGEN IN CATTLE

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Bovine viral diarrhoea virus (BVDV) is a member of the pestivirus genus, within the *Flaviviridae* family. This RNA virus is an important pathogen of cattle, causing significant economic losses world-wide. The E2 envelope glycoprotein is highly antigenic and elicits the production of neutralizing antibodies in the host after vaccination with live or killed vaccines. In previous reports, we expressed E2 in Sf9 insect cells. When mice and rabbits were immunized with the recombinant E2, it demonstrated to have immunological properties similar to those of the native viral protein. The aim of this work was to determine the serological responses of cattle to the recombinant E2 glycoprotein and the protection against challenge. Animals were immunized with cellular extracts obtained from Sf9 infected with recombinant baculovirus expressing E2 and they were challenged with NADL strain of BVDV. Clinical parameters were recorded daily and samples of serum were evaluated by neutralization test. Viral isolation was performed from buffy coat, nasal and ocullar swabs. Animals immunized with recombinant E2 developed higher neutralizating antibodies titers which increased in shorter time than control groups (one of which included an inactivated vaccine). These results indicate that E2 expressed in Sf9 cells stimulated a specific BVDV neutralizing response that lasted for at least 5 months post vaccination.

MI-P24.
A NOVEL SYSTEM TO SCREEN TRYPANOSOMATID ADENYLYL CYCLASE INHIBITORS IN SCHIZOSACCHAROMYCES POMBE

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Adenylyl cyclases have a key role in the differentiation of *T. cruzi*. Therefore, these enzymes could be exploited as therapeutic targets for the treatment of Chagas Disease. However, the adenylyl cyclase multiplicity of these parasites exclude the possibility of characterizing the inhibition of a single isoform. To overcome this problem we decided to express TczAC in a *S. pombe* mutant strain that contains the endogenous adenylyl cyclase gene deleted and the *lacZ* and *URA4* genes inserted under a cAMP repressible promoter. Thus changes in cAMP levels can be monitored by a decrease of â-gal activity and by growth in 5´-FOA (a toxic drug for *URA4* expressing cells). *TczAC* was first expressed in mutant yeasts under the control of a strong promoter. Transformed cells showed less â-gal activity and cells were longer than the control ones. *TczAC* was then expressed under two weaker promoters. Similar to the adenylyl cyclase activity, the length of the cells diminished with the decrease of promoter strength. On the other hand, the â-gal activity showed the opposite behavior. All together these results suggest that the expression of trypanosomatid adenylyl cyclases in this *S. pombe* strain could be useful to screen specific inhibitors of single enzyme isoform.

MI-P25.
GROWTH-PHASE MODIFIED THE UVA RESPONSE OF *E. coli*: INFLUENCE OF CONDITIONED MEDIA, ACETATE AND HYDROGEN PEROXIDE

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Results reported here indicate that UVA radiation induces deleterious effects in *E. coli*, depending on the growth phase. Stationary-phase cells recover faster from a sub-lethal UVA exposure and have a higher resistance to lethal effect of the radiation than exponential growing cells. Although pre-incubation in spent medium supernatant increased the resistance of log-phase cells to lethal UVA effects, this pre-treatment considerably prolonged the duration of the radioinduced sub-lethal growth delay. Our purpose was to investigate the effect exerted by the *E. coli* conditioned media and the influence of nutritional stress, hydrogen peroxide and acetate was determined. Preincubated in conditioned medium, exponential growing cells were irradiated and the induced effects were compared with those found when catalase, high culture densities and acetate were added. Unexpectedly, the duration of growth delay in cells submitted to these treatments was shortened in comparison with control cells incubated in conditioned medium with no modifications. Enlargement of the growth delay was mimicked when exponentially growing cells were incubated in fresh medium supplied with 5ì M H_2O_2. The effects of spent medium on wild type and *rpoS* (oxidative stress response) were similar, indicating that this response is independent of RpoS controlled functions. We assumed that an oxidative component of the spent medium, probably H_2O_2, could be involved in the observed phenomenon, this effect is specific of *E. coli* and independent of *rpoS*.

MI-P26.

MUTATIONS OF MICROCIN J25 THAT AFFECT ENTRY OF THE ANTIBIOTIC INTO *Escherichia coli* CELLS

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Microcin J25 (MccJ25) is a 2,107-Da cyclopeptide antibiotic of 21 amino acids produced by *Escherichia coli.* MccJ25 can enter sensitive cells by binding to specific membrane receptors: FhuA, in the outer membrane, and TonB and SbmA, in the inner membrane. *E. coli* RNA polymerase is the intracellular target of MccJ25. We are interested in identifying which residues within MccJ25 are critical for interaction with the target site and with the membrane receptors. MccJ25 shows a predominance of uncharged and hydrophobic residues over the charged residues Glu and His. We have mutagenized the unique His residue in MccJ25 in order to obtain 3 different analogs in which His has been replaced by Ala, Lys or Arg, respectively. This mutant peptides were purified by HPLC and their effect on RNA polymerase was assessed *in vitro.* No significant difference on RNA polymerase activity was noted as compared with the control, wild-type microcin. We also performed *in vivo* assays to calculate the minimal inhibitory concentration (MIC) of each one of the mutant microcins against *E. coli* strains where the different membrane receptors were overexpressed or not. From these experiments we found that *E. coli* cells are more resistant to the mutant microcins HisxAla and HisxArg than to the wild-type microcin and the HisxLys mutant. Additional experiments showed that the interaction with the inner membrane receptor SbmA is affected in these mutants.

MI-P27.

ABUNDANCE OF *nagAc* NAPHTHALENE DIOXYGENASE GENE MEASURED USING REAL-TIME PCR IS CORRELATED WITH NAPHTHALENE CONCENTRATIONS IN FRESHWATER SEDIMENTS

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Chattanooga Creek is one of the most polluted waterways in the southeastern United States. Chemical analysis of creek sediments indicated moderate to high levels of polycyclic aromatic hydrocarbons, with naphthalene concentrations between 0.1 and 105.64 mg/kg of dry sediment. The objective of this study was to quantify genes encoding for the naphthalene dioxygenase enzyme using TaqMan real-time PCR. An assay was designed and optimized for the quantification of *nagAc*-like genes using conserved regions in this gene cloned from *Ralstonia* sp. U2 and 41 related sequences from pure cultures and uncultured bacteria. The assay indicated the presence of $4.10 \pm 0.66 \times 10^3$ to $2.87 \pm 0.34 \times 10^5$ copies of *nagAc*-like gene per ?g of DNA extracted from sediments of Chattanooga Creek. These values corresponded to $1.15 \pm 0.56 \times 10^5$ to $5.35 \pm 0.41 \times 10^7$ copies of this target per g of dry sediment. A positive correlation was found between naphthalene concentrations and copies of *nagAc* target per µg of DNA ($r^2 = 0.747$) and per g of dry sediment ($r^2 = 0.586$). A real-time PCR system targeting the 16S rRNA gene of most bacteria indicated the presence of approximately equal concentrations of this target ($1.31 \pm 0.42 \times 10^9$) per µg of DNA in all analyzed samples. These results indicate significant differences in the population of Bacteria carrying *nagAc*-like genes in sediments of Chattanooga Creek with different naphthalene concentrations.

MI-P28.

DEVELOPMENT OF A COMPETITIVE ELISA TEST FOR *BABESIA BOVIS* INFECTIONS BASED ON MEROZOITE SURFACE ANTIGEN-2C

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Previous studies have shown that *Babesia bovis* merozoite surface antigen-2c (MSA-2c) has a high degree of genetic and antigenic conservation among geographically distant strains. This antigen thus appears as an adequate diagnostic candidate for bovine babesiosis. We have produced a set of monoclonal antibodies against recombinant MSA-2c (rMSA-2c) from the Argentine R1A strain, expressed in a prokaryotic system. One of them, MAb H9P2C2, was shown to react in Western blots with a parasite protein of the expected MSA-2c size. MAb H9P2C2 and rMSA-2c were employed to develop a competitive ELISA test. This test essentially consisted of binding of rMSA-2c to Immulon 2HB plates, blocking, incubations with a) serial dilutions of different sera from control or *B. bovis*-naturally or experimentally infected bovines, b) MAb H9P2C2, c) peroxidase-conjugated anti-mouse IgG and d) OPD-H_2O_2 colorimetric substrate, and optical density readings at 490nm. All tested *B. bovis* positive sera from experimental and natural infections significantly reduced binding of MAb H9P2C2 to rMSA-2c, while no reduction was observed with negative sera. These results indicate that this cELISA could be an adequate diagnostic tool for bovine babesiosis. Standardization of the test conditions and validation with a larger number of samples are currently under progress. *(Supported by ANPCyT PICT 98-083838 and Fundacion Antorchas, Argentina).*

MI-P29.

NOVEL GENES INVOLVED IN THE RESISTANCE OF ANTIMICROBIAL PEPTIDES IN *Salmonella enterica*

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Studies that provide new insight into the relationship between the host and their pathogens are necessary to solve the growing problem of resistance to conventional antibiotics. The bacterial genes involved in resistance to antimicrobial peptides are ideal targets for the action of novel antibacterial agents. The detailed knowledge of their mechanism of action will entail the development of alternative therapies. In *Salmonella enterica* serovar Typhimurium it has been demonstrated that resistance to antimicrobial peptides is essential to colonize the tissues of the infected host. We performed a genetic analysis of an operon that increased the bacterial resistance to the antimicrobial peptides protamine and cecropin P1, and that is essential for virulence. Interestingly, an insertional mutant of this operon showed increased resistance to polymyxin B, resembling a PmrA/PmrB-dependent effect. As expected, the LPS extracted from this strain showed an electrophoresis pattern similar to the one that characterizes PmrA constitutive strains. Surprisingly, its expression was independent of either PmrA-PmrB, or PhoP-PhoQ. In addition, the mutant was able to replicate in macrophages, indicating that resistance to antimicrobial peptides is not associated to macrophage survival. The analysis of this novel locus will broaden the knowledge of the actions taken by *Salmonella* to resist the initial antimicrobial assault in order to prosper in the infection process.

MI-P30.

ROLE OF QUORUM SENSING MECHANISM ON THE RESPONSE OF *Pseudomonas aeruginosa* TO UVA

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Our previous results demonstrated a high UVA sensitivity of *P. aeruginosa*. Together with reproductive cell death, alterations of several membrane associated functions were informed. In the present work the participation of Quorum Sensing (QS) in *P. aeruginosa* UVA response is shown. This cell-cell communication mechanism is involved in the regulation of transcription of around 600 genes including catalase and superoxide dismutase (SOD). When *P. aeruginosa* cells were harvested at exponential phase strains carrying *rhlI* mutation were more sensitive than the wild type strain or *lasI* mutant, indicating a specific involvement of QS system II. A linear correlation has been found between survival fraction and catalase residual specific activity, irrespective from which strain was analysed. This suggests a possible participation of this enzyme in the resistance to UVA. SOD activity was not modified by UVA treatment and the levels of SOD were similar for wild type and QS mutants. Our previous reports also informed that a short nutritional stress before irradiation protects *P. aeruginosa* from the UVA lethal effect. This protection is dependent on protein synthesis during starvation. In the present study it has been found that the mentioned protection was independent on QS system. In conclusion this study shows the participation of QS II and possibly catalase activity in the modification of the UVA lethal effect, and the independence of the radioprotection induced by nutritional stress from QS.

MI-P31.

A 20.9 kDa NOVEL PUTATIVE LIPOPROTEIN FROM *Mycobacterium paratuberculosis* IS STRONGLY ANTIGENIC

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Mycobacterium avium subsp. *paratuberculosis* (MPTB) is an important cause of ruminant animal disease such as paratuberculosis. The sequence of MPTB genome has recently been finished however a large proportion of the putative genes still have no identified functions. To identify a novel potential diagnostic antigen molecule we analyzed an expression library with serum of animals naturally infected with the bacteria or immunized mice. We identified an ORF of 611 pb that codes a hypothetical protein of 20872 MW. The sequence has 99% of identity with the corresponding ORF from de genome of *M.avium* subsp. *avium* (BLAST search). The presence of the gene was evaluated by Southern Blot and PCR in other species of mycobacterium genera. The results demonstrate that this sequence was only presents in these species and as well in *M. plhei*. Database of protein families and domains searching (PROSITE) revealed that the protein could have prokaryotic membrane lipoprotein lipid attachment site. The localisation to the bacterial membrane is hypothesized. The ORF was cloned an expressed in pRSET vector, and a recombinant peptide of 32.7kDa was obtained and purified. Mice were immunized to obtain policlonal sera to perform cellular localization. Humoral response was evaluated in sera and milk serum samples from different animals (bovine, ovine) by immunoblot, demonstrating that this protein is strongly recognized.

MI-P32.

FERREDOXIN FROM *Trypanosoma cruzi* AND ITS POTENTIAL ROLE IN BIOREDUCTION OF ANTIPROTOZOAL COMPOUNDS

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Ferredoxins (Frd) act as low potential electron carriers. This proteins can be classified according to their structure and the type and number of Fe-S clusters that contain. Vertebrate type ferredoxins contain one [2Fe2S] cluster and they are present in a wide variety of organisms, including prokariotes. Its biological function is poorly understood. In mammals, they are involved in the biosynthesis of steroids in mitochondria. However, the presence of this protein, and its reducing partner ferredoxin-NADP(H) oxidoreductase (FNR), in organisms incapable of sinthetizing steroids suggests that they may perform another function. They were implicated in and Fe-S cluster assembly in yeasts, in the metabolization of xenobiotics in prokaryotes and in induction of apoptosis in human cell lines. The observation that mammalian Frd and FNR can reduce *in vitro* compounds with antiprotozoal activity prompted us to search for this kind of enzymes in *T. cruzi.* We have previously identified sequences that code for FNR (TcFNR) and Frd (TcFrd). In the present work recombinant TcFrd was expressed fused to thioredoxin from *Escherichia coli*. The [2fe-2S] cluster was characterized by UV-visible spectroscopy and circular dichroism. The expression of the enzyme in *T cruzi* was analyzed by northern blot and western blot. We were able to confirm the ability of the recombinant enzyme to act as electron carrier. This results make TcFrd an interest candidate for the bioactivation of trypanocidal compounds.

MI-P33.

ANTI-*Trypanosoma cruzi* ACTIVITY OF GREEN TEA (*Camellia sinensis*) CATECHINS

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Flavan-3-ols compounds known as catechins posses a strong trypanocidal activity being the most active gallocatechin gallate (GCg) and epigallocatechin gallate (EGCg). Both compounds were assayed against three different *Trypanosoma cruzi* stages: IC_{50} values lower than 1 pM were obtained against blood-stream trypomastigote form; Vero cell culture infection by amastigote was 50% inhibited at approximately 100 nM without visible host cell damage; and growth of epimastigote in liquid cultures was 50% at 150 µM. Trypomastigotes morphological alteration and normal erythrocytes stages from 1 nM EGCg treated Balb/c mice blood at 15 and 60 min were confirmed by electronic microscopy. In toxicological assays no hepatic damage was observed under histological techniques in mice treated with 100 µM EGCg but reversible alteration with 400 µM EGCg were detected. Catechins mode of action in the parasite cell are under studies. This results allows us to suggest that these compounds could be used to sterilize blood and continuous working on the possibility to use they on the Chagas' disease chemotherapy.

MI-P34.
STRUCTURAL ANALYSIS OF XANTHAN

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The biosynthesis of the extracellular polysaccharide xanthan in *Xanthomonas campestris* pv. *campestris* is directed by a cluster of 12 genes, *gumB–gumM*. Several xanthan-deficient mutants of the wild-type strain 8004 which carry Tn*5* insertions in this region of the chromosome have previously been described. One of the mutants, the strain 8397, showed that the transposon insertion was located 15 bp upstream of the translational start site of the *gumB* gene and was unable to synthesis xanthan. A subclone of the *gum* gene cluster carrying *gumB* and *gumC* restored xanthan production of strain 8397 to levels approximately 28% of the wild-type, demonstrating that *gumB* and *gumC* are both involved in the polymerization and translocation of xanthan across the bacterial membranes. But it is not clear whether this low recovered activity is due to the formation of few long polysaccharide chains or to shorter chains. Another Tn5 insertion mutant, with a reduced slimy phenotype has been characterized. This mutant failed to produce the pentasaccharide repeating-unit of xanthan. Only three sugars were transferred to the prenyl phosphate intermediate and it was the precursor of a new polymer, a polytrisaccharide, that was detected *in vitro* and *in vivo*. It is shown that the *gumB* and *gumC* overexpression modify the degree of polymerization of both xanthan and polytrisaccharide. To achieve these results new techniques like pulse field electrophoresis and atomic force microscopy were applied.

MI-P35.
MOLECULAR IDENTIFICATION AND TYPING OF LACTOBACILLI ISOLATED FROM KEFIR GRAINS

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Kefir is a fermented milk prepared from kefir grains. The grains are clusters of microorganisms held together by a matrix of polysaccharides and proteins that include primarily lactic acid bacteria, yeasts and acetic acid bacteria. The aims of this work were the molecular identification and typing of 17 heterofermentative lactobacilli isolated from kefir grains (CIDCA, Cs. Exactas, UNLP). The identification of two isolates was made by sequencing of the PCR product of 16S rDNA gene. In all the isolates, this gene was analyzed by ARDRA (*Amplified Ribosomal DNA Restriction Analysis*), using the restriction enzymes *Hae* III, *Dde* I, *Alu* I and *Eco* RI. The genotyping was performed by analysis of RAPD-PCR (*Random Amplified Polymorphic DNA*) patterns using primers M13 (GAGGGTGGCGGTTCT), Coc (AGCAGCGTGG), ERIC-2 (AAGTAAGTGACTGGGGTGAGCG) and 1254 (CCGCAGCCAA). The analysis of 16S rDNA subunit sequences showed that both isolates belong to the species *Lactobacillus kefir*. The patterns obtained by ARDRA were identical in all the isolates and in the reference strain *Lb. kefir* JCM 5818, whereas a homofermentative lactobacillus showed a different pattern. *Lb. brevis* JCM 1059 showed a different ARDRA pattern with the enzymes *Dde*I and *Alu* I. The RAPD-PCR patterns obtained with the four primers used allowed the differentiation of lactobacilli isolates. The resulting unrooted phylogenetic tree grouped the 17 isolates in six clusters.

MI-P36.
PRE-mRNA PROCESSING IN TRYPANOSOMATIDS: SR NETWORK PROTEINS INVOLVED IN CIS- AND TRANS-SPLICING REACTIONS

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Regulation of gene expression in trypanosomatids is mostly exerted at the post-transcriptional level, including pre-mRNA maturation, stability and translation. Cis-splicing has recently been described in trypanosomatids. The *trans- and cis*-spliceosome contain conserved elements, that include snRNPs and non-snRNPs. SR proteins, which are the main non-snRNPs components of this structure, and its corresponding kinases conform the SR-Network. The charcharacterization of *Trypanosoma cruzi* SR protein (TcSR) and SR protein kinase in *Trypanosoma cruzi* and *Trypanosoma brucei* have been reported by our laboratory. TSR1 (*T. brucei* SR-like) is highliy homologous with TcSR, but has not functional activity in splicing reaction. Nowadays, three main issues are being implemented: 1) We are finishing the adaptation of a *T. brucei* splicing system, to study cis-/trans-splicing in *T. cruzi*, particulary in strains overexpressing SR-Network proteins. 2) TSR1 functions are being tested, both in HeLa splicing and *S. pombe* complementation assays. 3) To overcome the difficulty in obtaining RNA knockdown mutants in *T. cruzi*, we take advantage of the success of RNAi in *T. brucei,* to study how the depletion of SR-Network proteins affects pre-mRNA processing in the parasite.

MI-P37.
COMPARATIVE ANALYSIS OF THE N- AND C-TERMINAL REGIONS OF *Escherichia coli* AND *Pseudomonas aeruginosa* MutL PROTEINS

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In *Escherichia coli*, DNA mismatch repair (MMR) is initiated by MutS binding to a mismatch followed by MutL binding. MutL activates the endonuclease MutH which cleaves the unmethylated daughter strand at GATC sites. We showed that *Pseudomonas aeruginosa mutL* gene restore approximately 80% the MMR system of an *E. coli mutL* mutant strain. This is an interesting result considering that *mutS* and *mutL*, but not *mutH* homologues have been found in the genome of *P. aeruginosa*. The first 327 residues of *P. aeruginosa* and *E. coli* MutL proteins have 63% of sequence homology and 87% of homology in the predicted secondary structure. In this region, 22 of the 23 amino acids found to be important for the function of MutL in *E. coli* are present in the *P. aeruginosa* MutL protein. By contrast, the 300 C-terminal residues have only 18% of sequence homology, but 85% of homology in the predicted secondary structure. MutL chimeric proteins containing the *E. coli* N-terminal region and the *P. aeruginosa* C-terminal region or vice versa, also complement (~80%) the MMR system of an *E. coli mutL* mutant strain. We are analyzing *in vitro*, the capacity of the wild type and chimeric MutL proteins to interact with and to stimulate the MutH endonuclease activity. These results support the hypothesis that the N-terminal region of MutL contains most of the functional activities for the MMR system, while the C-terminal region is involved principally in the dimerization of the protein.

MI-P38.
INTERACTION OF P36 PROTEIN WITH *Mycobacterium tuberculosis* PROTEOME

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Tuberculosis, a chronic illness caused by *Mycobacterium tuberculosis,* is still a major worldwide disease. According to the World Health Organization, tuberculosis is a considerable public health problem in Latin America, Asia and Africa.
P36 is a secreted 36 kDa protein with a central domain containing several PGLTS repeats. This protein has been shown as associated to virulence since the disruption of its gene impaired multiplication of virulent *M. tuberculosis* and *M. bovis* BCG in cultured macrophages and immunocompetent mice. In this study, to further elucidate the function of P36, we searched for P36-binding proteins by screening a *M. tuberculosis* DNA library with full-length *P36* using a bacterial two-hybrid system. Two different genes were obtained whose products interact specifically with P36. These genes are Rv1417 and Rv2617c, which encoding for a possible conserved membrane protein and probable transmembrane protein respectively. The function of these proteins is currently unknown. Bioinformatical analyses revealed some similarity to hypothetical or membrane proteins from others bacterial species, e-g. *Corynebacterium ammoniagenes*, *Streptomyces coelicolor* and *Rhizobium meliloti*. Interesting, there is a putative transport protein from *Rhizobium meliloti* similar to Rv2617c, suggesting that Rv2617c could be involved in P36 transport.

MI-P39.
VP1: SEQUENCE VARIABILITY OF LAST FMDV OUTBREAKS IN ARGENTINA

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Foot and mouth disease virus (fmdv) belongs to the picornaviridae family. The single stranded rna codes for a unique polyprotein that is subsequently processed into different non-structural and structural proteins (vp1-4). Among them, vp1 is the mainly exposed one therefore it posses the major antigenic determinants and it is also the most variable of the structural proteins. Here we analyze this variability of different isolates in four national outbreaks: serotype o (1993-1994 and 2000); serotype a (2000 and 2001). 28 sequences of vp1 a2001 were aligned and we found a nucleotide difference, between pairs, from 0 to 3,86%. 11 isolates analyzed of a2000, 5 of o2000 and 12 of o1993/4 resulted in a nucleotide divergence up to 1,1; 0,63 and 2,91 respectibly. When we study differences in the amino acid sequence they slightly grew to 6,1; 2,3; 0,9 and 3,3% in the same order used above. Taking the four groups together, we found 103 nucleotide changes. 54% of them are synonymous changes and 46% are non synonymous, however more than a half of them were non conservative (57%). If we analyze the antigenic sites described for both serotype we found that, not surprisingly, 54% of the changes were located in these regions of the genome although they represent just 1/5 of their total length. A detailed analysis of the variability of different isolates in the same outbreak in combination with the phylogenetic analysis can give us some hint of the evolution of the virus in a short-term period.

MI-P40.
IDENTIFICATION AND CHARACTERIZATION OF β-OXIDATION PATHWAY REQUIRED FOR TESTOSTERONE DEGRADATION IN *COMAMONAS TESTOSTERONI*

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We have identified a new steroid inducible gene in *C. testosteroni*, designated *teiR* (**te**stosterone **i**nducible **R**egulator) that is required for testosterone degradation. Nucleotide sequence analysis of *teiR* predicts a 391-amino-acid protein, which bears homology between residues 327 and 380 to the LuxR helix-turn-helix DNA binding domain and between 192-227 residues to the PAS sensor domain. In addition, three ORFs transcribed in the same direction sequence that *teiR* were cloned. These genes encode proteins with high similarity to β**k**eto-**t**hiolase (*tekt*), MaoC acyl dehydratase and 3-oxoacyl reductase enzymes respectively, all of them involved in a β-oxidation pathway. Analysis of the gene expression indicated that at least *teiR* and *tekt* genes are tightly controlled at the transcriptional level by testosterone. *teiR*-disrupted mutant is completely unable to use testosterone as the sole carbon and energy source. Also, the expression of several steroid-inducible genes was abolished in this mutant. Moreover, when *teiR* was provided in *trans* to the TeiR-disrupted strain, the transcription level of these genes was restored. These results indicate that TeiR positively regulates the transcription of genes involved in the initial enzymatic steps of steroid degradation as well as the β-oxidation pathway and these genes are essential for the complete mineralization of testosterone.

MI-P41.
PSYCHROTOLERANT HYDROCARBON-DEGRADING RHODOCOCCUS STRAINS ISOLATED FROM ANTARCTICA

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When hydrocarbons are spilled on pristine soils, bioaugmentation represent the most promising option to reduce hydrocarbon contamination. In these cases, temperature is one of the most important factor affecting biodegradation activity and only psychrotolerant microorganisms are adequate for designing bioremediation processes in extremely cold areas as the Antarctic continent. Seeing as *Rhodococcus* strains seem to be one of the most efficient hydrocarbon-degrading bacterial group in Antarctica, we reported here the taxonomic and physiological characterization of three Antarctic *Rhodococcus* strains (ADH, DM1-21 and DM1-22) with hydrocarbon degradation activity. Isolation was made from polluted soils from Jubany and Marambio stations. *Rhodococcus* strains were characterized by biochemical and molecular test. ADH and DM1-22 were related to *R. erythropolis* whereas DM1-21 was related to *R. erythreus*. Strains showed high hydrocarbon-degrading capacity on aliphatics and complex mixtures but not on aromatic hydrocarbons. Specific growth rate in cultures with n-hexadecane ranged between 0.028 h^{-1} (ADH) and 0.038 h^{-1} (DM1-21) and 84-86% of the hydrocarbon was removed in all cases. Bioremediation performed in microcosms systems in Antarctica showed that the presence of the isolated strains enhances the biodegradation activity of the natural microflora. These results showed that the isolated psychrotolerant Antarctic *Rhodococcus* are useful for application in hydrocarbon bioremediation processes in Antarctica and other cold areas.

MI-P42.

SUCROSE ENZYME EXPRESSION IN AN *ANABAENA* MUTANT IMPAIRED IN NITROGEN FIXATION

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Cyanobacteria constitute a diverse group of prokaryotes characterized by their ability to perform oxygenic photosynthesis. Most of them are able to use nitrate or ammonium as a nitrogen source and some strains are capable of N_2 fixation. Nitrogen control is mediated by a unique transcription factor that acts as a global regulator of nitrogen and carbon assimilation. Sucrose is involved in the diazotrophic metabolism of the heterocyst-forming cyanobacterium *Anabaena* sp. PCC 7120. It is synthesized by sucrose-phosphate synthase and sucrose-phosphate phosphatase, and cleaved by sucrose synthase, enzymes encoded by *sps*A and *sps*B, *spp,* and *sus*A respectively. During N_2 fixation, sucrose synthesis is increased and sucrose cleavage diminishes. The aim of this work is to study the expression of the enzymes involved in sucrose metabolism in *Anabaena* sp. PCC 7120 and in a mutant strain (CSE2) lacking the global nitrogen transcriptional regulator and the capacity of N_2 fixation. Sucrose level was about 5-fold lower in the mutant than in the PCC 7120 strain, 24 h after combined-nitrogen step-down. Accordingly, expression in the CSE2 strain of *sps*B decreased, while of *sus*A increased, both at the mRNA and enzyme activity level. Moreover, there were detected putative binding sites for the transcriptional regulator in the promoter sequences of sucrose metabolism genes. Taken together these results suggest that sucrose metabolism may be coordinated with nitrogen assimilation through the action of the nitrogen transcriptional global regulator.

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MI-P43.

VP7 AND VP4 GENOTYPING OF BOVINE GROUP A ROTAVIRUS

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Group A rotaviruses, members of the Reoviridae family, are important viral diarrheal agents and have been recognized as the major etiologic agents of acute gastroenteritis in cattle (1969) and young children worldwide (1973). Two outer capsid proteins, VP4 and VP7, elicit independently, neutralizing antibodies and specify the virus P and G serotypes, respectively. At least, 14 G and 20 P serotypes or genotypes of group A rotaviruses have been described in humans and animals, being G6, G10, P1, P5 and P11 the most common strains found in cattle. Determination of the serotype specificity and characterization of the genetic and antigenic diversities of circulating bovine rotavirus strains (brv) in the field is important to develop more efficacious vaccines. The genetic variation among G types for human and bovine rotavirus strains has been described, but little information is available for P types. Group A rotavirus was unequivocally demonstrated in 95% of the samples tested by enzyme-linked immunosorbent assay (ELISA) for detection of VP6 protein, and reverse transcription-PCR (RT-PCR) for amplification of the VP7 and VP4 genes. G and P typing was carried out by nested amplification of variable sequences of both genes using two G- and three P-type-specific primers. Results obtained by this method showed the prevalence of the following combinations: P[5]G6: 65%; P[11]G6: 14%; while mixed infections with more than one type were found in 15% of the samples.

MI-P44.

AUTOANTIBODIES INDUCED IN MICE INFECTED WITH MOUSE HEPATITIS VIRUS

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Mouse hepatitis virus (MHV) diversely affects immune response, depending on the viral strain and the mouse genetic background. We have shown that sera from mice infected with MHV A59 contained autoantibodies (autoAb) directed toward a 40 kDa protein present in mouse liver and kidney extracts. Reactive immunoglobulins were detected from 10 days up to 12 weeks after infection. No correlation was found between the development of hypergammaglobulinemia that followed viral infection and the occurrence of the autoAb. The 40 kDa protein was purified from mouse liver and identified as fumarylacetoacetate hydrolase (FAH). To further characterise the autoimmune response, homologous aminoacid sequences from FAH and viral proteins were synthesized by the PEPSCAN technique, and each peptide was incubated with individual sera from either MHV-infected or FAH-immunized mice. Results indicated that three homologous sequences were recognised by the autoAb induced by the viral infection, whereas the anti-FAH samples reacted heterogeneously towards the various peptides. Moreover, ELISA and Western-blot competition experiments indicated that the reactivity of sera from infected mice depended on the conformational states of both FAH and MHV proteins. These observations suggest that the autoAb elicited by MHV-infection recognised mainly cryptic FAH epitopes and that the response differed from one animal to other.

MI-P45.

ORNITHINE AND ARGININE DECARBOXYLASE ACTIVITIES AND EFFECT OF SOME POLYAMINE SYNTHESIS INHIBITORS ON *Gigaspora rosea* GERMINATING SPORES

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The aim of the present study was to increase the knowledge on polyamine metabolism in AM fungi and to determine whether inhibitors of polyamine synthesis (which could be used as fungicides) may cause negative effects on spore germination and hyphal growth of *Gigaspora rosea*. *In vivo* decarboxylation of radioactive substrates, showed that the ODC pathway is active and ADC activity occurs in *G. rosea* spores. Results showed that urease is active during spore germination and provided evidence on the activity of NCPase, downstream the ADC. Effect of polyamine synthesis inhibitors on free polyamine content of spores, germination and germinating tube growth was studied. Spermine and spermidine were reduced by DFMA at concentrations 0.05 and 0.5 mM, whereas CHA or DFMO did not affect spermine and spermidine levels. DFMA and DFMO, either alone or in combination, and putrescine, exerted no significant effects on spore germination at any of the assayed concentrations. In contrast, CHA reduced spore germination by far than 70%. On the base of our results, the use of polyamine biosynthesis inhibitors to control fungal pathogens would not affect germination and growth of *G. rosea*.

MI-P46.

CHARACTERIZATION OF THE POLYHYDROXY-ALKANOATES (PHAs) DEGRADATION ACTIVITY BY AN ISOLATED PSEUDOMONA SPP. FROM EARTH-WORM COMPOST

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Polyhydroxyalkanoates (PHAs) are accumulated as an intracellular organic carbon storage by different bacteria, under unbalance growth conditions. The thermoplastic characteristics of these polyesters and the biodegradability has attracted much attention as environmentally degradable plastic materials.
An extracelular PHAs depolymerase of *Pseudomona* spp. isolated from earth worm compost was characterized. The enzyme was secreted by the bacterium to the culture medium only when was cultivated on poly 3-hydroxybutyrate-co-3-hydroxyvalerate (p3HB-co-3HV) as the sole carbon source. When yeast extract was added to the culture broth, *Pseudomona* spp. grew well but the depolymerase activity was not detected in the culture supernatant. The secreted enzyme was purified by chromatographic method fast performance liquid chromatography (FPLC). The molecular mass was determined as 45.000 by polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate. The purified enzyme was stable at temperature below 35ºC and at pH value of 7,0 and can depolymerized PHAs. Kinetics of enzymatic activities were measured on PHAs films and commercial PHAs powders (they were used after purification and aged). The purified enzyme has higher affinity for PHAs films.

MI-P47.

ACTIVITY OF ENTEROCIN CRL35 DISPLAYED AT SUB-INHIBITORY CONCENTRATIONS. POSSIBLE IMPLICATIONS

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The *in vitro* interaction between eight conventional antibiotics namely ciprofloxacin, nalidixic acid, cephalexin, ampicillin, vancomycin, tetracycline, erythromycin, and chloramphenicol with sub-lethal concentrations of Enterocin CRL35 was evaluated. The combination studies showed positive interactions between tetracycline, erythromycin, and chloramphenicol with the cationic peptide. The three antibiotics are excluded by efflux pumps dependent on the membrane proton gradient as could be demonstrated in everted vesicles derived from *Listeria* cells. Enterocin CRL35 even at sub-lethal concentrations induced the dissipation of both components of the proton motive force (Δp) i.e. transmembrane electrical potential and pH gradient, as could be demostrated by the inhibitory activity of this bacteriocin on two Δp-driven transport processes: the leucine transport and the active efflux of ethidium bromide. Moreover, the growth rate of *Listeria innocua* 7 was noticeably altered when this fluorescent was present alongside sub-lethal concentration of Enterocin CRL35, meanwhile, both compounds did not affect the bacterial growth when they were added separately, indicating a positive interaction between them, presumably as a consequence of the Δp depolarization. Taking into account all these results, we propose that the cationic peptide increase the effectiveness of some clinical antibiotics through the depolarization of Δp, indispensable factor for the extrusion of these compounds.

MI-P48.

CHARACTERIZATION OF AN *ANAPLASMA MARGINALE* EXPORTED ANKYRIN-LIKE PROTEIN

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Anaplasmosis is the tick - borne disease caused by *Anaplasma marginale* (order Rickettsiales, family Anaplasmataceae), an obligate intraerythrocytic parasite. The disease is clinically characterized by fever, marked haemolytic anaemia, haemoglobinaemia, icterus, weight loss, abortion and often death. Using the PhoA fussion system we identified several exported proteins. Among them we selected an ORF coding for a protein with a conserved domain of ankyrin. Ankyrins were first identified as protein mediating interactions between cytoeskeletal and transmembrane proteins, mainly in red blood cells. However a conserved domain of ankyrin was found to occur in a large number of functionally diverse proteins. The selected ORF was expressed in *E coli* and the purified recombinant protein was used to obtain specific mouse serum. Polimorfism studies were performed in different isolations by PCR amplification and restriction enzyme analysis and each of them were confirmed by sequence. Southern blot analysis was developed to verify the presence in the weakly virulent related organism *Anaplasma centrale.* Futher funtional studies will be developed in order to figure out the role of the ankyrin like protein in *A marginale*.

MI-P49.

CORDOBA NATIVE PGPR STRAINS: BIOCHEMICAL AND GENETIC CHARACTERIZATION

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Over 120.000 has. of cultivable lands on Rio Cuarto region are under salt effects due mainly to the deficient drainage of superficial and subterranean water. The objective of the present study was to isolate salt tolerant strains, native of Cordoba soils, able to promote the growth of important regional cultures. Wheat, maize and agropyro were used as tramp hosts, from whose rizosphere and endorhizosphere bacteria were isolated. These strains were tested in order to establish their PGPR role *in vitro* (N_2 fixation, siderophore and AIA production, phosphate solubilization). Those strains showing two or three PGPR characteristics were selected and studied on their ability to promote wheat, agropyro and maize growth in greenhouse conditions. Some isolates produced a significant increment in root and shoot fresh and dry weight parameters. From the 16s DNA sequencing analysis, one of the strains isolated from agropyro was assigned to the genus *Microbacterium* (99% homology) whereas two isolated from wheat showed high homology with *Pseudomonas* genus. *Microbacterium* demonstrated to be salt, pH and temperature tolerant. Other strains inhibited *Sclerotinia sclerotiorum* and *S. minor.*

MI-P50.

INTRAVENOUS LIPOSOMAL BENZNIDAZOL AS TRYPANOCIDAL AGENT: INCREASING DRUG DELIVERY TO LIVER IS NOT ENOUGH

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With the aim of investigating if delivery of benznidazole (BNZ) to liver could be increased by incorporating the drug in multilamellar liposomes, single bolus of free BNZ, or liposomal BNZ formulations (MLV-BNZ) composed of HSPC:DSPG:Chol 2:1:2 mol:mol at 0,7% w/w drug/total lipid ratio, were injected by intramuscular (i.m.),subcutaneous (s.c.) and intravenous (i.v.) routes, at 0.2 mgBNZ/kg, in rats. The resulting blood concentrations were followed along 9 h post injection (p.i.) and drug accumulation in liver was determined after 4 and 9 hours pi. Only upon i.v. injection of MLV-BNZ, a threefold higher BNZ accumulation in liver was obtained, together with blood BNZ concentrations of 1,1 µg/ml (30% lower than the BNZ blood concentration achieved upon iv administration of free drug). The increased uptake of BNZ by liver raised every 56 h showed to exert no effect on parasitaemia levels of mice infected with a RA strain of *Trypanosoma cruzi*. Our results indicate that relationship between increased selectivity for an infected tissue and therapeutic effect is not straightforward, at least for the MLV-BNZ regimen used in the present study.

MI-P51.

GLUTATHIONE DEFICIENCY INDUCES A PLEIOTROPIC RESPONSE IN *Rhizobium Tropici* CIAT899

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Glutathione is involved in many cellular functions including stress tolerance. In the common bean symbiont *R. tropici* it is essential for protection against environmental stresses, such as acidity, salinity and oxidative stress. We have previously cloned the *gshB R. tropici* wild type gene (which encodes glutathione synthetase) and demonstrated that its transcription is transiently activated when shifted to acidic conditions. We have now mapped the transcription initiation of *gshB* gene by primer extension analysis, and found it to be located 71 bp upstream from the predicted start codon. Centered at position –40 we have found a palindromic sequence of the general form $TTGN_{13}CAA$, similar to the $TTGN_{11}CAA$ palindromic sequence found in the *Rhizobium etli, Sinorhizobium meliloti* and *Agrobacterium tumefaciens rec*A promotor, known as the SOS box. *rec*A gene is activated in response to DNA damage, suggesting that *gshB* gene should be regulated by a SOS type response. We also show that catalase activation by oxidative stress is impaired in glutathione deficient mutant, which we propose occurs in an OxyR mediated manner. We have also studied the effects of glutathione deficiency in symbiosis. We have found that, although *gshB* deficient mutant is able to nodulate *Phaseolus vulgaris*, the resulting nodules are small and in a higher number compared to plants inoculated with the wild type. We have also found that bacterial glutathione is necessary to maintain an efficient symbiosis, since lack of rhizobial glutathione is associated to premature nodule senescence.

MI-P52.

CONSTRUCTION OF A SINGLE-CHAIN FV ANTIBODY FRAGMENT WITH *Trypanosoma cruzi trans*-SIALIDASE NEUTRALIZING ACTIVITY

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Trypanosoma cruzi trans-sialidase (TS) is considered a key factor in the establishment of the chagasic infection. TS is able to direct transfer sialyl residues among macromolecules an, activity of interest in glycoconjugates research. Specific inhibitors are required to understand both, TS involvement in the chagasic infection and its biochemical properties. TS-neutralizing antibodies constitutes the only inhibitors available. Then, defined inhibitory antibodies-derived peptides provide a useful tool for the further rational development of TS-targeted drugs. We report here the construction of a single-chain Fv (scFv) derived from a mouse monoclonal antibody displaying TS-neutralizing activity. By employing the Recombinant Phage Antibody System, the variable regions of the heavy and light chains of the IgG from the 13G9 hybridoma were cloned, sequenced and assembled into an sc Fv. This construction was cloned into pCANTAB vector, and expressed both, as phage displayed in *Escherichia coli* XL1Blue and in the soluble form cleaved to the supernatant in *E. coli* Top10F' cultures. Both constructions retained the TS-neutralizing activity.

MI-P53.

INTERACTION BETWEEN NATURAL POLYAMINES AND SYNTHETIC DEOXYOLIGONUCLEOTIDES: AN NMR STUDY

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Putrescine, spermidine and spermine are natural polyamines distributed in living organisms. The intracellular concentration of polyamines has been shown to influence cell growth and carcinogenesis. The structural rationale for the physiological effects of polyamines has relied in the understanding of the polyamine-nucleic acid interactions. Nuclear Magnetic Resonance (NMR) spectroscopy has provided valuable insights into tRNA-polymine interaction, giving support and enhancing structure-based drug design. In this work we extended our studies to explore the interaction between natural polyamines and synthetic deoxyoligonucleotides. The ^{1}H 2D NMR spectra of $d(CGCGAATTCGCG)_2$ are typical of a B DNA conformation. The ^{31}P NMR spectrum of $d(CGCGAATTCGCG)_2$ remains unchanged upon addition of putrescine and spermidine. However, the interaction with spermine reveals changes in the chemicals shifts of a discrete number of ^{31}P resonances. ^{1}H NMR titration experiments of $d(CGCGAATTCGCG)_2$ with polyamines exhibits a similar behavior. The ^{15}N relaxation times of ^{15}N-labeled spermine are strongly affected by the presence of the deoxyoligonucleotide, whereas purtrescine and spermidine show similar relaxation properties in the absence and presence of $d(CGCGAATTCGCG)_2$. These results clearly show that spemine is the natural polyamine exhibiting binding features in the presence of the $d(CGCGAATTCGCG)_2$ fragment. These conclusions are relevant for understanding the molecular basis of polyamine affinity and specificity and for enhanced polyamine analogs design.

MI-P54.
RADIATION SURVIVAL STRATEGIES OF A GRAM NEGATIVE YELLOW PIGMENTED BACTERIUM

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We isolated a yellow pigmented nonfermentative Gram negative bacterium which exhibited high resistance to the lethal effects of sunlight. In order to study the mechanisms responsible for radiation resistance in this organism, we assayed its ability to survive under UV exposure. Survival curves obtained with the pigmented bacterium irradiated at 254 and 313 nm showed increased resistance when compared with those obtained for *E.coli* K12, and a similar response was found at 365 nm. HPLC analysis of pigments extracted from the isolated bacterium suggested the presence of carotenoids. Protective effects of pigments were evaluated studying survival curves obtained with bacteria grown in the presence of an inhibitor of carotenoids synthesis and with a color-less mutant derived from the isolated organism by chemical mutagenesis. Pigments depletion had no influence at 254 and 313 nm, and slightly reduced resistance at 365 nm. Similar photo-reactivation capacity was found in *E.coli* and the isolated organism, which exhibited higher levels of catalase activity. The lethal effects produced at the assayed wavelengths depend on different targets and action mechanisms. The resistance at 254 and 313 nm suggests that the ability to overcome DNA damage plays a key role in radioresistance of the isolated bacterium.

MI-P55.
***yhdO* GENE OF *Bacillus subtilis* ENCODES AN ACYL-ACP ACYLTRANSFERASE**

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The first step in phospholipids biosynthesis is the acylation of glycerol-3-phosphate to give phosphatidic acid, which is the precursor of all membrane phospholipids. That step has been characterized in Gram negative bacteria but the knowledge in Gram positive microorganisms is scarce. That is why we begun the study of *Bacillus subtilis* acyltransferases. In *E. coli*, the genes *plsB* and *plsC* encode for glycerolphosphate and acylglycerol phosphate acyltransferases, respectively. The analysis of *B. subtilis* genome revealed only one gene homologue to *plsC*, called *yhdO*, and no homologues to *plsB*. To study the role of *yhdO*, we constructed the strain LP15, which contains the sole copy of *yhdO* under the control of an IPTG inducible promoter. In the absence of IPTG, growth of strain LP15 stops and also does lysophospholipids and phospholipids synthesis, as determined by one-dimensional TLC. Expression of *yhdO* in *E. coli* cells allowed growth of *plsC* but not of *plsB* mutant cells. By gel shift assays we demonstrated that the promoter region of *yhdO* is able to bind FapR, the global regulator of fatty acid biosynthesis of Gram positive bacteria, and by Western blot assays we found that YhdO is accumulated in *fapR*$^-$ cells. With these results we conclude that *yhdO* is the only gene of *B. subtilis* that encodes for an acylglycerolphosphate acyltransferase, that YhdO probably also has glycerolphosphate acyltransferase activity, and that expression of *yhdO* gene is tightly coordinated with expression of genes involved in fatty acid biosynthesis.

MI-P56.
IN SILICO* NORTHERN OF THE PROTEOLYTIC MACHINERY OF *PHYTOPHTHORA INFESTANS

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Members of the oomycete genus are the major pathogens of innumerable crops, among them *P. infestans* that causes the most important economic looses on potato fields around the world. Thus, the basic knowledge of the *P. infestans* biology remains a priority for many plant pathologists. Proteolysis is an essential metabolic process required for protein processing and turnover. In particular, proteases of pathogenic microorganisms are key component of developmental stages and pathogenesis. Previously, we have analyzed the intra and extracellular proteolytic activities in *P. infestans*. We have found an extracellular serine activity and an aspartic intracellular one. We have also cloned and expressed a cDNA with acidic *in vitro* activity. Nowadays, the important amount of information generated by genomic projects is demanding to be deeply studied. In this work we analyzed a databank of 18 different *P. infestans* EST libraries (SYNGENTA-NCGR). A search for high protease-homology rendered 110 entries, of 72822, with homology to proteases [numbers of entries]: aspartic [5], serin [12], cystein [28], subtillisin [7], metallo [5], ubiquitin specific [18] and not identified [35]. The "*in silico* northern" analysis predicts the differential expression of each clone during developmental stages, potato-*P. infestans* interaction and stress conditions. Results showed a broad view of the putative proteolityc machinery of *P. infestans* and could be of interest for planning further research directions. *Supported by UNMdP, Conicet and ANPCyT, Argentina.*

MI-P57.
PHENOTYPIC CHARACTERIZATION OF IRON TRANSPORT MUTANTS (FUR$^-$) OF *BORDETELLA BRONCHISEPTICA* SUGGESTS THAT VIR90 IS FUR REGULATED

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Bordetella pertussis and *B. bronchiseptica* undergo phenotypic changes modulated by the *bvgAS* locus, which regulates the expression of many virulence genes. We previously reported the n-terminal sequence of vir90, a 90kda *bvg*-regulated omp of *B. Pertussis*. *vir90* encodes for a protein with similarities to ferrisiderophore receptors. We scanned the *vir90* promoter region and found a potential fur box. Vir90 was detected under high-iron growth conditions in immunoblotting of OMPs, and its expression increased 4-fold under low-iron conditions. In order to test if the expression of *vir90* is repressed by Fur, we selected manganese-resistant deregulated iron transport mutants (Fur$^-$). 40 mutants were checked for positive Csaky assay for hydroxamate class siderophore and hemolysis (Hly+ indicates Bvg+) in media with iron. Two Hly+/Csaky+ mutants were cultured under both high-iron and low-iron conditions and tested for constitutive production of siderophore. Iron-regulated protein expression in OMPs from these cultures was monitored by SDS-PAGE as well as immunoblotting. The amount of Vir90 is incresed in these mutants independently of the iron condition used, in agreement with mutation in *fur*. These results suggest that expression of Vir90 is fur regulated.

MI-P58.
CHARACTERIZATION OF ROTAVIRUS VP6 PROTEIN AS CARRIER TO HETEROLOGOUS EPITOPES

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The general goal of this project is to evaluate the use of virus like particles (VLPs) based on VP6 protein from simian rotavirus as presentation system for heterologous antigens. Due to the spatial nature of VLPs, the antigenic sites are presented to the immune system in a multimeric way, associated to immunogenic proteins. Indeed, VLPs do not replicate and are non-infectious vehicles. In order to investigate the putative sites for insertion of foreign sequences into VP6 protein, the 14 amino-acid epitope V5 was chosen. Six putative sites for insertions were selected comprising amino-acidic positions 14, 101, 146, 235, 382 and the amino terminus. Recombinant baculovirus were constructed and chimaeras VP6-V5 were expressed in insect cells, rendering high expression levels all of the assessed versions. Version $VP6_{14}$ was able to form trimers, but it was not recognized by a polyclonal rabbit serum anti-rotavirus, whereas an anti-VP6 monoclonal antibody strongly reacted with $VP6_{14}$, indicating that a major antigenic site into VP6 is being interrupted by the insertion. Version $VP6_{235}$ was not able to form trimers, whereas the rest of the versions exhibited trimer formation and were recognized by both the rabbit serum anti-rotavirus and the monoclonal antibody anti-V5. Immunogenicity of the different chimaeras is being evaluated.

MI-P59.
EVALUATION OF ANTIGENICITY OF THE RECOMBINANT E2 GLYCOPROTEIN OF CLASSICAL SWINE FEVER VIRUS

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Classical swine fever (CSF) is a highly contagious disease that affects pigs, causing great economic losses. Argentina uses a lapinized chinese isolate for vaccination and the vaccinated animals cannot be differentiated by serology from those infected. Obviously, a vaccine that allows the discrimination between vaccinated and infected pigs is essential to eradicate this disease.
CSF virus genome consists on a molecule of RNA of positive polarity with a single open reading frame coding for a polyprotein flanked by highly conserved non-coding regions. This polyprotein is processed by viral and cellular proteases to generate structural and nonstructural proteins. The infection with the CSFV stimulates the production of antibodies against the proteins E2, Erns and NS3. Nevertheless, only E2 induces the production of neutralizing antibodies in pigs. This fact reveals that a vaccine based on E2 would allow to discriminate those animals vaccinated from those infected. The purpose of this work was the expression of the protein E2 in procariotic and eucariotic systems to be used either as a vaccine or as a diagnostic reagent. The genomic region that codes for the E2 glycoprotein was amplified by RT-PCR and cloned in vectors for expression in E. coli BL21 and in the system baculovirus/ insect cells. Both vectors incorporate a 6 histidine residues (His_6) in the NH_3 terminal end of the E2 to allow the purification. In tests of western-blot, purified proteins were revealed with Mab against CSFV E2, Mab anti-His_6 and with serum from infected and non infected pigs.

MI-P60.
PHOTOPROTECTIVE COMPOUNDS (CAROTENOIDS AND MYCOSPORINES) IN FRESHWATER *Patagonian yeasts*

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The synthesis of carotenoids and/or mycosporines (MYC) which have respectively antioxidant and UVR sunscreen properties is a common strategy in the photoprotection of microorganisms. The production of these compounds by freshwater yeast strains grown under PAR and UVR was assessed. In most red species, caretonoid synthesis was stimulated under PAR or UVR+PAR irradiation. This response was higher when constitutive levels of pigments (in the dark) were lower. This suggests that cells with higher constitutive levels of carotenoids are less responsive to induction by UVR-PAR. Four yeast strains were able to produce a UV-absorbing compound (309-310 nm) when exposed to PAR or UV-PAR identified as mycosporine-glutaminol-glucoside. This is the first time that the production of MYCs by yeasts is reported. All strains that developed under UVR-PAR were able to synthesize carotenoids either constitutively or in response to PAR exposure, and a few of them also produced MYCs. Collectively, our results suggest that the presence of carotenoids, either alone or in combination with MYCs, are required for sustaining yeast growth under exposure to UVR-PAR.

MI-P61.
BIOLOGICAL CHARACTERIZATION OF A MONOCLONAL ANTIBODY THAT INHIBITS THE *trans*-SIALIDASE FROM *Trypanosoma cruzi*

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To overcome its inability to synthesize sialic acids de novo, *Trypanosoma cruzi* acquires it by transference of the sialyl residues from the host to parasite glycoconjugates, a reaction catalyzed by the *trans*-sialidase (TS). The TS is considered a virulence factor since it prevents the attack by complement, is involved in the escape from the phagolysosoma and alters the immune system of the host. It is also suggested as involved in the invasion of mammalian cells. No specific inhibitors are already available. After deleting spurious epitopes from the catalytic region of the TS, a monoclonal antibody (mAb) collection against the enzyme was obtained, where only one displayed neutralizing activity (typed as an IgG2a). This mAb, was tested both in *in vivo* and *in vitro* assays. Differences in infected HeLa cell counts among mAb-treated cultures and controls were about 25-50%. Passive transfer of mAb to mice challenged with bloodstream trypomastigotes (RA strain) failed to significantly reduce levels of parasitemia and mortality. In contrast, the damage on spleen, thymus and peripheral ganglia were highly diminished. Our results strongly support the proposed role of the systemically disseminated TS in the attack of the immune system.

MI-P62.
MANGANESE TRANSPORT EXPRESSION IN *Sinorhizobium meliloti*

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The intracellular concentration of transition metals such as Fe, Mn, Co, Cd, Ni and Zn are precisely controlled through the regulation of its transport across the membrane. Bacterial manganese transport systems identified so far comprise: the Natural Resistance-Associated Macrophage Protein (NRAMP)- type transporters; the ABC-type transporters and the P-type ATPase transporter found only in *Lactobacillus plantarum.* In the genome of the symbiotic á-proteobacterium *Sinorhizobium meliloti*, genes homologous to NRAMP and ABC type manganese transport systems have been identified. In previous works we demonstrate that the ABC type MntABCD system is involved in manganese transport in *S. meliloti.* Bioinformatic studies have revealed a putative *fur* (*f*erric *u*ptake *r*egulation) gene at 122 bp from the presumed *mntA* start codon in the complementary strand, and Fur regulation of *mntA* expression was studied. In order to evaluate the expression of this transport system and to elucidate its relevance during the symbiosis with alfalfa plants, transcriptional fusions with the reporter *gfp* gene and the promoter regions of *mntA* and *fur* genes were constructed. Nodules were harvested from plants inoculated with *S. meliloti* cells carrying the *gfp* constructions. Fluorescent microscopy of nodules showed that the *mntA*:*gfp* construction was active suggesting a role for this transporter in the symbiotic interaction. The expression of *fur:gfp* construction is actually under study.
Supported by CSIC and PEDECIBA- Uruguay.

MI-P63.
CHARACTERIZATION OF *Herbaspirillum seropedicae* MUTANTS RELATED TO IRON METABOLISM

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Herbaspirillum seropedicae is a grass endophyte that belongs to the β subgroup of proteobacteria. In association with some rice cultivars, it is able to express nitrogenase and to promote plant growth. Nitrogenase complex contains about 40 iron atoms highlighting the importance of iron in this process. With the aim to elucidate iron uptake systems in *H. seropedicae* and to evaluate its contribution to the plant growth promotion, a generalized mutagenesis was done with a *minTtn5gus-o-gfp* transposon. Transconjugants screening was based on siderophore production in CAS solid medium. Five mutants that accumulate siderophores and twenty that did not produce halo in CAS plates were obtained. The interrupted genes of three of those mutants presented high homology with: *exb*D (a gene that encodes a component of the TonB complex implicated in iron internalization), *acn*A (the gene of aconitase A) and a putative gene for sulfite reductase (*cysJI*). The *gusA* (β-glucuronidase) gene present in the mini-transposon was inserted in the same orientation of the interrupted genes. In the $ExbD^-$ mutant (which overproduce halo in CAS plates), *gusA* was induced in cells grown on iron-chelated medium. The putative mutants $AcnA^-$ and $CysJI^-$ did not produce halo in CAS plates. GUA expression of cultures grown in different media was evaluated.
Supported by RITE-Japan.

MI-P64.
THE CGM GENE OF B. ABORTUS ENCODES A MEMBRANE PROTEIN THAT IS REQUIRED FOR SUCCINYLATION OF CYCLIC β-1,2- GLUCAN

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Brucellae periplasmic cyclic β-1,2-glucan provides important function during bacteria host interaction. In addition to the well-characterized neutral cyclic glucan, periplasmic glucans contain anionic components not previously reported. The presence of highly substituted forms of the cyclic β-1,2-glucan may be important in the *Brucella* infection process. In the present study, we describe that the anionic cyclic β-1,2-glucan are substituted with succinyl residues. We have identified a *B. abortus* 1.203-kb open reading frame with 39% identity to *Rhodobacter sphaeroides* succinyl transferase, named *cgm* for Cyclic Glucan Modifier. *Cgm* mutant produces neutral glucans without succinyl residues, confirming the identity of this protein as the *B. abortus* cyclic glucan succinyl transferase. Despite the absence of subtituted in cyclic β-1,2-glucan, mutant *cgm* was shown to effectively infect Balb/c mice, indicating that substitution of cyclic β-1,2-glucans with succinyl residues is not essential for *Brucella* infection.

MI-P65.
PHYLOGENETIC ANALYSIS OF ARGENTINEAN MUMPS VIRUS ISOLATES

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Mumps virus (MuV) is a member of the genus Rubulavirus in the family Paramyxoviridae. The single-stranded genomic RNA contains seven genes including the small hydrophobic (SH) gene, which encodes a protein of 57 amino acids. This gene has been used to genotype MuV strains, since it is the most variable portion of the genome. Purified RNA specimens isolated from throat swabs samples of acute mumps patients, confirmed by IgM capture ELISA and collected between 1997 and 2000, were amplified by RT-PCR using specific primers of the intergenic regions flanking the SH gene. All samples were subjected to nucleotide sequence analysis. Phylogenetic analysis of the sequences obtained and previously published sequences from USA, Canada, Portugal, UK, France, Germany, Switzerland, Denmark, Sweden, Russia, China and Japan showed the existence of the 11 genotypes (A-J) described. All the local strains studied belonged to genotype A, which also includes some vaccine strains, like Enders and Jeryl Lynn. When the deduced 57 amino acid sequences were analyzed the signature sequence motifs specific of this genotype were identified, being the amino acids at positions 28-30 the most characteristic motif. The local isolates showed more than 95% of nucleotide identity when compared with Enders and Jeryl Lynn strains and about 80% with Urabe strain. These variations induced non-synonymous changes at the amino acid level only when compared with Jeryl Lynn and Urabe strains.

MI-P66.
COMPETITIVE REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION ASSAYS FOR QUANTIFICATION OF HEPATITIS G VIRUS (HGV) RNA STRANDS

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Objective: To design and develop quantitative PCR-based assays for detection of either positive (genomic) or negative (replicating) HGV RNA strands. *Material and methods*: A 329 bp c-DNA fragment from the 5' UTR of HGV genome was cloned in a vector where a 67 bp deletion was introduced. Clones harboring both insert orientations -corresponding to [+] and [-] strands- were selected by sequencing. Linearized plasmids served as templates for T7 RNA polymerase activity. Fully purified competitive RNA copies were quantified by UV absorbance. HGV [+] RNA strand was quantified in serum samples (n= 5) by competitive RT-PCR (cRT-PCR). Each RNA sample was mixed with different amounts of competitor RNA and RT-PCR amplified with the highly specific *Tth* enzyme. PCR products were separated by gel electrophoresis. Digital images were analyzed by *Scion Image* (NIH) software. *Results*: The sensitivity of RT-PCR was 10^3 and 10^4 molecules for [-] and [+] competitor RNAs, respectively. This method has a discrimination of at least 5 $\log_{10}$ between both RNA strands. Titers obtained by cRT-PCR correlated exactly with those obtained by a limiting dilution method. Negative HGV strand proved undetectable in serum samples. *Conclusion*: The availability of an in-house made HGV [+] and [-] strand quantification method will shed some light on future studies concerning its poorly understood pathogenesis.

MI-P67.
CELL WALL MODIFICATIONS BY OSMOTIC STRESS IN *Lb. casei* ATCC 393

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Lb. casei was more sensitive to mutanolysine (MtLz) and antibiotics with target in the cell wall when grown in high salt medium (MRS+1M NaCl, condition N). EM showed that in N cell were 60% bigger than those grown in the control condition (MRS, C) and cell wall was detached from the cytoplasmic membrane. Purified cell wall of both conditions obtained by SDS-PronaseE treatment also showed the differential sensitivity to MtLz. Therefore differences observed would be related to structural modifications. In particular a decreased in peptidoglycan (PG) cross-linking was found by FACE analysis and DNF-derivatization. Treatment with 10% TCA or 0.1N HCl that enabled extraction of wall associated polymers such as Teichoic acid (WTA) showed that C condition produce 8 fold greater WTA than N. Alterations in PG could be attributed to the biosynthetic process dependant of the Penicillin-binding proteins (PBP). PBP are associated to the membrane with is also altered by osmotic stress. Membranes isolated from both conditions were developed with Biocillin that specifically binds PBP. Saturation assays with increasing Pen G concentrations showed a modified pattern between both conditions. Three of the 9 PBP here first described, were fully saturated in N at lower Pen G concentrations, and related to a different functionality *in vivo*. Taken all together these results showed that growth in high salt modified the cell wall (PG and WTA) and its related functions (PBP).

MI-P68.
USE OF *Brucella abortus* AS A VECTOR FOR THE EXPRESSION OF HETEROLOGOUS ANTIGENS

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Brucella abortus S19 and RB51 are attenuated strains used as live vaccines to control bovine brucellosis. Due to the strong cellular and humoral immune response that they elicit, they are particular attractive vectors for the delivery of heterologous antigens. The objective of the present study is to express antigens of veterinary pathogens that require for their control the same type of immune response elicited by *Brucella*. We have cloned *esat-6* and *cfp-10* of *Mycobacteium bovis*, *rap-1* of *Bavesia bovis* and *msp-1a* of *Anaplasma marginale* into the replicative plasmid pBBR1MCS and evaluated their expression in *Brucella abortus* S19 and RB51. We have also cloned *esat-6*, *cfp-10* and *msp-1a* under the control of *bp26* promoter and in frame with its signal peptide in order to express the heterologous protein in the periplasmic space. Immune response against the heterologous antigens and against *Brucella* is at present being evaluated. We expect to obtain valuable information about the use of *Brucella abortus* as a vector for the expression of heterologous antigens.

MI-P69.
SIMULTANEOUS EXPRESSION OF *VAG* AND *VRG* GENES DOES NOT ALTER *Bordetella bronchiseptica* –HOST INTERACTION

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In *Bordetella* the expression of the main virulence factors is regulated by *bvgAS* locus, a two-component transduction system. This locus enables *Bordetella* to alternate between two distinct phenotypic states. The virulent state (Bvg^+) arise when BvgAS induces the expression of genes that encode for most of the virulence factors (*vag* genes), while at the same time represses other genes called vir repressed genes (*vrg* genes). By contrast, the avirulent state (Bvg^-) is characterized by the expression of *vrg* genes and occurs when BvgAS activity is suppressed. Although the role of *vag* genes in the infection process is undisputed, the involvement, if any, of *vrg* genes in virulence as well as the molecular mechanism of their repression remain to be determined. Only in *B. pertussis* the involvement of a repressor gene called *bvgR* was recently reported. Therefore, we investigated whether *bvgR* has a role in *B. bronchiseptica*. Using PCR, sequencing methods, and Southern blot we could determine the presence of *bvgR* in *B. bronchiseptica*. On this basis we constructed a defective mutant by site-specific insertional mutagenesis. This mutant (*Bb:bvgR*) expresses at the same time *vag* and *vrg* genes when BvgAS system is activated. We could observe *in vitro* that both adhesion and intracellular survival in epithelial human cells were not affected. *In vivo* assays using intranasal Balb/C mice infection, showed colonization kinetics of *Bb:bvgR* comparable to the parental strain. Our results showed that the *vrg* gene products together with the already known *vag* gene products do not interfere in the infectious cycle.

MI-P70.
AN ALTERNATIVE MECHANISM OF ACTION OF MICROCIN J25 IN *Escherichia coli* STRAINS

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By using serial dilution assays we have observed striking differences in sensitivity to microcin J25 (MccJ25) between *E. coli* strains. We have previously established that RNA polymerase (RNAP) is the target of MccJ25 and that the antibiotic inhibits transcription *in vivo* and *in vitro*. *E. coli* strain SBG231 harbors a mutation in rpoC (the gene encoding largest subunit of RNAP) which makes this strain completely resistant to the antibiotic. The mutation was transduced to the MccJ25-hypersusceptible strain AB1133. One of the transductants, named PA232, was studied further. Transfer of the mutation was confirmed by genetic complementation tests and *in vivo* transcription experiments. Notably, strain PA232 did not become completely resistant to MccJ25. This could be explained by assuming the existence in strain AB1133 of an alternative mode of action of the antibiotic, which would not be operative in strain SBG231. This result could also explain the differential sensitivity of *E. coli* strains. Previous work from our laboratory showed that in *Salmonella* strains MccJ25 acts on the cytoplasmic membrane by dissipating the electric potential and affecting the cell respiration. In the present study we have not detected an effect on cell respiration in PA232. However, the possibility that the membrane be the putative second target in hypersusceptible *E. coli* strains cannot be discarded.

MI-P71.
PHYSIOLOGICAL AND MOLECULAR CHARACTERIZATION OF YEASTS PRESENT DURING SPONTANEOUS CIDER FERMENTATIONS

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The transformation of must apple into cider by spontaneous alcoholic fermentations is the result of the sequential development and metabolic activity of various species of yeasts originated from apple and cidery surfaces. The aim of this work was to isolate, to characterize and to compare yeast microbiota from fermenting apple musts in two cideries. Initial musts from both cideries, were also fermented in laboratory. The samples were took at three stages of spontaneous alcoholic fermentation: initial, middle and end. Yeast characterization at species and strain level was performed according to conventional physiological, morphological and molecular methods (RFLP of the ITS1-5.8S-ITS2 and mtDNA-RFLP). Differential killer sensitivity was also used as a tool for fingerprinting at strain level. *Pichia membranaefasciens, Pichia kluyveri, Dekkera anomala, Torulaspa delbrueckii* and *Kloeckera apiculata* were identified in the initial stages of fermentation from both cideries. *Saccharomyces cerevisiae* turned out the most among those isolated at middle and final stages. The killer phenotype (K^+) was observed in 80% of cider yeasts. The dominant strains in both industrial and laboratory vinification processes were just a few and the same. The quality of the final products was very similar, with the exception of the volatile acidity. We can conclude that there exist a great diversity of wild yeast in the cideries with particular roles in the fermentation processes.

MI-P72.
BIOCHEMICAL CHARACTERIZATION OF CITM RESPONSIBLE FOR THE OXALACETATE DECARBOXILASE ACTIVITY IN *Lactococcus lactis* CRL264

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Only a few numbers of malic enzymes have been studied in bacteria. These enzymes are in some cases associated with operons involved in several cellular processes. In *Lactococcus lactis*, *citM* encodes for a malic enzyme and it is co-transcribed with other genes of the citrate metabolic pathway (*citMCDEFXG* operon). In this work the *citM* enzyme from *L. lactis* was expressed in a heterologic host and was purified. *In vitro* studies let us determinate its oxalacetate decarboxylase activity and the kinetics parameters (Km 1.53 mM, Sp Act 12,6 μmol . min^{-1} . mg of $protein^{-1}$). *E. coli* EJ1321 (*dme, tme, pck*) unable to grow in minimal medium with succinate as the sole carbon source was transformed with the plasmid pQE264 (*citM* is under the control of IPTG). *E. coli* cells grow with succinate as sole carbon source only in the presence of IPTG. Summing up, the obtained results provide evidence to conclude that *citM* gene which is a part of *cit* operon, encodes the malic enzyme with oxalacetate decarboxylase activity necessary to piruvate production in the citrate metabolic pathway in *L. lactis*.

MI-P73.
CAROTENOID PIGMENTS IN RHODOCOCCUS OPACUS PD630

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Rhodococcus opacus PD630 is a pigmented actinomycete bacterium able to produce and accumulate triacylglycerols into intracellular inclusions. The aim of this study was to determine the identity and location of pigments in strain PD630.The production of salmon-pink pigmentation by strain PD630 was constitutive and non-light induced. Different chemical techniques were performed for the identification of pigments. A mixture of different compounds like carotenes (γ- carotene) and oxocarotenoids (xanthophylls) has been detected on the basis of their UV/visible spectra in hexane in comparison with references and thin layer chromatography. Several colourless mutants was produced by chemical mutagenesis suggesting a common biosynthetic pathway for the different kind of pigments in *R. opacus* PD630. Lipid inclusions containing triacylglycerols and cellular envelope were the main site of localization of pigments in strain PD630 as revealed by centrifugation of free-cell extracts in discontinuous glycerol density gradients. Preliminary results suggested that strain PD630 has a sophisticated antioxidant defence system protecting different lipophilic compartments since mixtures of carotenoids are more effective than single compound.

MI-P74.

BIOFILM GROWTH OF *Pseudomonas aeruginosa* MISMATCH REPAIR DEFICIENT CELLS CORRELATES WITH EMERGENCE OF MUCOID AND SMALL-COLONY ADHERENT VARIANTS

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Pseudomonas aeruginosa is an opportunistic human pathogen capable of forming specialized communities known as biofilm. Particularly in Cystic Fibrosis (CF), a biofilm mode of growth is thought to be responsible for persistent lung infections conferring protective advantage against antibiotic therapy, oxidative stress, and immune system attack. Moreover, emergence of mucoid and highly adherent small-colony variants within CF lung have been demonstrated to play a key role in biofilm formation and consequently in *P. aeruginosa* pathogenesis. Recently, the observation of a high proportion of mutator *P. aeruginosa* isolates from CF patients leads to the association between hypermutability and *P. aeruginosa* adaptability. In this work we show new evidences supporting this postulation. By performing an analysis of *P. aeruginosa* growth in continuous-flow culture chamber, a higher frequency of small adherent variants emerged from biofilms formed by a hypermutator *P. aeruginosa* mutant compared with isogenic wild-type strain biofilms. In addition, when biofilms were exposed to sublethal levels of H_2O_2, treatment known to induce the formation of mucoid variants *in vitro*, we observed the emergence of *mutS* mucoid variants at a frequency significantly high respect to the wild-type strain. Our results suggest that hypermutability favor the acquisition of the more adaptable characteristic *P. aeruginosa* CF phenotypes and could explain the high frequency of hypermutability observed in *P. aeruginosa* CF isolates.

MI-P75.

NEW INSIGHTS INTO THE CONTROL OF mAGNESIUM HOMEOSTASIS IN *Salmonella enterica*

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Magnesium plays a fundamental role in *Salmonella* pathogenesis. It controls the activation of the PhoP/PhoQ regulatory system, signaling the bacteria its specific location inside the host. In response to Mg^{2+} limitation, the PhoP/PhoQ regulatory system triggers the expression of two high-affinity Mg^{2+} transporter genes, *mgtA* and *mgtCB*. Mutations in *mgtA* or in *mgtCB* greatly affect the ability of *Salmonella* to grow in low Mg^{2+} environments.
We uncovered a novel checkpoint in the Mg^{2+} deprivation response. This mechanism can detect this cellular stress bypassing PhoQ and posing an additional control over the expression of the Mg^{2+} transporters. We have demonstrated that in *mgtA* the responsive element for the PhoQ-independent regulatory mechanism maps to the 5'UTR. We further characterized this region using site directed mutagenesis, and examined the level (transcriptional/ posttranscriptional) at which this system exerts its control. The study of this homeostatic network will provide new insights in the mechanisms that allow *Salmonella* to proliferate within its host.

MI-P76.

ISOLATION AND CHARACTERIZATION OF A SUCROSE - PHOSPHATE PHOSPHATASE FROM *AGROBACTERIUM TUMEFACIENS*

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Agrobacterium tumefaciens is an α-protebacterium able to induce the crown gall disease in plants. It has a chemoorganotroph metabolism, exploiting a variety of carbohydrates, organic acids and amino acids as carbon sources. Sequence analysis of *A. tumefaciens* C-58 genome revealed the existence of a Glucosyl-Transferase-Domain (GTD) and a Phospho-Hydrolase-Domain (PHD), characteristic of sucrose biosynthesis related proteins present in oxygenic photosynthetic organisms. The *Agrobacterium* PHD is encoded by a 747 bp *orf* whose deduced amino acid sequence shares 22-28 % identity with cyanobacterial and plant sucrose- phosphate phosphatase (SPP), enzyme that catalyzes the last step in sucrose synthesis. The present study describes the first isolation, structural analysis and functional characterization of a prokaryotic SPP gene present in a non-photosynthetic organism. The 747-*orf* expressed in *E. coli* produced a fully active SPP (248-amino-acid). Kinetic, biochemical and molecular properties of the recombinant protein were similar to those of SPP purified from *Agrobacterium* cells. It is a monomeric enzyme as its predicted M*r* (27,262 Da) was similar to the native M*r*. *Agrobacterium* SPP, similarly to orthologous enzymes, is highly specific for sucrose-6-P (K*m* 1.0 ± 0.1 mM) and it is inhibited by common phosphatase inhibitors (fluoride, molibdate and vanadate). Moreover, the conserved sequence motifs identified in all described SPP, are present in *Agrobacterium* SPP. *Supported by CONICET, Fundación Antorchas, Univ. Nac. de Mar del Plata, and FIBA.*

MI-P77.

THROMBOCYTOPENIA DURING THE ACUTE PHASE OF CHAGAS' DISEASE IS INDUCED BY THE TRANS-SIALIDASE

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Thrombocytopenia and bone marrow hypoplasia have been described during the acute phase of Chagas' disease. At present there is no molecular explanation for these observations. The parasite sheds an enzime, the *trans*-sialidase (TS), which transfers sialic acid residues between glycoproteins. By another hand, it is known that the sialic acid content of the platelet surface is related to their rapid removal from circulation. Also, platelets were involved in parasite clearance. Here we investigated whether the TS shed by the parasite is associated with the thrombocytopenia. The iv. administration of 10 µg of TS induces transient thrombocytopenia and the passive transfusion of pre-treated platelets results in their rapid removal from blood. Both experiments were reproduced in splenectomized mice, suggesting that Kuppfer cells are the major responsible of platelet clearance. To test this hypothesis, mice were depleted from phagocytes with clodronate-containing liposomes. The absence of thrombocytopenia in these mice, after TS injection, demonstrates the active participation of Kuppfer cells in the removal of altered platelets by the enzime action. Finally, a correlation between the percentage of desialilation of platelet surface and their disappearance from blood was observed. All this results contribute for the comprehension about the molecular mechanisms involved in Chagas' disease pathogenicity.

MI-P78.
PROTECTIVE ACTION OF ppGpp ON MICROCIN J25 SENSITIVE STRAINS

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Stringent response is mediated by the alarmone ppGpp. Many reports have shown that ppGpp has a significant role in growth rate control and gene expression during stationary phase. Sensitive strains exhibit an increased resistance to the peptide antibiotic microcin J25 in stationary phase. This result led us to suppose that accumulation of ppGpp during stationary phase could have a protective action against the antibiotic. To test this hypothesis we transformed *E. coli* AB1133, which is hypersusceptible to microcin J25, with a plasmid that overproduces ppGpp (pALS13). We observed that for AB1133 colony counts dropped five orders of magnitude, while AB1133 (pALS13) remained unaffected. The experiment was repeated using another ppGpp-overproducing plasmid, pCNB0209R, with similar results. A direct interaction of ppGpp with the microcin molecule can be ruled out, since extracts of ppGpp-overproducing strains did not exert any protective effect against microcin *in vitro*. It is known that MccJ25 targets RNA polymerase and reduces transcription. Therefore, we did *in vivo* transcription experiments, using AB1133 y AB1133 (pALS13) in the presence and in the absence of microcin. In contrast to the control, microcin had no effect on transcription activity in the strain harboring the ppGpp-overproducing plasmid. We conclude that ppGpp, either directly or indirectly, protects RNA polymerase from inhibition by microcin J25.

MI-P79.
DEVELOPMENT OF A NEW SYSTEM FOR *Babesia bovis* STRAIN IDENTIFICATION USING MOLECULAR MARKERS

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Prevention against the bovine hemoparasite *Babesia bovis* is achieved by vaccination with attenuated strains. Vaccine failures could be attributed to vaccine mishandling or to infection with field strains against which the vaccinial strains are not protective. Strain-specific molecular markers could be useful tools to discriminate this and, also, they could serve for epidemiological studies. The *B. bovis* Variable Merozoite Surface Antigen-2 (VMSA) family is encoded by five genes in the Mexican Mo7 strain, namely *msa-1*, $-2a_1$, $-2a_2$, *-2b* and *-2c*. Homologues of some of these genes are present in the Argentine strains R1A, S2P and M1A and the Texan strain T2B. We hypothesized that polymorphism among members of the VMSA family could be reflected in different restriction patterns. We tested our hypothesis by PCR-amplifying $msa\text{-}2a_1$, a_2 and *b* from DNA of the Mo7, R1A, S2P, M1A and T2B strains. PCR-products were digested with *BsPM*I and analyzed by polyacrylamide gel electrophoresis followed by silver staining. The results showed that all strains produced differential banding patterns. These data strongly suggest that these genes may be adequate molecular markers for strain comparison. Analysis of a larger number of samples from cattle from different regions of Argentina and its neighboring countries is now under way.
Supported by ANPCyT and Fundacion Antorchas, Argentina.

MI-P80.
NATIVE FLUORESCENT *Pseudomonas* AS BIOCONTROL AGENTS OF ALFALFA SEEDLING DISEASES

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Alfalfa plays an important rol in the Uruguayan agriculture. Seedling diseases caused by soilborne fungal pathogens affect alfalfa establishment. Biological control of these pathogens using native fluorescent *Pseudomonas* can be an excellent alternative. A collection of fluorescent *Pseudomonas* isolates was obtained from the rhizosphere of alfalfa and its antagonistic activity *in vitro* against *Pythium debaryanum* was evaluated. We found that 5.1% of isolates from Colonia, 5% from Paysandú and 25.5% from Tacuarembó were antagonist. The presence of biosynthetic loci for antibiotics with biocontrol activity found in fluorescent *Pseudomonas* was screened by PCR. Only 5% of isolates exhibit these biosynthetic loci. Production of biosurfactant compounds, screened by drop collapse test, was observed in 52% of isolates from Colonia, 60% from Paysandú and 40% from Tacuarembó. Growth chamber assays conducted to evaluate the ability of selected isolates to suppress damping-off *in vivo* revealed that 33% are able to protect alfalfa against *P. debaryanum.* Selected isolates were analyzed by fingerprinting using rep-PCR with primers BOX and ERIC. Distinct genomic fingerprints were observed, whit little correlation regarding phenotypic characters. The development of a bacterial inoculant, based on antagonistic strains, will have a relevant impact on alfalfa establishment.
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MI-P81.
GENETIC AND PHENOTYPIC STABILITY OF NDV ISOLATES

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In 1997, Argentina was declared virulent Newcastle Disease Virus (NDV)-free for commercial poultry. However, the disease has a global distribution with a wide host range, and wild birds are considered natural reservoirs for the virus. The difference in virulence between strains is determined primarily by the fusion (F) protein cleavage site aa sequence (less virulent has fewer basic aa) and, by the size of the viral hemagglutinin-neuraminidase (HN) protein. The objective of the present study was to evaluate the stability of NDV isolated from wild birds, during sequential passages in chicken embryo. The isolates were inoculated in the allantoic cavity of embryonated SPF chicken eggs. After incubation for 5 days, the eggs were chilled and the allantoic fluids collected and inoculated as described up to 20 passages. La Sota, which is an NDV low virulent strain, was included in the experiment as a control. The F protein cleavage site amino acid sequence changed from [112]**GKQGRL**[117] to [112]**GRQKRF**[117] after 6/7 passages. The HN C-terminal nucleotide sequence changed from a 616 aa HN to a 571 aa HN after 20 passages. Mean Death Time (MDT) of 83 hs was obtained from viruses after 20 passages whereas original isolates couldn´t kill all the embryos inoculated in the same test. F protein cleavage site, HN C-terminal and MDT of La Sota strain were not modified after twenty passages. This results showed an increment in virulence of NDV isolated from wild birds after these viruses were passaged in a different host.

MI-P82.

MOLECULAR DIAGNOSIS AND TYPING OF *T. CRUZI* IN DIFFERENT EPIDEMIOLOGICAL AND CLINICAL SCENARIOS

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Infection by *T. cruzi* was diagnosed, monitored and characterized by PCR-based techniques in different settings; I) congenital transmission, II) etiological treatment, III) parasitic reactivation due to immunosupression IV) re-emergence of infection in domestic-peridomiciliary vectors of an endemic area under surveillance. Molecular typing was done by lineage-specific PCR, cross-hybridization of amplicons with radiolabeled probes, LSSP-PCR, RFLP-PCR and sequencing. Results: I) We observed major incidence of congenital transmission from pregnant women with positive PCR results ($p<0.05$). The parasitic signatures were similar within each pair of mother-newborn and different among cases of different endemic areas, II) 16 infected infants, 24 children and 60 adults were followed-up after benznidazol treatment; 100% infants, 87.5% children but 60% adults remained persistently PCR negative during 3 years of post-treatment monitoring ($p<0.05$). III) The reactivation of two chagasic patients who received orthotopic heart transplantation was detected 30-42 days earlier by PCR than by routine monitoring. Chagasic encephalitis was diagnosed by PCR in brain biopsy of an AIDS patient with presumptive diagnosis of toxoplasmosis, who improved following benznidazol treatment. The parasitic profiles were characterized. IV) PCR analysis of faeces from *T.infestans* collected in domestic and peridomestic sites from Amamá showed infection by *T.cruzi* group II strains.

MI-P83.

STUDY OF QUINONE OXIDOREDUCTASE (QOR) ACTIVITIES PRESENT IN *TRYPANOSOMA CRUZI* AND THEIR ACTION ON LIPOPHILIC *O*-NAPHTHOQUINONES WITH CYTOTOXIC PROPERTIES

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Lipophilic *o*-naphtoquinones such β-lapachone, are endowed with antibacterial, antifungal, trypanocidal and cytostatic properties. The *o*-naphthoquinones redox cycling in the presence of reductants and oxygen yields "reactive oxygen species" (ROS) including O_2, HO•, and H_2O_2, whose cytotoxicity explains the *o*-naphthoquinones lethal effects in cells. The first step in ROS generation is either the one electron or the two electron reduction of the quinone to semiquinone or hydroquinone respectively, catalized enzymatically by the "quinoneoxido reductases" (QOR). We have investigated the presence in *T.cruzi* of QOR enzymes that can account for that activity. Cell fractionation of epimastigotes of *T.*cruzi, by silicon carbide grinding or by sonication showed that most of the QOR activity, measured with NAD(P)H as electron donor and 9,10 Phenanthrenequinone as electron acceptor, was in the soluble fraction while at least 10% of the total activity was in the membrane-bound fraction. Digitonine treatment of intact cells suggested the presence of at least three QOR activities with different subcellular localizations: cytosolic, microsomal and the third with un unique localization since it is liberated from the cell at a digitonine concentration considerable lower than the one necessary to liberate the cytosolic proteins. In addition, searching on the *T.cruzi* draft genome we were able to find two genes encoding a ξ cristalline protein with QOR activity.

MI-P84.

STUDIES ON CAROTENOID PIGMENT PRODUCTION BY *Rhodotorula. mucilaginosa* CRUB 0138. EFFECT OF C/N RATIOS

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The carotenoid pigments (â-carotene, torulene, etc.) produced by certain yeast species possess potential biotechnological aplications in the cosmetic, farmaceutic and alimentary industries. The *Rhodotorula mucilaginosa* yeast strain CRUB0138 isolated from a northwestern patagonian high altitud lake (1700 m.a.s.l.) was shown to be a good biomass and carotenoid pigment producer at lab scale using semi-synthetic media at low glucose concentrations (1%). Here we studied the behavior of this strain employing higher glucose concentrations (4%) and the effect of different C/N (5, 20 and 100) ratios on biomass and carotenoid pigments yields. Assays were carried out in an incubator shaker INNOVA 4000. Analytical measurements consisted in dry weight, remaining glucose, pH and carotenoid pigment content. The extraction of the pigments was achieved using the DMSO method and their were quantitatively and qualitatively analyzed through spectrophotometer and TLC based tests. High biomass volumetric productivity was obtained using low C/N ratios (5), however, carotenoid pigment yields nor their composition were significantly altered by the different C/N ratios tested. A maximum of carotenoid pigments was achieved (2,32 mg L^{-1}) at 96 hs of culture using a C/N of 5 and 4% of glucose.

MI-P85.

EVALUATION OF cDNA LIBRARIES OF THE EUGLENOID *Euglena gracilis*

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Directional cDNA libraries were constructed from mRNA of the commercial strain *E. gracilis* UTEX753 (U). One thousand *Expression Sequence Tags* (EST's) of an average size of 600bp. were generated. By searching on public databases similarities with several genes were found: 20% with photosynthetic genes, 2% with transcription / translation factors, 11% with membrane and cellular structure genes, 3% with metabolism genes, 3% with mitochondria and 2% with ribosomal proteins. Furthermore, we came up with a 12% of similarities with ESTs and a 47% with unknown identity. Microarrays were constructed for the study of gene expression changes in *E. gracilis* grown in the presence of different stress environmental conditions. The purified and amplified ESTs were fixed on mirror slides with the aid of a Generation III microarray spotter. Four hybridization conditions with RNAs labelled with *Cy5/Cy3* were assayed: i)U/wild type strain (MAT1), ii)U/U grown with streptomycin supplementation, iii)U/U grown in the dark, and iv) U/U grown in the presence of chromium. A dual laser scanner was used for the generation of images. Our results, using ArrayVision software (Imaging Research Inc.), indicated that 92, 75 and 153 clones from arrays ii, iii and iv respectively showed changes on the expression level with respect to the control. The sequencing and expression data obtained on this work are the first molecular data generated from the nuclear genome of *E. gracilis* and the starting point for future comparative studies among Euglenozoa.

MI-P86.

CHARACTERIZATION OF A COMBINATORIAL PHAGE DISPLAY LIBRARY DERIVED FROM A CCHD PATIENT

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Chronic Chagas´ Heart Disease (cChHD) patients present antibodies against the *Trypanosoma cruzi* ribosomal P proteins (anti-P Abs) which are able to stimulate cardiovascular receptors. To better understand this complex response we constructed a Fab combinatorial library from bone marrow from a cChHD patient. The size of the library was >1 x 10^8 cfu/μg. The phage library was panned against *T.cruzi* lysate and P proteins obtaining specific clones (T and P respectively). The reactivity profile of anti-T Fab resembled the one observed for the serum of patient. Furthermore, we analyzed the repertoire and distribution of rearranged VH genes to elucidate similarities to and differences from autoimmune processes. We sequenced 93 clones from the unselected library, 36 anti-T and 56 anti-P clones. The VH family usage in the library and amongst the T-clones was not different than in healthy adult repertoire. However, there was a VH4 overrepresentation in P clones, possibly indicating an autoimmune component in the anti-P response. The CDR3 regions were significantly longer in the unselected and panned libraries than in healthy repertoire. Analyzed Abs present a high degree of somatic mutation. In this study, we report the characterization of the first combinatorial antibody library derived from a patient with cChHD and an analysis of its repertoire. This approach may help to better understand the humoral response against the parasite and to obtain human monoclonal Abs against different *T. cruzi* antigens.

MI-P87.

ISOLATION AND CHARACTERIZATION OF A BACTERIOPHAGE FOR PATHOGENIC *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is a rod-shaped, motile, Gram-negative bacteria. Lung infection with this bacterium is the major cause of mortality for patients suffering cystic fibrosis. The attempts to eliminate the bacteria from lungs with antibiotics were not always successful and antibiotic multiresistent *P. aeruginosa* emerges, abating patient's health. Bacteriophages are viruses that infect bacteria. In the first decades of the twentieth century they were used as antibacterial agents, using a procedure called phagetherapy. Later phagetherapy lost importance against antibiotic based treatment of bacterial infections. However, a few years ago the phagetherapy's importance increase as multiresistent bacteria appears. In this work we show the isolation, from environmental samples, and the characterization of a new bacteriophage, specific for a clinical isolate of *P. aeruginosa*, called Wilde1. Using plaque-cloned and purified samples of Wilde1, we studied its biological action, optimized its virulence *in vitro* and analyzed its host range. Furthermore, we characterized its morphology by TEM and its genome at molecular level. The genome is contained into a unique double stranded linear DNA molecule of approximately 50 kbp. With the collected data Wilde1 was included in the family *siphoviridae* of the order *caudovirales*. Finally, we design an *in vivo* assay to determine its potential use in phagetherapy against *P. aeruginosa* infecting cystic fibrosis lungs.

MI-P88.

COMPLEXITY OF P PROTEINS INTERACIONS IN THE RIBOSOMAL STALK OF *TRYPANOSOMA CRUZI*

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In all eukaryotic cells, ribosomal P proteins form a lateral protuberance, which is directly involved in translation process. The number of classes and subclasses of P proteins is variable from species to species. In *Trypanosoma cruzi*, P1, P2α and P2β genes have been cloned. Interactions among these P proteins were evaluated by native PAGE and yeast two hybrid assays, showing strong interaction between P1 and P2β. However, the spatial arrangement of the pentameric P complex remains uncertain. Here we report evidences for the presence of other P proteins. Western blot assays of *T cruzi* lysates developed with a monoclonal antibody against the conserved C terminal peptide (R13) showed four bands in the 14-20 kDa range. One of these bands was identified as P2β by a specific monoclonal antibody. Moreover, algorithms corresponding to the consensus C terminal region of P proteins were used to search in the last version of the nucleotide TIGR database. This search showed the presence of at least one new class of P protein in a locus containing two copies in tandem. The new P protein was found in the EST database suggesting that this gene is expressed, at least at mRNA level. The protein sequence identity is 40% with P1, 35.8% with P2α and 28.8% with P2β.

MI-P89.

CHARACTERIZATION OF CIT, A NEW MEMBER OF THE TRANSCRIPTIONAL ACTIVATOR FROM *Weissella Paramesenteroides*

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In Lactic acid bacteria the genes involved in the citrate transport and catabolism are organized in operons. We previously demonstrated that transcriptional regulation of the citMCDEFGP operon (cit operon) in W. paramesenteroides, is mediated by CitI. CitI is member of the transcriptional regulator protein family SorC and it is expressed from a bi-directional extraordinary A-T rich promoter region located upstream cit operon. Gel shift and footprinting assays revealed that CitI recognizes at least three operator sites in the cit operon DNA sequence. The binding of the regulator to DNA is independent of the presence of citrate. However, *in vitro* transcriptional studies using the intergenic region, citI and the RNApolymerase from E. coli, suggest that citrate increase the transcription from both promoter region, PcitI and Pcit. Site directed mutagenesis and transcriptional fusion techniques allowed the characterization of the DNA binding site of CitI.

MI-P90.

TURNOVER OF RIBOSOMES IN THE HALOALKALIPHILIC ARCHAEON *Natronococcus occultus*

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Protein synthesis is in part regulated throughout ribosome turnover. Thus, external stimuli of cellular protein demands modify ribosome concentration, more than changing translational velocity. A better assessment of protein turnover in the haloalkaliphilic archaeon ***Natronococcus occultus*** requires an analysis of ribosome synthesis and breakdown. These parameters were assessed *in vivo* by labelling of rRNAs with ^{3}H-orotic acid and ^{14}C-uracil. The synthesis of rRNAs was maximal during exponential growth and minimal at stationary phase. The synthesis of mRNAs followed a similar behaviour, but the difference between exponential and stationary stage values was higher than that observed for rRNAs. The degradation of rRNAs was in inverse relationship with synthesis. Thus, it was low during early exponential growth and increased thereafter.
The ribosome content in the different growth phases was also estimated. It increased dramatically during the exponential growth phase while decreased thereafter. These data were coherent with their corresponding synthesis and degradation rates. In addition, the amount of protein associated with the translational system decreased with the age of the culture in the same way that the ribosome concentration. These regulatory mechanisms are similar to those displayed by eubacteria.
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MI-P91.

A PHOSPHORYLATED NON-ANTENNA COMPLEX PROTEIN IS PRESENT IN *Rhodovulum sulfidophilum* PURIFIED MEMBRANES

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In *Rhodovulum sulfidophilum* intracytoplasmic membranes appears a low molecular weight protein (6 kDa aprox, concordant to the antenna complex proteins), that is phosphorylated *in vivo* under different condition. We analyzed the phosphorylation in three strains: the wild type strain *Rhodovulum sulfidophilum* W4, an LHII- mutant EK281 and the LHI- mutant rsLRI constructed in our laboratory. All of them showed the phosphorylated protein, determining that this protein did not correspond to any antenna complex protein described until now. To determine if this protein is associated to one photosynthetic complex in particular, we treated the purified membranes with a non ionic detergent (LDAO) and separated the different complexes with a sucrose gradient. Three fractions were obtained: the heaviest corresponding to a fraction enriched in CR-LHI complex (fraction 1) the second corresponding to the LHII complex (fraction 2) and the lightest (fraction 3) corresponding to a heterogenic fraction that could contain the intracytoplasmic growing initiation sites. The phosphorylated protein appears only in this lightest fraction.

MI-P92.

CHARACTERIZATION OF THE VIPER'S RETROELEMENT FAMILY OF *T. cruzi.* GENOME

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The Vestigial IntersPersEd Retroelement, VIPER, is a retrotransposon of *Trypanosoma cruzi*. This is 2,359 bp long fragment that has been isolated from the λZapII genomic library. VIPER's 5´ and 3´ end consist of the first 182 bp and last 226 bp of SIRE (Short Interspersed Repetitive Element) respectively. Both SIRE's moieties are connected by a 1,924 bp long fragment that carries a unique ORF encoding reverse transcriptase and RNase H motive. The last 15 C-terminal amino acids derive from codons specified by SIRE's moiety 3'. The amino acid sequence of this protein share significant homology with the RT-RNAse H genes of differents retroelements. By performing public databases searching, new VIPER-like elements were found, with similar structure and an approximately size of 4 kb. From these sequences a longer ORF was deduced, that also confirm the RT and RNAsa H motifs described for VIPER. A CCHC motif can be use to define a probable reading frame for a gag protein. One of these new elements were found on a 123kb. fragment of the *T. cruzi* chromosome 3 that is syntenyc with genomic regions of *T. brucei* chromosme 1 and *L. major* chromosome 12.
Analysis of the RT-RNAse H motifs and the insertion sites of these elements led to the confirmation of its definition as a LTR-like retrotransposon, different to the non–LTR described for *T. cruzi* up to date.

MI-P93.

CONSTRUCTION OF ARTIFICIAL CHROMOSOMES FOR *TRYPANOSOMA CRUZI*

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Available vectors for stable transfections in *Trypanosoma cruzi* include those maintained as circular episomes and those that integrate into the endogenous ribosomal *locus*. To investigate chromosomal behaviour in this parasite, we have developed several linear vectors that stably function as *T. cruzi* Artificial Chromosomes (TACs). We have previously reported the construction of pTAC and pTACodc: these vectors generated linear molecules that were stably maintained even in absence of selective pressure. Cell lines transfected with pTACodc, which carries the ornitin decarboxilase gene from *C. Fasciculata*, could grow in SDM79 medium reverting the natural auxotrophy of *T. cruzi* for polyamines. In the present work, we have replaced the odc gene in pTACodc by a modified version of GFP with an internal EcoRI site to simplify the selection process of transfected lines. This feature makes the new vector, pTACgfp, a very interesting cloning tool. As the previous vectors, pTACgfp retains the genes for resistance to G418 and puromycin in opposite orientation. Clones grown in presence of both antibiotics showed an adequate level of fluorescence that persisted for more than 60 generations when the selective pressure was relieved. Interestingly, TACs seems to increase its molecular weight with sucesive generations. Finally, we have cloned a large EcoR I DNA fragment (aprox. 80 kb) from *Trypanosoma brucei* at the EcoRI site of pTACgfp .The transfected lines are currently under selection.

MI-P94.

SCREENING AND CHARACTERIZATION OF ALKALINE PROTEASE PRODUCING BACTERIA FROM PATAGONIA ARID SOILS, ARGENTINA

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A screening program for alkaliphilic bacteria producers of extracellular proteases was performed using samples from Patagonia arid soils. Isolate PAT 05, a gram-positive sporulating bacterium, was selected for proteolytic activity/stability properties. Its 16S rDNA gene sequence had the closest match (96% homology) with that of *Bacillus clausii*. This isolate grew at pH between 7 and 10 with an optimum around pH 9-10, and with up to 20% (w/v) NaCl at pH 10. The range of temperature for growth was from 5 to 40°C, with an optimum around 20°C. Optimal proteolytic activity was detected in the range of pH between 9-13 and the optimal temperature showed to be around 60°C. SDS-PAGE and casein zymogram analyses confirmed two protein active bands. Purification steps included supernatant protein precipitation with ammonium sulfate (70%) and desalting by Sephadex G-25 column chromatography. According to the pI value of the active fraction (>9.3), the desalted eluate was partially purified by cation exchange chromatography (CM-Sepharose Fast Flow equilibrated with 20 mM Tris-HCl buffer, pH 8.0, containing NaCl).

MI-P95.

CONSTRUCTION, SELECTION, EXPRESSION AND REFOLDING OF SINGLE CHAIN ANTIBODIES DERIVED FROM A MONOCLONAL ANTIBODY AGAINST TCP2ß OF *T.CRUZI*

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Patient sera with chronic Chagas heart disease show high levels of antibodies against the *T. cruzi* ribosomal P proteins, in particular against the acidic C terminal region. These antibodies exhibit high affinity for these peptides and can cross react with the acidic regions of the second intracellular loop of the cardiac Beta 1 Adrenergic receptor (BAR) and muscarin collinergic receptor with ability to induce chronotropic alterations in cardio miocytes cultures. Mice immunization with recombinant TcP2β protein allowed us to obtain the monoclonal antibody 17.2 specific for the last 13 amino acid with functional activity against BAR. We report here the construction of 4 different types of single chain recombinant antibodies in the pHEN 2 vector, selection of reactive clones by phage display, expression of soluble antibodies in the periplasmic space and refolding assays to recover reactivity in ELISA test and Western blot. We plan to use this cloning strategy to obtain recombinant antibodies with punctual mutations by random mutagenesis, emulating specific maturation in recognizing cardiac receptors.

BT-P1.

PURIFICATION OF RECOMBINANT PEROXIDASE FROM INSECT CULTURE MEDIUM BY LECTIN CHROMATOGRAPHY

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One of the most appealing features of baculovirus expression system has been the eucaryotic protein processing capabilities of the host. Accordingly, these systems are widely considered to be excellent tools for recombinant glycoprotein production. However, typical insect cell lines have only a limited capacity to produce complex *N*-glycans. The insect pathway includes all the enzymes involved in *N*-glycan trimming, but few of the enzymes involved in *N*-glycan elongation. These minimally elongated structures known as paucimannose structures (Man_3-$GlcNAc_2$-N-Asn). Recombinant horseradish peroxidase (a glycoprotein) was expressed at 40 mg/L in Sf9 culture medium. In this work, we describe the purification of the enzyme on ConA-agarose. ConA is a lectin that presents high affinity by mannose residues. After conditioning into equilibration buffer, enzyme was successfully adsorbed on the matrix in batch mode. A purification yield of 94-98% was obtained with 0.4M mannose in the elution buffer. A purification factor of 29-33 in one step was achieved. The fetal bovine serum presents in insect cell medium complicated the purification strategies commonly used, so lectin chromatography is a good choice for selective purification of recombinant glycoproteins.

BT-P2.

CYCLODEXTRIN GLUCOSYLTRANSFERASE: PURIFICATION IN AQUEOUS TWO-PHASE SYSTEMS

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The partition behavior of Cyclodextrin Glucosyltransferase (CGTase) from *Bacillus circulans* DF 9R in aqueous two-phase systems (ATPSs) was examined for simultaneous extraction-purification of the enzyme from culture broth. Different ATPSs composed by polyvinylpyrrolidone (PVP), hydroxypropyl starch (Reppal), polyethyleneglycol (PEG), sodium and potassium phosphate, sodium citrate and magnesium sulphate at pH 7.0 were assayed. In PVP/Reppal systems, the CGTase partitioned to the bottom phase. In PEG/phosphate systems no changes in CGTase partition coefficient were observed with the increment of PEG molecular weight (K_{CGT} PEG600=2.31, K_{CGT} PEG1000=2.63, K_{CGT} PEG4000=2.0, K_{CGT} PEG8000=1.77, K_{CGT} PEG10000=2.24). Suitable conditions for enzyme purification were found in PEG 4000/phosphate at pH 7.0 where a clear upper phase containing the partially purified enzyme was obtained (PF: 5.63, Y% : 94). Also PEG/sulphate and PEG/citrate systems were suitable for enzyme purification, displaying similar partition coefficients into the top phase. The enzyme could be extracted from the upper phase of PEG4000/phosphate system by the addition of fresh phase of magnesium sulphate and different proportions of Reppal. The use of 0.020 g/ml Reppal $_{200}$ led the enzyme to concentrate into the bottom phase (K_{CGT}: 0.36). Based on these results a two-step purification method was designed that allows 71% recovery at high purity enzyme, PF: 37.

BT-P3.

SYNTHESIS OF NUCLEOSIDES ANALOGUES USING IMMOBILIZED WHOLE CELLS

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Whole immobilized cells have been used as efficient biocatalysts in the production of many pharmaceutical compounds.
Sugar and/or base modified nucleosides have been extensively employed in cancer and antiviral therapies and, more recently, for antisense strategies. Traditionally, nucleosides are prepared by chemical methods that often involve difficult, inefficient and time-consuming multistage processes. Microbial preparations of both natural and unnatural nucleosides are an alternative methodology, which offer regio and stereoselectivity and simple experimental conditions.
Recently, we have demonstrated the high efficiency of immobilized *E. coli* BL21 for the synthesis of adenosine. The results showed that this biocatalyst maintained high activity for long periods both at 4ºC and 37ºC and previde good reuse capacity and high productivity. In this work the results obtened using different immovilized biocatalysts for the synthesis of nucleosides analogues with optimal yield are shown.

BT-P4.

CYCLODEXTRINS SYRUP OBTAINED FROM STARCH BY ENZYMATIC CONVERSION

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Cyclodextrins (CD) are cyclic oligosaccharides containing six (α), seven (β) or eight (γ) glucose units α-1,4 linked made from starch by cyclodextrin glucanotransferase (cgtase).They are able to form inclusion complexes with many hydrophobic molecules, changing their physical and chemical properties. for this reason, CD are used in food industry for stabilizing flavors, masking odors, emulsifying oils and protecting substances from decomposition induced by light, temperature and air.
In this work, an enzymatic process was developed for the production of CD syrup from starch. the enzyme was produced by bacillus circulans df 9r, isolated in our laboratory, purified by starch adsorption and preserved by lyophilization.
The production of CD was optimized using experimental designs. the fixed conditions were 5% (w/v) cassava starch, 20 ue/g of starch, ph 6.4, temperature 56°c and 90 minutes of reaction under continuous shaking (100 rpm). the conversion of cassava starch to CD achieved was 22.8% (w/w).
The product was concentrated under reduced pressure. the syrup obtained was analyzed by hplc and the ratio of α: β: γ -CD was 0.65 : 1.00 : 0.18.

BT-P5.

MILK CLOTTING AND PROTEOLYTIC ACTIVITIES OF PLANT PROTEASES - A COMPARISON TO CHYMOSIN

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The milk clotting and proteolytic activities of a new plant protease, named onopordosin, isolated from *Onopordon achantium* L. (Asteraceae), have been determined and compared to those of chymosin using cow and goat milk. Fresh flowers were grinded under liquid nitrogen, suspended in 0.1 M citrate buffer at pH 5.5 and centrifuged at 10.000*g* for 5 min (crude extract). Clotting activity was measured on both bovine and caprine milk at 30ºC and pH 6.5. The substrate (10 ml) was mixed with 0.2 ml of crude enzyme extract. Aliquots (100 μl) were taken at different times, added to 700 μl of 6M urea and 3 % β-mercaptoethanol, and then mixed with an equal volume of denaturing electrophoresis buffer. Tricine-SDS gels were used, as they are suitable to resolve mixtures of small peptides. Characteristic and differential proteolytic patterns on the main casein components (α_{s1}, α_{s2} and β fractions) were obtained for each substrate. After 10 min minimum degradation rate was observed for α_{s2}, α_{s1} and β caseins for both substrates. New peptides appeared in all cases after 60 min (20 kDa, 16 kDa and 14 kDa). The patterns could be correlated with the modification of functional properties and the production of bitter peptides.

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BT-P6.

HIERONYMAIN II, A NEW PEPTIDASE FROM FRUITS OF *BROMELIA HIERONYMI* MEZ (*BROMELIACEAE*)

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The best known family of cysteine peptidases is the papain family, which contains endopeptidases with broad specifity (such as papain), or a narrow specifity (such as glycyl endopeptidase), aminopeptidases, and peptidases with both endo- and exopeptidase activities. Most plant cysteine peptidases belong to the papain family, including those of Bromeliaceae. From unripe fruits of *Bromelia hieronymi* Mez (*Bromeliaceae*) a new peptidase, named hieronymain II, was purified to homogeneity by acetone fractionation followed by anionic exchange chromatography (FPLC) on Q- Sepharose HP and rechromatographied employing the same exchanger but different saline gradient. Homogeneity of the enzyme was confirmed by isoelectric focusing-zymogram. Hieronymain II is a basic peptidase (pI 8.3) and its molecular mass was 25 kDa (SDS-PAGE). Maximum proteolytic activity on casein (more than 90% of maximum activity) was achieved at pH 7.5-9.0. The enzyme was completely inhibited by E-64 and iodoacetic and activated by the addition of cysteine; these results strongly suggest that the isolated protease should be included within the cysteine group.

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BT-P7.

PRODUCTION OF RECOMBINANT ANTIBODIES TO GENERATE SPECIFIC ENZYME INHIBITORY ANTIBODIES

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In addition to conventional antibodies, camelids produce unusual immunoglobulins devoid of light chains. Their binding site is formed solely by one variable region (VHH). The binding strategies of these antibodies are very particular, their CDR3 region forms long extensions that can extent into cavities on antigens, e.g. the active site crevice of enzymes. Thus VHHs are a suitable fragment for the development of enzyme inhibitors.

We chose the Brucella Lumazine synthase (LS) as a target due to its high inmunogenicity and its use as carrier to elicite antibodies against others peptides or proteins.

Llamas inmunized with LS elicited high antibody titers. The cDNA isolated from lymphocytes was used as template to generate a VHH library composed of 6x106 transformants. Phage display was used for panning and screening of the library, getting an enrichment of binders through consecutive rounds of panning. Five binder clones were isolated and soluble proteins produced. Characterisation of these clones by sequencing, affinity measurements and inhibitor displacement assays will be discussed.

BT-P8.

CONSTRUCTION AND CHARACTERIZATION OF RECOMBINANT CANARYPOXVIRUSES EXPRESSING THE E2 PROTEIN OF BOVINE VIRAL DIARRHEA VIRUS OR THE gD PROTEIN OF BOVINE HERPESVIRUS-1

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Poxviruses have been used as expression vectors for a variety of foreign genes inserted into non-essential regions of the virus genome by homologous recombination. Canarypoxvirus (CaPV) is a member of the avipoxvirus genus. It has been demonstrated that recombinant avipoxviruses inoculated into non-avian cells can express foreign genes in the absence of productive viral replication and that their inoculation into mammals elicit protective immune responses. The main goal of our project is the development of recombinant vaccines to prevent diseases in cattle using non-replicative CaPV vectors. Previously, we showed that *CaPV-PC1* gene is non-essential for viral replication in cell culture and that can be used as a suitable target site for the obtainment of recombinant CaPV. Here, we report the construction and characterization of two canarypox virus-based recombinants, Ca-GUSE2 and Ca-GUSgD, each expressing the *uid A* gene*,* as a reporter gene, and either the *E2* gene of bovine viral diarrhea virus (BVDV) or the *gD* gene of bovine herpesvirus-1 (BHV-1), interrupting the *CaPV-PC1* gene. The integration of these genes into the CaPV genome was corroborated by PCR and Southern blot and their transcription was confirmed by Northern blot. Finally, the viral replication of the recombinants and the genetic stability of foreign genes were characterized in cell culture. In the future, the capability of both CaPVs recombinants to elicit specific immune responses will be evaluated in mice.

BT-P9.

HETEROLOGOUS GLUCOSE OXIDASE PURIFICATION BY IMMOBILIZED METAL ION AFFINITY CHROMATOGRAPHY

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Immobilized metal ion affinity chromatography (IMAC) is now a widely accepted technique for the purification of natural and recombinant enzymes and is beginning to find industrial applications. The effects of the pH, composition and ionic strength of the chromatographic buffer and immobilized metal on the purification of fungal heterologous glucose oxidase were studied using IMAC. A recombinant strain of the fungus *Aspergillus nidulans* were used to produce the enzyme. This work reports a study of the effect of above variables on the performance of the purification system, and the determination of the relevant parameters for the process scale-up by means of batch equilibrium isotherms and breakthrough curves.

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BT-P10.

CHARACTERIZATION OF THE CHOLESTEROL DESATURASE COMPLEX FROM TETRAHYMENA THERMOPHILA

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Two cholesterol desaturase activities that convert cholesterol into D7 and D22 dehydrocholesterol were isolated in Tetrahymena microsomal fractions. Both desaturases react on cholesterol forming the double unsaturated derivative 7,22 bisdehidrocholesterol as the major product in live cultures. We have characterized both enzyme activities present in the microsomal fractions with respect to substrates, cofactors and lipids requirements. From the saturation curve for cholesterol, we have calculated a Km = 30 uM and Vmax = 0.087 nmol/min for delta 7. The stability of the desaturase was assayed with respect to temperature and pH. Optimal conditions were 30ºC and pH 7.2-7.4. Both enzymes have an absolute requirement for oxygen and reduced pyridine nucleotides and are inhibited by cyanide. The microsomal fraction also displays NADH-Cytochrome b5 reductase activity and contains cytochrome b5, both required for the enzymatic activities. The desaturases were solubilized from microsomal membranes by means of a detergent and their activities reconstituted in artificial liposomes.

Similar properties with respect to cofactors, oxygen and cytochrome dependence has been observed in 4-methyl sterol oxidase and D5 sterol desaturase in plants, mammals and yeast. We assume therefore that the desaturase complex from Tetrahymena is microsome-bound and consists sequentially of NADH-Cytochrome b5 reductase, cytochrome b5 and the final oxidase.

BT-P11.
HYDROLYSIS OF CASEINS BY EXTRACTS OF SILYBUM MARIANUM FLOWERS

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The flowers of *Cynara* spp. (*Asteraceae*) contain aspartic proteinases and for this reason they are used in the Iberian Peninsula for curd formation in the production of farmhouse cheeses. In this work the proteinases present in flowers of *Silybum marianum* (L.) Gaertn. (*Asteraceae*) were characterized in terms of specificity toward caseins from milks of different species.
Proteolytic enzymes were extracted from N_2-frozen fresh flowers at pH 3.0. Gel filtration on Sephadex G-25 was needed to remove non protein low molecular weight contaminants. Whole caseinates (substrates) were prepared via isoelectric precipitation and freeze-drying of bovine, caprine and ovine milks. The substrates were dissolved at 10 g/l and pH 6.5. The reactions between the proteolytic enzymes and the caseinates were quenched at different times by addition of trichloroacetic acid. Urea-PAGE and their densitograms were employed to analyzed the profile of hydrolysis of caseins. Bovine β-casein was gradual and partially degraded during 5 hours of hydrolysis, bovine α_{s1} fraction was rapidly and extensively degraded after 3 hours of incubation. On the other hand, caprine and ovine β and -α_s caseins showed limited degradation after 24 hours of hydrolysis
Supported by CONICET, CIC, ANPCyT and UNLP.

BT-P12.
PRODUCTION OF A CHIMERA OF LUMAZINE SINTHASE FUSED TO THE RBD3 DOMAIN OF STAUFEN PROTEIN AND ITS USE AS IMMUNOGEN

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Oligomeric proteins produce a strong and specific response in immunocompetent hosts. This behaviour is attributed to the highly repetitive array of epitopes that exist in this kind of antigens, which produce an efficient cross-link of B cell receptors. *Brucella Abortus* Lumazine Sinthase (LS) is a protein which has an oligomeric structure formed by a 18 KDa subunit arranged as a dimer of pentamers (180 KDa). This protein is highly immunogenic and was successfully used as a carrier to enhance the antigenicity of short peptides fused to its N terminal. In this work, we describe the production of a chimeric protein of LS fused to a 9 KDa double stranded RNA binding domain (RBD3) derived from murine Staufen. After its cloning and recombinant expression as inclusion bodies in bacteria, the chimeric protein (RBD3-LS) was purified in denaturant conditions and refolded. Its conformational state was analysed by CD spectroscopy, light scattering and immunotechniques, demonstrating that was well folded and organised as a decameric structure of 250 KDa. The protein was then inoculated in mice and rabbits and produced a strong immunologic response toward both LS and RBD3. Finally, the results presented in this work demonstrate the feasibility of the LS carrier approach to deliver small proteins to the immune system in a highly order array and suggest the usefulness of this method for the production of antibodies with biotechnological and therapeutic applications.

BT-P13.
LIPID-DNA FORMULATIONS: AN APPROACH TO BIODISTRIBUTION ASSAYS

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Nowadays, stability determinations of lipid formulations require as mandatory the use of radioisotopes. This method is very expensive and toxic. To override this problem a cDNA fragment of VP7 inserted into pGEMT cloning-vector was selected as plasmid-DNA probe. VP7 is a protein of the outer envelope of the rotavirus capside. The objective is to develop a sensitive technique to study lipid bio-distribution. With this in mind, the viral dsRNA of the bovine rotavirus strain UK was extracted and a RT-PCR reaction was performed to obtain the cDNA corresponding to VP7. The plasmid DNA probe was encapsulated into several liposome formulations (Cationic, Non-Charged and Polymerized). The Lipid:DNA ratio was 300:1. Several assays were performed to characterize the formulations such us optical microscopy, electronic microscopy and spectrometry (osmotic volume determination). We observed that the non charged liposomes with DNA were half the size than the liposomes without DNA. The polymerized and cationic liposomes with DNA were bigger when compare to those without DNA. Finally, the cationic formulation had the highest efficiency of encapsulation, followed by the polymerized liposomes and the non-charged ones. Based on these studies we can use the selected formulations of liposomes as DNA carriers to study bio-distribution and *in vivo* stability.

BT-P14.
ANALYSIS OF POLYGALACTURONASE GENE EXPRESSION DURING STRAWBERRY FRUIT RIPENING

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Pectins are important constituents of plant cell wall polysaccharides and are composed by polymers of galacturonic acids. Polygalacturonases (PGs) and other hydrolases like pectinmethylstereases, endoglucanases and β-galactosidases are involved in depolymerization and solubilization of cell wall polymers during fruit ripening. PGs catalyzes the hydrolysis of α (1→4) glycosidic bonds of galacturonic acid residues of pectins. In this work, we have evaluated PG gene expression during ripening of three varieties of strawberry with different softening rates. The expression of a strawberry PG gene (spG) was analyzed by Northern Blot. We found that spG expression starts in white stages and increases gradually during ripening of fruits with low (Toyonaka) and intermediate (Pajaro) firmness, while spG expression starts in more advanced ripening stages (75% red) in firmer varieties (Camarosa). Additionally, we have cloned a gene (PGX8) from a cDNA library of ripe strawberry fruit. This clone shares sequence homology with spG, but it lacks of an 84 bp region, and probably presents a reading frameshift. Using semi-quantitative RT-PCR technique, we obtained the expression patterns of both genes during ripening of the three varieties. The results suggest that PGX8 expression is higher in the firmer variety (Camarosa), while the spG gene expresses preferably in the softer variety (Toyonaka).

BT-P15.
DETECTION OF A DEFENSE RESPONSE ELICITOR AGAINST A FUNGAL DISEASE IN STRAWBERRY

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Plants and pathogens have coevolved in the competence to defeat themselves by using highly specific attack and defense strategies. When plants recognize a pathogen and halt the invasion by activating a defense mechanism, the disease is prevented and the pathogen behaves as avirulent. In these cases the interaction established is of the type incompatible otherwise is compatible. The defense response in plants is normally induced by signaling molecules called "elicitors". The aim of this work was to evaluate the capacity of conidial extracts of an avirulent isolate of *Colletotrichum fragariae* to elicit in strawberry a defensive response against fungal disease. Experiments were conducted with plants of the cultivar Pájaro and with local isolates of *C. fragariae* and *C. acutatum* that induce incompatible and compatible interactions, respectively. The disease severe rate (DSR) was evaluated in petioles according to a scale ranging from 1 (no disease symptom) to 5 (maximum severity). Plants were firstly sprayed with conidial extracts of the avirulent *C. fragariae* strain, obtained by different procedures and after 72 hrs with the virulent *C. acutatum*. Results indicate that conidial extracts do not inhibit the growth of *C. acutatum* on PDA plates, but it does on strawberry plants. These outcomes suggest that extracts contain an active compound that induces a defensive response against a virulent fungal pathogen. The elicitor may be used as a novel strategy for the biocontrol of the antracnosis disease in strawberry.

BT-16.
PROTECTION AGAINST *XANTHOMONAS* INFECTIONS THROUGH HYPER-PRODUCTION OF DSF

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The synthesis of extracellular enzymes and extracellular polysaccharide (EPS) in black rot-causing *Xanthomonas campestris* pv *campestris (Xcc*) is regulated by a cluster of nine genes, *rpfA-I* (for *r*egulation of *p*athogenicity *f*actors). Several of these genes are involved in regulation mediated by small diffusible signal factor (DSF). Previous tests showed that mutation (through transposon insertion) of either *rpfF* or *rpfB* halted production of DSF, while mutation of *rpfC* leads to an overproduction in DSF. Wild-type *Xcc* strain 8004 was previously shown to induce necrotrophic symptoms on *Nicotiana benthamiana (Nb)* leaves. To investigate DSF-mediated regulation during plant-pathogen interaction, mutants derived from 8004 were subjected to leaf-infiltration assays. It was founded that EPS-deficient mutant 8397, DSF-deficient *rpfF* 8523 and DSF-hyper producing *rpfC* 8557 were all severely compromised in their ability to cause disease in *Nb* leaves compared with the wild-type. Co-infiltration assays showed that the 8397 but not 8557 was able to complement 8523. 8557 induced higher expression of defence gene PR1 and inhibited the reaction caused by 8004 when both were co-inoculated. These results suggest that DSF-hyper producing mutant 8557 triggers a resistance response that prevents the wild-type interaction. 8557 has also been shown to protect against *X. axonopodis* pv *citri* in citrus; thus, further study of DSF's role in the plant immune response may allow us to develop tools to control *Xanthomonas* infection that function across plant species.

BT-P17.
CONSTRUCTION AND UTILIZATION OF AN INTERNAL CONTROL (IC) DERIVED FROM Qβ PHAGE IN RNA VIRUSES DIAGNOSES

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RT-PCR is an important tool for the detection of RNA viruses in clinical and research laboratories. Due to RNA liability, it is necessary to have a control to verify the efficiency of both, sample processing and amplification reaction. We present the development of an IC derived from Qß phage to be spiked into the specimen, processed and co-amplified with the same target primers. Qß is a coliphage with single strand RNA genome. The primers KY78/KY80, which are commonly used in HCV detection, were inserted in the phage genome flanking a phage sequence. They were cloned into the open reading frame of A1 coat protein. The recombinant phage (Qß78-80) was tested in infection experiments and particles viability was demonstrated. To confirm the *in vivo* results, RT-PCR was performed with the Qß78-80 and HCV RNAs as templates and the KY78 and KY80 primers. Products obtained were the expected ones and they were confirmed by hybridization with specific probes and detection by colorimetric method. Experiments of RNasa-resistance and stability were performed. Results showed this IC is RNasa–resistant and stable at 4ºC for at least 125 days. The IC-HCV was used in RT-PCR co-amplifications together with HCV (+) samples with different viremia using the same pair of primers. The data obtained indicated that Qß78-80 was an useful tool, suggsting that this strategy could be used for the construction of IC for others RNA viruses of clinical relevance.

BT-P18.
DEVELOPMENT OF A POTENTIAL LIPOSOME – DNA VACCINE AGAINST ROTAVIRUS INFECTION

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Rotaviruses are the major cause of severe diarrhoea and dehydration among children worldwide. It has been estimated that an effective rotavirus vaccine might prevent between half and one million deaths of young children annually. Rotaviruses are also an important cause of diarrhoea in animals and are responsible for significant production losses in some livestock species.

VP7 is the major protein of the outer capsid of the rotavirus particle. Antibodies against VP7 can neutralize the virus and protect against homologous virus challenge *in vivo*. VP7 is a primary candidate for inclusion in a subunit vaccine. Liposome mediated DNA vaccination is a promising alternative to achieve protection against rotavirus infection as it avoids the potential difficulties of live vaccines.

dsRNA was extracted from bovine rotavirus strain UK and RT-PCR reaction was performed to obtain *vp7* gene without the glycosilation signal. The amplified gene *vp7* was cloned into pcDNA3 vector under the control of CMV immediate early promoter. APC cells are a preferred alternative as targets for DNA vaccine uptake and expression. Entrapment of pcDNA3-VP7 into a stable liposome formulation would be suitable to deliver pcDNA3-VP7 to such cells. Liposome entrapment would also protect pcDNA3-VP7 from deoxyribonuclease attack.

BT-P19.

RANDOM SEQUENCE ANALYSIS OF A *GEOBACILLUS SP*. ISOLATED IN ARGENTINA

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A thermophilic bacillus isolated from a thermal spring in Argentina was classified as a *Geobacillus sp*. by sequencing of 16S ribosomal encoding gene (16S rDNA). A genomic library was constructed using Sau3A digested DNA with fragment size ranging from 2.5-3 kb in pGEM3Z plasmid vector. Two hundred and eighty-five random clones were selected and plasmid DNA was purified. Sequences from both sides of the inserts were obtained (570 sequences) giving approximately a total of 256,000 bp sequenced. A low number of sequences gave significant identities (<10% of clones) using BLASTN to search NCBI databases. Using BLASTX most sequences (>90% of clones) gave similarities with different proteins including several with biotechnological relevance like alpha-amylase, glycogen synthase, DNA topoisomerase, catalase, DNA methylases, phosporylases, and peptidases. Other group of genes involved in metabolic pathways and transport suggest the existence in this microorganism of such processes. The discovery and availability of al least 30 plasmids with the complete gene sequence will facilitate their subcloning and expression in heterologous systems.

BT-P20.

DETECTION AND CHARACTERIZATION OF ANTICARSIA GEMMATALIS MNPV IE-0 GENE

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During the early phase of infection of *Autographa californica* nuclear polyhedrosis virus, different immediate-early transcripts from the IE-0/IE-1 region are expressed which encode the IE-1 (nonspliced transcripts) and IE-0 (spliced transcripts) gene products. These two gene products differ only in that IE0 contains an additional stretch of 54 amino acids at the aino terminus. IE1 was expressed early and late, whereas IE0 was expressed only early in infection. The ie1 gene product stimulated the expression of the ie1 promoter-directed expression but down regulated expression from the ie0 promoter. The ie0 gene product also transactivated the ie1 promoter but did not affect the expression from its own promoter. In a previous work of our group, the ie-1 gene of *Anticarsia gemmatalis* nuclear polyhedrosis virus (AgMNPV) was cloned, sequenced and biochemically characterized. Examination of ie-1 gene leader region revealed sequences similar to published consensus splice acceptor site.
In this study we show the presence of spliced transcripts in AgMNPV determining the splicing site between ie-0 and ie-1 sequences using 5' RACE experiments. The same experiments allow us to determine the transcription initiation site for ie-0 transcripts.
Furthermore, we detect the ie-0 coding sequences in a genomic library from AgMNPV. These clones were purified and sequenced.

BT-P21.

CHARACTERIZATION OF *RACHIPLUSIA NU* MULTIPLE NUCLEAR POLYHEDROSIS VIRUS

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Baculoviruses are dsDNA viruses that infect insects. Based on the occlusion body (OB) morphology and location of OBs in the infected cell, these pathogens are divided into two genera: nucleopolyhedroviruses (NPVs) and granuloviruses (GVs). In addition, the NPVs are subdivided in other categories: based on the number of nucleocapsids enveloped per virion, the NPVs has been classified in multicapsid (MNPVs) or single capsid (SNPVs); and according to type of the fusion protein present in the envelope of the budded virions (BVs), philogeny, and gene order, the NPVs has been grouped in Group I and Group II.
The looper *Rachiplusia nu* (Guenée) (Lepidoptera: Noctuidae), is a polyphagous leaf-feeder species, with a broad distribution in South America. The larvae cause damage in several economically important crops, such as sunflower, soybean, alfalfa, and beans. The present management of his pest is based on applications of chemical insecticides. A nucleopolyhedrovirus was isolated, and evaluated as a candidate for the biocontrol of *R. nu* larvae. To increase the knowledge of this virus we generate two genomic libraries by digestion of viral DNA isolated from occlusion bodies obtained from infected larvae. The EcoRI and HindIII libraries were used to identify several genes. In this work, we also present the characterization of RanuMNPV occlusion bodies by electron microscopy, the molecular characterization of its genome, and the nucleotide and amino acid sequences of p74 and polyhedrin genes.

BT-P22.

A COMMON mRNA 3'-END PROCESSING IN THE *BACULOVIRIDAE* FAMILY?

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Baculoviridae are the main viruses infecting lepidoptera. Their genome consists of a circular dsDNA molecule ranging in size from ~80 to 180 kbp.
Baculoviral gene expression is regulated in a temporal pattern in which immediate- and delayed-early genes are transcribed by host cell RNA polymerase II, while late and very late genes are transcribed by a virus-encoded RNA polymerase. All viral mRNAs have typical methyl-7-guanosine caps at their 5' ends and poly(A) tails at the 3' ends. The correct processing of the 3' end of mRNA precursors depends on several proteins and binding elements. From the point of view of the binding elements, it has been found that the 3'-end processing requires at least of two sequence signals: (i) an A-rich positioning element (PE) (optimally AAUAAA), upstream of the cleavage site (CS), and (ii) a downstream lss sequence-specific U- or UG-rich element, designated downstream element (DE).
In this work, we show a 3' RACE analysis of the AgMNPV *gp64* mRNAs, and computational comparisons with the other group I baculovirus. In addition, a bioinformatic analyses of all predicted genes in the 18 complete genomes reported to date shows that these signals exists in most of them, allowing the postulation of a common model for the 3'-end processing.

BT-P23.

APOPTOSIS AND NECROSIS IN BACULOVIRUS-INFECTED AND NON-INFECTED INSECT CELL CULTURES

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Baculoviruses are attractive biological agents for control of insect pest in crops. In addition, these viruses can be reprogrammed for the production of recombinant proteins, vaccines and gene therapy. The baculovirus production is achieved in insect cell cultures. Viral infection begins differents kinds of cell death processes. The most important mechanisms of cell death in cultures are necrosis and apoptosis. Virus infection is a strong stimulous of apoptosis. The aim of this work was the characterization of cell death phenomena in two Lepidopteran cell lines: IPLB-Sf 21 and UFLAg 286. The study was carry out employing both cultures with and without infection. The *AgMNPV* was employed for infection. Cultures were done in media with fetal calf serum and serum free medium. Cell death was characterized and quantified by Trypan blue method, LDH activity and epifluorescence microscopy (acridine orange and ethidium bromide). Necrosis was the predominant cell death process in cultures with and without infection. In non-infected cultures, apoptotic cells reach a 40% of the whole population. Apoptosis was culture media dependent but independent of cell line. Highest apoptosis values were achieved in serum-supplemented cultures. In infected cultures, apoptosis was less than 10%. Polyhedra production was directly affected by the cell death process.

BT-P24.

IDENTIFICATION OF A *Bacillus thurigiensis* STRAIN WITH ENTOMOPATHOGENIC ACTIVITY AGAINST HORN FLY (*Haematobia irritants*)

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The horn fly is a common pest of cattle that causes important economic loses. This hematophagus parasite is distributed throughout much of the tropical and temperate areas of the Western and Eastern Hemispheres. The horn fly was introduced into Argentina from Brazil in 1991 and has since spread to cover important regions of the country. Adults are about 1/2 the size of house flies, they spend most of their life on cattle and feed on blood and tissue fluids. Females leave the host to lay eggs only in fresh bovine dung where larvae feed on microorganisms.

We developed a system to produce adult flies in the laboratory using a semisynthetic medium culture. Using this medium we tested the toxic ativity of several sporulated *B. thuringiensis* strains against horn fly larvae. We found one strain with toxic activity. This activity was temperature sensitive indicating that the toxic effect was not produced by a β-exotoxin. Microscopy examination of the sporulated strain showed the presence of bipiramidal paraesporal crystals. Analysis by SDS-PAGE of purified crystals by urografin gradient centrifugation, showed the presence of a unique protein of MW ~ 130 kD. At present we are working in the identification of this protein, the cloning of the corresponding gene and the typification of the *B. thuringiensis* strain.

BT-P25.

ISOLATION AND CHARACTERIZATION OF AN INSECT TRANSPOSON

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Transposons are moving genetic elements spread in eukaryotic and prokaryotic organisms. The requirement for transposition change in every case, although much of those seem not to need host factors, showing its moving capacity in heterologous organisms. One of these, named *Piggybac*, is a transposon isolated from a *Trichoplusia ni lepidopteran* cell line. Its genetic constitution is very simple and its moving capacity was reported with success in different insects, including *diptera* and *lepidoptera* species and was used as system to generate transgenic insects. In this work we present a new isolation of the transponible element from the *Hife Five* cell line, the analysis of the nucleotide and amino acid sequence, the characterization of the coded protein expressed using the pFastBac system and the study of the insertion site in different target DNA. With all of this information we will project to design a plasmid system to generate transgenic cell lines and a new *in vitro* cloning system.

BT-P26.

BACTERIAL BIORREMEDIATION OF TOXIC POLYCYCLIC AROMATIC HYDROCARBONS (PAH) FROM THE INDUSTRIAL POLUTION

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The industrial development and the continually expanding human population generate important quantities of hazardous chemical waste. The disposal of new generated waste and the elimination of toxic compounds already accumulated over the last decades are problems that our Global Society can not omit. The use of microorganisms (bacteria) to treat contaminated environments (bioremediation) constitutes the best option because it is clean, safe and cost-effective. Here, we report the isolation and selection of natural microbial isolates and their use to bioremediate contaminated environments. Among the natural isolates that degraded a wide range of PAH (naphthalene, biphenyl, ethyl-benzenes, fluorene, and others) we identified members of *Pseudomonas aeruginosa* and *Pseudomonas putida*. The presence of plasmids, and the production of biosurfactants (rhamnolipids) and swarming motility to improve the poor-water solubility of the PAH and hence to make better the bio-availability and consequently the biodegradability of the xenobiotic were characterized for each isolate. Each isolate of *P. aeruginosa* and *P. putida* (a total of fifteen isolates) was able to grow in synthetic media using PAH as the unique energy and carbon source. Furthermore, the isolates were able to bioremediate complex samples of petrochemical-derived hydrocarbons (more than a hundred of different PAH) in pure cultures or in the presence of the indigenous flora of the effluent. These results acquire biotechnological importance for their use in the resolution of the environmental pollution.

BT-P27.

BIODEGRADATION OF 2,4-DINITROTOLUENE BY *Ralstonia eutropha* JMP134

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2,4-dinitrotoluene (2,4-DNT) is a priority pollutant used as intermediate in the synthesis of explosives and polyurethane foams. Although it is resistant to biological treatment, strains that mineralize this compound has been isolated. *Burkholderia cepacia* strain DNT was the first described bacteria able to grow in 2,4-DNT, and its biodegradation pathway has been extensively studied. *Ralstonia eutropha* JMP134 can grow in several chlorinated aromatic compounds. Furthermore, this bacterium utilizes nitrobenzene, 3-nitrophenol and 2-chloro-5-nitrophenol as sole carbon, nitrogen and energy source by a recently elucidated reductive pathway. Because of the demonstrated metabolic versatility of *R. eutropha*, the objective of our research was to investigate if this strain was also able to degrade 2,4-DNT. In this work we report that *R. eutropha* can grow in media with 2,4-DNT as sole carbon, nitrogen and energy source under aerobic conditions. Chemical analysis confirmed the degradation of 2,4-DNT and the generation of $N0_2$. *R. eutropha* genome analysis indicated the presence of several genes homologous to those encoding the biodegradative pathway described in *B. cepacea*. We are performing experiments to know if *R. eutropha* uses the same 2,4-DNT degradation pathway as *B. cepacia*.
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BT-P28.

ANALYSIS OF 2,4-DINITROTOLUENE BIODEGRADATION BY A GENETICALLY MODIFIED *Pseudomonas fluorescens* STRAIN

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The objective of our work is to generate an innocuous bacterial strain able to mineralize 2,4-DNT, a known priority environmental pollutant. We previously shown (SAIB, 2001) that a *P. fluorescens* strain modified by chromosomal insertion of genes encoding the upper 2,4-DNT biodegradative pathway present in the pathogen *Burkholderia cepacia* was able to efficiently denitrify this compound. However, in contrast to *B. cepacea*, in liquid cultures this strain did not grow using 2,4-DNT as a sole carbon source and showed an incomplete DNT degradation with accumulation of toxic intermediate metabolites. In this work we show that the additional insertion of the recently described *B. cepacia* lower biodegradative pathway genes allowed an efficient 2,4-DNT biodegradation without the accumulation of toxic intermediates. This new modified strain efficiently uses N from 2,4-DNT when the media was supplemented with a simple C source. Moreover, determination of the biodegradation products by HPLC indicated that the growth and the 2,4-DNT degradation rae were maximal when the media was supplemented with a complex C source, suggesting that this strain produces a 2,4-DNT cometabolic mineralization. In addition, in the absence of 2,4-DNT the modified *P. fluorescens* strain, in contrast to *B. cepacea*, stably maintains the *dnt* inserted genes after many generations. These results open the possibility to develop an innocuous and phenotypically stable strain for biodegradative processes.

BT-P29.

BIODEGRADATION OF 2,4-DINITROTOLUENE BY TRANSGENIC TOBACCO PLANTS EXPRESSING A BACTERIAL FLAVODOXIN

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Contamination of soil and groundwater at manufacturing sites with nitroaromatics such as 2,4-dinitrotoluene (DNT) poses serious environmental risks, since they are animal and human carcinogens. Flavodoxins (Fld) are small electron transfer flavoproteins present in bacteria and algae, but not in vascular plants. They are low-potential promiscuous electron carriers that can catalyse the NADPH-driven reduction of many aromatic compounds including nitroderivates. Since these chemicals are readily taken up by plants, we investigated whether transgenic tobacco expressing a bacterial Fld was able to degrade DNT more effectively than wild-type (wt) plants. Fld was targeted to chloroplasts (*pfld*) or cytosol (*cfld*) by *Agrobacterium*-mediated leaf disck transformation. Accumulation of the alien flavoprotein in chloroplasts, but not in the cytosol, resulted in augmented tolerance to DNT, with reduced symptoms of DNT toxicity and higher amounts of chlorophyll per fresh weight than in wt or in *cfld* lines. *Pfld* plants expressing high Fld levels removed more than 80% of DNT after 40 days of exposure. In contrast, susceptible lines mobilized less than 20% of the original amount of xenobiotic. These observations suggest that Fld expression in chloroplasts of transgenic tobacco plants allowed more effective detoxification of DNT, opening possibilities for their use in phytoremediation of contaminated sites.

BT-P30.

AN EXPERIMENTAL MODEL TO PREVENT CADMIUM BIOACCUMULATION BY AQUATIC ORGANISMS

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The aim of this work was to investigate the usefulness of several artificial particles in decreasing the bioavailability, and hence the bioaccumulation, of cadmium by aquatic organisms. Acute standard bioassays (48 h) were performed using the oligochaete *Lumbriculus variegatus* exposed to 0.1 mg Cd/l in test systems containing water only or water plus artificial particles in the presence or absence of humic acids. *L. variegatus* has been widely recommended and adopted by the US Environmental Protection Agency as standard animal model for toxicity tests. Different beads, commonly used as chromatographical resins, were used as artificial particles: a cationic exchanger, an anionic exchnger, and a resin for hydrophobic interactions. Pure sand particles were also included. After the treatments animals were allowed to depurate to purge gut contents. The results showed that the metal accumulation was 4.19 ± 0.47 mg Cd/g wet tissue when the organisms were exposed to water only. A similar value was obtained when the test system contained sand particles. However, all the artificial particles tested induced a decrease in cadmium bioaccumulation. A solution of 20 mg/l of humic acids promoted an increase in Cd bioaccumulation only in the absence of particles. Therefore, the artificial particles selected may constitute a useful tool in chemical remediation processes.

BT-P31.

EVALUATION OF rhIFN-β PRODUCTION BY CHO CELLS UNDER DIFFERENT CULTURE CONDITIONS

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Human beta interferon (hIFN-β) is indicated for the treatment of multiple sclerosis. The recombinant protein is produced either in mammalian cells (IFN-β−1a) or in *E.coli* (IFN-β−1b). IFN-β−1a is a glycoprotein structurally indistinguishable from the natural one, whereas IFN-β-1b lacks the carbohydrated chain. As a result differences have been found in their specific biological activities and in the plasmatic clearance rate. Minor doses of IFN-β-1a are required diminishing the immunologic compromise in patients.
To express hIFN-β in mammalian cells, an expression vector was constructed cloning the human gene into the plasmid pCIneo. Several transfections of CHO cells were performed using LipofectAMINE 2000 and pCIneorhINF-β. Three positive clones were selected (2A51D7, 2A51D5 and 2A61G5).
With the aim of improving cell productivity, different culture conditions were assayed by changing the amounts of fetal calf serum (FCS) and sodium butyrate. We found a higher production of rhIFN-β by CHO cells cultured in the presence of 0.1% (v/v) FCS and 5 mM sodium butyrate.
To determine the biological activity of the supernatants, we developed a rapid cytophatic effect inhibition biossay which could be completed in 24 h, with a detection limit of 0.75 UI/ml.

BT-P32.

A SINGLE MONOCLONAL ANTIBODY AS PROBE TO DETECT THE ENTIRE SET OF NATIVE AND PARTIALLY UNFOLDED rhEPO GLYCOFORMS

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Human erythropoietin (hEPO) is a highly heterogeneous glycosylated protein that requires well characterised-immunochemical reagents to evaluate its glycoform profile. Five antirecombinant hEPO monoclonal antibodies (mAbs) were analysed in terms of their abilities to bind to rhEPO, with the aim of selecting the appropriate mAb to develop several immunochemical approaches with no glycoform selectivity. The antibodies mapped two spatially distinct epitopes and neutralised the *in vitro* biological activity of the cytokine. All of them were able to bind to both, the partially folded and the native form of the protein. Isoelectric focusing analysis followed by immunoblotting confirmed that all he mAbs were able to bind to each glycoform. Nevertheless, only mAb 2B2 preserved the ability to bind to the complete set of soluble rhEPO glycoforms when it was immobilised onto polystyrene or chromatographic matrixes. Therefore, mAb 2B2 was useful as a capture antibody to perform an accurate, specific and fast sandwich ELISA to quantify rhEPO with a detection limit of 7 $ng.ml^{-1}$. mAb 2B2 was also satisfactorily employed as affinity ligand to purify rhEPO.
Our work led us to find a suitable reagent to perform a variety of immunochemical approaches where the binding of native and partially unfolded rhEPO glycoforms is required.

BT-P33.

CHARACTERIZATION OF N- AND O-GLYCANS OF rhGM-CSF PRODUCED BY CHO CELLS

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Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) is essential for regulating and differentiating hematopoietic progenitor cells. It contains two potential N glycosylation sites and is O-glycosylated near the N-terminus. The recombinant human GM-CSF secreted by CHO cells growing in suspension and in adherent culture was submitted to a detailed study, including the identification of the glycosylation sites, glycan characterization, site occupancy analysis and protein integrity definition, employing mass spectrometry and high-pH anion-exchange chromatography with pulsed amperometric detection. The occurrence of Nglycosylation sites at Asn27 and Asn37 was established. Among the most abundant Nglycan structures, fucosylated tri and tetraantennary Nlinked oligosaccharides with and without Nacetyllactosamine repeat units and nearly 10% of biantennary oligosaccharides were found, showing both preparations similar patterns. A high degree of sialylation with NeuNAc was observed with 7% of NeuNGc. In the predominantly doubly O glycosylated glycoprotein forms, Ser7 and Ser9 or Thr10 were detected as occupied. In the triply modified forms, Ser5 was additionally Oglycosylated. MALDI/TOFMS of the desialylated glycoproteins revealed a higher proportion of forms with a single Nglycosylation site occupied in the preparation derived from suspension culture.

BT-P34.

INSECT CELL GROWTH AND BACULOVIRUS MULTIPLICATION IN A NEW LOW-COST SERUM-FREE MEDIUM

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Baculoviruses are attractive agents for the biological control of agricultural insect plagues. AgMNPV is a baculovirus widely used in South America to control one of the main plagues in soybean crops. Commercial production of AgMNPV is currently performed in infected larvae. However, such a process is difficult to control and scale-up. The alternative process, baculovirus production in infected insect cell cultures, requires to account with a low-cost medium capable to support both cell growth and baculovirus replication. The aim of this work was to study both the growth kinetics of suspension cultures of the UFLAg 286 cell line and the AgMNPV replication in UNL-8, a new serum-free medium which composition was specifically optimized for this cell line. Cell cultures were performed in spinner-flasks, seeded at different initial cell densities. The cell specific growth rate (0.024 h^{-1}) and the maximum cell density reached in UNL-8 medium were similar than those obtained in TC-100 medium supplemented with 10% fetal calf serum. However, virus replication, measured as polyhedra yield, was significantly higher in serum-free UNL-8 medium (3.8×10^8 $polyhedra.ml^{-1}$) tan in serum-supplemented medium (5.5×10^7 $polyhedra.ml^{-1}$). These results open the possibility to develope an economically feasible process to produce AgMNPV in insect cell cultures.
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BT-P35.

ESTABLISHMENT OF HAIRY ROOT CULTURES FROM TRANSGENIC TOMATO OVER-EXPRESSING A BASIC PEROXIDASE GENE

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Successive transformation of plant tissue with *Agrobacterium tumefaciens* and *A. rhizogenes* has been used as a reliable technique for obtaining stable, transgenic hairy roots. These systems offer tremendous potential for introducing additional genes in order to change metabolic pathways and production of useful metabolites or interesting compounds. We used transgenic tomato plants *(Lycopersicon esculentum* Mill. cv. Pera) previously transformed using *A. tumefaciens* with the binary vector pKYLX71 containing *tpx1,* a tomato basic peroxidase gene, under the control of the CaMV35S promoter. Explants of leaves and stems from these transgenic plants were infected with *A. rhizogenes* strain LBA 9402. The transformation frequency was enhance using 100 μm acetosyringone. Transgenic hairy root clones developed with the characteristic high degree of lateral branching in growth regulator-free MS medium. The PCR analysis of the clones showed that T-DNAs from both bacteria used were present in the sample. All transgenic hairy root clones showed higher peroxidase activity than non-transgenic hairy roots, ranging from 20 to 174% of increment. Isoelectric focusing showed the overexpression of an isoperoxidase with pI 9.6 in the ionically bound extract of the transgenic clones. These cultures were able to enhance the removal of phenolic compounds from aqueous solutions.

BE-P1.

REGULATION OF GLYCOSYLATION OF THE POTATO AUTOCATALYTIC GLYCOSYLTRANSFERASE BY OLIGOMERIZATION AND SUBSTRATE LEVEL

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Very little is known about the regulation of the steps that control plant polysaccharide biosynthesis. Self-glycosylating proteins, believed to be involved in polysaccharide synthesis, are capable of self-glycosylation using UDP-glucose, UDP-xylose and UDP-galactose, but their precise function is still unclear. To understand the exact role of autocatalytic glycosyltransferase in polysaccharide biosynthesis is essential to know the self-glycosylating mechanism of the enzyme. Earlier, we reported that the glucosylation of the pure potato tuber self-glycosylating protein was reversible, while a non-pure protein exhibited partial glucosylation reversibility. Here, we are investigating several factors involved in the modulation of its glycosylation in order to elucidate the regulation of polysaccharide synthesis. This study reveals that the soluble potato protein presents active oligomeric forms. Moreover, under conditions that favor the oligomerization of the protein (low ionic strength, and high protein concentrations) a minor level of its glycosylation is obtained. Furthermore, the substrate concentration seems to be critical for its glycosylation. A little variation in the substrate levels gives rise to a strong change on the self-glycosylation reversibility. Therefore, this protein could be found in two conditions: in a reversibly glycosylated state or in a permanent glycosylated state.

BE-P2.

PSEUDOBIOSPECIFIC CHROMATOGRAPHIC CONVECTIVE MATRICES VERSUS TRADITIONAL SOFT-GEL DIFFUSION SYSTEMS FOR PROTEIN PURIFICATION

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Taking into account that up to 80% of the biotechnological production costs of a protein is due to recovery and purification processes, development of integrative methods of high capacity, resolution and productivity is desirable. The utilisation of pseudobiospecific ligands such as triazinic dyes immobilised on macroporous membranes such as hollow fibres becomes an interesting alternative strategy for lowering the purification costs. Membranes were activated by direct grafting of monomers with gamma radiation and the triazinic dye Cibacron Blue F3G-A was immobilised prior epoxy group opening with ammonia. Different monomer mixtures were grafted on the membranes (GMA:DMAA 1:1, 1:2, 1:3). A cartridge was built with 8 fibres of 12.7 cm lenght using those having the best hydrodynamic properties (GMA:DMAA 1:3). The comercial proteolytic preparation Flavourzyme - composed of 4 main proteins, but only one displaying proteolytic activity- was chosen as study sample. The neutral protease from Flavourzyme showed affinity for immobilised Cibacron. As compared with the standard Cibacron-Sepharose matrix, productivity was raised from 318 UE/h to 2180 UE/h working at the maximum allowed volumetric flux (1 ml/min) and at 5 ml/min respectively.

BE-P3.

STRUCTURAL AND BINDING STUDIES OF THE PRIMARY IMMUNE RESPONSE AGAINST PROTEIN ANTIGENS

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The primary function of the humoral immune response concerns the recognition and neutralization of the foreign antigens by antibodies. Since B-cells generally recognize protein antigens in their native form, it is important to elucidate the molecular basis of this response against them. To study the structural characteristics of the primary immune response, we produced mice monoclonal IgM hybridomas against hen egg lysozyme (HEL). HEL was chosen as a model antigen due to the availabily of extensive studies on its structure and stability. We have cloned and sequenced the variable domains of the IgMs against HEL to determine the germline gene that they were originated from and to establish the structural relation with the previously studied mature IgGs. As was described, the primary IgM response is produced against the whole accessible surfaces on the antigen. To determine the epitopes that recognize our group of antibodies, we developed an ELISA using a panel of different avian lysozymes of known structure. It has been demonstrated that the germline antibodies undergo a substantial structural change in the course of binding to the antigen which are reflected by the low affinity constants measured by biosensor assay using monovalent IgM fragments and HEL. Binding and structural studies will allow to define the main structural characteristics of the primary immune response against protein antigens.

BE-P4.

DEPICTING THE CATALYTIC MECHANISM OF THE METALLO-β-LACTAMASE OF *B. CEREUS*

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Metallo-β-lactamases are enzymes with a zinc binding motif in their active site, that is essential for their hydrolytic activity towards different antibiotics. A rational design of new inhibitors should necessarily rely on a thorough knowledge of their catalytic mechanism, still unveiled.
Asp90 is conserved in all known metallo-β-lactamases. This residue binds zinc in enzymes containing two metal ions, whereas it is involved in a strong hydrogen bond interaction with the nucleophilic OH^- in mono zinc enzymes. Asp90 might participate in the mechanism of mono zinc enzymes, as is the case of *B.cereus* BcII, either: (1) by defining the appropriate orientation of the attacking nucleophile, or (2) through acid/base catalysis.
To analyse the mechanism of the mono and bi-metallic BcII, we evaluated the solvent kinetic isotope effect (KIE) on the hydrolysis of different substrates. The differences observed in solvent KIE of these two forms of the enzyme can be interpreted in terms of different rate determining steps. In the mono zinc enzyme, the rate determining step would be the nucleophilic attack of the Zn-bound OH^- to the β-lactam ring, whereas in the bi zinc enzyme, a proton transfer step woud be rate-limiting. Solvent KIE experiments carried out on the mutants D90N and D90S suggest that Asp90 does not have an acid/base role in catalysis.

BE-P5.

MOLECULAR CHARACTERIZATION OF THREE PROTEINS WITH METALLO-β-LACTAMASE FOLD FROM *SALMONELLA TYPHIMURIUM*

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Zinc dependent β-lactamases belong to a superfamily of enzymes characterized by the same folding and a conserved sequence motif of two metal ions in their active sites. Variations on the sequence elements of this motif have been correlated with the selectivity towards different metal ions, and with their biological function. The presence of members of this superfamily in bacteria suggests that they could be the precursors of metallo-β-lactamases (mbl's). We have identified in the salmonella typhimurium lt2 genome three orfs showing the putative metal binding motifs and protein fold. These orfs were pcr-amplified from chromosomal dna and cloned in expression vectors as fusion proteins to gst and pelb leader sequence. The resulting proteins were overexpressed and purified. We named these proteins as glob, zndipa and ssp. Glob was able to hydrolyze s-d-lactoylglutathione, confirming its predicted identity as glyoxalase ii. Glob contained an equivalent of zinc and iron per mol of enzyme. Instead, the putative metallo-β-lactamase ssp showed a weak hydrolytic activity against nitrocefin. Atomic absorption measurements showed that ssp binds only an equivalent of zinc per mol of enzyme. These data support the purported evolutionary link between these proteins and mbl's

BE-P6.

ENGINEERING A HISTIDINE IN THE Cys221 POSITION OF *Bacillus cereus* β-LACTAMASE

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Metallo-β-lactamases (mbls) are zinc enzymes that hydrolyze the amide bond of β-lactam antibiotics. The b. Cereus enzyme (bcii) is naturally active in the monozinc form, though it is capable of binding a second zinc ion. The zinc ligands are his116, his118 and his196 at the first site, and his263, asp120 and cys221 at the second site. This residues are conserved in almost all the enzymes of the family. The first site is considered as the catalytic site. Cys is not usually a zinc ligand in zn dependent hydrolases, however, it is conserved in mbls. In order to study the catalytic and structural importance of cys221 in mbls, we analyzed a bcii derivative (c221h) harboring a substitution of this residue with his, the most common zinc ligand.
The c221h mutant showed a significant reduction in hydrolytic activity towards nitrocefin, cephaloridine, penicillin g and imipenem, compared with the wild type enzyme. The decrease in catalytic efficiency was due in most cases to a reduction in k_{cat}, although the k_m value was also affected. The c221h mutant contained ~1 mol of zinc/mol of protein. The electronic and nmr spectra of the co(ii)-substituted c221h bcii suggest that this mutant is able to bind only one metal ion equivalent of both zn(ii) and co(ii). Our results indicate an octahedral coordination for the metal ion in the first site, with his221 becoming a ligand of the first metal binding site. The lower activity of this mutant could be due to a change in the orientation of the catalytic hydroxide.

BE-P7.

AXIAL LIGATION IN ELECTRON-TRANSFER COPPER SITES: A PARAMAGNETIC ^{1}H NMR STUDY

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Copper proteins are involved in electron transfer processes by using the mononuclear blue copper and the binuclear Cu_A centers. Previous studies on blue copper and Cu_A sites have revealed that metal-axial ligand interactions strongly affect the electronic structure of these centers.
To probe the effect of various axial ligands on the spectroscopic properties of these copper sites, we have applied paramagnetic NMR spectroscopy to two variants of P. aeruginosa azurin, Met121Leu and Met121Asp, and to four mutants from the CuA–containing soluble fragment of T. thermophilus cytochrome oxidase: Met160Asn, Met160Glu, Met160Gly and Met160Gln.
In the azurin mutants, a stronger interaction with the axial ligand results in a diminished electron delocalization onto the Cu-S(Cys) bond. In contrast, in the structurally more robust CuA center, the interaction with the axial ligand does not weaken the Cu-S(Cys) bond. Instead, the mutations shift the energy of a low lying electronic state. In addition, the axial ligand mutations in the CuA center induce subtle changes restricted to one half of the binuclear unit. This fact strongly supports the suggestion that axial ligand interactions determine the His orientation, and highlights the structural robustness of CuA sites, naturally exploited to provide a more efficient electron transfer machinery than blue copper centers.

BE-P8.

STRUCTURAL STABILITY OF BRUCELLA SPP. LUMAZINE SYNTHASE

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The penultimate step in the pathway of riboflavin biosynthesis is catalyzed by the enzyme lumazine synthase (LS). One of the most distinctive characteristics of this enzyme is the structural quaternary divergence found in different species. The protein exists as pentameric and icosahedral forms, built from practically the same structural monomeric unit. The pentameric structure (fungal M. grisea, yeast S. cereviseae and S. pombe) is formed by five monomers each of them (18 kDa) extensively contacts the neighboring monomers. The icosahedrical structure (B. subtilis, A. aelicus and plant spinach) consists of 60 LS monomers arranged as twelve pentamers giving rise to a capsid exhibiting icosahedral 532 symmetry. In all LSs studied, the topologically equivalent active sites are located at the interfaces between adjacent subunits in the pentameric modules. The B. abortus LS (BLS) sequence clearly diverges from pentameric and icosahedric enzymes. This unusual divergence prompted us to further investigate on the quaternary arrangement of BLS. In the present work, we demonstrate by means of solution Light Scattering and x-ray structural analyses that BLS folds as a very stable dimmer of pentamers, representing a third category of quaternary assembly for LSs. We also describe by spectroscopic studies the thermodynamic stability of this oligomeric protein, and postulate a mechanism for dissociation/unfolding of this macromolecular assembly.

BE-P9.

CRYSTALLIZATION, DATA COLLECTION AND REFINEMENT OF LUMAZINE SYNTHASE FROM *BRUCELLA ABORTUS* BOUND TO A SUBSTRATE ANALOGUE INHIBITOR AT 2.90 Å

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Lumazine synthase is an enzyme which catalyses the formation of 6,7-dimethyl-8-ribityllumazine, the penultimate product in the synthesis of riboflavin in *Brucella abortus*, the causative agent of brucellosis. It has been shown that this enzyme can bind several substrate and product analogues and even riboflavin. We cocrystallized lumazine synthase in the presence of the substrate analogue inhibitor 5-nitro-6-(D-ribitylamino)-2,4(1H,3H)-pyrimidinedione to yield diamond-like crystals which diffracted to a resolution of 2.90 Å and belong to the trigonal space group $P3_121$. Initial phasing was carried out applying Molecular Replacement procedures using previously known free lumazine synthase as model. We obtained one molecule per crystal asymmetric unit with a solvent content of ca. 70%. First Fourier difference maps built showed a strong electronic density near the active site of the enzyme, which corresponded to the bound substrate analogue. Refinement of the crystallographic structure is being performed to describe the protein-ligand interaction. Preliminary analysis showed a strong hydrophobic stacking between the substrate analogue and residues Trp22 and Pro8.

BE-P10.

TRYPSIN PARTITION BEHAVIOR IN POLY(ETHYLENE GLYCOL) AND MAGNESIUM SULFATE AQUEOUS TWO-PHASE SYSTEMS

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Aqueous Two-Phase System (Atpss) Is An Integrative Purification Process That Combines Clarification And Coarse Purification In One Step Reducing The Downstream Processing Cost. This Extraction Technology Offers High Capacity, High Activity Yields And Easy Scale Up. Polymer-Salt Systems Have The Advantage Of Low Viscosity And Low Cost Compared To Polymer-Polymer Systems.
Trypsin Is A Proteolytic Enzyme Usually Employed In Pharmaceutical, Food And Leather Industries As Well As In Biochemical Research.
Trypsin behavior in poly(ethylene glycol) (PEG) and magnesium sulfate aqueous two-phase systems was characterized in order to find a suitable system for its purification.
Phase diagrams of PEG-magnesium sulfate for different PEG MW were described. The influence of PEG MW, pH and NaCl on the partition coefficient of trypsin (K_{TRY}) and total proteins (K_{TP}) of a pancreatic extract was studied. In the systems studied, trypsin partition was only slightly influenced by tie-line length and did not show any regular trend with pH. The K_{TRY} decreased from 0.4 to 0.15 and the K_{TP} from 0.85 to 0.47 by increasing the PEG MW from 4000 to 10000. The NaCl addition changed dramatically the K_{TRY} from 0.15 to 23 but no effect on K_{TP} was evidenced. These results may be considered as an interesting alternative for the use in trypsin purification from crude extract.

BE-P11.

á-CHYMOTRYPSIN SUPERACTIVITY AND CONFORMATIONAL CHANGES UPON BINDING TO CATIONIC MICELLES

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Aqueous micelles of hexadecyltrimethylammonium bromide (CTABr) increase the catalytic efficiency of á-chymotrypsin (á-CT). Whereas CTABr monomers have no effect on the catalytic behavior of á-CT, the micelles enhance both the maximum velocity and the affinity for the substrate p-nitrophenyl acetate. á-CT activity shows a bell-shaped dependence on increasing CTABr concentrations. A multiphase model, which considers different microenvironments in the micellar system, suggests that the bound enzyme reacts with the free substrate. Enzyme activation is accompanied by changes in á-CT structure. The intensity of á-CT fluorescence spectrum is increased and is red-shifted in the presence of CTABr micelles suggesting the annulment of internal quenching and a more polar localization of the tryptophans. Near-UV CD spectra indicate that aromatic residues are transferred to a more flexible environment. Far-UV CD spectra show an increase in the intensity of the positive and negative bands at 198 and 222 nm, respectively, suggesting higher á-helix content. This is confirmed by FTIR experiments where a higher absorption at 1655 cm^{-1} corresponding to á-helix was evidenced upon micelle binding. This change was accompanied by a reduction of â-sheet and unordered structures. Our data suggest that the higher catalytic efficiency of micelle-bound á-CT results from conformational changes.

BE-P12.

INHIBITION OF *TRYPANOSOMA BRUCEI* ARGININE KINASE GENE EXPRESSION BY RNA INTERFERENCE

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Arginine kinase catalyzes the reversible transphosphorylation between ADP and phosphoarginine, which is involved in temporal and spatial ATP buffering. The molecular and biochemical characterization of arginine kinase in *Trypanosoma cruzi* has been reported by our laboratory. Here, we report that three distinct arginine kinase genes are expressed by the African trypanosome *T. brucei*. The three genes are clustered together in chromosome IX and show a high degree of identity with one another, suggesting that the family has evolved following a tandem duplication event. The three proteins differ only in the nature of their N and C termini raising the possibility that each gene is targeted to a different cellular site or provide different cellular functions. Interestingly, although the expression of all three genes in procyclic trypanosomes can be efficiently silenced by RNAi, using a 400 bp fragment common to all three genes, our initial phenotype studies show there is no growth phenotype in the RNA knockdown mutants. Nor do these mutants show an increased sensitivity towards sodium azide, an inhibitor of the classical respiratory chain, or SHAM which is an inhibitor of trypanosome alternative oxidase.

BE-P13.

NMR CHARACTERIZATION OF THE INTERACTION BETWEEN CYTOCHROME c_{552} AND THE Cu_A-SOLUBLE FRAGMENT OF THE ba_3 OXIDASE FROM *THERMUS THERMOPHILUS*

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The structural analysis of protein-protein interactions is one of the most challenging tasks nowadays in structural biology. The protein-protein (transient) complexes between the various components of the respiratory chain are characterized by a lifetime on the order of milliseconds and K_D´s in the milimolar to micromolar range, thus complicating the structural analysis of the complexes. Chemical shift mapping by NMR spectroscopy is a useful tool to investigate such transient contacts, since it can monitor changes in the electron-shielding properties of a protein as the result of temporary contacts with a reaction partner. In this study, we investigated the molecular interaction between two components of the electron-transfer chain from Thermus thermophilus: the engineered, water-soluble fragment of cytochrome c_{552} and the Cu_A domain from cytochrome oxidase. Comparison of ^1H-^{15}N HSQC spectra of the [^{15}N]-labeled Cu_A fragment in the absence and presence of the cytochrome c_{552} fragment showed chemical shift changes for the backbone amide groups of a discrete number of resonances. The mapped contact areas on the Cu_A fragment surface were comparable in the presence of both reduced and oxidized cytochrome c_{552} species, suggesting that the respective chemical shits changes represent biologically relevant protein-protein interactions.

BE-P14.

PROCESS ENGINEERING STRATEGY FOR RECOMBINANT PROTEIN RECOVERY FROM BACULOVIRUS EXPRESSION SYSTEM

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A genetically engineered horseradish peroxidase isozyme c (hrpc) gene was constructed through the addition of charged polypeptide fusion tail by pcr strategy. A 72-pb primer encoding the c-terminal region of hrpc was specially designed. This primer included five original codons of native hrpc, a trombine site and a tail of six arginine residues (agg agg aga aga agg aga). Pcr product was inserted into bamhi and ecori restriction sites under the polyhedrin promotor into an specific baculovirus transfer vector pacgp67b. Spodoptera frugiperda sf9 cells were cotransfected with the vector containing the polyarg-hrpc gene and linearized baculovirus dna using calcium phosphate precipitation. After 4 days, it was possible to detect peroxidase activity (1.83 guaiacol units per ml) in the culture medium. Isoelectic focusing and specific hrp activity detection using 3,3' –diaminobenzidine as substrate provided evidence concerning the integrity of the tail as well as its altered charge characteristic. The isoelectric point of the modified hrp6xatg is 9.3. Isoelectric patterns derived from insect cuture medium containing fetal bovine serum 10% and insect cell lysate were performed. Lack of basic contaminant proteins in both cases creates the opportunity for directed and selective product recovery employing fluidised cation exchanger beads.

BE-P15.

IDENTIFICATION OF FUNTIONAL DOMAINS OF THE GRANULOCYTE-COLONY STIMULATING FACTOR

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The granulocyte-colony stimulating factor (G-CSF) is a glycoprotein involved in the proliferation and differentiation of neutrofilic precursors. In order to characterize functionally domains of the G-CSF molecule, monoclonal antibodies (mAbs 8C2 and 6E3) against the recombinant cytokine were employed. The identification of the antigenic regions recognized by mAbs was performed by determining the immunoreactivity of different peptides obtained either after G-CSF proteolytic digestion or by solid phase synthesis. Results showed that epitope 8C2 is formed by amino acids 39-52 and 155-164, whereas mAb 6E3 recognized sequences 1-22 and 94-123. In spite of defining distinct molecular regions, both mAbs significantly inhibited the proliferative response induced by G-CSF on a myeloid cell line. In addition, ^{125}I-mAb 6E3 was unable to bind to G-CSF-receptor complexes preformed at 4°C, although ^{125}I-mAb 8C2 recognized an exposed G-CSF region. To further explore mAb behavior, we examined the influence of temperature on the accessibility of epitope 8C2. When cytokine:receptor complexes were incubated at 37°C under conditions preventing the internalization, a significant reduction in the amount of accessible 8C2 epitopes was evident, suggesting that receptor aggregation could account for epitope masking. Taken together, our results indicate that whereas the neutralizing effect of mAb 6E3 would be due to the recognition of the G-CSF receptor-binding domain, mAb 8C2 would be inhibiting a receptor oligomerization process required for cytokine signalling.

BE-P16.

METALLO-β-LACTAMASE DOUBLE MUTANTS: TWO IS A CROWD

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Metallo-β-lactamases (MBLs) belong to a broad superfamily of Zn or Fe-dependent metallo-hydrolases, sharing a common αβ/βα fold. While metal binding sites' structures are highly conserved among these enzymes, their reactivity is finely tuned by the residues in the first and second coordination spheres. We have changed some of these residues at the bi-zinc site of MBL BcII from *B. cereus*, giving rise to two double-mutants, namely BcII-R121H/C221S and BcII-R121H/C221D, which resemble the coordination mode found in other members of the superfamily. These mutants were cloned, overexpressed in *E. coli* and purified with high yields. The zinc content was determined by atomic absorption spectroscopy. Protein folding was analyzed by CD spectroscopy, and the zinc binding sites were probed with paramagnetic ^{1}H-RMN and electronic absorption spectroscopy on the cobalt-substituted proteins. Hydrolase activity was tested against β-lactam antibiotics and several esters. These mutants showed a reduction in MBL activity with respect to BcII and the simple mutants R121H, C221S and C221D; as well as additional esterase activities that were not detected in the wild-type enzyme.
These results corroborate the evolutionary potential of this protein scaffold and provide hints on the substrate recognition pattern of these enzymes.

BE-P17.

SUBSTRATE BINDING DETERMINANTS OF B.CEREUS METALLO-β-LACTAMASE

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Metallo-β-lactamases are bacterial Zn(II) dependent hydrolases that confer resistance to -β—lactam antibiotics. The most threatening property of these enzymes is their ability to hydrolyze a broad range of -β—lactam substrates, inactivating most clinically useful -β—lactam antibiotics. As an initial approach to find the determinants of these enzymes' broad substrate range, we studied the binding of penicllins, cephalosporins and carbapenems to Bacillus cereus metallo—β—lactamase (BcII). The pre-steady state of the reaction of BcII with the mentioned substrates was analyzed employing the enzyme intrinsic fluorescence as a probe for substrate binding.
Binding of benzylpenicillin occurs within the dead time of the stopped flow instrument, indicating a low stability of the E·S complex due to a high k_{off} rate. For the three cephalosporins tested, substrate binding could be described by a two step process. Finally, the carbapenem substrate imipenem binds the enzyme in two forms, one productive and one non-productive. The apoenzyme does not bind any of the assayed substrates. Substrate binding is partially recovered after reconstitution of the apoenzyme with substoichiometric amounts of Zn(II) and fully recovered in the presence of excess Zn(II). This indicates that the metal center itself is the main determinant of -β-lactam recognition and binding by BcII.

BE-P18.

THE GLUCOSE 6-PHOSPHATE DEHYDROGENASE FROM *TRYPANOSOMA CRUZI*. CLONING, EXPRESSION AND CHARACTERIZATION

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Trypanosoma cruzi is the parasitic flagellate which causes the American Trypanosomiasis, Chagas disease.
In most organisms the two important pathways for glucose utilization are the classical Embden-Meyerhof pathway and the Pentose Phosphate Pathway (PPP) which has usually two major roles, namely the production of ribose 5-phosphate to be used in nucleic acid synthesis and the generation of NADPH, necessary for biosynthetic reactions and also for the protection against oxidative stress imposed by reactive oxygen species. The glucose 6-phosphate dehydrogenase (G6PDH) is the first enzyme of the oxidative branch of this pathway and is present in the four major stages of *T.cruzi* Cl Brener clone. Several genes have been identified, most of them encoding proteins with high identity scores when compared with the *T. brucei* G6PDH. We have also identified a pseudo-gene, and some genes encoding proteins with an unusual highly charged N-terminal extension of 37 amino acid residues. None of the translated sequences showed a peroxisomal targeting signal (PTS) type 1 or 2. We have cloned and expressed in *E. coli* cells one of the short *T. cruzi G6PDH* genes, encoding a protein with 70.6 % identity with the *T. brucei* G6PDH. The active purified recombinant enzyme showed it to be dimeric and the apparent K_m value for glucose 6-phosphate and NADP were 77 μM and 5 μM respectively.

BE-P19.

NORMAL MODES OF METHYLAMINE DEHYDROGENASE THAT PROMOTE H TUNNELLING

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The enzyme Methylamine Dehydrogenase (MADH) catalyses the oxidative deamination of Methylamine to Formaldehyde and Ammonia. The rate determinant step of the reaction, that involves the cleavage of one of the C-H bonds of Methylamine, presents an anomalously large Deuterium kinetic isotope effect that is indicative of Hydrogen tunnelling. More recent experiments confirm that bond cleavage does occur by tunnelling, and suggest that the vibrational motion of the protein scaffold promotes the transfer. The aim of our study was to identify the normal modes of MADH that affect the Hydrogen transfer step, by reducing the distance between the H-donor and the active-site base responsible for proton abstraction.
Calculations were performed using the TINKER package with AMBER94 parameters. The low frequency normal modes of MADH were determined using the Diagonalization In a Mixed Basis method of Mouawad and Perahia. This algorithm was implemented into a FORTRAN program that was coupled to the rest of the programs of the TINKER package. With this methodology, we identified and characterised the modes that shorten the distance between the H donor and acceptor. We also performed an analysis of the energetic distribution of the relevant normal modes in order to distinguish the residues that dominate those motions. From this analysis we inferred which mutations could affect the enzymatic activity of MADH by restricting/enhancing the vibrational motions that promote tunnelling.

BE-P20.

BIOCHEMICAL AND STRUCTURAL STUDIES ON ALANINE AMINOTRANSFERASE FROM *TRYPANOSOMA BRUCEI*

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T. brucei depends on glycolysis for energy generation. Pyruvate is the major end product in the bloodstream forms whereas alanine and succinate are mainly excreted in the procyclic stage. Degradation of aromatic amino acids (AAds) was also observed to lead to an abundant excretion of aromatic lactate derivatives. In *T. cruzi*, a closely related parasite, an aromatic aminotransferase (TAT) is responsible for the initial step of AAds degradation, represents 3% of the total soluble proteins and possesses a broad substrate specificity using efficiently alanine, leucine, methionine and AAds as substrates. Data bases of the *T. brucei* genome project were screened with *T. cruzi* TAT sequence using BLASTP programme in search of a potential TAT in *T. brucei*. The highest sequence similarity identified showed a score of 38%. The corresponding ORF was amplified by PCR and cloned into an expression vector, pET28. The putative protein, functionally expressed in *E. coli*, is almost specific for the pair alanine/2-oxoglutarate and has app K_m values for alanine and 2-oxoglutarate of 2,7 mM and 0.38 mM, respectively. The Arg33Ala mutation increased ten fold the app K_m value for 2-oxoglutarate however the catalytic constant for alanine was not affected at all. These results showed that Arg33 in *T. brucei* ALAT may be involved in dicarboxylic substrate binding.

BE-P21.

SURFACE BEHAVIOR AND PEPTIDE-LIPID INTERACTION OF M1 SECTION OF THE NACHR

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We have studied the surface properties and the protein-lipid interaction of the transmembrane M1 sequence of the nAChR by using the lipid monolayer technique. Previous works report that M1 peptide adopts á-helical structure in a membrane-like environment (Corbin and Méthod 1998). In absorption experiments, M1 shows a spontaneous adsorption into a lipid free interface reaching a maximal lateral surface pressure of about 27 mN/m and a surface potential of about 200 mV. Pure peptide monolayers show a molecular area of 210 Å^2/molecule compatible with an á-helix structure at the interface at maximal packing. The high stability under lateral compression and the low surface potential could be induced by proline residues that distort the helix. Compression isotherms of mixed peptide-lipid monolayers show a dual behavior in the peptide-lipid interaction. The peptide was immiscible when lipids form a condensed interface (DPPC, DPPG) at low peptide proportion, but it was miscible at high peptide concentration. On the other hand, the peptide was miscible in all proportions if the lipid is in the liquid-expanded state (POPC, POPG). For immiscibility or miscibility behavior no significant lipid-peptide interactions were seen (ideal behavior). In peptide penetration experiments, M1 peptide interacts preferentially with POPC compared with the penetration of M1 into POPG monolayers. The results show that transmembrane M1 peptide behaves as an “ideal partner” with zwitterionic PC in liquid state.

BE-P22.

HEME OXYGENASE AND OXIDATIVE STRESS IN RAT HEPATIC CIRRHOSIS

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A model of hepatic cirrhosis through biliary duct double ligation and section was used, to evaluate the relationship between heme oxygenase-1 induction and oxidative stress generation. Female albino Wistar rats (200-250 g) were operated and after 28 days were killed. Liver and lung were excised and used for determinations. In liver, an increase in thiobarbituric acid reactive substances (TBARS) (130% over controls) was found, and GSH, the most important soluble antioxidant defense, showed a significant decrease (85%). The antioxidant enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were also decreased (30, 90 and 60%, respectively). In contrast, in lung, neither TBARS content nor soluble (GSH) and enzymatic defenses (CAT, SOD and GSH-Px) were modified, compared to control animals. An increment in the HO-1 expression and activity (215% over control values) was observed only in lung, while in liver, the behavior of the enzyme was similar to control rats. The results obtained, clearly demonstrate that in lung, the HO-1 induction prevents the appearance of oxidative stress in the hepatopulmonary syndrome caused by liver cirrhosis. However, in liver, the marked cellular damage provoked by the cirrhotic condition, did not allow the induction of HO-1, and therefore a severe oxidative stress generation took place.

BE-P23.

OXIDATIVE STRESS AND ANTIOXIDANT DEFENSES IN *Biomphalaria glabrata* EXPOSED TO CADMIUM

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Fresh aquatic systems are fundamental for life on earth. They should be monitored in order to assess environmental conditions. Organisms exposed to contaminants may develop different metabolic responses in order to minimize the toxic effects. These primary responses may be used as biomarkers. In our laboratory, we worked with *Biomphalaria glabrata*, a pulmonate gastropod which showed to be a useful tool for this purpose as previously reported. Cadmium is one of the most popular and dangerous contaminants of our planet and is well known its pro-oxidant actions. The objective of this work was to study the oxidative stress: lipid peroxidation (LPO) and protein oxidation (PO) and antioxidant enzymes: catalase (CAT) and superoxide dismutase (SOD), elicited by acute and chronic exposure to 0,05 ppm cadmium concentration. We used pigmented and non-pigmented animals and the analyses were performed in different tissues (cephalopedal, lung, digestive gland and gonad). The levels of the bioaccumulated metal were also analyzed. Acute exposure (2 days): CAT and SOD presented increased values in the digestive gland (50% and 30% respectively) compared to control group. LPO and PO did not showed clear differences. Chronic exposure (14 days): In the digestive gland CAT and SOD activities decreased significantly (50%)? at 14 days, and non-pigmented snails showed increased LPO and PO levels at the end of the experience.

BE-P24.
MOLECULAR CHARACTERIZATION OF CARBAPENEM-HYDROLYZING β-LACTAMASE FROM *Chryseobacterium meningosepticum*

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β-Lactamases are bacterial hydrolases able to hydrolyze the four-membered ring of β-lactam antibiotics, rendering them inefficient. According to their primary structure, Ambler has divided them into four classes (A, B, C, D). Enzymes from classes A, C and D are serine-active, whereas those belonging to class B are metal-dependent. Most of the known metallo-β-lactamases (MBLs) are chromosomally encoded, highly divergent, and have been detected in pathogenic species of clinical relevance. Their usually broad activity spectrum, which includes carbapenems, together with their resistance to the existing clinically useful inhibitors, indicate the high potential risk of their existence.
From a clinical isolate of *C. meningosepticum*, we have amplified the gene coding for the periplasmic β-lactamase GOB. In the primary sequence we identified the metal-binding motifs and a predicted folding (αβ/βα) shared with the enzymes belonging to the superfamily of MBLs. GOB displays homology with the most divergent subclass B3 MBLs, displaying mutations in residues involved in the zinc-binding motif. The coding gene was subcloned into a PET-type vector. The metallo-enzyme was then overexpressed and purified in *E. coli*, rendering a 31 kDa protein. The content of the divalent ion Zn^{2+} was analyzed and the specific activities with different substrates were determined. These data further highlight the structural and functional diversity of MBLs from different sources.

BE-P25.
ISOLATION OF A TRYPSIN INHIBITOR FROM *PTEROGYNE NITENS* SEEDS

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Protease inhibitors are pseudosubstrates with variable affinity for enzyme catalytic sites. Many inhibitors of vegetal origin have been studied, especially those belonging to the Leguminosae family because of their economic importance. With the purpose of isolating a trypsin inhibitor from the legume *Pterogyne nitens* seeds, these were ground and extracted for 18 h with 150 mM NaCl, 5 mM $CaCl_2$. After centrifugation, the extract was submitted to affinity chromatography on a trypsin-agarose column, equilibrated with 20 mM Tris-HCl buffer, pH 8.2, 20 mM $CaCl_2$, washed thoroughly with the same buffer and eluted with 100 mM glycine-HCl buffer, pH 2.6, 100 mM NaCl. The protein was further purified by gel filtration on a Superdex G-75 column, equilibrated and eluted with 150 mM NaCl, 5 mM $CaCl_2$. A single band corresponding to a Mr of approximately 24 kDa was detected by SDS-PAGE. Tryptic inhibitory activity was measured using BAEE (N-Benzoyl-L-arginine ethyl ester) as a substrate, and chymotryptic inhibitory activity using BTEE (N-Benzoyl-L-tyrosine ethyl ester). The protein obtained was able to inhibit the activity of both trypsin and chymotrypsin.

BE-P26.
MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF RECOMBINANT FRAGMENTS OF SPARC, A TUMOR PROGNOSIS-RELATED PROTEIN

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SPARC is a matricellular glycoprotein that elicits changes in cell shape and proliferation. It was found to be overexpressed in different tumors, in association with tumor progression and metastasis. SPARC consists of three domains, named NT (for N-terminal), FS (for follistatin-like) and EC (for extracellular calcium-binding domain). Importantly, its NT domain consists in a highly acidic stretch of 52 amino acids, which is believed to be natively unfolded, that is, naturally lacking a defined secondary structure. In order to investigate the possibility that a disordered structure could account for some of many different SPARC activities, we decided to express recombinant human SPARC as well as fragments comprising the following domains: 1) FS-EC, 2) EC, 3) NT-FS. They were successfully expressed as soluble His-tag fusions by S3 *D.melanogaster* cells. Far-UV CD spectra of intact SPARC and the EC construction showed a substantial amount of α-helix. The NT-FS fragment is structured but the decrease in the elipticity and shift of the minimum from 208 to 205 nm, together with the decrease in elipticity at 195 nm, suggests a contribution from more extended or disordered regions. Initial studies on the effects of the fragments in cell proliferation show that, contrary to what is seen with intact protein, NT-FS enhances proliferation of tumor cell lines. Further studies are planned to confirm these observations as well as to evaluate the behavior of fragments on other aspects of tumor malignancy.

BE-P27.
A FERROCYANIDE-ZINC COMPLEX INHIBITS THE FERREDOXIN-NADP$^+$ OXIDOREDUCTASE ACTIVITIES

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Ferredoxin-NADP(H) reductases (FNR) are hydrophilic, monomeric enzymes that contain FAD. They catalyze the final step of photosynthetic electron transport from ferredoxin (Fd) to NADP$^+$ generating NADPH for biosynthetic pathways.
NADPH oxidation can be assayed using ferricyanide, 2,6-dichlorophenol indophenol (DCPIP), other substituted phenols as artificial electron acceptors (diaphorase), or the natural electron transport protein Fd.
In addition to its catalytic, co-catalytic, and/or structural roles in proteins, zinc inhibits several enzymes. We found that FNR ferricyanide-diaphorase activity is inhibited by zinc. Equimolar concentrations of the metal and ferrocyanide are necessary for inhibiting DCPIP-diaphorase activity and Fd reduction, suggesting that the complex is the true inhibitor. Kinetics studies showed that Zn-ferrocyanide behaves as a non-competitive inhibitor in all FNR enzymatic activities. Moreover, the dissociating constants (K_D) of the FNR-Fd and FNR-NADP$^+$ complexes do not change with inhibiting concentrations of zinc-ferrocyanide. Inactivation experiments vs time reflected that the diaphorase activity is considerably reduced in the presence of the inhibitor, probably due to the loss of the prosthetic group FAD. Finally, we found that FNR from E. coli (FPR) is inhibited by zinc-ferrocyanide similarly to the pea FNR. Our results depict a structural particularity likely to be present in most of the flavoenzymes, which reveals structure-function information about them.

BE-P28.

STRUCTURAL ANALYSIS OF THE AMINO TERMINI OF FUNGAL REGULATORY SUBUNITS OF PROTEIN KINASE A

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There are great differences among species in the affinity between regulatory (R) and catalytic (C) subunits of cAMP dependent protein kinase. These differences can not be explained by the present structural data. An acidic residue-cluster was found near the autophosphorylation site in the NH_2-terminal sequences of R subunits of several species. The degree of accumulation of the acidic residues apparently correlates with R-C affinity. Taking into account this and the ionic nature of the interaction, we propose that the acidic cluster is a region of interaction between R and C that would explain the affinity differences seen among species. To approach this issue we have decided to compare the ability of several peptides to inhibit the phosphorylating activity of *Mucor roxii*, *Saccharomyces cerevisiae* and bovine heart C subunit. The peptides sequences correspond to the autophosphorylation site of both fungi with the acidic cluster (long peptides) and without it (short peptides). Preliminary results show that *M. rouxii* short peptide is a better inhibitor than *S. cerevisiae* short peptide, assayed with bovine heart and *M. rouxii* C subunits.
An X-type four-helix bundle motif with a hydrophobic patch that modulates AKAP (A kinase anchoring protein) interaction and dimerization was described in the NH_2-terminal extreme of the mammalian R subunit. *In silico* analysis indicates that the same helix-turn-helix motif is present in lower eukaryotes. This finding prompts us to search AKAPs in fungi, not described yet.

BE-P29.

ORIENTATIONAL FLEXIBILITY OF THE NICOTINIC ACETYLCHOLINE RECEPTOR γM4 DOMAIN

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The topography of the nicotinic acetylcholine receptor (AChR) M4 membrane-embedded domain was studied using the intrinsic fluorescence signal from the single tryptophan residue (Trp^6) in a synthetic γM4 peptide (γM4-Trp^6) reconstituted in different lipid vesicles forming bilayers of known width in combination with differential quenching of its fluorescence with spin-labeled PC analogs or with the water-soluble quencher, acrylamide. The increase in hydrophobic mismatch between the peptide length and the bilayer thickness caused a change in the position of the Trp^6 inside the bilayer. Measured from the bilayer center, the distance to Trp^6 exhibited considerable changes, varying from 12.2 ± 0.1 Å in DPPC liposomes in the gel phase to 6.3 ± 0.6 Å in DLPC liposomes in the liquid-crystalline phase. The simplest interpretation is that the tilt angle of γM4-Trp^6 decreases as the membrane becomes thicker. Furthermore, the fluorescence data for the synthetic γM4-Trp^6 peptide were found to be compatible with an α-helical structure, which can be extrapolated to possible secondary structure of the γM4 segment in the native membrane-bound AChR protein.

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BE-P30.

THE N-TERMINAL DOMAIN OF THE HPV16 E7 ONCOPROTEIN DIPLAYS RESIDUAL AND EXTENDED STRUCTURE WITH POLY(L-PROLINE) TYPE II CHARACTERISTICS IN SOLUTION

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HPV16 E7 oncoprotein shares several properties with "natively unfolded" proteins. However, its far UV CD spectra, a cooperative unfolding, and the ability to form a stable dimer indicates that it cannot be unfolded in solution. The N-terminal domain of E7, E7 (1-40), displays apparently disordered CD and NMR spectra at neutral pH. With 33% of acidic residues, we have evidence that it is responsible for E7 pH conformational transitions and for its non-globular properties. At pH 4.0, the peptide shows a decrease in its hydrodynamic radius followed by molecular filtration and by pulse field gradient NMR experiments, and an increase in α-helix content can be observed by CD. CD spectra of E7 (1-40) at different temperatures are reminiscent of the poly(L-proline) type II structure and a large increase in helical content upon addition of trifluoroethanol is obtained at pH 4.0 but not at pH 7.0, suggesting a persistent residual structure at pH 7.0. Polyproline conformation is consistent with an increase of β–strand content at 70ºC relative to 2ºC. Similarly, this structure can be induced by sub-micellar concentrations of SDS at low pH. E7 (1-40) residual structure at neutral pH shows a polyproline II behavior and the neutralization of acidic residues is required to undergo substantial conformational changes. The natively unfolded-type properties of this domain could govern the known target promiscuity of E7.

BE-P31.

A SPECIFIC a-DNA ANTIBODY RECOGNIZES A DOUBLE AND SINGLE STRANDED DNA 18MER THROUGH DIFFERENT MODES

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We had previously obtained anti-DNA antibodies against a viral transcriptional regulatory site, 5'-GTA ACCG AAAT CGGT TGA-3', capable of discriminating single base replacements with affinities of 1×10^{-9} M. We present a characterization of the recognition mechanism, using a series of double and single stranded DNA oligos and the Fab fragment of the ED-10 antibody. Base replacements from both ends of the duplex, show a gradual change in binding energy down to 4.0 kcal/mol as the number of mutated bases increase. Mutation of the 5' base of strand B alone causes a 2.7 kcal/mol decrease, indicating that both the 5' terminus and neighboring bases participate in recognition. The ED-10 antibody binds strand A of the duplex very poorly, but it surprisingly binds the complementary strand B with affinity at least equal to the duplex. The affinity of the 18 mer duplexes is the highest (~1 nM), but 166 bp duplexes still bind with relatively high affinity (20 nM). However, single-strand B oligos of different sizes other than the 18 mer bind all very poorly (80 to 200 nM). Gel filtration confirmed that binding of the antibody does not dissociate the DNA. Thus, this antibody appears to recognise duplex or single stranded DNA through different modes, using an "indirect readout" mechanism where base mutations affect the structure of the DNA rather than direct contacts.

BE-P32.

LOCAL AND GLOBAL CONFORMATIONAL PLASTICITY OF THE HPV-16 E2 DNA BINDING DOMAIN

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Gene transcription in papillomavirus is controlled by the E2 protein. The high risk strain HPV16 E2 DNA binding domain is the only dimeric β-barrel structure that has been analyzed by NMR. Using NOE constraints and residual dipolar couplings, we have obtained a high resolution structure and we were able to address several issues related to folding and functional particularities of this unique fold. Despite being a highly stable and cooperatively folded dimer, H-D exchange rate measurements show that over 90% of the protons are exchanged after 15 hrs. Careful backbone dynamics measurements indicated that the apparent high mobility of the DNA recognition helix is restricted to its N-cap. We made conservative mutations of the first two residues of the N-cap. Surprisingly, A293G showed a dramatic increase in β-sheet content, but N294A displayed an increase in β-helical content. The few residues that remain unexchanged after 24 hs are I286 and V287 of β-strand 1, which suggest they are critical for the overall stability of the fold. However, all the backbone NHs of the main hydrophobic core exchange very fast. The properties of this DNA recognition and regulatory helix are mainly dictated by the core interactions with the β-barrel, rather than local sequence information in the helix. This recognition helix, far from being disordered as found in several transcriptional regulatory domains, acts as a structural hinge, providing an overall plasticity with a stable β-barrel.

BE-P33.

ISOLATION, PURIFICATION AND PARTIAL CHARACTERIZATION OF CYSTEINE PROTEASES FROM THE LATEX OF *ARAUJIA ANGUSTIFOLIA*

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Three new cysteine plant proteases have been detected by IEF and zymogram in a crude extract obtained from latex of fruits of Araujia agustifolia (Hook et Arn.) Decaisne. Latex was collected on citric-phosphate buffer (pH 6.4) containing 5 mM cysteine and EDTA. The suspension was centrifuged at 10,000g and the supernatant ultracentrifuged at 100,000g to obtain active soluble proteins. This crude extract exhibited an optimum pH (caseinolytic activity) between pH 7 and 9, was very thermo-stable and was inhibited by E-64 and iodoacetate, suggesting the presence of cysteine proteases. About eight bands could be detected by IEF, at least three of them (pI > 9) active (zymogram). The crude extract was purified by FPLC (SP-Sepharose, 55 mM Tris-HCl buffer, pH 7.4, NaCl linear gradient 0-0,5 M) affording three basic active fractions (araujiain aI, aII and aIII) with molecular masses around 25 kDa (SDS-PAGE). Kinetic parameters of araujiain aII (the most active fraction) were performed with PFLNA (L-Pyroglutamyl-L-Phenylalanyl-L-Leucine-p-Nitroanilide).

[1]ANPCYT fellow, [2]Member of the CIC Researcher Career. Work supported by the following grants: PICT 9-9916 and PIP 2813.

BE-P34.

ASCLEPAIN C I, A CYSTEINE ENDOPEPTIDASE ISOLATED FROM LATEX OF *ASCLEPIAS CURASSAVICA* L. (*ASCLEPIADACEAE)*

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In this work we report the isolation, purification and characterization of a new protease *(asclepain c I)* from latex of *Asclepias curassavica* L. The latex collected on 0.1 M phosphate buffer (pH 6.5) with 5 mM EDTA and cysteine, was centrifuged at 16,000 x g during 30 min at 4°C. The supernatant obtained was ultracentrifuged at 100,000 x g for 1 h yielding a "crude extract" containing soluble proteins. Proteolytic assays were made with casein as substrate. Crude extract was purified by FPLC (SP-Sepharose Fast Flow, buffer Tris-ClH 0.05 M, pH 8.25, linear gradient 0.0-0.6M NaCl). Two active fractions were obtained, both homogeneous by SDS-PAGE. The former, *asclepain c I*, has a molecular mass of 23,200 Da by mass spectrometry determination. When assayed on N-α-CBZ-aminoacid-*p*-nitrophenyl esters, the enzyme showed higher preference for the glutamine derivative. Determinations of kinetic parameters (Km and Kcat) were performed with PFLNA (L-Pyroglutamyl-L-phenylalanyl-L-leucine-*p*-nitroanilide). The N-terminal sequence (LPNSVDWRQKGVVFPIRDQGKCCGSXXTFSAV) showed a high similarity with other proteases from species of the *Asclepias* genus.

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BE-P35.

THE HIGH STABILITY OF THE EPSTEIN-BARR EBNA1 DNA BINDING DOMAIN IS BASED ON AN EXTREMELY SLOW UNFOLDING RATE

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The Epstein-Barr nuclear antigen 1 (EBNA1) antigen is a DNA origin binding protein (OBP). Its DNA binding domain binds to the EBV replication origin OriP and plays several roles such as activation of DNA replication, recruitment of host replication factors, destabilization of origin DNA, segregation of EBV episomes, transactivation of latent viral gene products, and repression of its own expression. The crystal structure of this domain (EBNA459-607) free or bound to DNA revealed a particular fold, the dimeric β-barrel, only shared with the papillomavirus E2 DNA binding domain. We have investigated its folding mechanism and found it is highly stable both to chemical denaturants and pH. The guanidine chloride denaturation midpoint is 6.0 M, and it can only be unfolded completely using guanidine isothiocyanate, with a midpoint of 2.5 M. The unfolding process is only partial at 2hr incubation with the denaturant, and it becomes completely unfolded after 16 hrs. EBNA(452-641) only denatures below pH 2, but we observed a fluorescence transition with a pH midpoint of around 4.5. Surprisingly, at pH 2.8, the protein retains its α-helical structure even at long incubation periods, as judged by far-UV circular dichroism; and gel filtration experiments strongly suggest it is a monomer. The particular topology of this intertwined dimeric β-barrel, makes the structural features of a folded and compact monomer most puzzling.

BE-P36.

STABILITY OF ENZYMES AND VITAMINS IN DEHYDRATED SYSTEMS: EFFECT OF SUNLIGHT SIMULATED CONDITIONS

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The purpose of the present work was to study the effects of sunlight simulated conditions on vitamins degradation and inactivation and structural damage of enzymes in dehydrated systems. Freeze-dried samples containing different enzymes (lactase, invertase and lysozyme) or vitamins (riboflavine and thiamine) were exposed to light using a medium-pressure metal halide lamp. The samples containing enzymes showed a significant reduction in the denaturation peak area, this could be attributed to protein denaturation caused by light exposure. For most of the enzymes, the loss of enzymic activity after 1 hour of light exposure was around 50%. When vitamins were added to samples containing enzymes, the effect of light on enzymic inactivation was reduced, probably due to a quenching effect. More than 50% degradation was observed for both vitamins after 3 hours of light exposure. We showed that the light exposure in dehydrated systems generated vitamin degradation, loss of enzymic activity and protein structural changes like denaturation (observed by DSC) and protein fragmentation and aggregation (observed by electrophoresis). Overall, we can conclude that a short exposure to the light produces dramatic changes on the function and structure of biological molecules in dehydrated systems with or without protective matrices.

BE-P37.

CONFORMATIONAL STUDY OF BETA-LACTAMASE USING DIAZIRINE

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Methylene carbene ($:CH_2$) is proposed as a general photoreagent to analyse conformational transitions in proteins. In particular, this probe was used to estimate solvent accessible surface areas (SASA), and to infer structural features of B. licheniformis β-lactamase (BL-βL). $:CH_2$ reacts with its immediate molecular cage, rendering methylated products. ^3H-$:CH_2$ is generated upon photolysis of ^3H-diazirine (^3H-DZN). This reagent is a gas similar in size to water, so that, from a geometrical standpoint it is expected to probe the same surface that a solvent molecule does. Along the conformational transition N→U of BL-βL, the observed increment in labeling is interpreted as an estimate of the SASA increment that takes place concomitantly with denaturation. In the N→I transition, the increment observed could arise from the facilitated access of ^3H-DZN to the protein core. By contrast, the small decrease in labeling observed for the I→U transition could arise from the balance of two opposite phenomena: the preferential partition of the reagent in hydrophobic zones of the protein occurring in the I state, and the SASA increment expected upon denaturation. Peptide patterns reveal a map of solvent accessibility along the polypeptide sequence. In general, peptides show an increased exposure in state U, however, a few exceptions to this rule occur. Causes underlying this behavior are the persistence of residual structure in state U and the putative preferential binding of ^3H-DZN to state N because of the existence of cavities and/or poor packing at a region between subdomains α and α/β of BL-βL.

BE-P38.

STRUCTURAL STUDIES OF Δ98Δ, A TRUNCATED BUT FUNCTIONAL FORM OF INTESTINAL FATTY ACID BINDING PROTEIN (IFABP)

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IFABP is a 15kDa intracellular lipid binding protein exhibiting a β-clam structure built of 2 perpendicular 5-stranded β-sheets and an intervening helix-turn-helix motif located in between strands A and B. Δ98Δ is the main product obtained after limited proteolysis of IFABP with clostripain. This fragment (29-126) lacks β-strand A, most of the helical domain, and also the last 5 amino acids belonging to the C-terminal β-strand. Despite this, it remains stable and soluble. Δ98Δ is purified in its apo form and free from any other proteolytic fragment. CD spectroscopy shows significant β-sheet content while near UV absorption and intrinsic fluorescence emission spectra reveal an identical tryptophan environment: $<\lambda>$em 341.3nm for both IFABP and Δ98Δ. These results are consistent with the conservation in Δ98Δ of all the hydrophobic amino acid residues (47,62,64,68,82,84 and 89) implicated in the nucleation event leading to the folded state. The fact that the Stokes radius of Δ98Δ is ~30% larger than that expected from its molecular weight (11kDa) and that this fragment unfolds less cooperatively and at a lower urea concentration (3.1 M) than IFABP, lends support to the view that Δ98Δ adopts a folded non-compact state. Δ98Δ is the smallest structure of this kind described so far preserving structure and binding activity.

BE-P39.

DYNACTINS P25 AND P27 WOULD BE LÂH PROTEINS

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Dynactin is a multimeric protein essential for the minus-end directed transport driven by microtubule-based motor dynein. Pointed-end complex in dynactin contains p62, p27, p25, and Arp11 subunits, which participate in interactions with membranous cargoes. Examples of cargoes are as diverse as mitochondria, endosomes, nuclei, mRNA particules, viral capsids, and lipid droplets. Here we present evidence that strongly support that dynactins p27 and p25 adopt the left-handed parallel beta helix fold (LâH). LâH proteins contain the isoleucine-patch or hexapeptide-repeat motif described as [LIV]-[GAED]-X_2-[STAV]-X and is usually found as an imperfect tandem repetition along the sequence. Using sequence similarity searches and threading analysis we found that dynactins p25 and p27 contain the hexapeptide-repeat motif and could adopt the LâH fold. Based on these results we build structural models using homology modeling techniques. These models were further analyzed and found to be reliable. It is interesting to note that it has been suggested that p25 could adopt the pleckstrin-homology fold, although no related fold has been found in our structural or sequence similarity searches. We expect that the structural models of the two dynactin subunits reported here could contribute to the understanding of the complex interactions that dynactins are able to establish with cargo particules or other dynactin or dynein subunits.

BE-P40.

PRODUCTION OF RECOMBINANT CAMELID IGG3 HINGE REGION PEPTIDE IN *E. COLI*

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Camelid antibodies constitute an exception to the common basic structure found throughout the vertebrate phylum as a considerable fraction of their functional antibodies are homodimers of heavy chains. Based on the differences in the hinge sequence, there are three IgG isotypes; IgG1 with two heavy chains and two light chains, IgG2 and IgG3 with two heavy chains and without CH1. Little is known about their constant region and immunological properties. The hinge sequence is a good choice for generating antibodies, thus is flexible and accessible. To separate IgG3 isotype for future analyses we used a DNA recombination strategy joining pGEX plasmid with a small fragment of DNA which codifies the hinge region of Camelids IgG3. The recombinant protein is GST-peptide and the amino acid sequence of the peptide is SGTNEVCK. This strategy has some advantageous features over synthetic peptides both to generate immune response in rabbits so as to obtain policlonal antibodies with specificity towards IgG3, and for peptide expression in *E. coli* resulting in good amounts without aggregation. This is a good approach for separating different isotypes for further studies.

BE-P41.

PURIFICATION AND PARTIAL CHARACTERIZATION OF PHILIBERTAIN I, A CYSTEINE PROTEASE FROM LATEX OF *PHILIBERTIA GILLIESII* HOOK. ET ARN., *ASCLEPIADACEAE*

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The crude extract obtained from latex of *Philibertia gilliesii* was partially purified by acetone fractionation as a first purification step that deprived the enzyme preparation of most soluble sugars, gums and low molecular weight peptides, retaining 73% of protein with 90% of proteolytic activity. Isoelectric focusing followed by zymogram analysis revealed the presence of several basic bands showing caseinolytic activity. According to pI values of the proteolytic components (8.75, 8.9, 9.8, and >10.25), the crude extract was purified by FPLC using a cation exchanger (SP-Sepharose Fast Flow equilibrated with 50 mM Tris-Gly buffer). To improve resolution, several saline gradients and different pH values were tested and the best resolution was obtained when a 0.1–0.4 M sodium chloride gradient at pH 9.0 was applied. The highest basic active fraction (philibertain I) was homogeneous by IEF, SDS-PAGE and mass spectrometry (Mr 23530).

[1]CONICET fellow; [2]CONICET Researcher Career; [3]CIC Researcher Career. Work supported by grants from ANPCyT, CONICET, CIC and UNLP.

BE-P42.

EXPRESSION OF HUMAN GLUTAMIC ACID DECARBOXYLASE IN ESCHERICHIA COLI: REFOLDING EXPERIMENTS

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Of the two homologous forms of glutamic acid decarboxylase, GAD65 and GAD67, only GAD65 is a common target of autoimmunity. This protein has special interest in biotechnology due its generalized use as an antigen in early detection of type 1 diabetes. Also it has been demonstrated in NOD mices that GAD65 administration slow down the appearance of diabetes. Different variants of GAD65 were expressed in *E. Coli* yielding mostly inclusion bodies. In an attempt to obtain a suitable protocol for the dissolution of the inclusion bodies and refolding, different aspects of the interaction of this protein with denaturant agents were studied. The greatest difficulty was to unfold completely the polipeptide chain. Urea, GdmCl, and SDS in isolation and under reducing condition were unable to extend the chain and make accessible all its Cys residues. A combination of DTT, urea and SDS at low temperature yielded the greatest degree of exposure: 60%. The results explain why commonly used strategies used to refold proteins from inclusion bodies failed so far in the case of GAD65. The study found that temperature and time were critical factors during the unfolding of GAD65 and that the protein in soluble partially folded state evolves slowly to irreversible nonnative conformations.

BE-P43.

PRESENCE OF TWO LIVER-TYPE FATTY ACID-BINDING PROTEINS IN THE DIGESTIVE GLAND OF *Mytilus edulis*

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Two fatty acid-binding proteins (FABPs) from the cytosolic fraction of the digestive gland of Mytilus edulis were found. The mollusk was collected in the San Jorge Gulf, Chubut, Argentina. Small gland pieces were suspended (1:1) in 50 mM sodium phosphate buffer (pH 7.4). These FABPs were purified by gel filtration on a Sephadex G-75 column, anion-exchange chromatography on a DEAE-cellulose column and reverse-phase high performance liquid chromatography. The presence of FABPs in each fraction was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Proteins bearing FABP characteristics were obtained from the DEAE-cellulose fractions eluted with 10 and 50 mM NaCl. Both proteins were submitted to Lys-C digestion.The amino acid sequence of several resulting peptides shows that both FABPs belong to the liver type subfamily. Although evolutionary-trees suggest that gene duplication - leading to the divergence of heart, liver and intestinal FABP differentiation - occurred before the vertebrate-invertebrate split, only the heart FABP has been reported so far for invevertebrates.

BE-P44.
***IN VIVO* AND *IN VITRO* STUDIES OF Pb^{2+}, Cd^{2+} AND Cu^{2+} EFFECTS ON CHICK EMBRYO URO-D AND CPG-ox ACTIVITIES**

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A number of chemicals, particularly metals and halogenated hydrocarbons, induce disturbances of heme synthesis in experimental animals. Trace minerals such as copper and cadmium are essential nutrients required in small amounts for normal growth., but excessive amounts of these minerals can be equally detrimental to the developing embryo. Increasing environmental pollution feeds soil and foods with heavy metals like Pb, Cd and Cu, which have shown to cause teratogenic defects in fetuses. The chick embryo has been used as a novel system in screening for the toxicological and teratogenic effects of many chemical substances. For the *in vivo* studies on day 12 of incubation, the eggs were injected in the yolk with two different doses of $Cd(ACOO)_2$, $Pb(ACOO)_2$ and $CuSO_4$. The eggs were opened 12 and 60 hours later, yolk sac membrane and liver removed and URO-D and CPG-ox activities assayed and products analyzed by HPLC. For the *in vitro* studies embryos of 12 days of incubation were used.
Pb^{2+} and Cu^{2+} administration induced much lower mortality rate than Cd^{2+} injection. The higher doses causes higher mortality rates, except in the case of copper administration.
The different effects observed *in vivo* and *in vitro* on the enzyme activities for these metals, could be due to its different capacity to cross vitelline membrane and the tissue ability for mobilization, uptake and short-term storage for these trace minerals.

BE-P45.
DNA BINDING SPECIFICITY OF HAKN1, A KNOTTED TRANSCRIPTION FACTOR FROM SUNFLOWER

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The Knotted family was the first group of homeodomain (HD) containing transcription factors identified in plants. The HD is a conserved compact domain that directly contacts DNA. In order to study the DNA binding properties of HAKN1, we constructed mutants in specific positions or regions of the HD. These mutants were employed in EMSAs and footprinting assays with synthetic oligonucleotides containing the core sequence bound by this class of proteins, TGAC, or variations of it. Replacement of the wild type I50 by S results in a preference for TGAC*T* versus TGAC*A*, while the I50K mutant binds TGAC*C* with highly increased affinity. When V is substituted for K in position 54, the mutant HD has a greater ability to discriminate against TGAC*T*, and still binds TGAC*A*, but with less affinity. Replacing the N-terminal arm of HAKN1 by that of the related yeast HD protein, MATα2, does not change binding specificity. Footprinting assays performed with the wild type HAKN1 and the I50K mutant indicate that both proteins contact similar positions in the DNA, namely, the TGAC core and some bases 3' to it.
In conclusion, combinatorial interactions between positions 50 and 54 in plant knotted HD containing proteins define target DNA sequence specificity.

BE-P46.
EXPRESSION OF PROPERLY FOLDED IA-2IC IN *E. COLI* AND ITS CHARACTERIZATION

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The intracytoplasmic domain of IA-2 (IA-2ic), a major autoantigen in autoimmune diabetes, is highly valuable for specific detection of autoantibodies. We optimized the IA-2ic gene for expression in *E. coli* and fused a His-tag to its C-terminus. Furthermore, we characterized the protein product (IA-2icH_6). Up to 80 mg of pure protein per liter of culture were obtained, improving previously reported yields. In size-exclusion chromatography, IA-2icH_6 eluted as a properly folded 44 kDa-monomer. As expected from its homology with protein tyrosine phosphatases (PTPs), far-UV CD spectra indicated a predominantly α-helix conformation. Near-UV CD spectra were strongly structured. In radioimmunoassays, using [^{35}S] IA-2ic (synthesized in an eukaryotic system) as tracer and an anti-IA-2ic polyclonal serum, IA-2icH_6 showed an apparent global affinity similar to that of soluble IA-2ic confirming that main conformation-dependent epitopes were not affected by the His-tag. IA-2icH_6 had eight free thiols in the native as well as in the unfolded state. In addition, one cysteine showed an unusually-high second-order rate constant in the Ellman's reaction, characteristic of a stabilized thiolate present in PTPs. In conclusion, we have expressed a properly folded protein product, suitable for immunochemical and diagnostic purposes.

BE-P47.
ELUCIDATING STRUCTURE AND CONFORMATION OF CARBOHYDRATES INVOLVED IN *Euphorbia milii* LECTIN RECOGNITION

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Glycans are key structures involved in different biological processes such as cell attachment, migration and invasion. The glycocode information is frequently deciphered by proteins, well-know as lectins, which recognize specific carbohydrate topology. The ligand carbohydrate conformation recognized by *Euphorbia milii* lectin (EML) is described in the present work. EML purified by affinity chromatography shown a homogeneous band by SDS-PAGE. From competitive assays can be seen that hydroxyl axial position of C4 and the N-acetamido on C2 of GalNAc are critical points involved in EML interaction. In addition, a hydrophobic locus adjacent to GalNAc is an important region on EML binding, clearly evidenced by using pNPβGalNAc as ligand. Direct binding assays of EML revealed a conformational requirement of adjacent structure to GalNAc. Results suggest that the presence of hydroxyl polar residues neighboring to GalNAc on oligosaccharide structure hindrance carbohydrate-EML interaction. These results are in accordance with preferential EML interaction to β-hydrophobic GalNAc derivative on competitive assays. The EML capacity to recognize epithelial tumor cells reveals to be a potential useful tool on the study of over-expressed GalNAc glycoconjugates.

BE-P48.
QUATERNARY STRUCTURE SEQUENCE DETERMINANTS IN LUMAZINE SYNTHASE

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Lumazine synthase catalyzes in microorganisms, fungi and plants the penultimate step of the riboflavin biosynthetic pathway. The most distinctive characteristic of this enzyme is that it is found in different species in two different quaternary structures, pentameric and icosahedral, built from practically the same structural monomeric unit. Despite this difference, the active sites are virtually identical in all structurally studied members.
We present a combined analysis that includes sequence-structure and evolutionary studies to find the sequence determinants of the different quaternary assemblies of this enzyme. Using similarity searches, phylogenetic clustering, sequence conservation and patterns of three-dimensional contacts derived from the known 3D structures we found a significant shift in structural constraints of certain positions. To analyze changes in structural/functional constraints, site-specific evolutionary rate shifts were also performed. We found that the positions involved in icosahedral contacts are not significantly more conserved than the rest when icosahedral sequences are compared. However the comparison between icosahedral and non-icosahedral representatives reveals that the icosahedral contact positions suffer a larger constraint increment than the positions not involved in this type of contact. Among the 27 icosahedral contact positions, 8 are postulated as the most important icosahedral sequence determinants.

BE-P49.
PRELIMINARY CHARACTERIZATION OF A NEW METALLOCARBOXYPEPTIDASE OF *T. CRUZI.*

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A search on unassembled shotgun reads derived from *T. cruzi* Genome Project reveled two open reading frames with homology to metallocarboxypeptidases of the M32 family. The ORFs had 1512pb and 1506pb, and predicted two proteins with 64% of identity between them: **TcCP-1** and **TcCP-2**. These enzymes belong to a new family of peptidases whose members had been found so far exclusively in archaea and bacteria. Homologous genes in eukaryotic organisms have been detected only in trypanosomatids (*L. major* and now *T. cruzi*) to date. This fact may offer a new possible target for the chemotherapy of Chagas disease. We designed specific primers to amplify TcCP-1 gen by PCR and expressed it in *E. coli* cells, as an NH_2-terminal His_6-tagged protein. Purification of TcCP-1 with an IMAC column charged with Co^{+2} gave an active protein with a molecular mass of 61.6 KDa (by MALDI-ToF MS) which was inhibited by 1mM EDTA, thus showing its metallopeptidase nature. Despite this fact, metal ions such as Ni^{+2}, Zn^{+2}, and Co^{+2} to a lower extent, also inhibited the recombinant enzyme. TcCP-1 presented a pH optimum around 6, when acting on the N-blocked synthetic dipeptides furylacryloyl(FA)-Ala-Lys and FA-Phe-Phe. Preliminary kinetic studies were not consistent with a simple Michaelis-Menten kinetics, showing a biphasic behavior and positive cooperativity (*n=1,74*). This is the first enzyme of this family to be reported in a eukariotic organism.

BE-P50.
CLONING, EXPRESSION AND CHARACTERIZATION OF THE 6-PHOSPHOGLUCONOLACTONASE (6PGL), RIBOSE 5-PHOSPHATE ISOMERASE (RPI) AND RIBULOSE 5-PHOSPHATE EPIMERASE (RPE) FROM *TRYPANOSOMA CRUZI*

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The pentose phosphate pathway (PPP) maintains a pool of NADPH, which serves to protect against oxidant stress, and generates carbohydrate intermediates used in nucleotide and other biosynthetic pathways. It may play an essential role in the host-parasite relationship in Trypanosomatids. We have detected activity of 6PGL in all the biological stages of *T. cruzi* CL Brener clone. We have cloned and expressed in *E. coli* cells, a gene of 950 bp encoding a protein with 52% identity with the *T. brucei* 6PGL. The latter enzyme has a glycosomal targeting signal (PTS-1) at the C-terminus (AKF), while we have found a PTS-1 like internal sequence (SLL) in the *T. cruzi* 6PGL.The RPI gene may be present in a single copy in the genome of the *T. cruzi* CL Brener clone. It has high identity with the RPI type B family, so far described only in procariotic organisms. The *T. cruzi* RPI is the first enzyme of this class found in an eucariotic organism. We have cloned and expressed the active recombinant *T. cruzi* RPI, and we have obtained antibodies from mouse and rabbit to be used in immunolocalization experiments. We have detected at least two genes encoding the *T. cruzi* RPE, which share 55% identical residues. One of them has a PTS-1 targeting signal at the C-terminus (SHL); we have cloned this gene and expressed the active recombinant enzyme in *E. coli*.
These authors contributed equally to the present communication.

BE-P51.
ANTIGEN 5 FROM POLYBYA SCUTELLARIS, A HYPOALLERGENIC VARIANT OF THIS PROTEIN FAMILY

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Vespid venoms contain antigen 5. The primary structure and allergenic properties of antigen 5 from vespids of the northern hemisphere are already known. In this work antigen 5 from the south american polybia scutellaris, has been studied: a) it has a lower ability to generate antibodies of the ige type than any other member of the family; b) was purified to homogeneity; c) amino acid composition, glycosylation degree and complete primary structure were determined; d) sequence alignment indicated a few amino acid changes which could be related to its lower allergenic activity; e) secondary structure prediction and solvent accessibility studies were performed; f) the recombinant protein was obtained. The structural knowledge acquired made possible the building of a molecular model by using the structure of vespula vulgaris antigen 5 as the template. This model will contribute to define differences and similarities with the antigen from the only family member with a known tertiary structure, said differences and similarities could be related to the different allergenic activity of the proteins. Results obtained, taken as a whole, indicate that antigen 5 from p. Scutellaris is a variant with conserved immunogenic properties but a reduced allergenic activity which could be useful for immunotherapy.

BE-P52.
CHARACTERISATION OF A RIBULOSE 5-P EPIMERASE ACTIVITY FROM *TRYPANOSOMA CRUZI* EASILY EXTRACTABLE WITH DIGITONIN
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The pentose phosphate pathway (PPP) has as major functions the generation of NADPH, required for protection against oxidative stress and for reductive biosynthesis, and of ribose 5-phosphate, required for nucleic acid synthesis. We have recently shown that the PPP is operative in *Trypanosoma cruzi* and that all its enzymes are present in the four major stages of the parasite. Digitonin extraction experiments showed that one of these enzymes, ribulose 5-P epimerase, has a very odd behaviour, since more than 80% of the total activity was extracted at detergent concentrations lower than those required to extract the cytosolic marker, pyruvate kinase. Moreover, subcellular fractionation experiments indicated that nearly half the enzyme activity was associated with particles sedimenting in the large granule fraction, showing latency, and banding at a density of 1.24 g/ml in an isopycnic sucrose gradient. Taken together, these results suggest that most of the epimerase activity is located in a subcellular compartment peripheric and dense. Treatment of whole epimastigotes with digitonin (0.16 mg of digitonin/125 mg of cells) extracted about 2% of the total protein, including almost half of the enzyme activity. The enzyme was partially purified by Mono S and BioSil chromatography. Since the sequence of two genes encoding enzyme isoforms in *T. cruzi* is already known, we are attempting the identification of the enzyme using in gel tryptic digestion and mass spectrometry.

BE-P53
ULTRASTRUCTURE AND LIPID COMPOSITION OF I-Z-I FRACTION FROM MUSCLE OF HAKE IN DIFFERENT GONADAL CONDITION
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The ultrastructure and lipid composition of cytoskeletal proteins from muscle hake it had been investigated in relation to the reproductive cycle of the fish. Myofibrils were depleted of thin and thick filaments with IK 0.6 M, pH 7.5 and visualized by electron microscopy and SDS-PAGE 10%. Lipids bound to I-Z-I fractions were extracted with chloroform-methanol 2:1 and the residues extracted three times at 37ºC during 20 minutes, with chloroform-methanol 2:1 containing 0.25% of concentrate HCl. The cytoskeletal proteins from pre and postspawned hake shown characteristics structures with sets of longitudinal (FL) and parallel filaments regularly spaced connecting successive Z disks. Typical transversal filaments connecting Z disks from different sarcomers were also observed. The integrity of FL were higher in postspawned hake. Z disks from prespawned hake appear as two circular rings held together by connecting filaments while that those of postspawned fish was represented by an unique ring. SDS-PAGE 10% profiles shown that α-actinin is the major component of Z disk. Densitometric analysis of the gels shown a lower α-actinin content in prespawned hake. I-Z-I fraction from prespawning hake contains 84% of non polar lipids (NPL) and 16% of polar lipids (PL) while that only a 32% of NPL was present in that from postspawning. Similar acidic PL level was observed in both gonadal conditions.

BE-P54.
PURIFICATION OF *GARLICIN I*, A NOVEL CYSTEINE PEPTIDASE FROM *ALLIUM SATIVUM* BULBS
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Peptidases play an essential role in many biological processes involved in growth and development of plant cells. The aim of the present study was to purify to homogeneity a novel peptidase named *Garlicin I* from *Allium Sativum* bulbs. It is well known that garlic bulb extracts contain numerous pharmacologically active substances, however, their mechanism of action is poorly defined. The peptidase was purified to homogeneity from bulbs of *Allium sativum* by ion exchange and gel filtration chromatographies. By SDS- PAGE and zymograms (gelatin-included gels) an apparent molecular weight of approximately 29-kDa was observed. The peptidase activity has an optimal pH of 5.5 and at least one cysteine residue in the active site was found by titration with E64. Moreover, its activity responds to proteinase inhibitors as a classic cysteine peptidase and it was activated by thiol protease activators, detergents and cations. The K_M for *Garlicin I* using α-casein and N-Cbz-Ala-Arg-Arg-4 methoxy-β-naphtylamide were 23 μM and 55.24 μM respectively, therefore it shows a high catalytic efficiency en relation to papain. To study protein expression anti *garlicin* I monoclonal and policlonal antibodies were performed. By western blot analysis it was found that the policlonal antibody recognizes a dimmer of 29-kDa as well as a monomer of the protein in storage bulb tissues.

BE-P55.
IDENTIFICATION OF AN ACTIVE GLUCOSYL-CERAMIDE TRANSFERASE IN EPIMASTIGOTE FORMS OF *TRYPANOSOMA CRUZI*
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Biosynthesis of glycosphingolipids involves the sequential action of glycosyltransferases. The key step involves the transfer of glucose from UDP-glucose to ceramide catalyzed by a UDP-Glucose:glucosylceramide transferase (glucosyl ceramide synthase, GCS) with special characteristics. This work shows the partial characterization of the glucosyl ceramide transferase from epimastigote forms of *Trypanosoma cruzi.* The enzymatic assay was performed by adding a fluorescent ceramide (BODIPY or NBD analogues) pre-coupled to BSA, UDP-glucose and β-NAD to tubes pre-coated with dipalmitoylphosphatidylcholine. Parasite lysates used as the enzyme source (50-100 μg protein) in Hepes buffer pH 7.4 were added and the reaction was incubated at 37ºC. The mixture was extracted with Chloroform: methanol and analysed by TLC in different solvents. Two strains (Tulahuen 2 and CL Brenner) were assayed using different concentrations of the fluorescent ceramides. The optimal incubation time was 30 min for Tul 2 and 120 min for CL strain. Different protein concentration of lysates were tried and the effect of the use of different detergents was compared. *T.cruzi* GCS uses saturated and unsaturated ceramides as substrates at variance to malarial GCS that presents specificity for the saturated one. Another component, with lower Rf was detected. An inositolphosphoceramide structure, already reported in *T. cruzi,* was evidenced after Phosphatidylinositolphospolipase C treatment.

BE-P56.

IDENTIFICATION OF SULFATED-OLIGOSACCHARIDES RELEASED FROM CRUZIPAIN, THE MAJOR CYSTEINE PROTEINASE OF *TRYPANOSOMA CRUZI*

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Sulfated N-linked-oligosaccharides have been reported as components of glycoproteins from mammalian cells and a few other organisms: birds, Dyctiostelium discoideum and some viruses. In most cases, these glycans are involved in adhesion processes. In the present work we report for the first time the presence of sulfated N-linked oligosaccharides as components of a lysosomal glycoprotein of the parasitic protozoan *Trypanosoma cruzi*. Analysis by HPAEC-PAD, of the oligosaccharides, released from cruzipain by PNGase F digestion, before and after mild acid hydrolysis, suggested the presence of an acidic group different from sialic acid previously reported. Phosphate groups had been discarded after in vivo labelling with ^{32}Pi. Thus, the presence of sulfate groups was investigated by different methods. The UV-MALDI TOF analysis of the released oligosaccharides, in the negative-ion mode, using nor-harmane as matrix, showed the presence of sulfated high-mannose type oligosaccharides. Signals with m/z values from $GlcNAc_2Man_3$ to $GlcNAc_2Man_9$ were detected. Interestingly, the signal at m/z 990 corresponding to the sulfated core structure indicated that the sulfate group is linked to a core-GlcNAc at variance to sulfated high mannose oligosaccharides of D. discoideum where sulfate group is linked to terminal-mannose residues. These finding triggers the study of the role played by these glycans in the parasite.

BE-P57.

CHICK EMBRYO ALA-D AND PBG-D ACTIVITIES: *IN VIVO* EFFECTS OF Pb^{2+}, Cd^{2+} AND Cu^{2+}

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The chick embryo has been used as a novel system in screening for the toxicological and teratogenic effects of many chemical substances. Heavy metals like Pb, Cd and Cu are environmental pollutants and have shown to cause teratogenic defects in fetuses. On day 12 of incubation, the eggs were injected in the yolk with 100 and 200 µl of $Cd(AcOO)_2$ 10^{-3} mM, 50 and 100 µl of $Pb(AcOO)_2$ 10^{-2} mM, 100 and 200 µl of $CuSO_4$ 10^{-3} mM. The eggs were opened 12 and 60 hours later, yolk sac membrane and liver removed and ALA-D and PBG-D activities assayed. Pb^{2+} and Cu^{2+} administration induced much lower mortality rate than Cd^{2+} injection. The higher doses causes higher mortality rates, except in the case of copper administration.
The acute loading of cadmium induced teratogenic effects in the chicken embryo more marked than for lead and copper, but has no effects on ALA-D and PBG-D activities.
Lead administration caused an important decrease on yolk sac membrane ALA-D activity, much more marked than in liver enzyme activity, and reduced poorly PBG-D activity. Copper injection showed an inhibitory effect only on yolk sac membrane ALA-D enzyme at the higher dose.
The different effects observed in vivo on these enzyme activities for these metals, could be explained due to its different capacity to cross vitelline membrane. The more markedly effect observed on yolk sac membrane enzyme activity could be due to this tissue ability for mobilization, uptake and short-term storage for these trace minerals.

BE-P58.

CLONING AND SEQUENCING OF A CYSTEINE PROTEASE FROM LATEX OF *ASCLEPIAS FRUTICOSA* L.

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The full amino acid sequence of asclepain f, a plant cysteine protease isolated from latex of *Asclepias fruticosa* L. (*Asclepiadaceae*), was established by cDNA cloning. Total RNA was extracted from latex and used for retro-transcription. Degenerated oligonucleotides for amplification reactions were designed according to N-terminal and internal peptides previously sequenced. The cDNA was submitted to PCR and Nested-PCR, and the amplification products corresponding to the cDNA *full length* (approximately 0.7-0.9 kb) were detected in 2% agarose gels with ethidium bromide. Bands were extracted with the QIAEX II Agarose Gel Extraction Kit, PCR amplified and cloned using pGEM-T Easy Vector and XL1 Blue *E. coli* strain. The translated from plasmidic DNA consensus sequence shows a high similitude degree with different plant cysteine proteases belonging to the papain family. Translation of the nucleotidic sequence results in a peptide whose physicochemical properties are in agreement with those obtained by experimental procedures (211 peptide residues, Mr=23.4 kDa; pI>9,3; seven Cys residues).
[1]CONICET fellow; [2]CIC Researcher Career; [3]CONICET Researcher Career. Supported by grants from ANPCyT, CONICET, CIC, UNLP and CYTED.

BE-P 59.

pH-INDUCED CONFORMATIONAL CHANGES OF LIVER BASIC FATTY ACID-BINDING PROTEIN (Lb-FABP) IN LIPID MEMBRANES

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Understanding the interactions that fatty acid-binding proteins establish with lipid membranes is relevant to know the biological functions of these proteins and the mechanisms for fatty acids transport. We have previously shown that Lb-FABP interacts with anionic lipid membranes. As a consequence, the protein acquires the characteristics of a partially folded intermediate: it maintains some secondary structure and looses the native tertiary structure [*Nolan V, Perduca M, Monaco HL, Maggio B, Montich GG (2003). Biochim. Biophys. Acta, 1611: 98-106*]. It can be proposed that partial unfolding under these conditions is facilitated both by the local decrease of pH in the proximity of the anionic membrane and by direct interaction with the lipids. To address this question we have studied the conformational changes of Lb-FABP in solution and in the presence of anionic membranes using Fourier transform infrared spectroscopy and circular dichroism. It is concluded that the observed conformational change is not due only to the acidic environment in the vicinity of the membrane but it is also mediated by direct interaction of the protein with the lipid.

BE-P60.

THE INTERACTION OF HD-ZIP PROTEINS WITH DNA IN SOLUTION STUDIED BY HYDROXYL RADICAL CLEAVAGE

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The homeodomain (HD) is a 61-amino-acid motif present in several eukaryotic transcription factors involved in development. It folds into a characteristic three-helix structure that binds DNA. HD-Zip proteins constitute a plant-specific HD family and bind the pseudopalindromic DNA sequence CAAT(A/T)ATTG as dimers. The pseudopalindrome can be divided into two T(A/T)ATTG half-sites, similar to those bound by animal HDs. In the present work, we have employed hydroxyl radical cleavage of DNA to study the interaction of the HD-Zip protein Hahb-4 with DNA. Footprinting studies indicate that a region of about eight nucleotides towards the 3'-end, including the T(A/T)ATTG core and adjacent nucleotides, is protected in each strand. Stronger protection is observed within the core. Missing nucleoside experiments suggest that the dimer does not make symmetric contacts with its target site. Binding interference is observed when the same nucleotides protected by footprinting are modified, but stronger effects are evident with the strand that contains a central T. It is also noteworthy that modification of the first nucleotide of the T(A/T)ATTG core influences binding more dramatically than modification of the third nucleotide, which base pairs with the first T of the other core. A model of the interaction of HD-Zip proteins with DNA has been derived from these observations.

BE-P61.

ASSOCIATION BETWEEN THE BOVINE SPERM PROTEASE BSp120 AND THE SPERM MEMBRANES

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BSp120 is a dimeric glycoprotein with trypsin-like activity located in the apical portion of cryopreserved sperm. The aim of this work was to investigate whether BSp120 was associated to sperm membranes. Different chaotropic agents were tested during the extraction of proteins from cryopreserved bovine sperm:1- 50mM Tris-HCl pH 7.4 (buffer A); 2- 0.6M KCl in buffer A; 3- 1% Triton X100 in Buffer A; 4- 1% SDS in Buffer A. Crude extract was loaded onto a DEAE column. Fractions containing BSp120 were assayed by zymography, azocaseinolytic activity and Western blotting. The yield of proteolytic activity, protein concentration and specific activity for each condition were as follows: 1- $1.22x10^{-4}$U, 0.625µg/µl, $1.95x10^{-4}$ U/µg; 2- 1.61 $x10^{-4}$U, 0.625µg/µl, 2.57 $x10^{-4}$ U/µg; 3- 1.08 $x10^{-3}$U, 0.5µg/µl, 2.16 $x10^{-3}$ U/µg; 4- Nd, 0.67µg/µl, Nd. Condition 3 showed the highest activity corresponding to the electrophoretic mobility of BSp120. The chromatographic profile of BSp120 extracted with Triton X100 on DEAE-Sephacel differed from that previously reported as it was not retained at pH 7.5.

Protein obtained with mild chaotropic agents (KCl, acidic buffers, alkali) are superficially related to membranes, while proteins obtained with strong agents (detergents) are integrated to the lipidic bilayers. Therefore, we suggest that BSp120 is tightly associated to the plasmatic or acrosomal membrane of the bovine sperm. Besides, the net charge of BSp120 in sperm extracts changes depending on the condition of the extraction.

BE-P62.

ENTROPIC CONTRIBUTION TO THE LATERAL PRESSURE IN LANGMUIR MONOLAYERS

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We present a series of molecular dynamics simulations of tetradecanol monolayers at the air-water interfase. The compression isotherm was obtained in two ways. In the first method the alcohol molecules (a total of 1800) were arranged in a hexagonal lattice at the desired lateral area per molecule. In this way we constructed systems at 40, 35, 30, 27, 24, 22 and 20 $Å^2$ / molecule, which were simulated for 1 ns in the NVT ensemble. In the second method, the equilibrated system at 27 $Å^2$ /molecule was allowed to compress by coupling the system to a pressure bath. In all cases, the calculated isotherm was in qualitative agreement with the experimental results. Some discrepancies were observed in the region of low molecular area, where the simulations are probably trapped in a metastable state. As the molecules compress, the fraction of dihedrals in the trans state increases, following the lateral pressure-area curve. Also, the potential energy decreases continuously as the lateral pressure increases. This indicates that the increment in the larteral pressure is not due to the van der Waals repulsion between the chains, but to the appearance of an entropic pressure. We propose that this entropic pressure is a consequence of the redistribution of velocities along the aliphatic chain in the condensed state as compared with the expanded state.

BE-P63.

mARNs TRANSCRIPTS OF GLYCEROL-3-PHOSPHATE DEHYDROGENASE (E.C. 1.1.1.8) GENE IN ADULTS THORACIC MUSCLES OF *Triatoma infestans*

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Triatoma infestans (*T. infestans*), vector of Chagas's disease, acquire wings and the ability to fly, which is important for their dispersal, after the last molt from fifth instar nymph to adult. In previous studies, in our laboratory, we have demonstrated that glycerol-3-phosphate dehydrogenase (GPDH), enzyme involved in glycerophosphate shuttle, increases 30 fold its activity in adults thoracic muscles. Adult muscles would have higher glycolitic and respiratory capacity than those of fifth instar nymphs for flight. Two different isozymes forms were studied by electrophoresis from extracts of adults and nymphs thoracic muscles, showing a slower mobility the last one. In order to study the expression genetic regulation of GPDH we have started to study RNAs transcrips from adults muscles. We have extracted total RNA and realized RT-PCR with four degenerated primers. We have amplified in higher amount transcripts with molecular weighs about 1.0 Kb and 2.0 Kb. The transcript about 2 Kb seems to be a primary transcript and the other, a mature transcript, may corresponds with adult enzyme form of GPDH. This transcripts were sequenced and compared with Data Bases on-line with GPDHs of other known flight insects muscles.

BE-P64.

EFFECTS OF HEXANE ON SECONDARY STRUCTURES OF *ARAUJIAIN* AS OBSERVED BY INFARRED SPECTROSCOPY

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The amide I band of the FTIR spectrum, which is due to the C=O stretching vibrations of the protein backbone weakly coupled with C-N stretching and in plane N-H bending, has been the most useful probe for determining the secondary structures of proteins in solution. The object of this work was to study the effect of hexane in a biphasic system on the secondary structure of the *araujiain* hI, by means of FTIR studies. These results were compare with those happened in Tris-HCl buffer pH 8.
By means of FTIR studies we demonstrated that hexane modified the secondary structure of the *araujiain* hI in biphasic medium. According to FTIR spectra is clear that the noncovalent forces, which maintain the secondary and tertiary structures of enzymes, were modified as a result of addition of the hexane to an aqueous enzyme solution. In the second-derivative analysis of the original spectrum, *araujiain* hI showed a stronger β-sheet band, a more random character and a greater α-helical character of the enzyme in buffer than in hexane-buffer. Larger amount of antiparallel β-sheet residues indicates the formation of tighter intermolecular hydrogen bond and enzymatic aggregate in buffer than in hexane-buffer. Then, *araujiain* hI in hexane-buffer is more flexible than in buffer. By other hand, the conformational differences of enzyme between both media were too readily detectable and they resulted in a native-like conformation but more active one.

BE-P65.

MODELED OF THE EFFECT OF DIFFERENT ORGANIC SOLVENTS ON THE RESIDUAL ACTIVITY OF *ARAUJIAIN* AND *PAPAIN* IN BIPHASIC SYSTEM

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In this work we study the effect of different water-immiscible organic solvents on the residual caseinolytic activity of *araujiain* and *papain* in aqueous-organic biphasic systems.
Those enzymes showed much greater residual activity in some biphasic media than in water and an important decrease of the autolysis degree in the presence of some organic solvents. This effect could be related to the variation of the flexibility and/or conformational changes of enzymes in the absence of a pure aqueous phase. Exceptionally, some organic solvents inactivated those enzymes due to the toxicity of the organic solvent molecules dissolved in the aqueous phase (molecular toxicity) and/or the presence of a separate organic phase (phase toxicity). We modeled the residual activity of *araujiain* and *papain* in aqueous-organic biphasic systems as a function of physico-chemical properties of the organic solvents. The generalized belief that organic solvents with log P > 4 causes less inactivation than hydrophilic ones could not be verified. A single–parameter approach was limited in its ability to reflect the possible enzyme-solvent interactions but correlation which combines dielectric constant (ε), dipole moment (μ), solvent dipolarity/polarizability (π*) and refraction index (η_{25}), were useful for predicting residual caseinolytic activity of those enzymes in aqueous-organic biphasic media.

BE-P66.

ACTIVITY OF BETA-GALACTOSIDASE ENCAPSULATED IN A SILICATE MATRIX BY THE SOL-GEL METHOD

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Beta-galactosidase from *Escherichia coli* was encapsulated in a porous silicate glass according to Ellerby *et al*. (Science, 255 (1992) 1113-1115). The catalytic activity towards 4-nitrophenyl-beta-D-galactopyranoside hydrolysis was preserved after encapsulation. The kinetical parameters determined at 37°C and in 50 mM phosphate buffer pH 6.8, were $K_{M,SE}$=0.1220±0.014 (mM), $K_{M,EE}$=0.0836±0.015 (mM), $V_{max,SE}$=0.455±0.014 AU/min and $V_{max,EE}$=0.188 ± 0.008 AU/min for the soluble enzyme (SE) and EE, respectively. EE showed a higher activity compared with SE at pH<4. The EE reused after washed and stored at 4°C for 10 days, conserved its activity profile within the pH range 2-10. Pre-incubation at 75°C for 30 min induced a decrement of 85% in the activity of SE but only a 40% for the EE. The activity against lactose in solution was similar between SE and EE but increased more than 3 times with SE and decreased a 50% with EE if the sugar was in an heterogeneous media like milk. In addition to diffusional and excluded volume effects from molecular confinement, other factors may be influencing protein conformation and/or the reaction mechenism in the pores of the glass. The difference in chemistries between a silica surface and the lipid-water interface may result in very different types of water structrue which may account for the opposite effects of these surfaces on the Vmax for substrate hydrolysis.
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BE-P67.

INTERACTIONS BETWEEN PMCA AND PHOSPHO-LIPIDS: A PHOTOACTIVATABLE APPROACH

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Active transport proteins are particularly sensitive to their phospholipid environment. Using hydrophobic photoreactive compounds we have examined the membrane embedded domains of this protein. In particular we are able to analyse the composition of the lipid annulus around the transmembrane domain. To this end, PMCA was solubilized from erythrocyte membranes with the detergent $C_{12}E_{10}$ and purified by affinity chromatography through a calmodulin column in the presence of glycerol instead of phospholipids. The delipidated protein was then labeled with the photoactivatable phosphatidylcholine analogue ([^{125}I] TID-PC/16) in the presence of different concentrations of phosphatidylcholine (PC). The incorporation of the probe was evaluated by the amount of radioactivity covalently associated to PMCA. By measuring the yield of the photolabeling reaction we calculated the number of PC molecules in direct contact with the protein. This value follows an hyperbolic function of the concentration of PC being the best fit parameter values: a = 100 ± 2 μM and b = 17 ± 1 PC/protein. This result indicates that 17 molecules of PC suffice to completely cover the transmembrane surface, a value that closely agrees with those measured for membrane proteins using spin-label electron paramagnetic resonance. This analytical development could find general application to the study of lipid-protein interactions in the membrane milieu and as a good alternative to other less sensitive methods.

BE-P68.

MODELLED OF THE EFFECT OF DIFFERENT ORGANIC SOLVENTS ON THE RESIDUAL ACTIVITY OF *MORRENAIN* IN BIPHASIC SYSTEM

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In this work we study the effect of different water-immiscible organic solvents on the residual caseinolytic activity of *morrenain* in aqueous-organic biphasic systems.
A statistical design that permitted the assembling of 70 organic solvents according to their physicochemical properties was carried out and one of each cluster (representative) was selected for the biphasic medium. Then, the residual caseinolytic activity of *morrenain* in biphasic media was modelled as a function of 10 physicochemical parameters of the organic solvents.
This enzyme showed much greater residual activity in some biphasic media than in water and an important decrease of the autolysis degree in the presence of some organic solvents such as (70:30) ethyl acetate. This effect could be related to the variation of the flexibility and/or conformational changes of enzyme in the absence of a pure aqueous phase. Exceptionally, some organic solvents, such as acetophenone, inactivated *morrenain* due to the toxicity of the organic solvent molecules dissolved in the aqueous phase (molecular toxicity) and/or the presence of a separate organic phase (phase toxicity). A single–parameter approach between dipole moment (μ) and solvent dipolarity/polarizability (π^*) was useful for predicting residual caseinolytic activity of *morrenain* in aqueous-organic biphasic media.

BE-P69.

A SIMPLIFICATION OF AOAC OFFICIAL METHOD 958.09- DIASTASIC ACTIVITY OF HONEY

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Diastasic activity in honey allows to know if the honeys have been centrifuged, warmed up or are mixture of artificial and natural honey; reason why it constitutes a form to value his quality.
The objective of this work was to modify the AOAC Official Method 958.09- Diastasic Activity of honey- 44.4.19 (AOAC Official Method of Analysis (1995)), in order to simplify some passages and to get more rapidity and simplicity, without the validity of the method is altered.
2.5 ml of acetate buffer (1.59 M) pH: 5.3 and 12 ml of distilled boiled and cooled water were added to 5 g of honey. The total content was quantitatively transferred to a 25 ml volumetric flask, containing 1.5 ml of solution of NaCl (0.5 M). It was mixed by investment. 5 ml from the solution were taken and they were placed in a bath at 40ºC, during 15 min, after which 2.5 ml of 2% starch solution were added. The tube was mixed by investment and stopwatch was started. Each 5 min, 0.5 ml were taken and spilled in a 50 ml test tube, containing 5 ml of a (7.10^{-5} M) iodo solution. This solution was measured in a spectrophotometer at 630 nm, using distilled water like blank. Samples are taken each 5 min until the value of absorbancia was smaller than 0.235 nm. The slope of the linear regression (absorbance vs time (min)) allowed to calculate ND (diastase number).

BE-P70.

OXIDATIVE STRESS PARAMETERS ASSOCIATED TO VITAMIN A DEFICIENCY IN RAT AORTA

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We had demonstrated increased expression of iNOS, eNOS, COX-2 and NF-kB in rat aorta under vitamin A deficiency. In this work we study the effect of such deficiency on antioxidant defense system in rat aorta. Wistar male 21 days old rats were fed during three months with free vitamin A diet (-A) and the same diet plus 8 mg of Retinol palmitate/kg of diet (+A). A group of -A rats received control diet fifteen days before sacrifice (-A refeeded). Serum TBAR'S and aorta SOD, GPx and CAT activities were measured. –A group showed increased serum TBAR'S and decreased SOD, GPx and CAT activities in aorta when compared to +A group. The restitution of Vitamin A to the Vit A deficient diet reverted TBAR'S levels and GPx activity to control values while SOD activity was partially reversed. No change on CAT activity was found. Vitamin A deficiency affects oxidative stress parameters in serum and rat aorta. Nutritional Vitamin A administration to –A rats reverts significantly those effects.

BE-P71.

CONFORMATIONAL ASPECTS OF EXENDIN-4 BY MOLECULAR DINAMICS SIMULATIONS

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Exendin-4,Ex4, is a natural 39-residue peptide from Gila Monster saliva that has some pharmacological properties similar to glucagon-like peptide-1. Thus, it was found to be a potential therapeutic agent for the treatment of diabetes. This work is focused on conformational aspects of EX4 in aqueous solution by molecular dynamics simulation (MDS) which has proved to be a powerful technique to predict properties of molecules in solution. The MDS was performed (GROMOS) for 600ps, with 3709 SPC/E water molecules surrounding an EX4 peptide. The protein mobility was analized from the trajectories collected along the MD runs. The root mean square departures from the simulated system when compared with the NMR original data for the C-α atoms were taken as a mobility parameter. It was found to be higher for the residues located around the beginning and the end of the chain. Related to the stiffness of the molecule another interesting data are the MD intra-chain hydrogen bonds, which gave characteristics of α-helix to the protein. Furthermore, the averaged final conformation of EX4 was found to be a monomeric α–helix (residues THR7-GLY29). These helicity results are in agreement with the experimental (NMR) evidence.

MCD is a Member of Research Career of CIC, and JRG is a Member of CONICET and UNLP.

BE-P72.

HYDROGEN BONDS IN ANTI-INFLAMMATORY DRUGS. A MOLECULAR DYNAMICS SIMULATION APPROACH

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The aim of this work is to gain a better understanding of role of the dynamics hydrogen bonds in anti-inflammatory drugs in aqueous solutions, by molecular dynamics simulation (MDS), which was found to be a powerful technique to predict properties of molecules in solution. Some authors have suggested that the presence of intramolecular H bonds in the orthohydroxybenzoic acid and its derivates is essential if these compounds have antirheumatic properties. Acetylsalicylic acid is the most representative molecule of the group. The MD simulations were performed (GROMOS 96 package) at constant pressure and temperature, for 500 ps. The MD calculations show the existence of intramolecular hydrogen bonds between the lateral neighboring groups of the molecule, in agreement with the ab-initio evidence. In particular, the strongest interaction observed is the H bond formed between the hydroxylic H and the carboxylic O, with a mean lifetime value of 97%. This H bonding forces the molecule to adopt a closed conformation like an eigth-membered ring, almost during the whole simulation. The nine conformers defined by MD H bonds, have been also found by ab-initio studies. These internal H-bonds inhibit the rotational movements of the lateral groups maximizing their repulsive interaction and playing an important role in the stabilization of the conformation.

MCD and JXO are Members of Research Career of CIC and CONICET respectively.

BE-P73.

SELECTION OF CAMELID ANTI-IDIOTYPIC VHHs BEARING DNA INTERNAL IMAGE BY PHAGE-DISPLAY

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In camelids, a subset of the immunoglobulins consists of heavy chain homodimers devoid of light chains, and are thus called heavy chain IgGs (hcIgGs). Their variable region (VHH) is the smallest antigen-binding fragment possible, and being just one polypeptide chain it is especially suitable for engineering. From llamas immunized with an anti-DNA mouse IgG, we sought to obtain anti-idiotypic VHHs able to back-elicit anti-DNA antibodies by molecular mimicry. Upon immunization of llamas with ED84, a monoclonal anti-DNA antibody with sequence specificity to a ds18-mer (Site35), high titers (>30000) towards ED84 Fab fragment were obtained. Total IgGs were purified and hcIgGs and conventional IgGs were isolated. By ELISA, both fractions showed significant binding to ED84. On the other hand, the VHHs were amplified from cDNA of mononuclear cells and were subsequently used to construct a phage display library of 10^8 individual clones. After two rounds of solid phase panning with specific elution (i.e., with Site35), a 58% of anti-idiotypic VHHs was obtained. Those monoclonal VHHs, and the idiotypic fraction of polyclonal hcIgGs and conventional IgGs were injected in mice. Preliminary results show that it is possible to obtain anti-DNA antibodies from the response to anti-idiotypic antibodies, confirming the hypothesis of molecular mimicry being attained from an «internal image» of the antigen.

BE-P74.

PRODUCTION OF RECOMBINANT ANTIBODIES TO GENERATE SPECIFIC ENZYME INHIBITORY ANTIBODIES

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In addition to conventional antibodies, camelids produce unusual immunoglobulins devoid of light chains. Their binding site is formed solely by one variable region (VHH). The binding strategies of these antibodies are very particular, their CDR3 region forms long extensions that can extent into cavities on antigens, e.g. the active site crevice of enzymes. Thus VHHs are a suitable fragment for the development of enzyme inhibitors.

We chose the Brucella Lumazine synthase (LS) as a target due to its high inmunogenicity and its use as carrier to elicite antibodies against others peptides or proteins.

Llamas inmunized with LS elicited high antibody titers. The cDNA isolated from lymphocytes was used as template to generate a VHH library composed of $6x10^6$ transformants. Phage display was used for panning and screening of the library, getting an enrichment of binders through consecutive rounds of panning. Five binder clones were isolated and soluble proteins produced. Characterisation of these clones by sequencing, affinity measurements and inhibitor displacement assays will be discussed.

BE-P75.

STRUCTURAL AND KINETIC STUDIES ON TWO ANTI-LYSOZYME MONOCLONAL ANTIBODIES RAISED AGAINST THE SAME EPITOPE

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Affinity maturation of anti-protein antibodies has been reported to be the result of small structural changes product of somatic hypermutation, mostly confined to the periphery of the antigen-combining site. However, little is understood about how these small structural changes account for the increase in the affinity towards the antigen. Monoclonal antibody F10.6.6 was obtained from a long-term exposure to the antigen lysozyme, whereas mAb D44.1 is the result of short-term exposure to the same antigen. These mAbs are the result of identical or nearly identical somatic recombination events. Nevertheless, different framework and variable region mutations from germline genes result in an approximately 10^3 higher affinity for the F10.6.6 antibody. The crystallographic three-dimensional structures of both free and complexed Fab F10.6.6 were solved by molecular replacement at a resolution of 2.0 Å, using D44.1 structure as template. The interaction area, the amount and distance of contacts and the number of the interface waters are increased in the F10.6.6 complex in contrast to D44.1. Biosensor assays were performed in solvent stress conditions to identify the role of the structural waters present at the antigen-antibody interface. Affinity maturation produces a fine-tuning of the complementarity of the paratope towards the epitope, explaining how the immune system is able to increase the affinity of an anti-protein antibody.

BE-P76.
DETERMINATION OF THE AUTOGLUCOSYLATING ACTIVE SPECIES CONTENT OF RECOMBINANT GLYCOGENIN PREPARATIONS
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The rate of glycogenin (Gn) autoglucosylation is dependent on the glucosylation state of Gn prior to incubation with UDPG and it reaches a plateau when the bound-oligosaccharide acquires 8-11 glucose units. The rate of transglucosylation is, under standardized conditions, proportional to the concentration of active Gn and expressed as units of activity has been used to measure the Gn content of different tissues (*Carrizo ME et al., 1997, Biochem. Biophys. Res. Commun., 240: 142-145*). Having Gn preparations isolated from proteoglycogen by digestion with different amylolytic enzymes we estimated their relative glucosylation states from the autoglucosylation/transglucosylation index (ATI), defined as the auto(14C)glucosylation from UDP-14C-glucose measured at the plateau, per unit of DBM-transglucosylating active enzyme. ATI was also used to determine the relative content of autoglucosylating active Gn of different recombinant Gn preparations. This required prior exhaustive amylolysis of Gn. Taking the ATI value for native glycogenin (coming from amylolyzed proteoglycogen) as 100% (all the Gn autoglucosilable), the ATI values obtained for three recombinant glycogenin preparations were 100%, 66% and 28%, indicating that the proportion of transglucosylating active glycogenin that was inactive for autoglucosylation were, 0%, 34% and 72%, respectively. This result is discussed in terms of possible factors affecting only the acceptor capacity of Gn.

BE-P77.
ROLE OF SURFACE FEATURES ON THE FUSOGENIC PROPERTIES OF GLOBULAR PROTEINS
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Several dehydrogenase enzymes namely malic (MDH), glutamic (GDH), glyceraldehyde-3-phosphate (G3PDH), sorbitol (SDH), xilulose (XDH) and alcohol (ADH) dehydrogenases were tested as globular soluble fusogenic proteins. All of them induced fusion of phospholipid vesicles, excepting ADH. In order to find any conserved motif involved in the process, the proteins sequence and structure were aligned. The unique conserved sequence motif was NAD^+ binding site, excepting in MDH. Furthermore, ADH showed this motif highly conserved indicating the absence of any relationship. We did not find any common structural folding related to the fusogenic activity. The electrostatic forces could be involved in the fusion process since the fusogenic proteins were more efficient with negatively charged vesicles at acidic pH and GDH and GAPDH diminish their fusogenic activity at high salt concentration. We test the electrostatic surface of all the proteins. All the fusogenic proteins show an overall positive charge in the accessible surface. On the contrary, the surface of non-fusogenic proteins like BSA, ConA, shows an overall negative charge. Also we detected superficial clusters of hydrophobic residues. High-density positive charged residues could serve as the initial site of contact with the negatively vesicles and the exposed hydrophobic residues essential for the lipid bilayer destabilization and its merging. The protein surface analysis could be useful in anticipate its properties upon at least pure artificial membranes.

BC-P1.
DIFFERENTIAL ACCUMULATION OF MUTATIONS IN PARTICULAR DOMAINS OF MUCIN GENES EXPRESSED IN THE TRYPOMASTIGOTE STAGE OF *TRYPANOSOMA CRUZI*
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The surface of *Trypanosoma cruzi* is covered by mucin-type glycoproteins involved in parasite protection, attachment and immunoevasion. The gene family coding for the mucins expressed by the parasite in the vertebrate host, named TcMUC, is composed of hundreds members and presents high variability. The genes encoding mucins expressed in the insect-dwelling parasite stages are part of a much more homogeneous family, named TcSMUG. Here, we addressed the organization and evolution of physically linked *T. cruzi* mucin genes by sequencing large chromosomal fragments. Specific accumulation of mutations was restricted to particular domains of TcMUC genes, showing that these regions have, or have had, an accelerated evolution rate. Sequence analysis of several TcMUC genes allowed for the identification of members sharing features of TcMUC I and II, thus evidencing that one group of genes was generated from the other. The highly conserved intergenic regions of both TcMUC and TcSMUG families contained TG-rich microsatellites, suggesting a role for homologous recombination in shuffling and/or amplification of *T. cruzi* mucin genes. The comparison of putative homologous TcMUC II genes from different strains of *T. cruzi* showed that their central variable domains are conserved. This conservation was always higher at the DNA level suggesting positive selection in these particular regions of TcMUC II genes.

BC-P2.
EFFECT OF REPETITIVE ALCOHOL DRINKING ON RAT BREAST ULTRASTRUCTURE AND EtOH METABOLISM TO ACETALDEHYDE (AC) AND MAMMARY TISSUE TENDENCY TO GENERATE REACTIVE OXYGEN SPECIES (ROS)
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Recent studies from our laboratory evidenced that the breast cytosolic xanthine oxidoreductase (XOR) activates EtOH to AC and free radicals and that there is a microsomal non-P450 dependent activation of EtOH to AC but not to free radicals, requiring NADPH and oxygen. Now we report that repetitve EtOH administration during 28 days through the standard Lieber & De Carli diet led to 80% increase of XOR pathway and to 27% induction of the microsomal one. The t-butylhydroperoxide promoted chemiluminiscence of breast homogenates in the EtOH treated animals was significantly enhanced in relation to controls, evidencing the tendency of the EtOH group to generate ROS. The XOR activity as evidenced by histochemistry was located in the epithelial cells. Electron microscopy observation of those cells in the EtOH treated animals revealed the presence of significant ultrastructural deleterious effects, including their alterated architecture. Their nuclei show irregular forms having invaginations of the nuclear envelope, margination of the chromatin and filamentous material oriented to cytoplasm. In summary, breast epithelial cells are able to bioactivate EtOH to reactive moieties, which may harm their ultrastructure and function.
Supported by grants from ANPCyT and UNSAM.

BC-P3.

EFFECTS OF SEVERAL PLANT POLYPHENOLS (PP) AND SULFUR CONTAINING CHEMICALS ON THE RAT TESTICULAR MICROSOMAL BIOTRANSFORMATION OF ETHANOL (EtOH) TO ACETALDEHYDE (AC)

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Deleterious effects of chronic alcohol consumption on testicular function are well known and considered to result from a toxic effect of EtOH and AC on the gonads. We previously described the presence of a several metabolic pathways present in testicular microsomal, mitochondrial and cytosolic fractions able to bioactivate EtOH to AC. In the present work we describe initial experiments on the inhibitory properties of several PP and of a few sulfur-containing compounds on these pathways. Some of the chemicals tested were quercetin (QT); naringenin (NG); catechin (CT); ellagic acid (EA); resveratrol (RV); gossypol (GO); epigallocatechin gallate (EPI); curcumin (CC); 2,3-dimercaptopropane-1-sulfonic acid (DMPS); penicillamine (PA); l-cysteine (CYST); allyl sulfide (AS); allyl disulfide (DDS); allyl mercaptan (AM) and 2,3-dimercapto-1-propanol (BAL). QT; EA; RV; GO; EPI and CC strongly inhibited microsomal NADPH-dependent bioactivation of EtOH to AC at concentration as low as 10 μM. PA and CYST fully inhibited microsomal metabolism of EtOH at 500 μM. AS or DDS were also strongly inhibitory. The other compounds did not inhibit the process at equivalent concentrations. The inhibitory properties of active compounds are mostly attributable to actions in situ on enzymes themselves or to AC trapping by the thiol compounds to give thiazolidine derivatives.

Supported by grants from SECyT and UNSAM.

BC-P4.

NIFURTIMOX NITROREDUCTASE ACTIVITY IN RAT BREAST TISSUE, ITS POTENCIAL TOXICOLOGIC RELEVANCE

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Nifurtimox (Nfx) is a chemotherapy agent used in the treatment of acute Chagas′ disease. Clinical and experimental studies with this nitroheterocyclic compound evidenced serious undesirable side effects. These were correlated with Nfx nitroreduction to a nitroanion radical followed by superoxide anion generation through a redox cycling process. The aim of this study was to verify that Nfx was present in the Sprague Dawley female rat breast tissue after treatment and the Nfx nitroreductase activity on subcellular fractions of the same tissue. The original compound reached a concentration of (18.5 ± 7.0), (17.4 ± 1.7) and (6.1 ± 2.7) nmol/g 1, 3 and 6 hours respectively after an intragastric Nfx administration (100 mg Nfx/kg) to rats. Studies on Nfx nitroreductase activity of breast tissue showed that the microsomal and cytosolic fractions had the ability to nitroreduce Nfx; its respective values were 173.6 ± 7.1 and 110.5 ± 7.3 pmol/mg prot. min. The Nfx nitroreduction was followed by the decrease in the drug concentration that was measured by HPLC at 400 nm. Nfx reactive intermediates produced during nitroreductive biotransformation of the drug in the breast tissue could interact with proteins and other cellular components to cause injury.

Supported by FONCYT grant.

BC-P5.

GENETIC ANALYSIS TO DEFINE THE ROLE OF YFR041C, A DNAJ HOMOLOGUE IN SACCHAROMYCES CEREVISIAE

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Reducing agents, glycosylation inhibitors, or mutations that cause hypoglycosylation, induce the Unfolded Protein Response (UPR) leading to enhanced expression of chaperones and enzymes involved in biosynthesis of secretory proteins in the endoplasmic reticulum (ER). In *S. cerevisiae*, two Hsp70s, Kar2p and Lhs1p, perform functions related to protein translocation and folding in the ER, acting in cooperation with different DnaJs. Three DnaJ homologues have been described: Sec63p, Scj1p, and Jem1p. An interaction between Sec63p and Kar2p was found necessary for protein translocation, while an interaction between Scj1p and Jem1p and Kar2p was found necessary for Kar2p-mediated protein folding and degradation.
To investigate the role of a putative new DnaJ induced by the UPR and predicted to be located in the ER, we generated deletions of the non-essential YFR041C gene. We are using two approaches to investigate the function of this gene: a) genetic crosses and tetrad analysis of Δyfr041c strains with mutants in genes encoding characterized functions of the ER. b) a synthetic lethal screen was set up to identify cellular functions that connect to the role of YFR041c. We scored growth at different temperatures and sensitivity to agents that produce stress in the ER in strains lacking YFR041C. Our results show that the absence of YFR041C aggravates phenotypes of strains with compromised functions in the ER, and suggest a role of this DnaJ homologue in the biosynthesis of secretory proteins.

Supported by Fundación Antorchas and FONCyT-PICT2000

BC-P6.

IDENTIFICATION OF A NOVEL DNAJ HOMOLOGUE ENCODED BY YFR041C IN *SACCHAROMYCES CEREVISIAE*

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Chaperones from the HSP70/DnaK family interact with Hsp40/ DnaJ chaperones to perform a variety of cellular functions. In the genome of *S. cerevisiae*, 19 ORFs encoding sequences with homology to DnaJ can be identified. One of the uncharacterized ORFs, YFR041C, encodes a hypothetical protein with a J domain and a predicted signal sequence for translocation across the endoplasmic reticulum (ER). Genomic studies pointed to YFR041C as one of the genes whose expression is induced through the Unfolded Protein Response (UPR), a pathway that modulates expression of enzymes and chaperones to support efficient folding and/or degradation of proteins accumulated in the ER. We performed Northern analysis in wild-type and mutant strains in DnaJ chaperones of the ER. We confirmed the enhanced expression of the YFR041C mRNA by DTT and tunicamycin, and found that strains lacking YFR041C show an induction of the UPR. To identify the protein, we generated strains with a HA-tagged Yfr041cp by integration in the genome of a PCR-generated module fussed to the carboxyl-terminus of the ORF.
We report here that YFR041C encodes a DnaJ homologue that enters the secretory pathway, has an amino-terminal signal sequence for translocation across the ER membrane, a single transmembrane domain preceding the J domain, and a carboxyl-terminal region located in the cytosol. Our results suggest that this novel DnaJ homologue whose expression is regulated by the UPR plays a role in the secretory pathway of yeast.

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BC-P7.

EFFECTS OF TYPE 2 PURINIC RECEPTOR ACTIVATION ON RVD OF TROUT HEPATOCYTES

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In trout hepatocytes, hyposmotic conditions induce cell swelling and subsequent efflux of ion and water, thereby promoting a down regulation of cell volume called RVD. It has been postulated that RVD is triggered by the interaction of extracellular ATP with P_2 purine receptors. We used trout hepatocytes to assess the importance of extracellular ATP in the regulation of cell volume. In hyposmotic medium, the cells swelled up to aprox. 160% of the isosmotic value, followed by 26.6 ± 0.8% and 53.5 ± 1.7% RVD after 20 and 40 min. Suramine and cibacron blue, two antagonists of P_2 receptors, generated an inhibition of 27-32% (20 min) and 48-50% (40 min) of RVD, whereas ATP and ATPγS (P_2 agonists) increased RVD by 66-77% (20min) and 12-17% (40 min). In separate experiments, we added an excess of apyrase and Na^+/K^+ATPase to cell suspensions to scavenge any possible ATP present in the extracellular medium. Incubation of cells in hyposmotic medium containing these scavengers decreased RVD by 70-100% with respect to controls.
Results indicate that ATP is an important factor enabling RVD of hyposmotically induced trout hepatocytes.

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BC-P9.

PHLOXINE B AFFECTS GLYCOGEN METABOLISM DURING *CERATITIS CAPITATA* DEVELOPMENT

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Several halogenated xanthene dyes act as photoinsecticides after ingestion by insects and sunlight excitation. The aim of this study was to examine phloxine B location at tissue level and to evaluate its pleiotropic biochemical effect that affects different steps of metamorphosis. In sub-lethal conditions, the chemical toxicity generated a delay in the jumping of larvae, thus extending the third larval-stage. The surviving larvae required more days to reach their optimal weight; probably due to a decrease in food intake, caused by damage of midgut cells. Phloxine B differently affected the accumulation of energetic storage molecules in larva III. Proteins in hemolymph decreased in treated larvae, as well as total proteins. Midgut fatty acids were not degradated as normally occurs during the jumping period, indicating a possible inhibition of lipases. Glycogen dramatically increased in larvae treated with 1 mM and or 3 mM Phloxine B showing rrespectively twice and 5 time more glycogen than controls. This finding is in agreement with our data demonstrating that fat body and midgut glycogen degrading enzyme (glycogen-phosphorylase) was partially inhibited whereas the glycogen synthesizing enzyme (glycogen-synthase) activity was increased. *In vitro* preliminary experiments seem to confirm that phloxine B directly interacts with glycogen phosphorylase, probably oxydating the histidine residue at the catalytic site.

BC-P8.

NOVEL METABOLISM OF â-ALANINE IN INSECTS

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During the last few years we studied the biosynthesis of N-â-alanyl-dopamine (NBAD), the main sclerotizing precursor of insect brown cuticles. Using free-cell systems of *Ceratitis capitata* we were able to show that the N-â-alanyl-dopamine synthase is induced in epidermis immediately before the larva to pre-pupa transition as well as before the pharate adult to imago transition. Preliminary experiments showed that this induction is regulated direct or indirectly by the molt hormone, 20-OH-ecdysone. These results have been confirmed in *Drosophila melanogaster* and other insects. We recently demonstrated (Pérez *et al.,* 1992) that the N-â-alanyl-dopamine synthase is, in fact, a wide substrate specificity cathecolamine-â-alanyl ligase, that can also synthesize N-â-alanyl-norepinephrine (NBANE) and other substances like sarcophagine (N-â-alanyl-tyrosine). Recent results, including those shown in this communication, have demonstrated that this enzymatic activity is able to *in vitro* synthesize carcinine (N-â-alanyl-histamine) and other â-alanyl-derivatives. Carcinine was first reported in the decapod *Carcinus maenas* and later in mammalian tissues. We have also demonstrate that a constitutively expressed NBAD-synthase enzymatic activity is present in the neural ganglia of insects. Using different criteria, including the characterization of the melanic mutants *niger* of *Ceratitis capitata* and *ebony*[4] of *Drosophila melanogaster* we confirmed that in these insects the brain and the epidermis enzymes are coded by the same gene, respectively *niger* and *ebony.*

BC-P10.

â-(1,2)-GLUCAN AS POTENTIAL CHEMOPREVENTIVE SUBSTANCES IN CARCINOGENESIS PROCESSES

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Cancer is perhaps the most progressive and devastating disease posing a threat of mortality to the entire world despite significant advances in medical technology for its diagnosis and treatment. Recently, considerable attention has been focused on identifying naturally occurring chemopreventive substances capable of inhibiting, retarding, or reversing the process of multistage carcinogenesis. Wide arrays of substances, particularly those present in diet, microorganisms, and medicinal plants, have been reported to possess substantial anticarcinogenic and antimutagenic effects. In particular, (1,3)-*â*-D-glucan and its derivatives have known to induce activation of several defense reactions in different organisms. The effects of three â-glucans on primary mouse keratinocytes and established cell lines from squamous cell carcinomas were analyzed. A sulfated polysaccharide (laminarin), consisting primarily of beta-(1,3)-glucan, and two cyclic â-(1,2)-glucans from *Agrobacterium tumefaciens*(At) and *Xanthomonas campestris*(Xcc) were active inhibiting cell proliferation and inducing morphological changes with cytological appearance of senescence cells. Cyclic-(1,2)-*â*-D-glucan from At and laminarin induced keratine-1 expression on the squamous cell carcinoma cell line (CH72), a marker of epidermal differentiation, suggesting that this reagent trigger keratinocyte differentiation. The stronger effect was observed with â-(1,2)-glucan from Xcc on primary keratinocytes and different cell lines. In this case, arrest of cell cycle result in decrease cyclin D1, but not in keratine-1 expression. These results suggest that distinct â-glucans trigger different mechanisms, but all of them results in lack of the replicative potential in mammalian cells. This is the first report showing a â-(1,2)-D-glucans as a potential chemopreventive compound.

BC-P11.
RETINAL ABLATION DECREASES CALBINDIN D_{28K} AND GABA EXPRESSION IN THE CHICK OPTIC TECTUM

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The effect of retinal ablation on qualitative and quantitative changes of calbindin D_{28k} and GABA expression in the contralateral optic tectum was studied in young chicks. Fifteen days old chicks had unilateral retinal ablation and after 7 or 15 days, calbindin expression was analyzed by Western blot and immunocytochemistry. Neuronal degeneration was followed by the amino-cupric silver technique. After 15 days, retinal lesions produced a significant decrease in calbindin immunostaining in the neuropil of layers 5-6 and in the somata of neurons from the layers 8 and 10 of the contralateral tectum, being this effect less marked at 7 days post-lesion. Double staining revealed that 50-60% of cells in the layers 8 and 10 were calbindin and GABA positive, 30-45% were only calbindin positive and 5-10% were only GABAergic neurons. Retinal ablation also produced a decrease in the GABA expression at either 7 or 15 days after surgery. At 7 days, dense silver staining was observed in the layers 5-6 from the optic tectum contralateral to the retinal ablation, which mainly represented neuropil that would come from processes of retinal ganglion cells. Tectal neuronal bodies were not stained with silver, although some neurons were surrounded by coarse granular silver deposits. In conclusion, most of calbindin molecules are present in neurons of the tectal GABAergic inhibitory circuitry, whose functioning apparently depends on the integrity of the visual input.

BC-P12.
A NOVEL METHOD FOR 3-D PARTICLE TRACKING IN BIOLOGICAL SYSTEMS

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We describe a novel method to track fluorescent particles in 3-D, applicable to the study of motion of fluorescent molecules in cells or other biological systems. In this method, the laser beam of a two-photon excitation microscope moves in a circular path with radius of half the width of the point spread function (PSF). When the fluorescent particle is located within the scanning radius of the laser, the precise position of the particle in the x-y plane can be determined by its fluorescence intensity distribution along the circular scanning path. A z-nanopositioner on the objective allows us to change the laser focus at two different planes located half the width of the PSF apart. The difference of the fluorescence intensity in the two planes is used to calculate the z-position of the fluorescent particle. With a fast feedback mechanism, the position of the laser beam is directed to the center of the fluorescent particle, thus allowing us to track a fluorescent particle in 3-D. Calibration experiments showed that this new method allows the tracking of particles with a time resolution of 64 ms in 3D and 16 ms in 2D. The standard deviation for the position of the particle was 20 nm in either the x, y or z axis. As an example of the applicability of this method to biological systems, we studied the dynamics of fluorescent particles in cells.

BC-P13.
EPITHELIAL-MESENCHYMAL INTERACTIONS AS A SYSTEM TO STUDY ALTERNATIVE SPLICING REGULATION BY EXTRACELLULAR SIGNALS

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Our group studies the effect of extracellular signals on the alternative splicing of the fibronectin (FN) pre-mRNA. In the case of the murine FN gene, a single primary transcript gives rise to up to 12 different mRNAs and polypeptides as the result of cell-specific alternative splicing, occurring in three different regions: EDII, EDI and IIICS. We have reported previously that a mammary mesenchymal cell line expresses higher proportions of EDI+ fibronectin than a mammary epithelial one. Furthermore, soluble factors secreted by mesenchymal cells (MC-conditioned medium) stimulate the inclusion of EDI in epithelial cells and induce cell scattering. These observations led us to speculate that the upregulation of EDI inclusion could be due to an epithelial-mesenchymal transition.
Preliminary results indicate that most of the cells remain epithelial after treatment with MC-conditioned medium, as judged by the maintenance of cytokeratin, an epithelial marker, and the isoform of the FGF receptor expressed.
We investigated the splicing pattern of the other FN alternative exons in these two cell types and asked whether the MC-conditioned medium had any effect on the splicing of this two exons. While mesenchymal cells express higher levels of EDII+ and IIICS+ FN than epithelial cells, only the alternative splicing of IIICS is regulated by this treatment.

BC-P14.
ENHANCED DELETERIOUS EFFECTS OF CARBON TETRACHLORIDE ON NUCLEAR AND MICROSOMAL COMPONENTS FROM LIVERS OF ANIMALS CHRONICALLY EXPOSED TO ALCOHOL

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It is known that chronic alcohol consumption induces CYP2E1-mediated liver microsomal metabolism of some xenobiotics. Our laboratory reported a CYP2E1 dependent ethanol metabolizing system in highly purified liver nuclei, leading to acetaldehyde and free radicals and inducible by chronic ethanol drinking. This could change the toxic and carcinogenic response of other environmental chemicals bioactivated by CYP2E1, such as CCl_4.
We found that, liver microsomes and nuclei from animals chronically exposed to alcohol showed enhanced protein carbonyl formation when these subcellular fractions were activating CCl_4 in either the presence or absence of NADPH. The microsomal and nuclear protein sulfhydryl groups content from alcohol treated animals was decreased in incubation mixtures bioactivating CCl_4 in the absence of NADPH and this effect was partially reversed by NADPH. Liver microsomal and nuclear proteins from alcohol treated animals showed increased formation of CCl_4 altered proteins in the presence of NADPH but no enhancing effects in the content of their altered lipids was observed. Results suggest that chronic exposure to alcohol might enhance the liver carcinogenic effects of CCl_4.
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BC-P15.

APOPTOSIS OF *TRYPANOSOMA CRUZI* IS INDUCED BY SESQUITERPENE LACTONES

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Trypanosoma cruzi, is a flagellate protozoa that causes the Chagas' disease. This parasite develops its life cycle between hematophage insects (*Triatoma infestans)* as intermediate vector, and mammals as definitive hosts, included the man. Numerous studies on this hemoflagellate have been made during the last century, most of them foccused to find an effective pharmacological agent with trypanocidal activity to erradicate this disease.
Sesquiterpene lactone is a group of compounds that exhibit diverse biological actions, as antitumoral and antiprotozoal. In the present study we tested the effect of two sesquiterpene lactones: dehydroleucodine (DhL) and helenaline (HLN) on cultured epimastigotes of T.cruzi, and demonstrated that these drugs inhibit the cellular proliferation, the protein and DNA synthesis and induce apoptosis, evaluated by the TUNEL technique.
On the other hand, extracts of the parasites exposed to the action of these compounds, display increased immunoreactivity against caspases 3 and 8. These evidences suggest possible pro-apoptotic actions of DhL and HLN and open new pharmacologic perspectives against Chagas' disease.

BC-P16.

HYDROXYLATED QUINONE ICETEXANE AFFECTS THE PROLIFERATION OF *TRYPANOSOMA CRUZI*

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Tripanosoma cruzi is the ethiological agent of Chagas' disease. Since decades, several trypanocidal substances have been used against the parasite, many of which have been isolated from plants. Here, we studied the effect of Icetexane 1 (ICTX), an hydroxylated quinone isolated from specimens of *Salvia gilliesi,* on the growth of cultured epimastigotes of *T.cruzi*. We observed that ICTX inhibited the proliferation of the parasites at concentrations between 1 and 3.5 ì g/ml with very low mortality. At higher concentrations the compound turned to be deleterious, with an estimated IC_{50} of 6 ì g/ml. The antiproliferative effect of ICTX was irreversible, even at short times of exposure. In order to know whether the quinone blocked a punctual step of the cell cycle we sudied the effect of the compound at different time points in synchronized parasites. They were exposed to hydroxyurea (HU) for 24 hs, and after remotion of HU, parasites re-iniciated their cell cycle from the phase S. We observed that the most important antiproliferative effect of ICTX ocurred when this drug is added during phase S (7-8 h), although we also observed an effect when the compound is added between 12-15 h after remotion of HU. Whether ICTX affects the activity of certain enzymes involved in DNA synthesis remains to be determined.

BC-P17.

SYNAPTOTAGMIN VI FUNCTION IN ACROSOME REACTION IS REGULATED BY PHOSPHORYLATION

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Acrosomal exocytosis is a calcium-dependent secretion event causing the release of the acrosomal contents and the loss of the membranes surrounding the acrosome. The synaptotagmins are a family of calcium-binding proteins that synchronize the calcium entry to exocytosis of vesicles, and share the same domain structure: an N-terminal single transmembrane domain, a spacer domain, two calcium and phospholipid binding domains (C2A and C2B), and a short carboxyl terminus. The ubiquitous synaptotagmin VI isoform was found in human sperm by Western blot. Immunocytochemistry localized the protein to the outer acrosomal membrane. Here we show that calcium-triggered acrosomal exocytosis in permeabilized sperm was completely inhibited by the C2A or C2B domains of synaptotagmin VI purified as fusion proteins with glutathione S-transferase. Surprisingly, phorbol ester-dependent *in vitro* phosphorylation of these recombinant proteins abolished their inhibitory effect. When two Thr adjacent to the C2B effector domain were mutated to Ala, the effect of the protein became resistant to phosphorylation. In addition, when these two Thr were mutated to Glu, mimicking the negative charges accomplished by phosphorylation, the protein lost its effect on exocytosis. Our results strongly indicate that synaptotagmin VI is a key component in acrosomal exocytosis. Interestingly, its activity is regulated by phosphorylation.

BC-P18.

ROLE OF NITRIC OXIDE IN THE INHIBITION OF PROLIFERATION BY SCAVENGING HYDROGEN PEROXIDE

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A role of H_2O_2 in the induction of cell proliferation has been previously demonstrated. The aim of the present study was to evaluate the mechanisms by which scavenging of H_2O_2 inhibits proliferation, particularly the role of nitric oxide in this process. For this purpose, the modulation of cell proliferation was evaluated after treatments with L-NAME, an inhibitor of nitric oxide synthase (NOS), and with ODQ, an inhibitor of guanylate cyclase, in several cell lines (PB, CH72-T4, HBL-100, MCF-7) treated with exogenous catalase and in MCF-7 cells stably transfected with the cDNA of human catalase. The inhibition of proliferation induced by exogenous catalase was reverted both by inhibiting NOS and guanylate cyclase in all the cell lines tested (50-100% control depending on the cell line). Stable expression of catalase in MCF-7 cells resulted in a decrease in H_2O_2 production and an increase in NO production, associated with a significant decrease in proliferative capacity and in malignant features. Treatments of catalase transfected cells with L-NAME or ODQ induced an increase in proliferation of 70±10% and 63±12% respectively.
In conclusion, the inhibition of proliferation by scavenging H_2O_2 could be mediated by the activation of guanylate cyclase via an increase in NO production.

BC-P19.
ANALYSIS OF THE FUNGICIDAL EFFECT OF VISCOTOXINS

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Viscotoxins (Vts) are basic low-molecular-weight proteins isolated from mistletoe, related to the type III of the thionin family. To date, six isoforms of Vts are known, namely A_1, A_2, A_3, B, 1-PS and U-PS. We have reported previously the antifungal activity of VTA_3 and VTB and their interaction with model membranes and suggested that their biological activity could be ascribed to membrane permeabilization. In the present report we describe, by using fluorescence spectroscopy, the effect of VtA_3 on spore membranes, where it produces a decrease in fluidity. We also report that VTA_3 induces leakage in the two membrane model systems used, liposomes of fungi-like synthetic lipids and liposomes composed of extracted fungal lipids. Being interested in the specific mechanism of action of VtA_3 on spores death, we report an increased level of H_2O_2 as well as a rise in internal Ca^{2+} concentration in fungal cells exposed to Vts. Ca^{2+} is involved in the spore death and in H_2O_2 increase induced by VTA_3, since in the presence of BAPTA-AM, an intracellular Ca^{2+} chelator, spore death and H_2O_2 production is drastically reduced. The possible mechanism resulting in the membranotropic effect of Vts and the relationship with fungal death is discussed.

BC-P20.
ROLES OF AN OVIDUCT-SECRETED GLYCOPROTEIN IN THE FERTILIZATION OF *BUFO ARENARUM*. MOLECULAR AND BIOCHEMICAL CHARACTERIZATION

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In *Bufo arenarum*, the success of fertilization depends partially on products secreted around the oocytes during their transit through the oviduct, forming what is known as the egg jelly coat. When immersed in a saline solution, the egg strings release a number of molecules into the medium. Along with other diffusible components, we found a 185 KDa glycoprotein named L-HGP which appears to be a heterodimer with a protective activity on the homologue acrosome. Isoforms of different molecular weight were observed in the Pars Recta, Pars Preconvoluta and Pars Convoluta portions of the oviduct. Furthermore, these isoforms of L-HGP appeared to have different protective effect on the acrosome stability. A direct demonstration of the site of synthesis of this protein was provided by inmunostaining using polyclonal antibodies against L-HGP. The staining was performed in slices from different levels of stimulated oviduct. In order to further characterize L-HGP, we sequenced the N-terminal region of the minor component of the heterodimer, and two distinct internal peptides from partial digestion. The isoelectric point, aminoacid and carbohydrate composition have also been obtained.

BC-P21.
MECHANISMS OF APOPTOSIS TRIGGERED BY $1,25(OH)_2D_3$ AND L-BUTHIONINE-S,R-SULFOXIMINE ON BREAST CANCER CELLS

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1,25-dihydroxyvitamin D_3 (1,25D) is a potent negative growth regulator of breast cancer cells, however the mechanisms involved are incompletely understood. Previous data indicate that of oxidative stress might contribute to 1,25D mediated growth inhibition and apoptosis. L-buthionine-S,R-sulfoximine (BSO) depletes GSH content increasing the effects of anticancer drugs. The aim of this study was to know the effects of 1,25D and BSO on apoptosis of MCF7 and vitamin D resistant MCF7 breast cancer cells. Cell lines were cultured in the absence or presence of 100 nM of 1,25D and/or different concentrations of BSO for 24h or 5 days. The data showed that both independently decreased cell growth, and the effect was higher when 1,25 D was combined with 20 ìM or higher BSO for 5 days. MCF7 ^{Dres}cells were not responsive to 1,25D but they were sensitive to BSO or BSO plus 1,25D. DNA fragmentation was increased by 1,25D or 1,25D plus BSO in MCF7 cells but was not detected on MCF7^{Dres}cells. ROS production was highly increased by 1,25D, and this effect was enhanced in the presence 20 ìM BSO in MCF7 cells. In MCF7^{Dres}, ROS production was only increased by 1,25D+BSO. Cytochrom c release from the mitochondria was observed in MCF7 cells treated with 1,25D or 1,25D plus BSO. In conclusion, 1,25D plus BSO produces cell growth suppression through apoptosis mechanisms that involve DNA fragmentation and disruption of mitochondrial integrity.

BC-P22.
THE ROLE OF EPAC –A NOVEL TARGET FOR cAMP- IN ACROSOMAL EXOCYTOSIS

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Cyclic adenosine 3´, 5´-monophosphate (cAMP) is produced by adenylyl cyclase in response to a variety of extracellular stimuli. cAMP regulates a wide range of biological processes, which include cell division, growth, differentiation, and secretion. Most of these processes were thought to be mediated by cAMP-dependent protein kinase A (PKA). It has recently been proposed that cAMP also directly activates Epac, a guanine nucleotide exchange factor for the small GTPases Rap1and Rap2. The acrosome reaction (AR) is a specialized type of regulated exocytosis leading to a massive fusion between the outer acrosomal membrane and the plasma membrane of sperm cells. The induction of the AR by cAMP has been proposed to be mediated by PKA. Here we report an alternative pathway for cAMP-AR involving Epac. In a SLO permeabilized human sperm model, the Epac specific cAMP-analogue 8-CPT-2Me-cAMP triggers the AR. As is the case with calcium-dependent exocytosis, 8-CPT-2Me-cAMP induced AR is sensitive to anti-αSNAP, anti-NSF and anti- Rab3A antibodies, to intraacrosomal calcium chelators, and botulinum toxins. Our results indicate a role for Epac in mediating the AR.

BC-P23.

INTERACTION OF CELLULAR NUCLEIC ACID BINDING PROTEIN (CNBP) WITH PUTATIVE CELLULAR TARGETS

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CNBP was identified as a cellular factor that interacts with single-stranded nucleic acids. It has been related to many mechanisms of gene expression regulation ranging from transcription to translation. However, its biological function reminds unknown. By band-shift assays we analysed the ability of CNBP and different mutant CNBP forms to interact with single-stranded nucleic acid probes (RNA and DNA from 5'-UTR of L4-ribosomal protein mRNA) in order to identify protein motifs involved in the interaction. We show that (i) an RGG motif is the most committed with the protein binding to RNA targets, but not to targets of DNA with the same sequence; (ii) the interaction with RNA, but not with DNA, requires addition of embryonic factors; and (iii) CNBP interacts with DNA as a monomer as well as a dimmer whereas its binding to RNA is mainly as a dimmer. This implies a differential mechanism of CNBP interaction with each kind of single stranded nucleic acids.
For defining whether CNBP has a regulative action in c-*myc* proto-oncogen expression we tested CNBP interaction with several c-*myc* putative regulatory sequences. Only the 5'-UTR of c-*myc* mRNA showed interaction following a binding pattern similar to that of L4-5'UTR. This suggests that CNBP may not be restricted to bind ribosomal protein mRNAs and could act in a more generalised mechanism of translational control.

BC-P24.

DEVELOPMENTAL BEHAVIOUR OF ZEBRAFISH CELLULAR NUCLEIC ACID BINDING PROTEIN (zCNBP)

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CNBP is widespread throughout the animal kingdom. This protein shows an striking sequence conservation and general structural organisation which suggests it plays a fundamental biological role in different species throughout evolution. It was suggested to be involved in the developmental control of ribosomal proteins translation as well as in *c-Myc* expression regulation. We used the zebrafish *Danio rerio* as an animal model to study the CNBP behaviour during early development. We cloned and analysed zebrafish CNBP. zCNBP contains seven retroviral CCHC zinc finger motif, an RGG box and putative phosphorylation sites. Although its mRNA is detected in several adult tissues, we could observe a higher level of expression in ovary. We performed *in vitro* phosphorylation assays on GST-zCNBP fusion protein in presence of zebrafish embryo extracts from different developmental stages. We observed that zCNBP is phosphorylated *in vitro* in a differential way depending on the embryo developmental stage used. Band-shift assays were performed to analyse the ability of proteins present in embryo extracts to bind single-stranded probes of ribosomal protein 5´UTR and c-Myc 5´UTR. All the stages analysed were able to bind *per se* both probes, but their binding capacity varied through early development. A similar band-shift pattern was observed using GST-zCNBP instead embryo extracts. Therefore, the differential behaviour of CNBP during early development might have regulatory implications.

BC-P25.

FORMATION OF M8B ISOMER IS NOT ESSENTIAL FOR DIVERTION OF MISFOLDED GLYCOPROTEINS TO PROTEASOMAL DEGRADATION IN *S.pombe*

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Cells have to continuously monitor whether newly synthesized glycoproteins are in the process of proper folding or if they are irreparably misfolded. Both folding intermediates and misfolded species are retained in the ER. It has been proposed that a glycan structure ($Man_8GlcNAc_2$ or M8B) could be the signal by which cells recognize irreparably misfolded glycoprotein. A mechanism for their recognition and diversion to degradation is necessarily required As M8B is produced by ER mannosidase I, a slow acting enzyme, it has been speculated that if a protein folds extremely slowly or if it is irreparably misfolded, it would display the particular M8B structure which in turn would be recognized by an ER lectin and thus diverted to proteasomal degradation. We have identified the genes encoding for de ER mannosidase I (*mns1*) responsible for the formation of M8B isomer and for the ER lectin (*mnl1*) in *S.pombe*. We analyzed the degradation of *S. cerevisiae* CPY*, (a mutant variant of the vacuolar carboxypeptidase CPY unable to fold properly) in cells lacking one or both of these proteins and in presence of mannosidase inhibitors. Disruption of *mns1* does not alter the half life of CPY*, but it is increased in cells lacking Mml1p. These results and those previously presented argue against $Man_8GlcNAc_2$ being a signal for glycoprotein proteasomal degradation.

BC-P26.

CHOLESTEROL EFFLUX AFFECTS THE MEMBRANE FUSION PROCESS IN HUMAN SPERM ACROSOMAL EXOCITOSIS

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After ejaculation mammalian sperm do not posses the ability to fertilize the egg. They acquire this ability during transit through the female tract in a process called capacitation. Cholesterol removal from the membranes is a required alteration of sperm cells for capacitation. Lipid composition also affects membrane fusion, hence it is likely that changes in lipid composition of sperm membranes may have a direct role in the membrane fusion process during the acrosomal exocitosis (AE). Here we demonstrate that the treatment of intact or permeabilized human spermatozoa with methyl-β-cyclodextrins (CD) increased the percentage of cells that underwent AE. The effect of CD on the AE was due to cholesterol removal from the plasma membrane since it was reverted by CD-preloaded with cholesterol. In addition the polienic antibiotic Amphotericin B, that is a specific sterol ligand, inhibits the AE in a dose-dependent manner in both, intact and permeabilized spermatozoa. Its effect is reverted by cholesterol. These data suggest that cholesterol efflux affects the fusion process itself and is necessary in an optimal concentration for the AE to occur. Making use of the caged calcium chelator NP-EGTA-AM we also demonstrated that cholesterol acts upstream of intracrosomal calcium release, in a very early step of the fusion process. Moreover, we have evidences that this lipid regulates the assembly of some fusion proteins involved in the AE.

BC-P27.

REVERSE TRANSCRIPTION (RT)-MULTIPLEX PCR ASSAY FOR β-ACTIN, BAX, BCL-2 AND TELOMERASE MESSENGER RNAs (mRNAs)

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Objective: To develop an RT- multiplex PCR for mRNA semi-quantitative detection of 3 genes involved in cell cycle regulation. *Materials and methods:* Specific primers for β-actin, bax, bcl-2 and telomerase mRNA were designed to span adjacent exons. RNA from Hela, H9, MT-2, Jurkat, Daudi and BL-2 cell lines was extracted and quantified by UV absorbance. cDNA synthesis was performed with oligo-dT primers by using M-MLV reverse transcriptase. PCR was carried out in two steps: 15 cycles with primers for bax, bcl-2 and telomerase (annealing Tº at 63ºC) followed by 25 cycles with the addition of β-actin primers (annealing Tº at 58ºC). PCR products were resolved by agarose gel electrophoresis. Digital images were analyzed by *Scion Image* (NIH) software to determine relative abundance of each mRNA. The bcl-2, bax and telomerasa mRNAs were normalized to β-actin mRNA for each cell line.
Results: The bcl-2 / bax ratio allowed classification of cell lines into 2 groups: ≤ 1 (HeLa, MT-2, and BL-2) or >1 (H9 and Jurkat). Daudi cells proved negative for bcl-2 mRNA. Telomerase mRNA was readily detectable in all cell lines, although at different levels. *Conclusion:* An in-house method for simultaneous analysis of genes' expression involved in cell cycle regulation was optimized and may be a useful tool for pathogenetic studies in oncologic diseases.

BC-P28.

MOLECULAR DETERMINATION OF â-GLUCOSIDASE ACTIVITY IN PSYCHROPHILIC MICROORGANISMS

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Psychrophiles are extremophiles microorganism that have the ability to grow and colonize environments where the temperature is close to 0°C. Cold-adapted microorganisms are interesting for manufacturers who need cold-storage room temperatures enzymes for food processors and cold-wash laundry detergents. The â-glucosidase is an enzyme able to hidrolize cellobiose and glucosides related. The main of this work was to isolate and characterize of â-glucosidases activity marine bacteria present in sea water and bentonic organisms samples.
The microorganisms were isolated from the South of Argentina sea water. Samples were taken from different sites of the Beagle Channel (55°S; 67°W) and intestinal content from bentonic organisms. They were cultivated in a solid medium added of cellobiose and incubated at 4ºC and 15ºC in shaking. In order to determine the presence of â-glucosidase gene PCR reactions were carried out. Microorganisms were selected by the growth on plate agar containing cellobiose and by yellow color reaction over the colony using an analogous PNPG.
â-Glucosidase activity was found in 32 colonies of the 48 isolates. â-glucosidase positive. According with the ITS profiles, the isolates were divided in 6 different groups. Microorganism of one group of its was sequencing the 1,500 bp of the 16S rDNA and of â - glucosidase gene, to determine their association with the groups and the class of bacterias. The obtained results are industrial interest because can provide new cold active products for biotechnological use.

BC-P29.

CELL-SPECIFIC REGULATION OF THE ZFHEP PROMOTER IN NEUROGENIC AND LYMPHOBLAST CELLS

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Zfhep transcription factor is involved in myogenesis, neurogenesis and T-cell differentiation. Zfhep protein is down regulated during neurodifferentiation in the embryonic brain, and in P19 embryocarcinoma cells. We have isolated the human Zfhep promoter, and aim to define the major regulatory elements in different cell types. Constructs contained 1000, 400, 212, or 12 bp of the promoter in a luciferase reporter vector. The reporter (0.7 μg) and 0.3μg of CMVβ-gal (to normalize transfection efficiency) were transfected into P19, CHO-K1 (ovary) or Jurkat (lymphocytic) cells. Data are expressed as activation relative to Z1p.12Luc (control) which contains only 12 bp of promoter. The Z1p.1000Luc reporter was active in each cell line assayed. The highest activity was obtained in Jurkat cells where the activity of Z1p.1000Luc and Z1p.400Luc was 31 times higher than Z1p.12Luc ($P< 0.001$). In Jurkat cells, Z1p.212 was activated 3-fold above Z1p.400Luc ($P < 0.01$), suggesting the presence of a negative cis-acting element in the promoter between -400 and -212. This element is not observed in CHO or P19 cells. Transfection of differentiating P19 cells showed decreased promoter activity compared to control P19 cells, indicating the presence of a neurogenic response element within the first 212 bp. Conclusion: We have localized two different regulatory regions of the Zfhep promoter utilized in either lymphocytes (-400 to -212) or in neuroblasts (-212 to cap site).

BC-P30.

ACUTE PORPHYRIA MODEL. A MECHANISTICAL APPROACH TO STUDY THE RESPONSE OF GLUCOSE METABOLISM ENZYMES TO POPHYRINOGENIC DRUG

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The acute hepatic porphyrias are metabolic diseases characterized by the accumulation of heme precursors. The glucose administration can prevent the symptomatology of these diseases. The aim of the present work was to study the relationship between glucose metabolism and the development of experimental hepatic acute porphyria, in order to investigate the role of reactive oxygen species (ROS) arising from 5-aminolevulinic acid accumulated. Female Wistar rats were administered with 2-allyl-2-isopropylacetamide (AIA) 100, 300 and 500 mg/kg bw, sc. and 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) 50 mg/kg bw, ip. The enzyme activities: ALA synthase (an acute porphyria marker), phosphoenolpyruvate carboxykinase (PEPCK, a gluconeogenic key enzyme), glucose 6 - phosphatase (G-6-Pase) and pyruvate kinase, (PK, a glycolytic enzyme) were measured. These enzymes were affected at different drug doses. At the higher doses PK and PEPCK activities (both cytosolic enzymes) decreased 43% and 45% with respect to control values. On the other hand, G-6-Pase activity (a microsomal enzyme) decreased only 13% respect to control values. The levels of carbonylated protein in cytosol, increased approx 49% at the highest AIA dose. These results suggest that the alterations on the glucose metabolism enzymes observed could be related with the damage on protein structure by ROS and could be linked to the beneficial role that glucose administration produces in this pathology.

BC-P31.

EFFECT OF GLUTATHIONE DEPLETION ON MOUSE LIVER GLUTATHIONE S-TRANSFERASES CONTENTS

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In mice fed with a protein free diet (PFD), liver glutathione S-transferase subunits mGSTM1 and mGSTP1 increase while mGSTA3 decreases. Whether these effects depend on a decrease of GSH level was examined in normal fed mice. For that, the GSH level was decreased using an inhibitor of its synthesis, buthionine sulfoximine (BSO). Simultaneously, it was also considered whether the contents of other proteins related to oxidative stress are modified. Groups of female mice fed with complete diet, received different BSO doses (2-6 mmol/kg body weight) during 3-6 days, while control groups received saline solution. The contents of mGST subunits were assessed after purification by affinity and HPL chromatography. Catalase (CAT) was determined by immunoblot analysis and superoxide dismutase (SOD) by its activity. The different BSO doses caused the following changes: a) GSH level decreased a range of 10-50%; b) mGSTM1 increased 14-25%; c) mGSTA3 decreased 14-18%; d) mGSTP1 increased (32-34%); e) CAT level and SOD activity decreased. It may be noted that the mGSTA3 and mGSTP1 changes elicited by BSO resembled those caused by PFD, but with rather low intensity. In general term, the results obtained suggest that the changes in mGST levels caused by PFD treatment occur throughout a GSH depletion and are, therefore, associated with an oxidative stress.
Supported by CONICET, and UNMDP

BC-P32.

AN ALTERNATIVE PATHWAY FOR GLYCOPROTEIN FOLDING IN *TYPANOSOMA CRUZI*. THE INTERPLAY BETWEEN CALRETICULIN AND Grp78/BiP

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The calnexin (CNX)/ calreticulin (CRT) cycle is responsible of promoting the folding of glycoproteins, retaining non-native glycoproteins in the endoplasmic reticulum (ER) and targeting misfolded glycoproteins for degradation. This system is operative in trypanosomatid protozoa but protein-linked monoglucosylated N-glycans needed to interact with CRT, are exclusively formed in these microorganisms by UDP-Glc:glycoprotein glucosyltransferase (GT)-dependent glucosylation. The gene coding for this enzyme in Trypanosoma cruzi was sequenced. Even though several of these parasite glycoproteins have been identified as essential components of differentiation and mammalian cell invasion processes, disruption of both GT-encoding alleles only partially affected these processes. The cellular content of one of the already identified *T. cruzi* glycoprotein virulence factors (cruzipain, a lysosomal proteinase) only showed a partial (5-20%) decrease in GT null mutants in spite of the fact that >90% of all cruzipain molecules interacted with CRT during their folding process in wild-type cells. No CRT-cruzipain interaction was detected in GT null mutants but secretion of the proteinase was nevertheless delayed due to a lengthened interaction with Grp78/BiP probably caused by the detected induction of this chaperone in GT null mutants. This result provides a rationale for the absence of a more drastic consequence of GT absence.

BC-P33.

TRANSCRIPTIONAL REGULATION OF THE GENE ENCODING THE KRÜPPEL-LIKE FACTOR KLF6

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The Krüppel-like transcription Factor 6 (KLF6) is highly regulated during cell proliferation and differentiation, while its aberrant expression is associated with tumor formation. To gain insight into the transcriptional regulation of KLF6 we have determined the KLF6 gene expression in 26 human cell lines representing 11 tissues by employing an array spotted with cDNA representing the entire mRNA message. Simultaneously we investigated the effects of DNA damage, oxidative stress, and various metabolic inhibitors on KLF6 gene expression. The survey of a total of 702 samples are presented, which in turn predicted interesting new functions for KLF6 in hypoxia and cell cycle control. To explore the mechanisms underlying KLF6 transcriptional control the 5´ flanking region of human KLF6 was analyzed. First, we found a high structural resemblance of the regulatory regions between human KLF6 and its orthologous genes in rodents that might also predict a high degree of evolutionary conservation, thereby reflecting similarity of converging signals in these species. Second, the potential regulatory regions of KLF6 were analyzed in transfection experiments. Results showed a strong activation of the fragment located between positions -580/-1, while deleted fragments devoid of TATA-box had lower activation rates. Our data suggests that KLF6 will emerge as a promising candidate involved in the transcriptional control of cell proliferation, differentiation and also in tumor development.

BC-P34.

ROLE OF THE HOST CYTOSKELETON IN THE FORMATION OF *COXIELLA BURNETII*-CONTAINING VACUOLES

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Coxiella burnetii, the agent responsible for Q fever, is a strictly intracellular microorganism that replicates in the host cell forming large vacuoles, likely phagolysosomes. However, very little is known about the trafficking and the survival of *C. burnetii* within this harsh environment. In order to characterize the role of cytoskeleton in the formation of the compartment where this bacterium replicates, *C. burnetii* infected CHO cells were treated with inhibitors of different cytoskeleton elements for 16 h. After 48 h infection, untreated cells showed numerous bacteria-containing vacuoles. Interestingly, by fluorescence microscopy we have observed that the membrane of the *C. burnetii*-containing vacuole was decorated with actin as determined by rhodamin-phalloidin labeling. When infected cells were treated with cytochalasin D, a microfilament depolimerizing agent, the number of the vacuole decreases significantly. In cells treated with latrunculin B, another actin-disrupting drug, the inhibitory effect was more dramatic. A further significant effect was observed in cell treated with butanedione monoxime, a myosin ATPase inhibitor. Whereas no effect on vesicles formation was observed in cell treated with nocodazole, a microtubule depolimerizing agent, at the concentration tested. These results suggest that actin and myosin play a critical role in the biogenesis of the parasitophorous vacuole where *C. burnetii* replicates.

BC-P35.

THREE PUTATIVE NEGATIVE REGULATORY PROTEINS ARE INVOLVED IN PSG5 GENE REPRESSION IN NON-PLACENTAL CELLS

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The human pregnancy-specific glycoprotein 5 gene (PSG5) expression is maximal in placenta. Its minimal promoter lacks TATA-box, but contains a GC-like box acting as a core promoter element, recognized by the ubiquitous Sp1 transcription factor in both PSG-producing and non-producing cell lines. The proximal 5´ regulatory sequence bears at least four footprint motives (FP1-FP4). To get further insight into the regulatory factors involved in PSG5 gene expression, we have now investigated the nature of the transcription factors that recognize the FP3 and FP4 *cis* elements in placenta as well as in different cell lines which do not express endogenous PSG genes. The incubation of protein extracts from these cell lines with FP3 and FP4 probes yielded, in both cases, specific DNA-protein complexes that were abolished by an excess of unlabelled competitor DNA but not by mutated FP3 or FP4, respectively, nor by unrelated oligonucleotides. However, these complexes were absent when extracts of human term placenta were employed. UV-crosslinking assays revealed that two proteins of 71 ± 2 KDa and 142 ± 6 KDa are involved in complex formation with FP3 *cis*-acting element, and a 115 ± 3 KDa protein is involved in FP4 complex formation. Disruption of these sequences in PSG5-CAT constructs showed increased transcriptional activity in transient-expression assays in COS7 cells. We propose that placenta specific expression of PSG5 gene is mainly achieved through an impairment of these repressing functions.

BC-P36.

KAZAL PROTEINS ISOLATED FROM THE SKIN OF *PHYLLOMEDUSA SAUVAGII*

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In acidic extracts of *Phyllomedusa sauvagii* skin we have found two new proteins, PSKP-1 and PSKP-2, of 6.7 and 6.6 kDa, respectively; which belong to the Kazal family of serine protease inhibitors. PSKP-1 and PSKP-2 exhibits the unprecedented feature of having proline at P_1 and P_2 positions of the active site. PSKP-1 was expressed in *E. coli*, purified from inclusion bodies, and oxidatively refolded to the native state. Native PSKP-1 is bacteriostatic and agglutinates red cells at micromolar concentrations, but it was unable to inhibit trypsin, chymotrypsin, V8 protease, and proteinase K. An engineered PSKP-1 with changes at P_1, P_4, P_5 and P_6 inhibits trypsin. The biological function of PSKP-1 may be to inhibit prolyl peptidases. Its microbicidal and membrane disturbing activity suggests also a role against microbial invasion.

BC-P37.

Rab22a CAUSES THE ACCUMULATION OF TRANSFERRIN IN ENLARGED ENDOSOMES

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Eukaryotic cells continuously endocytose membrane proteins, lipids, and solutes. Separation of recycling receptors from lysosomally directed proteins takes place within the early endosomes, also named sorting endosomes. The small GTPases Rab5, Rab4 and Rab11, members of the rab family of regulators of intracellular transport, have been implicated in the recycling of receptors. We qualitatively monitored the effect of Rab22a Wild Type and of the constitutively active mutant, Rab22aQ64L, tagged with the green fluorescent protein, on the distribution of Transferrin (Tfn) receptor in TRVb-1 cells by confocal immunofluorescence microscopy. High levels of Rab22aWT and Rab22aQ64L expression cause enlargement of endosomes rich in Tfn receptors. These enlarged endosomes were accessible to internalized Tfn as indicated by the colocalization of fluorescently labeled Tfn and Rab22a-containing vesicles. In control cells, the bulk of Tfn was recycled into the medium after a 60 minutes incubation. However, in cells overexpressing Rab22aWT or Rab22aQ64L, the rhodamine-Tfn was still found in the large vacuolar-like structures labeled with Rab22a. We speculate that increased homotypic fusion between early endosomes may interfere with the spatial organization of membrane domains that mediate transport to the recycling pathway. Alternatively, vesicles destined for recycling might fuse back with the early endosome in the presence of Rab22aWT or Rab22aQ64L.

BC-P38.

POLYAMINES AND LIPID PEROXIDATION IN *BIOMPHALARIA GLABRATA*. EFFECTS OF PARAQUAT

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Polyamines are small organic cations that are required for eukaryotic cell growth and differentiation. Paraquat (PQ) is an herbicide widely used in terrestrial and aquatic environments and extremely toxic to humans and certain animal species. PQ can be actively internalized by the polyamine transport system, especially in lung cells. It can also induce the production of reactive oxygen species. The aims of the present study were to determine polyamine levels in pigmented and non-pigmented freshwater gastropods *Biomphalaria glabrata* and to investigate some aspects of PQ toxicity. Polyamine levels were determined by HPLC in the total soft tissue and in different anatomical regions. The values were: 2687±104 and 2485±145 nmol putrescine/g wet total soft tissue, and 827±53 and 601±56 nmol spermidine/g for control non-pigmented and pigmented organisms, respectively. Spermine was not detected in either case. In all the tissues, excepting for digestive gland, putrescine levels were always higher than spermidine levels. Acute PQ treatment (0.5 ppm) did not modify polyamine levels in both organisms. Instead non-pigmented organisms exhibited a higher lipid peroxidation than pigmented ones. The values of LC_{50} were 2.0 and 8.8 ppm for non-pigmented and pigmented *B. glabrata*. These results suggest a possible protective role of melanin pigments in PQ toxicity.

BC-P39.

MOLECULAR DETERMINANTS OF NUCLEAR-CYTOPLASMIC LOCALIZATION IN PROTEIN TYROSINE PHOSPHATASE 1B (PTP1B)

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PTP1B is a non transmembrane protein tyrosine phosphatase anchored to the cytosolic face of the endoplasmic reticulum (ER) via a 35 amino acid C-terminal sequence. Here we show that a chimeric GFP-PTP1B lacking the 51 C-terminal residues (PTP1B-ΔC) still localizes in the ER suggesting that additional sites are involved. Mutation of Pro309/Pro310 to alanines (PA) on PTP1B-ΔC disrupts a type II ligand for SH3-containing proteins. This PA-PTP1B-ΔC mutant exhibits a diffuse distribution in the cytosol indicating that, in addition to the C-terminus sequence, the proline-rich motif of PTP1B contributes to its ER targeting. Interestingly, PA-PTP1B-ΔC accumulates into the nucleus, as two different substrate traps (STs) of PTP1B-ΔC, in which Cys 215 was replaced by Ser, and Asp181 by Ala in the catalytic domain. Nuclear accumulation of the STs is stimulated by serum, and cytosolic localization is promoted by starving conditions. Together, these results suggest that PTP1B may be transported to the nucleus in complex with a tyrosine phosphorylated substrate. Nuclear localization also require of an additional site that maps in the PTP1B N-terminus. Deletion of 18 residues of the N-terminus excludes the ST from the nucleus in 100% of the cells. This ST-ΔN/ΔC regains the association with the ER and suggests a hierarchy of PTP1B interactions.

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BC-P40.

REDOX MEDIATED CHANGES PRODUCED BY CHLORPYRIFOS IN *Bufo arenarum* LARVAE

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Examination of biochemical and whole organism responses to stress provide evidences into the ways in which animals manage to survive. Therefore, we have studied *in vivo* the effect of the organophosphorus pesticide Chlorpyrifos on the activities of Glutathione S- Transferase (GST) and Catalase, two important antioxidant cellular systems and GSH content. Groups of 200 larvae, (Stage 25: complete operculum), were exposed to sublethal concentrations (0.5; 1; 1.5; 2 and 2.5 mg/L) of Chlorpyrifos for 96 hours. The LC 50 of Chlorpyrifos was 7 mg/L. All the sublethal concentration assayed reduced the GSH level in 30%. The GSH-S-T activity in control groups was 0.12 moles/min mg protein and decreased significantly ($p<0.05$) in all the groups treated. Catalase activity was also decreased. The greatest reduction was obtained with the lowest concentration used. The reduction in the activity of GST contrast with the results obtained with other organophosphorus pesticides and may be produced by the chlorine atom present in this compound. The inhibition of Catalase could be assigned to the inhibitory effect of the O_2^- anion.

BC-P41.

INTRACELLULAR LOCALIZATION OF A LIPOPHILIC ALKYL LONG-CHAIN TETRAPHENYLPORPHYRIN AND CHLORIDE CHANNEL ACTIVATION IN *Psammobatis extenta* ELECTROCYTES

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We have investigated uptake, intracellular localization of 5,10,15,20-tetrakis(4-*n*-dodecylphenyl)-porphyrin (TPP), elemental composition and morphological changes in electrocytes of *P. extenta* (Rajidae), a weakly electric fish. Electrocytes are large cells with few organelles and this facilitates the study of the intracellular localization of the photosensitizer. The development of new generation photosensitizers to improve the efficiency of photodynamic therapy (PDT) is an area of intensive research. Electric organ cryostat sections were incubated with the systems: a) 7.8 $x10^{-5}$ M of TPP in a mixture of $CHCl_3$-EtOH-imidazole buffer or b) 3.9 $x10^{-4}$ M of TPP in xylene-EtOH-imidazole buffer, and observed with an epifluorescence microscope. In both cases, TPP was localized in electromotor nerves and in the nuclear chromatin of electrocytes. The mitochondria were slightly fluorescent. This localization is unusual for a photosensitizer of similar polarity. For microanalysis by energy-dispersive X-ray spectra (EDAX, EPXMA), segments of electric organ were treated with the TPP in similar conditions described above. In both cases the EDAX pattern shows a new peak corresponding to chloride anion. The semi-quantitative weight % (Kα) for this element was 47% (a) and 33% (b). Compared to negative controls, the peaks for Na^+ and Ca^{2+} were 5 and 2-fold larger, but for K^+ was 6-fold smaller. The massive intracellular accumulation of Cl^- and the influx of Na^+ lead to cell swelling, and the subsequently necrotic response of cells.

BC-P42.

SKIN DAMAGE INDUCED BY UVB RADIATION. ANALYSIS OF AN *IN VITRO* MODEL

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The Ultraviolet irradiation is divided in A, B and C, being the UVB the most harmful for health. This radiation is involved in the development of non melanoma skin cancer and in the cutaneous photosensitivity processes showed by patients suffering from autoimmune diseases, like Systemic Lupus Erythematosus. Failures in cell cycle control, as well as programmed cell death disturbances, could mediate tumor development. In the cutaneous photosensitivity process there are certain autoantigens involved, like Ro/SS-A 52 and 60; these are nuclear antigens, which are relocated towards the cell membrane after UVB light exposure and, once relocated, they could be recognized by circulating autoantibodies. Keratinocytes are the cells which are more exposed to natural UVB radiation, this is the reason why we have chosen HaCaT cells (human keratinocytes cell line) for the development of the present work. The cells were irradiated with variable doses of UVB light and the apoptosis morphology was evaluated by fluorescence microscopy. The cells irradiated with 50 mJ/cm^2 developed almost 100% of apoptosis within 24 hours after irradiation. In addition to the morphology analysis we will evaluate, by Western Blot, the expression of different proteins involved in the apoptotic pathways, like p53, caspases, Bax and Bcl-2 and the autoantigens Ro/SS-A 52 and 60.

BC-P43.

EFFECT OF RAB GTPASES ON THE AUTOPHAGIC DEGRADATION OF LONG-LIVED PROTEINS

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Autophagy is a degradative pathway by which cells sequester cytosolic proteins and organelles into double membrane vesicles (autophagosomes) that deliver their content to the lysosomes. This process plays an important role in cell maintenance and development and it is regulated by nutrient deprivation. Little is known about the proteins that regulate the autophagic pathway in mammalian cells. Map-lc3 (microtubule-associated protein 1 light chain 3) was the first mammalian protein identified that, after processing, specifically associates with autophagosome membranes. Rab gtpases are small gtp-binding proteins that control specific intracellular traffic events. We have shown that rab24 colocalizes with lc3 after induction of autophagy. Surprisingly, our results indicate that in cho cells, overexpression of lc3 and rab24 wild type proteins reduce the autophagy-induced long-lived protein degradation. In contrast, overexpression of disable membrane associated mutants (lc3 ^{g120a}, lc3 $^{g120a,\Delta c22}$ and rab24^{s67l}) have no effect on autophagic degradation. On the other hand, rab7 wild type and the active mutant rab7^{q67l} which control the aggregation and fusion of late endosomes/lysosomes induced protein degradation; while the inactive mutant rab7^{t22n} hampered the fusion of autophagosomes with lysosomes. These results indicate that rab proteins are key molecular components for the normal progression of autophagy.

BC-P44.

DIFFERENTIAL EXPRESSION OF THE h-DLG ONCOSUPPRESSOR IN HISTOLOGICAL SAMPLES FROM HPV-ASSOCIATED LESIONS AS A MARKER FOR PROGRESSION TO MALIGNANCY

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High risk HPVs play a causal role in the development of cervical cancer. HPV E6 oncoproteins target h-Dlg for ubiquitin-mediated proteolysis. h-Dlg oncosuppressor is associated with cell-cell interactions, and deregulation of these structures leads to defective cell adhesion, loss of cell polarity and unregulated proliferation. We evaluated the contribution of this E6 activity in the progression to malignancy in HPV infections by analyzing Dlg expression in HPV-associated lesions. We analyzed cervical, laryngeal, vulvar, colon and kidney histological samples by Dlg immunohistochemistry. HPV association was ascertained by a PCR-colorimetric method. Although Dlg was certainly expressed in the intraepithelial cervical, vulvar and laryngeal HPV associated lesions, its cellular and tissue distribution pattern was altered comparing with normal tissue. Interestingly, a critical reduction in Dlg levels was observed in HPV-positive cervical invasive carcinomas. However, Dlg loss was not observed in tumours not linked to HPV, such as colon and kidney carcinomas, even though changes in Dlg distribution archetype were detected. The loss of Dlg may be considered a late stage in cervical HPV carcinogenesis but alterations in its expression and localization take place during the different displastic stages.

BC-P45.

RIM IS PRESENT IN THE HUMAN SPERMATOZOA AND IS INVOLVED IN THE ACROSOME REACTION

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Acrosome reaction (AR) is a regulated exocytic process, essential during gamete interactions. There is information regarding the role of fusogenic proteins during this exocytosis. One of those proteins is RIM, a putative effector of Rab3A. We used human normal sperm samples (WHO). After swim-up separation, the concentration was adjusted to 7 x 10^6 cells/ml. Indirect immunofluorescent assay using a polyclonal antibody showed that RIM is present in streptolysin O permeabilized human spermatozoa, and is localized to the acrosome. Additionally, the N-terminal residues 1-399 of RIM inhibited the AR stimulated by calcium in a dose-dependent way. In brief: 1) RIM is present in the acrosomal region in human spermatozoa; 2) the N-terminal portion of RIM inhibits the AR. It is possible to postulate that the lack of the C terminus, where the conspicuous domains C2A and C2B are located, is responsible for the inhibitory effect. We will test this possibility by using the whole molecule from purified brain extracts.

BC-P46.

ALPHA2-MACROGLOBULIN (α_2M) MEDIATES INTRACELLULAR CALCIUM LEVELS VIA LOW-DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN (LRP) IN MACROPHAGE CELL LINE

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Alpha-2-Macroglobulin is the most important human proteinase inhibitor, upon binding to proteinases it undergoes a conformational change, activated α_2M (α_2M*), that exposes receptor recognition sites allowed its interaction with LRP receptor. LRP is a scavenger receptor which belongs to LDL receptor gene family that binds to a variety of ligands, some of which trigger signal transduction. The biological function of α_2M*/LRP interaction is not fully understood. Previously we have demonstrated that α_2M* has proliferative effects on macrophage cell line, J774, which could be blocked with RAP (receptor associated protein), a specific antagonist to LRP. In this work we investigate the α_2M* effect down stream to LRP interaction measuring intracellular calcium and MAPK phosphorylation on J774 cell line in the presence or absence of LPS. It is know that LPS down regulates the gene expression of LRP generating J774 cells fully depleted of LRP [J774(LRP-) cells]. By using Fura-2/AM and spectrofluorometric techniques we demonstrate that α_2M* increase intracellular calcium in J774(LRP+) but not in J774(LRP-) cells. Lactoferrin, another ligand of LRP could also increase intracellular calcium in J774 (LRP+) cells. In addition, by immunoblotting we observed an increase in MAPK phosphorylation in J774 (LRP+) cells treated with α_2M*. Thus, we propose that α_2M*/LRP interaction is involved in intracellular signaling events, suggesting that calcium could be a key regulator of cell proliferation.

BC-P47.

INCREASED OR REDUCED LEVELS OF SPARC LEAD TO ADHESION AND MIGRATION PHENOTYPES IN *Drosophila melanogaster* EMBRYOS

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The extracellular matrix (ECM) is an intricate arrangement of glycoproteins, collagens, proteoglycans and growth factors 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. SPARC is a 42 kDa ECM component which regulates cell-shape, adhesion, proliferation, migration and differenciation. 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 receptor and its associated signaling pathway using the *D melanogaster* model. This biological model shows extensive conservation of the main ECM components in the context of a smaller gene number. To carry out this project, we generated several UAS transgenic lines and used them to overexpress dSPARC in spatially and temporally restricted patterns. Ubiquitous or mesoderm-ectoderm specific overexpression produced phenotypes likely related to migration and adhesion failures, namely germ band retraction, dorsal closure and denticle-belts pattern. We have observed similar phenotypes studying chromosomal deficiencies that include the SPARC locus, as confirmed by single-embryo PCR. Our results are consistent with a model in which either increased or decreased activity of SPARC leads to defects in cell adhesion and migration.

BC-P48.

EXPRESSION OF EBAF GENE IN RAT OVIDUCT. EVIDENCES OF A REGULATION OF POLY A SITE RECOGNITION

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We have previously isolated an oviduct-uterus specific cDNA fragment (Pr14) (Accession No. AF202268) by using RNA arbitrarily primed PCR (RAP-PCR). This EST (**E**xpressed **S**equence **T**ag) corresponds to the 3' UTR (**U**n**T**ranslate **R**egion) of the *Rattus norvegicus* endometrial bleeding-associated factor (ebaf) (Locus Link Accession No. LOC289316) a novel member of the Transforming Growth Factor superfamily. Previous Northern analyses with the Pr14 probe showed two transcripts of about 2.9 kb and 4.1 kb in early pregnant rat oviducts. Analysis of the nucleotide sequence of the 3' UTR of this gene showed potential poly A signals at 1,028; 1,032; 1,240; 2,957 and 3,344 nucleotides from the end of the CDS (**C**oding **D**educted **S**equence) of the terminal exon. Taking into account that the predicted CDS of this gene is 1,101 bp in length, the different theoretical mRNAs could have 2,129; 2,133; 2,341; 4,058 and 4,445 nucleotides respectively. Interestingly, these sizes are in agreement with some of those calculated from the genomic sequence. Our hypothesis is that the 2.9 kb and 4.1 kb transcripts detected in Northern Blots could be originated by an alternative poly A site recognition mechanism. In order to confirm this idea we performed RT-PCR assays using specific primers to amplify the different transcripts. Results obtained are in agreement with our hypothesis.

BC-P49.

GLUTATHIONE REDUCTASE AND CATALASE IN TOAD EMBRYOGENESIS: PESTICIDE AND POLYAMINE INTERACTION

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We have demonstrated the relevance of glutathione (GSH) joined to GSH transferase induction in organophosphate (OP) detoxification for protection and response against cellular damage in aquatic species. Polyamines (PAs) potentiate OP toxicity in toad embryogenesis competing for GSH pool. We evaluate here GSH reductase (GR) and catalase (Cat) profiles in the embryonic development of *Bufo arenarum* and their alteration by OP and PA exposures.
Cat activity was high and constant through embryogenesis (100 IU mg protein^{-1}). Azinphos methyl elicited a biphasic effect, first inhibiting (20%) and then inducing Cat at last stages (30%), which was also observed for malathion. Larval stages showed up to 50% inhibition by OPs. Spermine inhibited Cat at middle development, while spermidine inhibited it in larvae. GR activity continuously increased along development (7 mIU mg protein^{-1}). Azinphos methyl caused early inhibition of GR (60%), followed by Malathion (45%). Spermine inhibited GR up to 70% in caudal circulation stages. Effects on exposed larvae were not significant.
GR inhibition by spermine and spermidine may contribute to the described redox shift to GSSG during PA degradation and consequently to oxidative stress derived from GSH depletion. Although lipid peroxidation was not enhanced in our experimental system, the early inhibition of Cat might be related to oxygen radical attack, while a compensatory induction would be elicited as response to alleviate oxidative stress.

BC-P50.

ANTIOXIDANT DEFENSE AND FUNTIONAL INTEGRITY OF ACROSOMAL MEMBRANE DURING RAM SPERM CRYOPRESERVATION

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Thawing of cryopreserved ram semen currently enhances reactive oxygen species accelerating semen aging. We have developed hypertonic diluents which result better cryoprotectants evaluated *in vitro* and *in vivo*, and inversely related to lipid peroxides. We compare here glutathione (GSH) content and GSH reductase (GR) activity in spermatozoa, and *in vitro* capacitation and acrosome reaction after freeze-thawing in isotonic (B1) vs. hypertonic diluents containing threalose (B2) or threalose+EDTA (B3).
GSH content was significantly lower in spermatozoa frozen in B1 ($p=0.05$), and 3h after thawing ($p=0.015$). GR activity was significantly decreased using B1 ($P=0.00008$) and 3h after thawing ($p=0.013$). Intact acrosomes were significantly lower using B1 when thawing in control medium ($p<0.0007$), with a high capacitation percentage (72%) which was lower for B2 and B3. Capacitation with seroalbumin increased acrosome reaction downshifting intact acrosomes, and additionally from capacitated ones in B1. Ca^{2+} ionophore A23187 increased reacted status to 60% for B2 and B3 decreasing capacitation to 34%.
These results suggest that the notable capacitation by freeze-thawing would be lower for semen cryopreserved in hypertonic diluents, maintaining besides a better response to chemical signals for acrosome capacitation and reaction. A better antioxidant status is inferred from GSH and GR which may be related to this action.

BC-P52.

OXYGEN SENSING IN DROSOPHILA: A PROLYL-4-HYDROXYLASE THAT MODIFIES THE bHLH-PAS PROTEIN SIMA/HIF-1α

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In mammals, the bHLH-PAS protein HIF-1α controls the transcriptional response to hypoxia. HIF-1α levels increase dramatically in hypoxia and largely depend on protein stabilization. Proteasomal degradation in normoxia requires hydroxylation of a specific prolyl residue that is catalyzed by oxygen-dependent prolyl-4-hydroxylases that operate as oxygen sensors. We have identified the *Drosophila* bHLH-PAS protein "Similar" (Sima) as the Hif-1α fly homologue. Furthermore, we have isolated the specific prolyl-4-hydroxylase that we have named Fatiga (Fga). We show here that *fga* loss of function caused stabilization of Sima in normoxia along with constitutive induction of the transcriptional response *in vivo*. We also show that *fga* gene generates three different transcripts originated by a combination of alternative splicing and alternative initiation of transcription (*fgaA, B, C*). RNA in situ hybridization experiments revealed that *fgaB* and *C* but not fgaA are induced in hypoxia or upon ectopic expression of Sima. We raised Fga-specific antisera in rabbits and immunofluorescense experiments in embryos showed that FgaA is the most abundant isoform, being localized mainly in the cytoplasm. We have generated several novel *fga* loss of function alleles that are lethal at several different stages ranging from late embryogenesis to pupal stages. Morphological analysis revealed that *fga* mutants have alterations in tracheal development and that such defects are phenocopied in hypoxia or upon Sima ectopic expression.

BC-P53.

***IN VITRO* INVESTIGATION OF THE MOLECULAR MECHANISM OF CYTOPROTECTIVE ACTIVITY OF DEHYDROLEUCODINE**

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Artemisia douglasiana Besser, used in folk medicine as a cytoprotective agent, was studied, and its active principle dehydroleucodine (DhL) was isolated (Giordano *et al.*, 1990). In the present study, we investigated the effects of DhL on lipid peroxidation on cultured rat hepatocytes, in an attempt to elucidate several factors involved in the mechanism of DhL-induced cytoprotection. Hepatocytes were isolated from Sprague-Dawley male rats by reverse perfusion of the liver with collagenase, as previously described (*Toxic in vitro* **5**:435-438, 1991). Lipid peroxidation was assayed in aliquots of culture medium after reaction with thiobarbituric acid (*Vitamins* **51**:21-29, 1977). DhL (0.5 μg/ml) reduced lipid peroxidation. DhL significantly prevents the formation of gastroduodenal lesions induced by ethanol in duodenum-ligated rats (Method of Melchiorri *et al.*, 1997). Lipid peroxidation was suggested to contribute to the ethanol-induced gastric injury (Nordmann *et al.*, 1992). The data reported here indicate that DhL reduced oxidative injury. Cytoprotective activity of DhL could be due, in part, to their capacity of decrease lipid peroxidation which may be involved in peptic ulcer.

BC-P54.

INSIGHTS OF PHOSPHOLIPID SYNTHESIS ACTIVATION BY c-FOS

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c-Fos is a transcription factor that is also capable of regulating phospholipid synthesis *in vivo* and *in vitro*. This capacity depends only on the protein itself, independently of its role as a transcription factor. The transfection of fibroblasts with *c-fos* is enough to increase phospholipid labeling assayed *in vivo* or *in vitro*. Recombinant c-Fos activates *in vitro* the following phospholipid synthesis enzymes: CDP-DAG synthase, PtdOH phosphohydrolase, PtdIns kinases and PtdInsP kinases. Each enzyme responds with maximum activity at different c-Fos concentrations. The molecular mechanism of this activation is not clear yet, although the co-localization of c-Fos with endoplasmic reticulum markers suggests an interaction between this protein and the enzymes studied and/or phospholipids. It should be noted that all the phospholipid synthesis enzymes are integral membrane proteins or translocate to the membrane. An example of the latter is PtdIns(4) kinase and c-Fos increases the activity of the immunoprecipitated enzyme in a cell-free system. Taking into account the importance of phospholipids as membrane constituents and as secondary messenger providers, the study of c-Fos´ new role as a phospholipid synthesis activator on proliferation and differentiation of cells was considered of interest. Deregulated expression of c-Fos or its viral counterparts v-Fos FBJ or v-Fos FBR results in morphological transformation of cells. Cells transfected to express v-Fos showed increased phospholipid labeling. It is postulated that phospholipid synthesis activation by cytoplasmic c-Fos participates in cell transformation and neoplasia.

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BC-P55.

MOLECULAR CLONING OF HUMAN EPIDIDYMAL E-CADHERIN

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Cadherins (cad) comprise a large protein superfamily with a vast structural diversity. Classical cad (within them epithelial: E-cad) have five extracellular domains, a transmembrane domain, and a highly conserved cytoplasmic domain. Other cad, such us T-cad, lack the last two domains. Immunolocalization and Western blotting studies show the expression of E-cad in human epididymis and spermatozoa, and biological assays suggest its involvement in gamete interaction.

The aim of the present study was to characterize the coding sequence of human epididymal E-cad.

A human epididymis cDNA expression library was developed using lambda ZAP express vector, and was screened using a polyclonal anti E-cad antibody. A group of positives were selected, cloned, and characterized.

A total of 19 positives were identified; 6 were cloned and partially characterized (insert size 2.9-6 kbp). Some of the clones showed a high similarity between their nucleotide sequence and that reported in other tissues (>95%). In addition, a set of clones were found to have a 34 bp deletion in the 3´region of the transcript. This sequence change disrupts the reading frame, which results in the generation of a novel highly basic amino acid domain, and a premature stop codon.

Molecular cloning of human epididymal E-cad suggests the presence of transcripts encoding a novel protein isoform. Protein expression analysis will help in assessing localization and function of this isoform.

BC-P56.
VASCULAR ENDOTHELIAL GROWTH FACTORS (VEGF-C/D) AND TISSUE-RELATED MATRIX METALLOPROTEINASES (MMPs) ASSOCIATION WITH TUMOR METASTASIS

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Metastasis stepwise progression involves matrix extracellular proteolytic activity as well as cytokines and growth factors expression, both compromised in cell-cell and cell-matrix interactions. In this work, by using an experimental tumor model developed in our laboratory, we evaluated the gene expression of VEGF-C and VEGF-D as well as the protein level of MMP2 in liver, spleen, lymph nodes and tumor biopsies. In this model, the down regulation of the cell adhesion molecules uPAR and ICAM-1 associated with tumor cell spreading to liver, spleen and lymph nodes was demonstrated. Herein, by semiquantitative RT-PCR analysis, we demonstrated that tumor cells were able to express VEGF-C and VEGF-D mRNA in culture and this expression was maintained in tumors derived from these cells. Liver biopsies from bearing tumor rats showed a significant increase in VEGF-C/D mRNA content compared with normal liver samples. The VEGF-C/D mRNA expression observed in spleen and lymph nodes samples did not show differences between tumor bearing and normal rats. MMP2 proteins were examined by immunohistochemistry on liver, spleen, lymph nodes and tumor biopsies. Tumor, spleen and lymph node samples were MMP2 negative. On the other hand, on liver samples, even though tumor cells were MMP2 negative, a strong MMP2 expression was detected in the hepatocytes. Thus, these results strongly suggest that growth factors and proteases from the stromal milieu participate in tumor cell anchorage.

BC-P57.
PLASMINOGEN ACTIVATORS IN PORCINE OVIDUCT: mRNA LEVELS IN EPHITELIAL CELL CULTURES UNDER SEXUAL STEROIDS INFLUENCE

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Proteolytic enzymes have been shown to participate in multiple phases of mammalian fertilization; some of these events occurring in the oviduct. In previous works we demonstrated urokinase type (u-PA) and tissue type (t-PA) plasminogen activators (PAs) activities in porcine oviduct. These enzymes are expressed in this organ and the u-PA mRNA levels vary during estrous cycle, being higher after ovulation. In order to know if u-PA is expressed in epithelial cells and if it depends of hormonal regulation, two types of experiments were designed: 1) Northern blot assays were performed in cells obtained by scraping the oviductal lumen of oviducts in follicular (FP) and lutheal phase (LP). The 2.4 Kb mRNA of u-PA was detected in both phases. Higher levels were observed during LP, when the predominant hormone in the sow is Progesterone (P). 2) Semi quantitative RT-PCR was employed to study mRNA levels of u-PA, t-PA and plasminogen activator inhibitor (PAI-1) in primary cultures of oviductal cells, under hormonal stimulation. After seven days of culture, cells were stimulated during 24 hs. with Estrogen (E) or P. The transcriptions levels of the genes corresponding to t-PA, u-PA and PAI-1 increased when cell cultures were under influence of P; no changes in their expression were observed during E stimulation. Our finding indicates that oviductal epithelial cells produce PAs and PAI-1 and transcription of these genes is up-regulated by P.

BC-P58.
c-FOS ACTIVATES PHOSPHOLIPID SYNTHESIS IN NORMAL AND TUMOR CELLS

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In PC12 cells induced to differentiate with NGF, c-Fos plays a dual role: it first releases the nuclear differentiation program (genomic activity) and then cytoplasmic c-Fos sustains neurite elongation. Upon NGF withdrawal, neurites stop growing and preformed neurites retract. However, NGF withdrawal has no effect on transfected cells expressing full-length c-Fos or c-Fos deletion mutant capable of activating phospholipid synthesis. c-Fos and these mutants co-localize with ER whereas those without phospholipid synthesis activating capacity do not. These results indicate that cytoplasmic c-Fos activates phospholipid synthesis for the genesis of membrane required for growth. Large scale phospholipid synthesis is required for proliferation and growth of tumor cells. We examined c-Fos expression, phospholipid synthesis activation and proliferation in the following tumor cells: C6, T98G, U87MG, HOG, NB41A3. Cells stimulated to proliferate showed an increased phospholipid synthesis whereas those stimulated in the presence of a c-Fos antisense oligonucleotide showed no phospholipid synthesis activation and proliferation ceased. Addition of recombinant c-Fos or of the deletion mutants containing the basic domain (aa 139-159) activated phospholipid synthesis whereas those without the basic domain did not. c-Fos expression was examined in 75 human brain tumors specimens and in 12 control slices- Fos immunoreactivity was positive in 100% of the tumors and negative in 100% of the controls. Only 18 (24%) tumors showed nuclear c-Fos whereas all showed ER/c-Fos co-localization. These results indicate that cytoplasmic c-Fos activates phospholipid synthesis to support both normal and exacerbated malignant growth.

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BC-P59.
CALCIUM, A KEY COMPONENT IN THE REGULATION OF THE RAB11-DEPENDENT EXOSOME PATHWAY

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Multivesicular bodies (MVB) are membranous structures that accumulate 60-100nm vesicles internally. MVBs are generated after invagination and pinching off of the endosomal membrane in the lumen of the vacuole. We have previously demonstrated that Rab11 is involved in exosome secretion, regulating the interconnection between endocytic, recycling and secretory pathways. In the present work, we have examined how an increase in cytosolic calcium affects the development of MVBs and exosome release in K562 cell overexpresing wt GFP-Rab11 or its mutants. We show that treatment of transfected cell with monensin, an agent that increases cytosolic calcium concentration, caused a marked enlargement of the MVBs, especially in mutant Rab11Q70L and wt Rab11 overexpresing cell. This effect was abrogated by the membrane permeant calcium chelator BAPTA-AM. We also examined the behavior of MVBs in living cell by time laps confocal microscopy. Interestingly, we observed that many MVBs decorated by wt or Q70L mutant GFP-Rab11 are docked and ready to fuse in the presence of a calcium chelator. This observation suggests that Rab11 is acting in tethering/docking of MVBs to promote homotypic fusion, but that the final fusion reaction required the presence of calcium. Additionally, a rise in the intracellular calcium concentration enhanced exosome secretion in Rab11 wt overexpressing cell and reverted the inhibition shown by the mutants. Taken together our result sauggest that Rab11 is involved in MVB biogenesis and that calcium is required for the homotypic fusion of MVBs.

BC-P60.
IN VIVO THYMOCYTE DEATH INDUCED BY AN ANDROGEN-DEPENDENT SIALYLATION QUALITY CONTROL

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Sialylation is considered among the most significant surface modifications during thymocyte maturation although its physiologic relevance is far to be completely understood. It is regulated by the concerted expression of sialyl-transferases along cell development. Alteration of the sialylation pattern induces thymocyte apoptosis inside the "nurse cell complex" as observed after the *in vivo* administration of *trans*-sialidase (TS), a virulence factor from *Trypanosoma cruzi* that is able to direct transfer the sialyl residue among macromolecules. A sexual dimorphic sialylation sensing me-chanism that operates only in males based on an androgen-signaling basis is reported here. No apoptosis was observed after TS administration in anti-androgen treated, gonadectomized or androgen receptor (AR) mutant male mice. Although no apoptosis was observed, mitosis was stalled in the AR^- mice after treatment. By bone marrow chimeric mouse approaches, the thymic epithelial cell was determined as the thy-mocyte apoptosis executor. The apoptosis progressed trough the TNF-α/TNFR1 deathly signaling and Caspase 3 activation was observed. No apoptosis increase was detected in the $CD43^{null}$ mouse and then the CD43 mucin seems to play a role as an altered sialylation sentinel molecule. Findings might be associated with the known different quality in the immune response among sexes.

BC-P61.
RAB COUPLING PROTEIN IS ASSOCIATED TO PHAGOSOMES AND REGULATES PHAGOCYTOSIS

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Rab coupling protein (RCP) is a novel identified protein, that belongs to the Rab11-FIP family. RCP interacts specifically with Rab4 and Rab11, small GTPases that function as regulators along the endosomal recycling pathway. We used fluorescence confocal microscopy and biochemical approaches to evaluate the participation of RCP during particle uptake and phagosome maturation. In macrophages, RCP is predominantly membrane-bound and displays a punctate vesicular pattern throughout the cytoplasm. RCP colocalizes with the transferring receptor and does not colocalize with lysotracker, a marker of lysosomes. Overexpression of H13, the carboxyl-terminal region of RCP which contains the rab binding domain, results in an abnormal endosomal compartment. We found that RCP is associated as discrete patches to early phagosomal membranes and to recycling vesicles departing from phagosomes. In macrophages, overexpression of full-length RCP stimulates the recycling pathway, while overexpression of H13 diminishes this vesicular transport step. This truncated form of RCP is probably acting in a dominant-negative manner by sequestering Rab4/Rab11 in a nonfunctional complex, thus preventing their interaction with endogenous RCP. RCP is an example of a putative effector protein that interacts with more than one RabGTPase and it is likely that RCP acting as an intermediate between Rab4 and Rab11, regulates the vesicular transport along the recycling pathway.

BC-P62.
VESICULAR TRANSPORT ALONG THE PHAGOCYTIC PATHWAY IN STREPTOLYSIN O PERMEABILIZED MACROPHAGES: ROLE OF SMALL GTPASE RAB 11

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Macrophages engulf large particles in vesicles called phagosomes. Phagosome maturation involves a series of membrane fusion and budding events resulting in phagolysosome formation and in the recycling to the cell surface of membrane and many phagosomal proteins. Our objective was to reconstitute phagosome maturation in macrophages permeabilized with streptolysin O (SLO) and to evaluate the participation of Rab11 in trafficking along the phagocytic via. In permeabilized cells, addition of GTPγs significantly enhances recycling from phagosomes, while GDPβs inhibits it, implying GTPases in this transport event. In agreement with the results obtained with macrophages overexpressing Rab11wt, addition of recombinant GST-Rab11wt to permeabilized cells stimulates recycling from the phagosomal compartment In contrast, overexpression of Rab11:S25N, a mutant preferentially locked in the GDP-bound state, decreases the recycling of proteins from phagosomes in a similar extent as does the addition of the recombinant GST-Rab11:S25N to SLO permeabilized cells. The results obtained demonstrate that SLO permeabilized macrophages could be an useful tool to study the molecular machinery involved in transport along the phagocytic pathway. In this system, trafficking could be manipulated by the addition of proteins or reagents that usually do not cross the plasma membrane. Our results show that recycling from the phagosomal compartment is regulated by the small GTPase rab11.

BC-P63.
DROSOPHILA MELANOGASTER SIMA/HIF-1α STABILIZATION AND NUCLEO-CYTOPLASMIC LOCALIZATION ARE BOTH REGULATED BY OXYGEN DEPENDENT UBIQUITINATION

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The *Drosophila* bHLH-PAS protein Similar (Sima) is homologous to the mammalian Hypoxia Inducible Factor alpha subunit (HIF-1α). We have found that Sima is stabilized in hypoxia and degraded in normoxia, destruction being dependent of a Von Hippel Lindau (VHL)-containing E3 complex that cathalyzes ubiquitination. By inducing ectopic expression in transgenic lines, we over-rode the rapid rate of Sima degradation and were able to determine that the protein remains cytoplasmic in normoxia and accumulates in the nucleus in hypoxia. We show here that nuclear import is constitutive and depends on an atypical bipartite nuclear localization signal. Furthermore, we show that Sima oxygen-dependent stabilization and nucleo-cytoplasmic localization are both controlled by hydroxylation of Pro850 and further ubiquitination. Replacement of Pro850 by Ala lead to constitutive localization of Sima in the nucleus, irrespective to oxygen levels. Consistent with this, mutations targeting the *Drosophila* HIF prolyl hydroxylase homologue or affecting the VHL E3 ubiquitin ligase complex exhibited both, stabilization and constitutive nuclear localization of Sima. Interestingly, mutations in the *Drosophila* CRM1 nuclear export receptor gene also lead to constitutive nuclear localization to Sima. Our results are consistent with the notion that subcellular localization of HIF-1α/Sima relies on ubiquitination that determines oxygen-dependent nuclear export.

TS-P1.

NOVEL ROLE OF THE MASTER TRANSCRIPTION FACTORS Spo0A AND σ^B DURING THE COLD SHOCK RESPONSE OF *BACILLUS SUBTILIS*

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Spore development and stress resistance in the Gram-positive paradigm *Bacillus subtilis* are governed by the master transcription factors Spo0A and σ^B respectively. Although *B. subtilis* is a soil bacterium, and hence temperature changes would constitute a common environmental stress, there is not any previous report on the role of Spo0A and σ^B during the adaptation of *B. subtilis* to low temperature. Here, we show that the coding genes for both regulatory proteins are dramatically induced after a temperature downshift from 37°C to 20°C. These transcriptional inductions correlated with a noticeable increment in the level and activity of Spo0A and σ^B at 20°C. Loss of σ^B reduces stationary-phase viability of cold-adapted cells 10 to 15-fold. Unexpectedly loss of σ^B also delays and decreases spore formation at low temperature. On the other hand, Spo0A loss dramatically reduces stationary-phase viability of cold-adapted cells 10,000-fold. Surprisingly, this effect is independent from the simple loss of the sporulation ability. Furthermore, Spo0A, and not the proficiency in sporulation or AbrB, is required for the development of complete stress resistance of cold-adapted cells to heat shock (54°C, 1h) since loss of Spo0A reduced the cellular survival to heat 100,000-fold. The overall results suggest new and important roles for Spo0A on the development of full stress resistance and for σ^B on the morphogenesis of the spore at low temperature.

TS-P2.

PROGRAMMED CELL DEATH IN *STREPTOCOCCUS PNEUMONIAE* IS INDUCED BY ACIDIC STRESS AND REGULATED BY QUORUM SENSING

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In bacteria, programmed cell death mainly lead to cellular lysis, and plays an important role in certain developmental process. In *S. pneumoniae*, lysis is produced by the major autolysin LytA. Our studies on acid tolerance response (*Cortes et al.*, poster presentation) showed that log-phase cells lysed when cultured at pH 5.6 after 3 Hs of incubation at 37°C. With the aim to understand the mechanism of acid-induced autolysis, we analysed mutants with an altered lytic phenotype. Previously, we have described a serine threonine kinase, StkP, whose mutant *stkP::km* showed autolysis in log-phase and to be essential for competence development activating *comCDE* transcription (*Echenique et al.*, in revision). We analysed the impact of *stkP::km* mutant in acid-induced autolysis, and it showed early autolysis compared with wild-type. Considering that *comCDE* transcription is not activated in the *stkP::km* mutant, we expected that *comE::km* mutant should present the same phenotype. Interestingly, this mutant presented no lysis at pH 5.6, however *ciaR::spc*, a response regulator mutant that present hyper-expression of *comCDE*, lysed as early as *stkP::km.* These results allow us to conclude that programmed cell death is induced by an environmental condition like low pH, and it's controlled by a quorum sensing mechanism as ComCDE. We also propose that StkP had a protector role against acid-induced autolysis, independently of ComCDE pathway.

TS-P3.

PHOSPHORYLATION OF THE MOVEMENT PROTEIN TGBP1 OF POTATO VIRUS X BY A PATHOGEN-INDUCED PROTEIN KINASE IN *NICOTIANA TABACUM*

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The potato virus X (PVX) is a potexvirus whose genome consists of a molecule of RNA encoding the replicase, three movement proteins (MPs: TGBp1, TGBp2 and TGBp3) and the capsid protein (CP). It was shown that MPs and CP are essential for viral mobilization and that CP is phosphorylated by plant protein kinases. In this work we demonstrate that the movement protein TGBp1 is phosphorylated *in vitro* by CK2 in a specific manner. In addition, *in vitro* studies using *Nicotiana tabacum* protein extracts show that TGBp1 is phosphorylated by a plant kinase. A significant increase of TGBp1 kinase activity is observed in plants infected with PVX or PVX plus PVY. A TGBp1 kinase is also present in plants that were inoculated with water as negative control. A preliminary characterization indicates that the kinases present in infected and non-infected plants are different. The kinase present in non-infected plants has CK2 characteristics: it is inhibited by heparin, activated by polylysine and its phosphorylation is efficiently competed by GTP. Both kinases are neither activated by Ca^{+2} nor inhibited by EGTA. Our results also show that TGBp1 is phosphorylated in serine and threonine residues in infected and non-infected plants. We will study the influence of phosphorylation in replication and mobilization of PVX.

TS-P4.

cAMP AND HEAT SHOCK INDUCE MAP KINASE PHOSPHATASE-1 (MKP-1) IN MA-10 LEYDIG CELLS

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MAP kinase phosphatase-1 (MKP-1) is a dual activity phosphatase involved in the inactivation of MAP kinases. We have demonstrated that in adrenocortical cells, ACTH and cAMP promote MKP-1 induction and it is known that its activity is required for the expression of steroidogenic enzymes. The aim of this work was to analyze the effect of cAMP and of stress conditions on MKP-1 expression in MA-10 Leydig cells. Since cAMP is the main second messenger involved in LH action on steroidogenesis in Leydig cells, we studied the effect of this nucleotide on MKP-1. Leydig cells are very sensitive to heat shock, a stressful treatment that notably and transiently reduces its steroidogenic capacity. Thus, we subjected the cells to heat shock (HS) (10 minutes, 45°C). In serum-starved (24 h) cells, both cAMP and HS raised MKP-1 protein levels after 2 h. Also, a rapid and transient increase of MKP-1 mRNA levels mediated by cAMP and HS (3 fold in both cases at 1 h) was detected by Northern blot analysis. In addition, cAMP and HS triggered MAP kinases activation. Taken together, our results indicate that both cAMP and HS prompt an off mechanism for MAP kinase action through MKP-1 induction. A possible physiological role for MKP-1 expression could involve the recovery of the steroidogenic capacity of the cells after HS and/or cAMP-induced steroid production.

TS-P5.

MODULATION OF MITOCHONDRIAL THIOESTERASE-I BY COLD

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In mammals, cold exposure involves changes in energy expenditure, heat production, activation of the sympathetic axis and remarkable activity of mitochondrial uncoupling proteins (UCPs). It has been proposed that members of the UCP family are associated with the mitochondrial thioesterase-I (MTE-I) -that releases arachidonic acid from arachidonoyl-CoA- in the export of long chain fatty acids from the mitochondrial matrix to the cytosol. We have demonstrated that β-adrenergic agonists regulate the levels of cardiac MTE-I mRNA at short times, but we couldn´t find any change in the protein expression. In this work we used the cold exposure model to study the expression of MTE-I at longer times. Rats were caged at 4°C for different days. Rat weight and food intake were measured. To estimate sympathetic activity, norepinephrine concentration was determined (497, 694 1283, 663 pg/ml at 0, 2, 10 and 24 days) and plasma nonesterified fatty acids were studied (210, 375, 450, 236 μM at 0, 2, 10 and 24 days). Miocardial tissue was isolated and mitochondrial proteins were analyzed by Western blot with a specific antibody against MTE-I. An increase in the protein levels was observed at the 8th day. Taking into account that cold exposure induces hypertension in a mechanism that is also mediated by angiotensin II and aldosterone synthesis, our results suggest that the increase in cardiac MTE-I levels can be due to a similar pathway.

TS-P6.

CHITOSAN-INDUCED ANTHRAQUINONE SYNTHESIS IN *Rubia tinctorum* INVOLVES MAPK ACTIVATION AND INTRACELLULAR CALCIUM MOBILIZATION

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Plants have acquired economical importance due to the synthesis of secondary metabolites, but the levels produced *in vitro* are generally too low for commercial applications. Elicitation with chitosan (200 mg/l) significantly stimulated (≅100%) anthraquinone (Aq) synthesis in R. *tinctorum* cultures. The elicitor's action could be blocked with 1 mM BAPTA-AM but not by 1 mM EGTA. When elicitation was performed in a free- calcium medium, Aq levels were ≅ 10 % higher in comparison to a medium containing calcium. Inhibitors of Ca^{2+} release from inner stores such as 15 μM 2-APB, 50 mM caffeine, 30 μM ruthenium red, 0.3 mM dantrolene, 100 μM neomycin and 10 μM U73122 abolished elicitation. Chitosan rapidly (7 min) stimulated MAPK, the enzyme remaining active at least for 60 min. This activation was blocked by BAPTA-AM and the calcium modulators mentioned above; however EGTA did not affect MAPK activity. These results show that chitosan elicitation involves MAPK activation and is dependent on intracellular but not on extracellular calcium.

TS-P7.

TYROSINE PHOSPHORYLATION AND DEPHOSPHORYLATION OF ENDOGENOUS PROTEINS IN STEROIDOGENIC CELLS STIMULATED BY cAMP

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It is very well known that in steroidogenic cells Ser/Thr phospho-dephosphorylation events are important regulators of hormone action. However, we have previously demonstrated that a protein basely phosphorylated in a tyrosine residue must be dephosphorylated after hormone action in order to obtain stimulation of steroid synthesis. Therefore, one can conclude that both tyrosine phospho and dephosphorylation may be also important events in steroid synthesis. In order to study the participation of both processes, we studied the action of hormones in the tyrosine phosphorylation and dephosphorylation of endogenous substrates in intact cells. It was possible to demonstrate a transient increase in the phosphotyrosine level of a 50 kDa protein in MA-10 cells, a well known Leydig cell line. The stimulation was maximal after 5 minutes of cAMP action and dephosphorylation rapidly occurs afterwards. A parallel analysis of ERK1/2 phosphorylation, also detected a hormone activation of this MAPK. In Y1 cells (adrenocortical cell line), the same stimulus provokes, after 15 minutes, the dephosphorylation of a protein of about 30 kDa, demonstrated by two dimensional gel electrophoresis. Taken together, these results show that after cAMP action, tyrosine dephosphorylation occurs together with tyrosine phosphorylation on different substrates, indicating that tyrosine kinases and phosphatases could be acting in parallel.

TS-P8.

BIOCHEMICAL CHARACTERIZATIOB OF CDPK ACTIVITIES IN POTATO LEAVES

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The sessile form of plants emphasizes the requirement of efficient adaptation and defense mechanisms. Calcium is a second messenger that plays a main role in plant physiology and Calcium-dependent calmodulin-independent protein kinases (CDPKs) are key intermediates in calcium signaling, that couple changes in Ca^{2+} levels to a specific response. Soluble CDPK activities, able to phosphorylate syntide-2, GS and Histone H1, were purified from potato leaves using ion-exchange and affinity chromatography. CDPK activity eluted at 0.2 M NaCl from a DEAE-cellulose column and the addition of 4M urea was necessary to elute the enzyme from a Phenyl-Sepharose column suggesting that the enzyme is highly hydrophobic. Western analysis of the purified extracts with an antibody against the CLD-domain of the soybean CDPK detected two polypeptides of 57 and 54 kDa suggesting that different CDPK isoforms or differentially phosphorylated versions of the same kinase are present in potato leaves. Protein extracts from different developmental stages of leaves were obtained and CDPK was assayed in crude and partially purified extracts (DEAE-cellulose column). CDPK activity from younger not fully expanded leaves was significantly higher than in older ones. The effect of biotic and abiotic factors on these activities will be analyzed.

TS-P9.

A SUSTAINED ACTIVATION OF PROTEIN KINASE A PROMOTES ISODIAMETRIC GROWTH, IN THE FUNGUS *MUCOR ROUXII*

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Polarized growth is impaired in *Mucor rouxii* spores incubated with cAMP analogs. In crude extracts of these cells PKA levels are significantly diminished compared to control cells, free catalytic subunit (C) is not detected and cAMP binding to regulatory PKA subunit (R) is similar to control cells. In order to dilucidate if these events are promoted by an up or downregulation of PKA activity we studied the effect of the addition of PKI ± dbcAMP to the culture medium of *M. rouxii* spores upon germination. PKI added after germ tube emission elevates the number of tubes per cell while PKI+ dbcAMP addition to culture medium reduces the number of germ tubes per cell to the one of control cells. Glycogen content decreases in *M. rouxii* cells treated with N_6cAMP analogs, while glycogen content is similar to control in cells incubated with C_8cAMP analogs. Both results suggest that PKA is upregulated. We purified PKA by DEAE-sepharose columns and detected C in the flow through or 0.12M NaCl fractions and holoenzyme in the 0.35M NaCl fraction. The study of control, +10µM or +100µM dbcAMP extracts of purified cultures performed during different times show: 1) C activity is elevated in cells cultured with dbcAMP. 2) C localization in the elution profile changes with the incubation time. 3) R molecular weight decreases after germination. 4) cAMP binding to R is also alterated. The results strongly suggest that a sustained PKA activation impairs polarized growth and C and R are post translationed modiffied and / or proteolized during this process.

TS-P10.

CHARACTERIZATION OF ESTROGEN BINDING PROTEINS FROM *Solanum glaucophyllum* CALLI

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We have previously detected estrogen binding sites for 17β-estradiol, estrogen like-compounds and information on the subcellular distribution profile of these estrogen receptor(ER)- like sites in *S.glaucophyllum* calli by immunoblot and ligand blot approaches. Our studies were now focused to resolve the nature of the estrogen binding structures present in *S. g* calli. Specific and saturable [^{3}H] 17β-estradiol binding sites of high affinity (k_d ~ 6.6 nM) were detected in callus tissues. Various other steroid hormones, phytosterols and the stereoisomer 17α-estradiol were significantly less or no effective than 17β-estradiol to compete with the radioactive ligand. The nonsteroidal estrogenic and antiestrogenic compounds diethylstilbestrol, tamoxifen and ICI 182, 780 were as effective as 17β-estradiol in competition assays. Lipid fractions obtained from callus were able to compete for these estrogen binding sites in a dose-dependent manner. The protein nature of the estrogen binding sites was clearly indicated by their sensitivity to trypsin degradation. An specific antibody against the steroid binding domain of the ER was an effective inhibitor for the specific radioligand binding. The results of this work are consistent with the existence of 17β-estradiol binding proteins in plants, structurally related to the ER, and endogenous ligands that suggest the operation of a receptor-ligand mechanism.

TS-P11.

CYTOSOLIC CALCIUM REQUIREMENT FOR FRUCTAN SYNTHESIS INITIATION

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In a previous work we have shown the involvement of protein kinases in the process that leads to the induction of fructan synthesis by sucrose in wheat. Experiments with the addition of specific Ser-Thr protein kinase inhibitors (staurosporine and W7), and cation chelating agents (EDTA, EGTA) strongly suggested the participation of calcium dependent protein kinases (CDPKs) in this process. Here we present further evidence of the role of Ca^{2+} in this sugar signaling cascade. The incubation of wheat tissues (root and leaf blades) with sucrose led to a rapid increase in cytosolic calcium (noticeable after 30 min), as shown by using a fluorescent probe (fluo3 AM). When blockers of calcium channels of either the plasmatic ($LaCl_3$) or intracellular (ruthenium red) membranes were added, the sucrose response (induction of fructan synthesizing enzymes) was inhibited in dose-dependent manner (IC_{50}= 0,19 mM and 1,15 ìM, respectively). On the other hand, calcium by itself did not mimic the induction effect of sucrose. The time course of calcium requirement seems to indicate that the signaling process takes place within 2-3 hrs after increased sucrose concentration in leaf tissues. In conclusion, our results suggest that cytosolic calcium is involved in this sucrose mediated signaling pathway, probably in relation with CDPK action.

Supported by ANPCyT and FIBA.

TS-P12.

BIOCHEMICAL CHARACTERIZATION OF THE REGULATORY SUBUNIT OF PROTEIN KINASE A FROM *SACCHAROMYCES CEREVISIAE*

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The regulatory subunit of protein kinase A (PKA) modulates kinase activity directly via binding of cAMP to two cAMP-binding sites (A and B). The two sites are clasically distinguished by the different dissociation kinetics of cAMP from both sites and by the selectivity towards N6- (site A) and C-8 (site B) substituted cAMP analogues. We present preliminary data on the biochemical characterization of *Saccharomyces cerevisiae* R subunit. The dissociation kinetics of cAMP from both sites is very similar. N6- analogues do not but C8-analogues do distinguish two sites in wt R. cAMP was competed more efficiently by C-8 than N6-analogues in a mutant of R subunit containing only site A (bcy1-16). The apparent affinity of the C8-analog for bcy1-16 only site was similar to the affinity of the site competed with higher affinity by C8- in wt R. These results suggest there may be an inversion of selectivity of cAMP analogues in yeast R subunit. Activation of PKA by cAMP analogues in an *in situ* permeabilized assay showed that C-8-analogues display higher activation potency than N6-analogues. Activating capacity of analogues combinations was measured *in situ;* the only combinations that were synergistic in the activation process were N6-benzoyl-cAMP with 8-thiobenzyl-cAMP, suggesting that there is a cooperativity between the two cAMP binding sites.

TS-P13.

SIGNAL TRANSDUCTION PATHWAYS INVOLVED IN ALTERNATIVE SPLICING REGULATION

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Alternative splicing generates distinct proteins from a single gene, and about fifty percent of the human genes undergo this process. Its regulation by extracellular signals is then critical for altering gene expression. Our laboratory studies the effect of extracellular signals triggered by epithelial-mesenchymal interactions on the alternative splicing of fibronectin pre-mRNA. We have previously shown that soluble factors present in a mammary mesenchymal cell-conditioned medium, as well as different growth factors, stimulate the inclusion of the alternatively spliced fibronectin EDI exon in mammary epithelial cells. We now show that the factors that induce the inclusion of EDI strongly activate different signalling cascades, resulting in the phosporylation of different protein kinases. Furthermore, the overexpression of a constitutively active form of Ras (RasV12) stimulates EDI inclusion in a dose-dependent manner. By using different pharmacological inhibitors we conclude that the PI3K pathway is the main responsible for the regulation of EDI splicing in this cellular context. On the other hand, inhibition of the JNK pathway potentiates the effects of different stimuli on the inclusion of this exon.
Linkage of transduction pathways to alternative splicing provides a poorly understood way to convert extracellular stimuli into changes in splicing patterns that can result in different physiological responses.

TS-P14.

cAMP DEPENDENT PROTEIN KINASE IS INVOLVED IN MORPHOGENESIS OF *MUCOR CIRCILLENOIDES*

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The cAMP signal transduction pathway controls many processes in fungi. The *Mucor circinelloides pkaR* and *pkaC* genes, encoding the regulatory (R) and catalytic (C) subunits of the protein kinase A (PKA), have recently been cloned. Expression analysis during the dimorphic shift and colony morphology suggested a role for R in the control of morphology and branching. We have used strain KFA121, which overexpresses the *M. circillenoides pkaR* gene, to quantify growth and branching under different aerobic growth conditions by computerized image analysis. An inverse relationship between the *pkaR* expression level and the hyphal growth unit length was observed, suggesting a central role for R in branching. A biochemical analysis of R by Western-blot and by cAMP binding demonstrated that the level of R is 3-fold higher in KFA121 under inducing conditions than in the vector control strain KFA89. The level of PKA activity determined in crude cell extracts showed that KFA121 had a 2-fold increase in C. These data suggest that PKA might be downregulated during hyphal tube emergence due to the increase in R levels resulting in increased branching. In Western-blot we observed an R isoform of higher MW than the theoretical one which had different subcellular localization than overexpressed R. Our results suggest that the R subunit has different isoforms with differential subcellular distribution, which could be important in the function of the enzyme during the germination of this fungi.

TS-P15.

CONSTRUCTION OF A GREEN FLURESCENT FUSION PROTEIN OF THE REGULATORY SUBUNIT OF PKA FROM THE FUNGUS *CANDIDA ALBICANS*

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Previous work from our laboratory indicates that a normal germinative behavior of the fungus depends on the nuclear localization of the catalytic subunit (C) of PKA which in turn seems to be determined by the subcellular localization of the regulatory subunit (R). To assess the subcellular localization of the R subunit, we tagged one of the alleles of the single R gene (BCY1) with the green fluorescent protein GFP, leaving the fused gene under the control of the natural promoter. The method used, developed by Gerami-Nejad, utilizes PCR primers with 5´-ends corresponding to the desired target gene sequences, and 3´-ends that direct amplification of the *GFP* gene along with the selectable marker *URA3*. The amplified DNA was transformed directly into *C. albicans* H2D strain *(ura/ura tpk2/tpk2)*, and recombinants carrying the inserted marker at the locus of interest were identified by PCR using appropriate primers. The three strains obtained harbouring the *BCY1-GFP* construction expressed the fused Bcy1-GFPp as assessed by western blot analysis using anti-R and anti-GFP antibodies. The subcellular localization of the fused protein will be investigated by fluorescence microscopy.
Supported by FONCyT, PICT 1999 and Fundación Antorchas.

TS-P16.

FLUCTUATIONS IN *TPK1* AND *TPK2* mRNA LEVELS DURING VEGETATIVE GROWTH IN *CANDIDA ALBICANS*

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Previous work from our laboratory has demonstrated that the PKA catalytic and regulatory subunits are differentially expressed during the *C. albicans* yeast-to-hypha transition. We considered it relevant to study the expression of the two catalytic subunits isoforms (*TPK1* and *TPK2* genes) during vegetative growth of the fungus.
The *TPK1* and *TPK2* mRNA levels were measured in the parental CAI4 strain and in the HD2 mutant strain (*ura/ura tpk2/tpk2*) during batch growth in YPD medium. There are remarkable differences concerning the relative abundance of both messengers during growth, being that of *TPK1* much lower than that of TPK2. The levels of both *TPK1* and *TPK2* messengers rise during vegetative growth: *TPK2* expression reaches its maximal value at 10-12 h of growth and decreases slowly later on, while *TPK1* mRNA level rises at very low rate up to 32 h growth. The H2D strain shows comparable results concerning level of the *TPK1* mRNA and a similar pattern of changes during growth. These results seem to indicate that there is not a compensation at the level of gene expression of one of the catalytic isoforms in the absence of the other.
These results indicate probably that the PKA catalytic isoforms play different roles in the biochemical mechanisms regulating vegetative growth.
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TS-P17.
DEVELOPMENT OF StCDPK1 TRANSGENIC POTATO PLANTS

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Tuberization in potato (*Solanum tuberosum*) is an ideal model system to study morphogenetic processes triggered in response to changing environmental conditions. Calcium and protein phosphorylation are involved at the onset of tuber development. *StCDPK1* encodes an active CDPK isoform (59 kDa) transiently induced in swelling stolons. This transcript appears before the induction of other tuber specific genes, such as Pin 2 and patatin, suggesting that this isoform could be involved in stolon to tuber transition. We developed transgenic potato plants (α, β and δ) to study the role of StCDPK1 in this process. The complete cDNA sequence was subcloned into pCB201 binary vector under the control of CMV35S constitutive promotor in sense orientation (lines α) or into pGUT binary vector under the control of GBSS exclusive tuber promotor in anti-sense orientation (lines δ). Lines β were generated subcloning StCDPK1 N-terminal fragment (300 bp) into the pGUT binary vector. All constructs were introduced into Agrobacterium tumefaciens strain GV2260-6. The presence of the transgene was confirmed by Southern blot and PCR analysis. Expression studies were done by RT-PCR and Northern blot. Different growth conditions are being used to analyze these transgenic lines.

TS-P18.
INVOLVEMENT OF TRPC3 IN THE 1α,25(OH)$_2$D$_3$-MEDIATED SOC INFLUX IN MUSCLE AND OSTEOBLAST CELLS

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In chick skeletal muscle and in rat osteoblast-like cells (ROS 17/2.8), 1α,25-dihydroxy-vitamin-D$_3$ [1α,25(OH)$_2$D$_3$] stimulates release of Ca^{2+} from inner stores and cation influx through both voltage-dependent and store operated Ca^{2+} (SOC) entry channels. We investigated the involvement of TRPC proteins in SOC influx induced by 1α,25(OH)$_2$D$_3$. Two fragments were amplified by RT-PCR, exhibiting >85% sequence homology with human TRPC3. Northern and Western blots employing TRPC3-probes and anti-TRPC3 antibodies, respectively, confirmed endogenous expression of a TRPC3-like protein. Both cell types transfected with anti-TRPC3 antisense oligodeoxynucleotides showed reduced SOC and Mn^{2+} entry induced by either thapsigargin or 1α,25(OH)$_2$D$_3$. In muscle cells, anti-VDR antisense inhibited steroid-induced Ca^{2+} and Mn^{2+} influx and co-immunoprecipitation of TRPC3 like protein and VDR was observed, suggesting an association between these proteins and a functional role of the receptor in 1α,25(OH)$_2$D$_3$ activation of SOC entry. In osteoblasts, two PCR fragments showing high homology with human INAD-like sequences were obtained. Northern blot and antisense functional assays suggested the involvement of the INAD-like protein in SOC influx regulation by the hormone. Therefore, we propose that an endogenous TRPC3 like protein mediates 1α,25(OH)$_2$D$_3$ modulation of SOC influx in muscle and osteoblastic cells, which seems to implicate VDR-TRPC3 association and the participation of a INAD-like scaffold protein.

TS-P19.
PRESENCE AND ACTIVATION OF PHOSPHOLIPASE D IN *Trypanosoma cruzi* EPIMASTIGOTES

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Several signaling cascades are triggered by the activation of phospholipid cleaving enzymes such as phospholipase C (PLC) and D (PLD). In our laboratory we demonstrated PLC activation in *Trypanosoma cruzi* epimastigotes in response to a wide variety of stimulus, such as stress osmotic and mastoparan, a protein G activator, but there is not yet evidence for PLD participation. In this study, we provide the first experimental evidence of presence and activation of PLD in this parasite. Using fluorescent microscopy and a fluorescein isothiocyanate-coupled goat anti-rabbit IgG secondary antibody, we demonstrated the presence of PLD in *T. cruzi* epimastigotes. Moreover, western blotting analysis showed an inmunoreaction with antibody anti-PLD2 from humans in citosol and fractions enriched in flagellar membrane, but not with anti-PLD1 isoforms. An increase in PLD-flagellar membrane fraction was observed in the presence of mastoparan or NaCl. Both effectors increased PLD activity in [^{32}P]Pi-labeled cells, determined by transphosphatidylation reaction, a specific measure of PLD activity. The results indicate that PLD activation must be considered as a potential signal transduction mechanism in *T. cruzi*, as occurs in animal and plants.

TS-P20.
REGULATION OF ACYL COA SYNTHETASE EXPRESSION BY CYCLIC AMP DEPENDENT MECHANISM IN MA10 CELLS

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We have previously described that an Acyl-CoA synthetase 4 (ACS4), an enzyme that has preference for Arachidonic Acid (AA) as substrate, and that an Acyl-CoA thioesterase (ARTISt) regulate in a concerted mode the intracellular levels of AA and steroid production.
In this work we show that inhibition of steroidogenesis by the combined action of Triacsin C and Nordihydroguaiaretic acid, inhibitors of ACS4 and ARTISt respectively, was reverted by the addition of AA.
The expression of both enzymes was analyzed by western-blot after treatment with cAMP in MA10 cells. ACS4 expression was increased while ARTISt expression remained unchanged. This increase correlates with the induction of StAR protein levels and steroid synthesis. The ACS4 increment was blocked in the presence of cycloheximide.
These results suggest that the limiting step in the AA release necessary for the steroidogenesis involves the hormonal induction of ACS4.

TS-P21.
MODULATION OF [Ca^{2+}] IN OSTEOBLASTIC CELLS BY OLPADRONATE AND AMINO-OLPADRONATE. PROTEIN PHOSPHATASES AS POSSIBLE TARGET OF BISPHOSPHONATES

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In rat ROS 17/2.8 osteoblasts the bisphosphonates (BPs) olpadronate (OPD) and NH_2-olpadronate (lidandronate; LID) induce an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) after prestimulation of the cells with ATP (5-10 µM). This ATP-dependent BPs Ca^{2+} response is due to calcium influx through voltage dependent calcium channels (VDCC) and required purinergic activation of the PLC pathway. The present work investigated the mechanism by which OPD and LID affect the ATP-dependent Ca^{2+} response in osteoblasts. Spectrofluorimetric measurements showed that stimulation of fura-2 loaded cells with protein phosphatase inhibitors (ortovanadate and NaF) at micromolar dosis (1-200 µM) after ATP prestimulation mimicked the OPD effect on calcium influx whereas at milimolar concentration evoked a more sustained cation influx and blocked the BPs action. p-Nitrophenylphosphate (PNPP), a protein phosphatase substrate, did not affect the [Ca^{2+}]i *per se* but blocked the calcium influx induced by OPD. Whole cell radioligand binding assays using [H^3]OPD suggest the presence of a saturable and high affinity binding site for OPD (Kd = 1 µM) which was similarly competed by LID, alendronate, etidronate and protein phosphatase substrates (PNPP and alfa Naphtyl phosphate) but not by purinergic agonists (ATP, ADP, AMP or UTP) or protein phosphatase inhibitors. Studies with plasma membranes showed location of BPs binding sites in the cell surface. These results suggest that BPs modulate purinergic Ca^{2+} signaling in osteoblasts stimulating calcium influx through VDCC by inhibition of protein phosphatases.

TS-P22.
PLC ACTIVATION VIA Na^+/H^+ EXCHANGER IN RESPONSE TO STRESS OSMOTIC IN *Trypanosoma cruzi* EPIMASTIGOTES

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Trypanosoma cruzi multiplies and differentiates in the digestive tract of triatomine insects. In the rectum, the parasites are confronted with an increase in the osmolarity caused mainly by an elevated content of NaCl in the urine. We demonstrated previously that 0,5 M NaCl and 0,5 M manitol induced an increase in the number of parasite intermediate forms and activation of PI-PLC. In addition, manitol induced Ca^{2+} signaling via Na^+/H^+ exchanger together with a Ca^{2+}/nH^+ exchanger from acidocalcisome. Electron microscopy revealed an increase in the size of acidocalcisomes in the presence of NaCl or manitol, as occurs in halotolerant cells. Under the same assay conditions, it was possible to detect the release of Acridine Orange previously accumulated in acidocalcisomes by measuring spectrophotometric and fluorescence microscopy. This effect was abolished by EIPA, an inhibitor of Na^+/H^+ exchanger, indicating the alkalinization of these vacuoles via a Na^+/H^+ exchanger. The IPs/IP_3 accumulation evoked by NaCl and manitol was inhibited with U73122, an inhibitor of PLC. A similar effect was observed with EIPA or BAPTA-AM, a calcium intracellular chelator. These data suggest that PLC activation induced by stress osmotic is mediated by Ca^{2+} release from the acidocalcisome and this process was mediated by alkalinization of the acidic vacuole via a Na^+/H^+ exchanger followed by Ca^{2+}/nH^+ exchanger.

TS-P23.
EFFECT OF CHRONIC EXPOSURE TO CADMIUM ON INVOLUTION MARKERS IN RAT PROSTATE

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Cadmium is a toxic element considered as an important environmental contaminant. Based on previous histological studies which have shown non proliferative changes in the prostate under a chronic exposure to cadmium *in vivo*, we wanted to study if there was an involution state in this organ.

Male Wistar rats (21 days of age) were divided into two groups: the exposure group (Cd) which received 100 ppm Cd as $CdCl_2$ in drinking water ad libitum for 12 weeks and the control group (Co) which received water without Cd. The rats were killed and prostates were obtained, frozen in liquid nitrogen and stored at –70°C until its study. 1 µg of RNA was transcribed to cDNA at 42°C using random hexamers as primers and RT-MMLV (Moloney Murine Leukemia Virus). Aliquots of 2 µl of cDNA were used in the amplifications by PCR using specific primers for the following genes: IGF-I, IGF-BP5 and TRMP-2/clusterin. Beta actin was used as an internal control. 100 mg of prostates were homogenized in the presence of protease inhibitors and 40 µg of proteins were separated in an 8% SDS-PAGE, transferred to PVDF membranes and incubated with an antibody against TGF-β1.

The levels of IGF-I, IGF-BP5, TRMP-2/clusterin mRNA were not modified under Cd exposure, while the expression of TGF-β1 decreased. Histological alterations should not be due to an involution state.

TS-P24.
THE ANTIFUNGIC LIPID TRANSFER PROTEIN HAAP10 IS PHOSPHORYLATED BY A MEMBRANE-BOUND PROTEIN KINASE

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Lipid transfer proteins (LTPs) are a class of small basic proteins, approximately 9 kDa, which are widely distributed in higher plants. Accumulated evidences suggest that LTPs can perform diverse roles. We have previously isolated, characterized and cloned Ha-AP10, a potent sunflower antifungal protein homologous to members of the LTP family.

The objective of this work was to study the phosphorylation of Ha-AP10 using *in vivo* and *in vitro* analysis. The incubation of sunflower seeds with [α-^{32}P] Pi followed by immunoprecipitation of the Ha-AP10 protein with an specific antibody showed that Ha-AP10 was radiolabeled *in vivo*.

Since Ha-AP10 was localized in soluble and membrane fractions of sunflower seeds, we analyzed the ability of both fractions to phosphorylate Ha-AP10. The recombinant protein Ha-AP10-gluthathion-S-transferase expressed in *Escherichia coli* and purified from bacterial extracts was used as substrate for protein kinases present in soluble and membrane fractions in pull down assays. Results obtained showed that Ha-AP10 is phosphorylated by a membrane-bound protein kinase present in germinating seeds. Using pull down assays a preliminary characterization of this kinase activity was performed analyzing calcium-dependence and inhibitors sensitivity. The results obtained suggest that the protein kinase responsible of Ha-AP10 phosphorylation is partially calcium-dependent and it is inhibited by H7, an inhibitor of cyclic nucleotide dependent protein kinase and protein kinase C. The putative role of LTPs phosphorylation is discussed.

TS-P25.

PHOSPHATIDIC ACID AND NITRIC OXIDE: CROSS-TALK BETWEEN THESE TWO PLANT MESSENGERS

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Phosphatidic acid (PA) is an emerging lipid second messenger (Munnik, 2001) that plays a role during the early signal transduction leading to the hypersensitive response (HR) in plant-pathogen interactions (Laxalt and Munnik, 2002). Nitric oxide (NO) is a novel plant signal molecule involved in stress responses (Lamattina *et al*., 2003). In Arabidopsis NO has also been implicated in the induction of HR (Clarke *et al*., 2000). Here we provide evidence for a cross-talk between NO and PA in elicited plant cells.
Tomato cell cultures treated with the general elicitor xylanase have previously been reported to produce a rapid PA accumulation via phospholipase D (PLD) and PLC/diacylglycerol kinase pathways (van der Luit *et al*., 1999). This accumulation was inhibited when cells were treated with the specific NO scavenger, cPTIO. Moreover, the NO donor SNAP induced PA accumulation within 30 minutes in a dose dependent manner and cPTIO prevented this accumulation. Xylanase-treated cells showed a fast (15 min) and maintained (3h) NO production detected by the fluorescent probe DAF2-DA and by EPR. This response was also blocked by cPTIO. In northern blot experiments, cPTIO showed to be able to prevent the xylanase induced PLDβ1 and PR1 transcript accumulation, characteristic events of HR (Laxalt *et al*., 2001). All together, these results strongly suggest that NO is upstream of PA accumulation in the signal transduction cascade leading to HR.
Supported by UNMdP, Antorchas, ANPCyT and TWAS.

TS-P26.

NITRIC OXIDE REGULATES GUARD CELL Ca^{2+}-SENSITIVE ION CHANNELS AND EVOKES A SUB SET OF THE ABSCISIC ACID SIGNALLING PATHWAYS

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The phytohormone abscisic acid (ABA) triggers a complex sequence of signalling events that lead to concerted modulation of ion channel activity at the plasma membrane to drive stomatal closure. Nitric oxide (NO) was described as a signal and effector molecule in diverse plant systems. Recent work has indicated that NO induces stomatal closure and it is a prerequisite for ABA signal transduction in *Arabidopsis* and *Vicia* guard cells. Previous results showed that NO induction of stomatal closures requires the participation of endomembrane Ca^{2+} channels such as ryanodyne and IP3 sensitive, however mechanism(s) of action of NO are still poorly defined. Here we provide direct evidence that NO selectively regulates the Ca^{2+}-sensitive ion channels of *Vicia* by promoting Ca^{2+} release from intracellular stores to raise cytosolic-free [Ca^{2+}]. NO-sensitive Ca^{2+} release was blocked by antagonists of guanylate cyclase and cyclic ADPR-dependent endomembrane Ca^{2+} channels, implying an action mediated via a cyclic GMP-dependent cascade. NO did not recapitulate ABA-evoked control of the Ca^{2+}-insensitive K^+ channels since NO scavengers failed to block the ABA-activation of these K^+ channels. These results place NO action firmly within one branch of the Ca^{2+}-signalling pathways engaged by ABA.
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TS-P27.

SPATIAL ANALYSIS OF INTRACELLULAR CALCIUM AND CHANGES IN ERK 1/2 PHOSPHORYLATION INDUCED BY 1,25(OH)$_2$-VITAMIN D$_3$ IN MAMMALIAN SKELETAL MUSCLE CELL LINES

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Agonist induced changes in intracellular Ca^{2+} levels ([Ca^{2+}]i) control several mammalian cell functions. 1α,25(OH)$_2$D$_3$ exerts direct effects on avian muscle cell Ca^{2+} metabolism. The hormone also induces tyrosine phosphorylation of ERK1/ERK2, PLCγ and c-myc. We investigated the effects of 1α,25(OH)$_2$D$_3$ on [Ca^{2+}]i and on phosphorylation and activation of ERK 1/2 in cultured rat skeletal muscle cells and murine normal NLT cell line in comparison with dystrophic mdx XLT 4-2 and dysgenic myotubes from the GLT cell line, which do not express the á$_1$ subunit of the DHPR. Neonate rat myotubes and muscle cell lines were loaded with fluo-3 AM followed by stimulation with 1-10 nM 1α,25(OH)$_2$D$_3$ and a temporal secuence of 100-200 images/2 seconds was recorded using a epifluorescence microscope. A marked increment in [Ca^{2+}]i was detected 50 -100 s after hormone addition, linked to oscilations with a 20 s period. Spatial differences were observed in fluorescence, which was greater in the cell nucleus. In normal cell lines, less differentiated, the fluorescence increase occurred a few sec after hormone stimulation. 1 nM of 1α,25(OH)$_2$D$_3$ induced rapid oscilations in [Ca^{2+}]i in all cell lines. Primary cultures of rat muscle cells show steroid hormone-dependent ERK 1/2 phosphorylation greater than the cell lines. Furthermore, 1α,25(OH)$_2$D$_3$ activation of ERK 1/2 was temporally correlated with Ca^{2+} changes in hormone stimulated cells.

PL-P1.

CHARACTERIZATION OF THE MOLECULAR RESPONSE TO A CHANGE IN THE PLOIDY LEVEL IN *PASPALUM NOTATUM*

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This work was aimed at the identification of genes up or down-regulated at the early stages of autopolyploidy in *Paspalum notatum*. The transcriptome response was studied by differential display in flowers and leaves of a diploid individual (2n = 2x = 20) and a synthetic autotetraploid (4n = 4x = 40). Around 4500 transcripts were analyzed in duplicated tests. The expression of 65 transcripts was reproducibly altered in the autotetraploid: 29 fragments showed putative differential expression in flowers while 36 did it in leaves. 6 transcripts showed reduced expression while 23 were activated in flowers. 12 transcripts were down-regulated while 24 were up-regulated in leaves. Differential expression was confirmed for 19 clones by reverse-Northern. Sequences of the fragments identified corresponded to genes involved in metabolism and cell-cycle regulation, rRNAs and retroelements. The genetic structure from the diploid and tetraploid isogenic lines was examined by the use of 296 RAPD markers and showed 13.51 % divergence. Data indicate that chromosome doubling affects gene expression inmediately upon autopolyploid formation and may direct some extent of genome modification.

PL-P2.

STRUCTURE AND FUNCTION OF AN α-GLUCAN FROM CELL WALLS OF NON-PATHOGENIC FUNGI ON POTATO SPROUTS

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In a previous work we have isolated and characterized an α-glucan from cell walls of binucleate non-pathogenic *Rhizoctonia sp*. isolate. This α-glucan induces glucanase activities in potato sprouts and may be important as a biocontrol factor. We report now, studies on the structure of this polysaccharide. The α-Glucan presents different degrees of aggregation. Chromatography on a Sephacryl S-200 HR column afforded an excluded (**a**) and a broad included (**b**) peak. The highest induction of ß-1,3-glucanase activity was obtained with peak **b**, which has a minor degree of aggregation. Methylation studies of peak b showed that glucose is mainly present as consecutive units linked á-(1-3). Other type of bonds are á-(1-4). Part of the glucan was hydrolyzed to glucose by amyloglucosidase. This suggests that the á-glucan is not composed by alternate á-(1-3) and á-(1-4) bonds. Some of the units linked á-(1-4) are branched in O-6. The products of hydrolysis with amyloglucosidase showed less induction of ß-1,3-glucanase activity than the original glucan. In addition, peaks **a** and **b** contained 9 and 10.3% respectively of uronic acid with respect to total sugar. The content of uronic acid and a minor amounts of other monosaccharides could be important on the elicitor activity of the α-glucan. On the other hand, an immunological analysis of crude extracts from potato sprouts treated with the α-glucan was performed using antibodies raised against PR proteins and showed that the level of PR proteins were increased when the sprouts were treated with the glucan.

PL-P3.

ISOLATION AND CHARACTERIZATION OF DISEASE RESISTANCE GENE ANALOGS FROM CULTIVATED AND WILD STRAWBERRY

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Cloned resistance (*R*) genes from a broad range of plant species share similarities in DNA sequence and structural motifs. The most prevalent class of *R* genes contains Leucine Rich Repeats (LRR) and a Nucleotide Binding Site (NBS) domain. LRR domains mediate protein-protein interactions and are the major determinants of recognition specificity. The NBS domain contains a P-loop (also called kinase 1-a), a kinase 2 and a kinase 3-a domains with ATP- and GTP-binding activity and are thought to alter the interaction between resistance proteins and other proteins downstream the signal transduction pathway. The experimental setup consisted in the utilization of degenerate oligonucleotide primers designed from conserved regions of the NBS domain to isolate resistance gene analogs (RGAs) from genomic DNA of three strawberry cultivars of *Fragaria ananassa* and one accession of the wild *Fragaria vesca*. A total of 40 clones were randomly picked and sequenced. 29 of them were RGAs and displayed high sequence similarities to NBS domain reported in the type NBS-LRR *R* genes (amino acid identity range between 38 and 50%). As expected, all clones were 510 bp size, except for the cultivar US-10 that presented a 17 bp insertion. Most of the sequences analyzed had stop codons except 10 clones that displayed continuous open reading frames. These results represent the first report of RGAs in strawberry.

PL-P4.

***IN VITRO* ASSAYS TO EVALUATE THE TOLERANCE OF AGRICULTURAL PLANTS TO ORGANOCHLORINE HERBICIDES**

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Phenoxy and triazinic herbicides (2,4-D and atrazine) are two of the most employed pesticides in Argentine agriculture. Due to their physicochemical properties and their extensive use there is a great risk of soil and water contamination. To investigate the tolerance of some important crops to these herbicides for phytoremediation purposes, *in vitro* assays with young plants (seedlings) were carried out. Alfalfa and maize seedlings were exposed to 2,4-D and atrazine. The seeds were surface sterilized before sowing in 390 ml glass flasks on Murashige-Skoog medium containing 10 g/l sucrose, 4 g/l agar and different concentrations of the herbicides. Incubation was carried out at 24±2ºC, under a 16/8 h photoperiod. After 10 days for maize and 13 days for alfalfa, the individual seedlings were harvested, washed and the following tolerance parameters were evaluated: root length, shoot length, shoot fresh weight and percent germination. The results showed a more marked toxic effect of 2,4-D in comparison with that of atrazine. 2,4-D concentrations of approximately 0.1 ppm and between 0.01-0.05 ppm diminished the plants parameters in 50% in maize and alfalfa, respectively. On the other hand, only high atrazine concentrations, approximately 25 ppm with alfalfa and 50-75 ppm with maize, produced a 50% reduction in the evaluated parameters. Knowing the herbicides concentrations that the plants can tolerate is a necessary step for developing phytoremediation strategies based on the eco-engineering the rhizosphere environment.

PL-P5.

AN ARABIDOSPIS MUTANT DEFFECTIVE IN UV-B-MEDIATED PHOTOMORPHOGENESIS

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Ultraviolet B radiation (UV-B, 280–315 nm) can cause damage and induce photomorphogenic responses in plants. In etiolated *Arabidopsis thaliana* seedlings of the Columbia ecotype (*Col*), a daily exposure to 2.5 h of low-fluence UV-B enhances the cotyledon opening response induced by a subsequent red light (R) pulse; an R pulse alone or 2.5 h of continuous R irradiation caused little cotyledon opening while exposure to 2.5 h of blue light (B) followed by an R pulse was highly effective. Analysis of phytochrome and cryptochrome deficient mutants in the *Col* background suggests that phytochrome-B is involved in the detection of R and cryptochrome1 is required for the response to UV-B and B. Using the cotyledon opening assay, we screened for Arabidopsis T-DNA mutants with reduced UV-B sensitivity. One of the mutants, *m62*, has greatly reduced UV-B sensitivity but normal responses to B and R. The pigmentation and morphological responses to UV-B in the mutant is similar to the wild-type. Genetic characterization of the mutation is currently in progress. The physiological characterization of the mutant suggests that the product of *m62* is involved in a UV-B-specific signaling cascade that influences seedling morphogenesis in the early stages of de-etiolation.

PL-P6.

DESIGN OF MICROCOSMS FOR PHYTOREMEDIATION RESEARCH

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Phytoremediation is a technology based on the use of plants and associated microflora in order to remove, degrade or stabilize environmental contaminants. The relationship between soil, plants and microorganisms is extremely complex due to the intricate network of physical, chemical and biological interactions. For carrying out phytoremediation research, it is necessary to develop experimental systems, which allow shrinking this complex framework and simplifying the handling of such variables. Those scale systems, in which a portion of the universe under study is represented and the main experimental variables can be controlled, are called Microcosms. The design of microcosms for studies with plants and microbes is not an easy task: on one hand, it is necessary to have a system which permits to discriminate the effects of plants and microbes, which may require to work under aseptic conditions. On the other hand, the system must permit to carry out all the technical procedures required for an experimental setup, such as working with sterile soil, spiking of soil with the contaminants under study, watering, plant and soil sampling and other procedures, which need to be carried out without disturbing the system. In strict relation to plants, proper protocols for seed sterilization, data on nutritional requirements, as well as preliminary data on the tolerance of plant species to the xenobiotics under study are needed. All the mentioned issues have been considered for the design of appropriate microcosm systems. The design itself and its applications will be presented.

PL-P7.

ANALYSIS OF EXPANSIN GENE EXPRESSION IN STRAWBERRY FRUITS

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Strawberry is a non-climateric fleshy fruit, which ripens quickly and has a short post harvest life. The fruit's progressive softening is one of the most important factors that determinate its susceptibility to pathogen attack. Fruit softening has been related to the cell wall disassembly through the action of cell wall enzymes and proteins. Increments in the expression of fruit specific expansins have been described and these proteins were proposed to be involved in the softening process. Therefore, in this work we cloned four strawberry expansin cDNAs and analyzed their expression through the ripening of three strawberry cultivars that differ in the softening rate. We found a correlation between the expression of FaExp1, FaExp2 and FaExp5 genes and the difference in firmness of the cultivars analyzed. No correlation was found in the case of FaExp6 gene expression. Previous reports had shown that FaExp2 gene expression was not regulated by auxins, which differs from most of the strawberry ripening related genes. In this work, we have analyzed the effect of sucrose and different growth regulators on the expression of FaExp2. We found a decrease in FaExp2 gene expression after ABA treatment and an increase after sucrose treatment, but there was no effect after treatment with NAA or GA_3 or after the elimination of fruit achenes.

PL-P8.

NITRIC OXIDE-DEPENDENT EFFECTS ON SOYBEAN LEAVES AND ISOLATED CHLOROPLASTS

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Nitric oxide (NO) supplementation in intact soybean (*Glycine max* var Hood) leaves and isolated chloroplasts was studied. The rate of generation of NO in solution by S-nitrosoglutathione was of 6 μM over the 15 min incubation period. NO incorporation in intact soybean leaves and isolated chloroplasts was assesed by the EPR signal of MGD-Fe-NO adduct. Lipid radical content was no significantly different between control and NO exposed leaves immediately after the treatment. The activity of ascorbate peroxidase (AP) was significantly inhibited (-17%) in intact soybean leaves immediately after exposure to NO (0.72±0.02 and 0.60±0.03 μmol min^{-1} mg^{-1} prot, for control and treated leaves, respectively). This effect was completely avoided if the incubation was performed in the presence of hemoglobin. Isolated chloroplasts exposed to GSNO 78, 169, and 255 μmol NO showed a significant decrease of AP activity (48, 53 and 54%, respectively), and a decrease in ascorbyl radical content, despite, the total content of ascorbate was not altered. A 3-fold increase in the generation of H_2O_2 by chloroplasts exposed to NO, as compared to control chloroplasts, was measured. The data presented here showed that ascorbate metabolism was affected by NO exposure. The effects on chloroplasts suggest that NO could be responsible for an H_2O_2 increase in the cells.

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PL-P9.

ISOLATION OF CANDIDATE GENES PRESENTING UP- OR DOWN-REGULATION IN FLOWERS OF *PASPALUM NOTATUM* DURING THE OCURRENCE OF APOSPOROUS INITIALS

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Apomixis is defined as asexual reproduction through seeds. During apomixis megagametophytes are formed through a series of mitosis and the embryo is generated by parthenogenesis. The molecular events that take place during the development of the trait are still at the initial steps of characterisation. Twenty-four primer pair combinations were used in duplicated differential display experiments to evaluate the expression of around 2500 transcripts in the flower at three different developmental stages. Five cDNA differentially expressed in apomictic and sexual plants were detected. Out of them, four were up- (*srp1*, *srp2*, *srp3*, *srp4*) and one down-regulated (*arp2*) in sexuals at the CM stage, coincident with the emergence of the aposporous initials. Differential expression was confirmed by RT-PCR or reverse northern blot. Sequencing revealed that one out of all the fragments isolated (*srp1*) presented homology at the protein level to the vertebrate transcription factor HoxA5 and a second (*srp2*) to a retrotransposonal sequence. The remaining three segments (*srp3*, *srp4* and *arp2*) showed no similarity to known sequences. Further characterization of the genomic structure and expression of *srp1* is presented.

PL-P10.

PUTATIVE COMPLEX I γCAS ARE INDUCED IN ENGINEERED MALE STERILE ARABIDOPSIS THALIANA

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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 three different promoters: CaMV 35S, apetala 3 and A9. The size and shape of transgenic plants bearing the apetala3: :u-atp9 and A9: :u-atp9 genes looked normal while the 35S: :u-atp9 transformed plants showed a dwarf morphology. All u-atp9 expressing plants, independent of the promoter used, exhibited a male sterile phenotype. We have recently identified three novel genes encoding putative γ carbonic anhydrases as subunits of mitochondrial complex I. These genes were named AtγCA2, AtγCA4 and AtγCA5. Molecular analysis of male sterile plants revealed the induction of the mitochondrial nuclear complex I (nCI) genes, *psst*, *tyky* and nadh binding protein (*nadhbp*) and the three identified novel genes, associated with a mitochondrial dysfunction. In addition, we present conclusive evidences showing AtγCA2, 4 and 5 are efficiently imported *in vitro*. These results are in agreement with the idea that γCAs are essential components of plant mitochondrial complex I.

PL-P11.

ROLE OF BARLEY ALEURONE PHOSPHATIDYL-INOSITOL 4-KINASE IN α-AMYLASE SECRETION

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Phosphatidylinositol 4-kinase catalyzes the phosphorylation of PtdIns in the D-4 position of the inositol ring. This is the committed step in the synthetic pathway $PtdInsP_2$ which is the precursor of intracellular messengers. In addition, the inositol lipids are important biochemical cues for regulating vesicle formation and trafficking, cytoskeletal dynamics and enzyme activity. We demonstrated previously that PLC is involved in GA response, since GA increases the polyphosphoinositides synthesis and turnover and IP_3/IPs levels. These responses and α-amylase secretion were inhibited by U73122, a PLC inhibitor. Here, we present evidences that the inhibition of PtdIns4-kinase by phenylarsine oxide (PAO) leads to attenuation of hydrolase secretion and it was correlated with changes in the membrane-associated PtdIns-4 kinase activity. PLC inhibition did not affect neither α-AMY and Rab21, a gen regulated by ABA, mRNAs nor SLN1 levels, a negative regulator of α-AMY expression. By using fluorescence microscopy the cell viability was measured and we determinated that U73122 effect was not due to toxic cause. Since, inhibition of PLC and PtdIns 4-kinase only affect the Ca^{2+}-dependent pathway in GA signal, the inositol cycle role appears to be at level of exocytosis. Our results suggest that PtdIns-4 kinase may be essential for sustaining the aleurone capacity to secrete hydrolases during GA stimulation and show, for the first time, the involvement of this kinase in plant secretion process.

PL-P12.

POTATO TUBER ASPARTIC PROTEASE INTERACTS WITH A 10 KDA PROTEIN

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Plant aspartic proteases (APs) have been demonstrated to process and degrade proteins *in vitro*, and in many cases, the localization of the protein would be consistent with such a role *in vivo*. We have purified an extracellular AP from potato tuber (APT) with antimicrobial activity which is induced by wounding and infection. This AP is able to interact in 1:1 molar ratio with a basic chitinase (with inhibitory proteolytic activity) *in vitro*. The aim of this work is to know the nature of proteins that interact *in vivo* with APT. To reach this objective, polyclonal antibodies raised against APT were bound to sepharose-4B, and intercelullar fluids from healthy, wounded and infected tubers were cromatographed. After elution, intercelullar fluids (IWF) from healthy and wounded tubers showed only one peak. Analysis of this peak in SDS-PAGE showed two protein bands of 10 and 40 kDa approximately. Only 40 kDa protein was detected by western blot analysis using IgG-anti APT as primary antibody, acordding with APT molecular weight. The isolated complex was chromatographed in gel filtration and only one peak corresponding to 80 kDa was obtained. These results would suggest that in IWF from healthy and wounded tubers, APT interacts with a protein of 10 kDa in a 1:4 molar ratio and that this interaction is affected by infection with a pathogen, as *P. infestan* infection so that no peaks were detected in the elution of IWF from infected tubers. At this time we are working in the sequencing of the 10 kDa protein.

PL-P13.

CHARACTERIZATION OF A NEUTRAL INVERTASE FROM WHEAT INVOLVED IN ENVIRONMENTAL STRESS ADAPTATION

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Neutral/Alkaline-Invertases (N/A-Inv) are cytosolic sucrose-hydrolysing enzymes with pH optima in the range of 6.8-8.0. They have been only identified in plants and cyanobacteria but their physiological function is not known yet, having suggested that they may regulate the entry of sucrose into different cytosolic pathways. We have recently reported that N/A-Inv activity increased in wheat seedling during environmental stress acclimation. The aim of this work was the isolation and characterization of a N/A-Inv from wheat, in order to gain knowledge about its role in plants. The enzyme has been purified 457-fold from wheat leaves (specific activity about 2.8 μmol.mg $protein^{-1}.min^{-1}$). The enzyme has a native M_r of about 330 kDa as estimated by gel filtration and 80 kDa on SDS-PAGE. Optimal activity was found at pH 6.8, and it hydrolized sucrose with hyperbolic saturation kinetics with a K_m of about 20 mM. Maltose was hydrolysed at a 10% rate relative to sucrose whereas the enzyme did not hydrolyse raffinose, melezitose or trehalose. The enzyme was strongly inhibited by fructose, Tris-HCl and heavy metals, such as Cu^{+2} and Hg^{+2}, with similar inhibition patterns to those observed for others N/A-Inv. The structural analysis of deduced amino-acid sequences of known N/A-Inv revealed special features related to their activity that are agree with our biochemical results.

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PL-P14.

AUXIN SIGNAL PATHWAY INVOLVED IN POTATO DEFENSE RESPONSES

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Indol 3-acetic acid (IAA) mediates an enormous range of plant developmental events, as well as environmental responses. More recently, it has been suggested that auxin homeostasis is one of the components participating in the regulation of the defense responses.
To investigate the role of auxin in the responses of potato tubers against fungal pathogen, we explored different biochemical components involved in potato tubers against *Fusarium* attack. We measured a putative fungal pathogenecity factor, corresponding to a Fusarium Extracellular Serine Protease (FESP), which has been previously characterized in our laboratory. The pattern of potato pathogenesis related proteins was also analyzed in tubers after fungal and hormone treatments. Our results indicated that the IAA application altered potato biochemical responses against fungal infection, suggesting that changes in auxin levels could serve to limit the pathogen spread and disease development by a fungistatic action or activating potato defense mechanisms. Finally, in order to investigate the IAA signal pathway related with the defense mechanism we are testing the expression of a member of Aux/IAA gene family. *St*IAA was isolated by a differential screening of an infected-tubers cDNA library and is up-regulated by stress conditions. These preliminary results may provide a new insight into the role of auxin in defense response.
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PL-P15.

NITRIC OXIDE: A PLANT STRESS MODULATOR?

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Nitric oxide (NO) attracts the attention of plant scientists since it is involved in many plant stress responses. NO treatment induces physiological and even anatomical responses that were earlier believed to be regulated by plant hormones such as ethylene, SA, JA, ABA (Poster Garcia-Mata *et al.*) and auxins (Posters Lanteri *et al.* and Correa-Aragunde *et al.*). NO has also been shown to mediate the activation of second messengers like protein kinases (Poster Lanteri *et al.*) and phospholipases (Poster Raho *et al.*). This suggests that NO can act as a plant hormone itself but also as a modulator of stress hormone action. Many stresses result in a quick, strong, local (i.e. hypersensitive response (HR)) change and/or generalized and abrupt metabolic adaptations. One of our working hypothesis is that NO might quench these strong stress-derived responses. In this work we use tomato mutants altered in production and/or sensing of ethylene, SA, JA, ABA in order to shed light on the role of NO-hormone cross-talk to modulate stress responses. The following systems were used: i) drought, wounding and senescence, three abiotic stresses regulated by at least one of the mentioned hormones and ii) among biotic stresses, *Botrytis cinerea* which is a necrotrophic tomato pathogen that seems to abuse senescene, wounding and HR and *Phythopthora infestans* which is a hemibiotrophic pathogen of tomato. The latest results will be shown and discussed.
Supported by STW (Netherlands) and UNMdP, Conicet and ANPCyT (Argentina).

PL-P16.

FUNCTIONAL GENOMICS IN OXIDATIVE STRESS RESPONSE OF *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* (24.000 genes) under oxidative stress conditions. We identified 621 genes with expression levels two- fold greater under ROS treatment than in the control samples. Expression profiles of several genes were confirmed by Northern blot analysis. Most of the identified genes do not have an obvious direct role in oxidative stress and there are a considerable number of genes with unknown function. These gene products can be classified into two groups. The first group includes proteins that function or are probably functional in stress tolerance. The second group contains regulatory proteins, that is, protein factors involved in regulation of signal transduction and gene expression related to general stress responses. A subset of genes that belong to both groups were considered to be interesting candidates for further functional analysis and we are currently investigating their roles in ROS response.

PL-P17.

MAIZE RECOMBINANT NON-C4 NADP-MALIC ENZYME: A NOVEL DIMERIC ENZYME WITH HIGH SPECIFIC ACTIVITY

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In plants, different isoforms of the NADP-malic enzyme (NADP-ME) are involved in a wide range of metabolic pathways, being the most abundant the C4-enzyme, which has evolved from C3-NADP-ME. In maize, the most recent ancestor of C4 NADP-ME is a plastidic non-photosynthetic NADP-ME, whose cDNA is supposed to codify for a 72 kDa NADP-ME. In the present work, we express a cDNA encoding for this maize root non-photosynthetic NADP-ME in *E. coli*. However, in contrast to the enzyme previously purified from maize roots, the recombinant NADP-ME thus obtained presents different monomer molecular mass (66 kDa) and kinetic and structural properties, presenting very high NADP-ME activity. Using antibodies against this recombinant enzyme, an immunoreactive band of 66 kDa is detected in different maize tissues, which expression pattern correlates with that of the 72 kDa protein, indicating that the 66 kDa enzyme is in fact an *in vivo* expressed protein. The recombinant NADP-ME assembles as a dimer *in vitro*, although the tetramer found in maize roots *in vivo* is made up by this 66 kDa NADP-ME, probably in association to the 72 kDa protein. In this way, in the present work we characterize a novel maize NADP-ME not identified until now. This contribution will be of great importance for studies concerning the equilibrium between the oligomeric states and the evolution towards the C4-isoenzyme in maize.

PL-P18.
POLYAMINE METABOLISM OF TOBACCO PLANTS INFECTED BY *SCLEROTINIA SCLEROTIORUM*

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Polyamines are polycationic compounds that are found in all living organisms. In plants, they have been shown to be essential for growth and developmental processes, and recently, a defense–related role was suggested. Regarding this, high levels of spermine were observed in tobacco plants after TMV infection and spermine itself was able to induce the expression of pathogenesis-related proteins in a salicylic-independent way. Furthermore, an increase of polyamines in plants infected by biotrophic fungi has been reported but information about nechrotrophic fungi is lacking This work was aimed to evaluate the role of polyamines in tobacco plants in response to the infection of the nechrotrophic fungus *Sclerotinia sclerotiorum*, the most important pathogen of cultivated sunflower in Argentina. Tobacco plants inoculated with *S. sclerotiorum* mycelium showed higher levels of putrescine than controls in whole tobacco leaves, but when intercellular spaces was studied considerable changes in putrescine, spermidine and spermine was observed. In order to evaluate the specific role of each polyamine, we infiltrated tobacco leaf discs with solutions of these compounds before the inoculation. In this case, polyamine infiltration resulted in an increase of necrotic area of inoculated leaf discs, suggesting that either polyamines are deleterious to defense response of tobacco plants or they are able to support fungal development. The probable role of polyamines in tobacco-*S. sclerotiorum* interaction will be discussed.

PL-P19.
GLOBULIN-P CHARACTERISTICS THAT PROMOTE ITS AGGERGATION

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Globulin-P, 11S-globulin and glutelin are the main protein fractions in Amaranth seeds. Although they are legumins, (oligomers with subunits composed of a 30 kD and a 20 kD disulfide linked polypeptide) they differ in their aggregation state: Globulin-P presents some peculiarities which make it a hallmark of amaranth. It revealed a strong trend towards polymerization and contains high amounts of a monomeric subunit of 56 kDa (P56). To obtain information about the structural characteristics that promote globulin-P aggregation, our objective was to compare conformational aspects of globulin-P and other legumins and to analyze the purified globulin-P polypeptides. Globulin-P, soybean and amaranth 11S-globulins, and gobulin-P polypeptides were purified and analyzed by electrophoresis, isoelectrofocusing, fluorescence spectroscopy and RP-HPLC. Fluorescence results suggested that the three globulins contained two kinds of tryptophan locations, one surrounded by non-polar elements (λ_{max}=320nm) and another close to the protein surface (λ_{max} =340nm). Nevertheless, subtle differences were found. In the three legumins the 30 kDa polypeptides were the most acidic, but their pI ranges were different for each globulin. Five P56 subunits of different pI were found in globulin-P. These subunits showed the most hydrophobic character by RP-HPLC. The purified globulin-P polypeptides formed aggregates linked by disulfides. They showed high retention times in RP-HPLC. The presence of the hydrophobic P56 and the trend of its polypeptides to establish disulfide bridges may promote globulin-p polymerization. These polypeptide properties may not confer to globulin-P molecules very different conformational characteristics from other 11S-globulins.

PL-P20.
SIGNALS OF 11S STORAGE PROTEINS THAT ARE SUFFICIENT TO TARGET PROTEINS OF DIFFERENT SIZE TO VACUOLES

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Storage proteins do not have any conserved motif that can function as vacuolar sorting determinants and it has been suggested that aggregation itself can function as a targeting signal. The C-terminal tetrapeptide of phaseolin has been shown to be necessary and sufficient for targeting to vacuoles, but there are also indications, that this is not the only signal involved in the process and that the sorting machinery require cumulative information. The aim of this work was to identify signals involved in the vacuolar targeting of the Amaranth 11S protein. Two different aminoacid sequences of amaranth 11S globulin were chosen. The first sequence is the C-terminal pentapeptide of amaranth 11S globulin, KISIA and the internal sequence: GNIFRGF (Amh-H1N) that is similar to the sequence-specific vacuolar sorting determinant NPIR, responsible of directing proteins to lytic vacuoles. This motif was named Helix 1 because corresponds to an alpha helix region found in the crystal structures of both 7S and 11S storage globulins. These signals were fused at the C terminal region of the genes encoding for green fluorescent protein (GFP) or GFP-GUS. To target these proteins to the secretory pathway a mouse immunoglobulin signal peptide was fused to their N-terminal sequence. These constructs were transient expressed in *Arabidopsis* protoplasts and were observed by confocal microscopy at different times after transfection. A typical vacuolar pattern was observed 36-48 hs after transfection, therefore both signals are sufficient to target both GFP and GFP-GUS to vacuoles.

PL-P21.
***γca1* AND *γca5* NULL MUTANTS SHOWS MALE STERILE PHENOTYPE**

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We have identified mutants for five AtγCA genes. In this report we present two null mutants mapping at γCA1 and γCA5 loci. Homozygous and heterozygous null mutants were isolated and examined for phenotypic characteristics over at least two generations. Vegetative growth is not apparently affected while reproductive phase is impaired, mainly in pollen development. Anthers are full of pollen grains that apparently are arrested at tetrad stage. Accordingly, these immature pollen grains are unable to fall on stigma at anthesis. Consequently, fertilization is not produced, and fruit development is stopped. This phenotype is known as fruit abortion. The degree of this phenotype may vary along the floral bolt and in secondary bolts, suggesting some redundancy mechanisms. These male sterile flowers are able to be pollinated with wild type pollen grains and fertilization occurs, showing a nearly normal fruit set, indicating that female organs are not affected. Furthermore, 35S::γCA1 plants show a stronger male sterile phenotype and sometimes premature senescence of flowers. These plants were also pollinated with wild type pollen grains and seeds could be harvested. Physiological parameters were measured as respiration and photosynthetic capacities. These results suggest that mitochondrial complex I γCAs are essential component of the respiratory chain in plants.
**both authors contributed equally to this work.*

PL-P22.

EFFECT OF CADMIUM IONS ON PROTEOLYTIC ACTIVITY IN SUNFLOWER COTYLEDONS

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Proteolysis removes abnormal or damaged proteins from the cell, regulates protein processing and intracellular protein levels, working as a cellular housekeeper. By means of proteolysis, cell can control the short-lived regulatory proteins that affect processes such as signal transduction and reception, transcription, division and cellular growth. The aim of the present study was to elucidate the effect of cadmium ion (Cd) on protease activity in sunflower cotyledons. Plants were grown in vermiculite, and after 10 days they were transferred to hydroponic medium with cadmium in Hoagland´s nutrient solution during 4 days. Treatments with 100 and 200 μM Cd produced a 20% increase in cotyledon protease activity, respect to the controls. Protease isoforms were separated by gel electrophoresis (SDS-PAGE 10%, 0,5% gelatin). Two isoforms were modified by cadmium treatments as compared to controls. The highest molecular weight isoform (less mobility) showed lower activity in cadmium-treated cotyledons, while the lowest molecular weight isoform showed higher protease activity in cadmium-treated cotyledons. These results suggest a direct relationship between cadmium stress and protease activity in sunflower cotyledons. The types of proteases involved in cadmium-induced senescence will be studied in order to establish the role of these isoforms in sunflower cotyledons under cadmium stress and their relationship with the proteases involved in natural senescence.

PL-P23.

ISOLATION AND CHARACTERIZATION OF FUNASTRAIN C II, A THIOL ENDOPEPTIDASE FROM *FUNASTRUM CLAUSUM* LATEX

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Funastrain c II, a cysteine endopeptidase, was purified and characterized from the latex of *Funastrum clausum (Asclepiadaceae).* Ultracentrifugation and cation exchange chromatography by FPLC at pH 6.5 were the main purification steps. The molecular mass (mass spectrometry) of the protease was 23.636 kDa. The analysis of funastrain c II by SDS-PAGE revealed a single polypeptide chain, and IEF showed that its pI was higher than 9.3. The cysteinic nature of the protease was demonstrated by inhibition with 10 μM E-64, whereas activation was achieved with reductor agents such as 12 mM cysteine and 5 mM DTT. An important percentage of residual caseinolytic activity after incubation at temperatures up to 70°C was conserved. Funastrain c II enzymatic activity optimum pH varied according to the substrate used: for casein, it was 9.0-10.0, and for the synthetic substrate L-pyroglutamyl-L-phenylalanyl-L-leucine-*p*-nitroanilide (PFLNA) was 6.3-6.8. K_M and k_{cat} kinetic parameters were calculated for the synthetic substrates N-α-CBZ-Ala-*p*-nitrophenyl ester (K_M =0.0243 mM, k_{cat}=1.5 seg $^{-1}$) and PFLNA (K_M=0.1011 mM, k_{cat}=0.86 seg $^{-1}$). Funastrain c II N-terminal sequence (LPNSVDWRQKGVVSAIRNQGKCGSCWAFSAV) showed a considerable similarity to other proteases isolated from latex of different *Asclepiadaceae* species, and also to cysteine proteinases belonging to the papain family.
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PL-P24.

MOLECULAR CHARACTERIZATION OF PRECURSOR ACCUMULATING VESICLES IN SUNFLOWER DEVELOPING SEEDS

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Vacuolar proteins are transported to protein storage vacuoles (PSV) via vesicle-mediated transport system that has unique features. This pathway has been studied only in pumpkin and in pea, being the vesicles different in size and origin: endoplasmic reticulum (ER) or Golgi apparatus (GA). The aim of this work was to study the vesicular transport pathway in developing sunflower seeds. To this end, two methods were used to characterize the pathway: sucrose and percoll gradients. Dense vesicles (DV) were isolated by sucrose gradients, in similar conditions to that described for pea DV and analyzed by Western blot. Sunflower dense vesicles have precursor of 11S proteins, tonoplast intrinsic proteins α-TIP (marker of PSV), BiP (ER marker). Similarly by separation of developing sunflower seeds in Percoll gradients, vesicles with the same density of pumpkin precursor accumulating vesicles (PAC) were obtained. Sunflower PAC also has precursor of 11S proteins, α-TIP, BiP and VP72 (pumpkin BP-80 homologue). Both method are useful to isolate vesicles with precursor of storage proteins. Presence of BiP in the vesicles might be indication that vesicles are derived from the ER, although a recycling mechanism from AP to ER can not be excluded. VP72 were found in PAC but not in DV what might be an indication that there are some differences among these vesicles. Preliminary data indicates that sunflower PAC are able to bind the vacuolar targeting sequence of an 11S storage protein in a calcium dependent manner. This result confirmed the role of PAC in storage protein transport to PSV and the role of sunflower VP72 homologue in storage protein sorting. BP-80 or VP72 could not be detected in sunflower DV what might be an indication of these vesicles are not equivalent to PAC.

PL-P25.

IN VIVO ANALYSIS OF GLYCOGEN BIOSYNTHETIC PATHWAY IN *ANABAENA* sp. AND *E. COLI*

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Cyanobacterial and *E. coli* cells were permeabilized using a mixture of toluene:ethanol or toluene, respectively. Permeabilization was confirmed after addition of fluorescein diacetate and microscopic visualization. After the incubation of the treated cells with ADPGlc PPase substrates, ATP and [14C]Glc1P; or glycogen synthase substrate, [14C]ADPGlc, labelled alpha-1,4-glucan was recovered. Addition of external non radioactive ADPGlc in the incubation media with ATP and [14C]Glc1P did not affect the [14C]Glc incorporation into the glucan. Moreover, there was only 40% of the total incorporation of [14C]ADPGlc when the cells were in the presence of ADPGlc PPase non-labelled substrates. Structural data from Blue native PAGE, western blot analysis and co-immunoprecipitation, suggest the existence of protein-protein interactions between the enzymes involved in glycogen biosynthesis in *Anabaena* and *E. coli*. Results suggest a direct transfer of ADPGlc between ADPGlc PPase and glycogen synthase (metabolite channeling). This is a process by which a metabolite is directly transferred from one enzyme active site to the next without being released free into solution. The direct channeling of an intermediate between enzymes catalyzing consecutive reactions in a biochemical pathway offers the possibility of an efficient and exclusive metabolite delivery. This mechanism may allow to maintain a high flux of substrates in the glycogen biosynthetic pathway under different physiological conditions.

PL-P26.
SAPOSIN-LIKE DOMAIN IS PRESENT IN MONOMERIC PLANT ASPARTIC PROTEASES

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Plant aspartic proteases (APs) have an extra domain generally named plant-specific domain (PSI), it is present in the precursors of APs but is absent from the mature heterodimeric forms and there are not reports about its presence in mature monomeric forms. PSI has high homology with saposin like proteins, a group of lipid binding proteins and it is able to interact with phospholipid membranes and induces membrane permeabilization. In this work we show that recombinant PSI was recognized by polyclonal antibodies raised against monomeric plant AP, isolated from potato tubers (APT). This result would suggest the presence of PSI in monomeric mature forms. On the other hand, we have studied the capacity of potato monomeric APs (APT and APH2 form leaves) to produce leakage of liposomes. The leakage activity of both APs was higher at pH 4.5 and requires the presence of acidic phospholipids. We have previuosly reported the antimicrobial activity of potato APs. In view of these results the enzymes were conjugated with FITC and then incubated with conidia of *Fusarium* sp. The results show that both APs have bind to the conidial surface. These findings indicated that PSI present in monomeric plant APs would mediate an interaction of these enzymes with phospholipid membranes and induces membrane permeabilization. It is therefore possible that the PSI, alone or in conjunction with the proteolytic activity of plant APs, may have a function either as a defensive weapon against pathogens or in late autolysis of plant cells.

PL-P27.
RESPONSE OF TWO TOMATO PEROXIDASE GENES AGAINST BIOTIC ELICITORS

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When plants are attacked by pathogens, they defend themselves against such invasion with different mechanisms, which include the production of phytoalexins, pathogenesis related proteins and reactive oxygen species, lignification, etc. Such responses are initiated by host recognition of molecules called elicitors, which are directly or indirectly released from an invading pathogen. Peroxidases (Px) are involved in some of these mechanisms. In tomato, two genes, *tpx1* and *tpx2*, which codify for cell-wall Px, constitutively expressed in roots, were described. *Tpx1* gene, has been related in the response to different types of stress and recently it was reported as being involved in the synthesis of lignin and suberin in specific root cells. These peroxidases could be involved in the cross-linking of the cell-wall proteins in response to pathogen attack and we have decided to study gene expression in tomato hairy root cultures exposed to biotic elicitors (chitosan and a specific root pathogen) trough electrophoresis, IEF and Northern blots. An increase of 50 % of Px activity was observed in samples exposed to 200 mg/l of chitosan, 2.5 hours post-elicitation together with the over-expression of a Px, presumably TPX2, with pI higher than 9.6. The treatment with fungal spores produced an increment of the *tpx2* transcripts and Px activity 5 h post-elicitation with 105 spores/ml. In addition, *tpx1* messenger was induced 48 h post-elicitation in treatments with 0.8 % P/V of fungal extract.

PL-P28.
RELATIONSHIPS BETWEEN *TDR4* AND GIBBERELLIN IN TOMATO FRUIT DEVELOPMENT

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Gibberellin class of plant hormones influences a wide variety of developmental processes. GAs have been implicated in the control of flowering in several species, as well as sex determination, control various aspects of seed germination, and fruit set. Arabidopsis can be used to identify the genes controlling carpel morphogenesis and hormone signal transduction and their development is dependent on fertilization and seed set. Gibberellin is an essential component for silique development, because both seed and fruit development in the *ga1* mutant were dependent on the application of exogenous GA_3 following pollination. GA_3 primarily influences mesocarp cell division in a similar manner to that observed following pollination in *Arabidopsis*. In tomato, GA_3 treatment has been observed to induce mesocarp cell expansion with restricted cellular division, while auxin treatment stimulated cell division. Exogenous application of GAs at anthesis increases *TDR4* mRNA levels in wild-type *Lycopersicon esculentum* var *Microtom.* A tomato gib3 mutant deficient in the synthesis of GAs shows a reduction *TDR4* transcript. Moreover, transgenic plants of *Arabidopsis thaliana* expressing *TDR4* ectopically show a phenotype *spy-like* (*spy* plants have altered GA perception). Plants 35S::*TDR4* phenotype includes longer internodes, early flowering, highly branched inflorescence-like flowers, partial replacement of sepals with carpel structures, production of ectopic secondary axillary flowers and parthenocarpy as *SPY* mutants. Taken together, these results suggest the relationship between *TDR4* and GAs in fruit development.

PL-P29.
FROST DAMAGE AND CARBOHYDRATE METABOLISM IN CITRUS FRUIT

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Freezing temperatures can cause severe damage and economic loses to the citrus industry by reducing yield and by afecting the external and internal parameters of fruit quality. The activities and levels of the enzymes involved in the carbohydrate metabolism of fruit from orange trees (*Citrus sinensis* L., cv. Valencia late) exposed to different temperatures were analized. The aim of the work was to find biological markers to develop an early diagnostic technique for frozen fruits. The enzymatic activity measurements (in terms of U/mg and U/g FW) showed a significative and moderate increase in frozen fruits compared to control in pyrophosphate-dependent phosphofructokinase (1.85-fold), Ala amino transferase (1.6-fold), phosphoenolpyruvate carboxylase (1.75-fold) and pyruvate kinase (1.75-fold). A more pronounced rise was observed in lactic and alcohol dehydrogenase activities (4.4 and 4.7-fold, respectively). In contrast, only NADP-malic enzyme diminished (40%). These results were consistent with the profile in the immunoreactive proteins levels. Another important feature to emphasize was the marked decrease in total soluble protein in frozen fruits. These studies suggest that freezing temperatures induce the activation of the glycolytic and fermentative metabolisms.

PL-P30.
NITRIC OXIDE MIMICS THE AUXIN ROLE IN THE PROMOTION OF LATERAL ROOT DEVELOPMENT

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Nitric oxide (NO) is a bioactive molecule that functions in numerous physiological processes in plants, most of them involving a cross talk with traditional phytohormones. In this work we report that NO promotes lateral root (LR) development, an auxin-dependent process. Application of the NO donor sodium nitroprusside (SNP) to tomato seedlings induced LR emergence and elongation in a dose-dependent manner. Depletion of endogenous NO with the specific scavenger CPTIO resulted in a complete abolition of LR emergence, confirming a physiological role for NO in the regulation of lateral root initiation. Detection of endogenous NO by the specific probe DAF-2 DA revealed that the NO signal was specifically located in LR primordia during all stages of its development. In another set of experiments, SNP was able to promote LR development in auxin-depleted seedlings treated with the auxin transport inhibitor NPA. Moreover, it was found that LR formation induced by the synthetic auxin NAA was prevented by CPTIO. All together, these results suggest a novel role for NO in the regulation of LR development, probably operating in the auxin signaling transduction pathway. We are currently analyzing the NO involvement on the regulation of cell cycle and auxin-induced genes during LR development to support the hypothesis suggested and contribute to our understanding of the molecular mechanisms that regulates root morphogenesis.
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PL-P31.
NITRIC OXIDE IMPROVES IRON UTILIZATION AND REGULATES ADAPTIVE ROOT RESPONSES TO IRON DEFICIENCY

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Iron is an essential nutrient for chlorophyll synthesis and several metabolic processes in plants. Nitric oxide (NO) is a small signal molecule that has proven to be involved in iron nutrition in maize plants (Graziano *et al.*, Plant Physiol, 2002, 130: 1852-9). Here we show that exogenous NO application to tomato plants was also able to avoid the iron deficiency-induced chlorosis, while endogenous NO blockage resulted in chlorotic plants even at high iron supply. The chlorophyll/iron ratio in leaves was 2-fold higher in iron-deficient NO-treated compared to iron-sufficient tomato plants. NO also reverted the chlorotic phenotype of the tomato mutant *fer*, unable to uptake iron from soil. Roots are plant organs that sense iron in soil. Iron deficiency induced an accumulation of NO in roots as was detected by DAF-2 DA, an NO specific fluorescent probe. Lateral root promotion, a root response to iron deficiency, was stimulated by NO even in iron-sufficient plants (see poster Correa-Aragunde et al.). On the other hand, Fe(III) reducing activity in roots, which is increased by low iron supply, was not stimulated in iron-deficient NO-treated plants. All together, these results suggest that NO is probably acting on iron nutrition at two levels: improving the efficiency of iron utilization and regulating root adaptive responses to iron deficiency.
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PL-P32.
ISOLATION AND CHARACTERIZATION OF SNAKIN-1 PROMOTER

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Snakin-1 (SN1) is a newly characterized antimicrobial peptide isolated from potato tubers that has been found to be active against bacterial and fungal pathogens from potato and other plant species**.** Snakin-1 causes aggregation of both gram-positive and gram-negative bacteria. Since gene expression has been detected in tubers, stems, axillary buds, and young floral buds, the expression pattern of gene StSN1 suggests that protein SN1 may be a component of constitutive defense barriers, especially those of storage and reproductive plant organs. Its regulatory region has not been studied until now. We report here the cloning, sequencing and analyis of the promoter region placed upstream of Solanum tuberosum snakin-1 gene. A 671 pb fragment was amplified by Inverse polymerase chain reaction (IPCR) using potato Kennebec germplasm as template. After cloning into pGemT easy vector, it was fully sequenced and probableTATA and CAAT motives were identified. Two upstream sequences: a 671 bp fragment corresponding to the whole cloned sequence and a short version of 448 pb were used to transiently express betaglucuronidase (GUS) in bombarded potato explants. GUS expression was detected in stem apex and axillary buds. This suggests that the isolated promoter sequence conserved its tissue specifity. In addition we obtained transgenic potato and tabacco plantas that express GUS under the control of the same. cloned fragments. Further studies will clarify the influence of differents growth regulators on this new promoter in order to better understand its physiological functions.

PL-P33.
GAMMA CARBONIC ANHYDRASES IN PLANT MITOCHONDRIAL COMPLEX I

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Three genes from Arabidopsis thaliana with high sequence similarity to gamma carbonic anhydrase (γCA), a Zn containing enzyme from *Methanosarcina thermophila* (CAM), were identified and characterized. Evolutionary and structural analyses predict that these genes code for active forms of CA. Phylogenetic analyses reveal that these Arabidopsis gene products cluster together with CAM and related sequences from α and γ proteobacteria, organisms proposed as the mitochondrial endosymbiont ancestor. Indeed, *in vivo* experiments indicate that these gene products are addressed into the mitochondria as occurs with several mitochondrial protein genes transferred, during evolution, from the endosymbiotic bacteria to the host genome. Moreover, putative orthologous CAM genes are detected in other plants and green algae and were predicted to be imported to mitochondria. Modeling and sequence analysis performed in more than a hundred homologous sequences show a high conservation of functionally important active site residues. Thus, the three histidine residues involved in Zn coordination (His 81, 117 and 122), Arg 59, Asp 61, Gln 75, and Asp 76 of CAM are conserved and properly arranged in the active site cavity of the models. Two other functionally important residues (Glu 62 and Glu 84 of CAM) are lacking, but alternative amino acids that might serve to their roles are postulated. Accordingly, we propose that photosynthetic eukaryotic organisms (green algae and plants) contain γCAs and that these enzymes codified by nuclear genes are imported into mitochondria to accomplish their biological function.

PL-P34.

HELIANTHUS ANNUUS L. RESPONSES TO CADMIUM STRESS. ANTIOXIDANTS AS MARKERS OF THE METAL TOXICITY

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Cadmium is an important environmental pollutant present in soils, toxic to living cells and strongly phytotoxic. Cadmium toxicity was evaluated in relation to antioxidant parameters such as antioxidant enzymes, polyamines (Pas) and proline in sunflower plants during 10 days of growth under 0.1 mM and 1 mM $CdCl_2$. Plant growth and root length were significantly inhibited by the higher cadmium concentration from the third day. Except for guaiacol peroxidase, all the antioxidant enzymes (catalase, ascorbate peroxidase and glutathione reductase) showed a significant increase under 1 mM $CdCl_2$ from day 7 in shoots and roots, though in roots the rise in the enzymes activity was lower. Glutathione content also increased by day 7 in roots and shoots. Polyamines and proline increased from day 7 in shoots and roots under both cadmium concentrations. Thiobarbituric acid reactive substances measured as a marker of oxidative damage increased in roots and shoots only by 1 mM $CdCl_2$. This increase was higher in roots than in shoots. Cadmium stress induced by the highest metal concentration produced an evident growth inhibition and oxidative damage, in spite of the increment of all of the measured parameters. The increase in polyamines and proline, postulated as antioxidants, offered an additional protection. However, other parameters, in addition to growth and oxidative damage, could add information about the exact magnitude of the damage exerted by the metal.

PL-P35.

BEHAVIOR OF PEROXIDASE ISOFORMS IN SUNFLOWER PLANTS SUBJECTED TO UV-B RADIATION

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Ozone depletion results in an increase in the biological harmful solar ultraviolet-B radiation (280-320 nm) reaching the surface of the earth. UV-B radiation has deleterious effects in plant growth and fundamental physiological processes. Recent studies confirm that hydrogen peroxide (H_2O_2) is a signaling molecule in plants that mediates responses to abiotic and biotic stresses. Peroxidases (POD) are heme proteins that catalyze the oxidation of a range of substrates by H_2O_2, and are important in the H_2O_2 turnover. The objective of this work was to determine the effect of UV-B radiation and H_2O_2 on peroxidase isoforms as a potential mechanism of UV tolerance in plants. Seven and fifteen day-old sunflower plants were treated with UV-B doses 30 kJ/m^2 or increased H_2O_2 concentrations (0.5-100 μM). Peroxidase isozymes were analyzed in cotyledons and leaves by native-PAGE and FPLC. In cotyledons two POD isozymes were enhanced (pI: 5.0 and pI: 5.2) and appeared a new isoform (pI: 5.4) in UV-B treated plants, compared with control plants. Similar results were obtained with H_2O_2 treatments. UV-B treatment induced pI: 5.0 and pI: 5.2 POD isoforms in leaves. Our results are indicating that the UV-B signaling in plants are mediated by H_2O_2 and POD plays and important role in this response.

PL-P36.

ARABIDOPSIS ACTIVATION-TAGGED MUTANTS TOLERANT TO OXIDATIVE STRESS

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Plants have evolved a dynamic network of antioxidant defenses that serve to reduce the oxidative damage caused by environmental stress. To identify new components of this network, we had performed large-scale gain-of-function screenings of Arabidopsis mutants. These activation-tagged mutants were generated by Weigel´s group, who constructed a vector (pSKI015) with four copies of an enhancer element from the cauliflower mosaic virus 35S gene. Seedlings (8.850 lines; segregated into 86 pools) were subjected to oxidative stress by spraying with the herbicide Paraquat (methyl viologen, MV). MV generates superoxide by leakage of electrons at photosystem I in chloroplasts under illumination. Mutants more tolerant to oxidative stress were easily identified in comparison with wild-type lines, which were not able to survive. In a primary screening, 18 putative pools with higher tolerance to MV were isolated. In a secondary screening, 11 lines of mutants displaying strong tolerance to MV were confirmed. Plasmid rescues were carried out to identify the segment of plant DNA adjacent to the activation-tagged border, and the nearly gene expressed, which could be responsible for the tolerant phenotype. The function of the newly identify sequences in the antioxidant defense network will be discussed.

PL-P37.

SCREENING A TOBACCO cDNA LIBRARY WITH PURIFIED MAIZE NADP-ME ANTIBODIES AND EXPRESSION OF THE PRODUCT OBTAINED IN _E. COLI_

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Previous studies indicated that different antibodies´ batches against the purified photosynthetic NADP-malic enzyme (NADP-ME) from maize leaves cross-react with a 72 kDa protein, detected in different tissues of several plant species. A 72 kDa protein with low NADP-ME activity was also purified from several plant sources. Thus, this 72 kDa-protein was pointed out as a non-photosynthetic NADP-ME. Nevertheless, we recently found that the cDNA supposed to encode for this NADP-ME in maize roots, codifies in fact for a novel highly active 66 kDa NADP-ME. In this way, in order to identify the nature of the 72 kDa protein, we used the maize purified NADP-ME antibodies to perform a screening of a cDNA tobacco library; as Western blot analysis of tobacco leaf protein with these antibodies detected only a 72 kDa-signal. After the screening, we obtained 8 independent clones, 4 of which were completely sequenced. Blast analysis of the sequence obtained indicated that it was 80 to 93% identical to Hsp70 from different plant sources. Prediction analysis of the sequence obtained (AY253326) showed that codifies for a possible cytosolic 70,876 Da protein. The cDNA for this Hsp70 was connected in frame to a pET32 expression vector to over-express the protein in *E. coli*. The association between the recombinant NADP-ME and Hsp70 proteins *in vitro*, suggest that such association can be the reason for the cross-reaction of the anti-purified maize NADP-ME antibodies.

PL-P38.

ENZYMATIC BROWNING INHIBITION IN SOLANUM TUBEROSUM USING RNAi GENE SILENCING TECHNIQUES

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The enzymatic browning of fruits and vegetables that takes place when they are cut and exposed to air, is caused by the action of the enzyme Polyphenol Oxidase (PPO) and the reaction that catalyses is called Enzymatic Browning. PPO has also been reported to play a role in the defense against pathogens and pests produced by insects. There are known at least five PPO genes in potato plants, each with a specific spatial and temporal pattern of expression. The Pot 32 gene is strongly expressed in tubers and roots and Pot 33 gene is mainly expressed in the outer cortex of tubers. Due to this fact we have designed an RNAi silencing vector against the 5' region of Pot 32 gene in order to silence it only the tuber and avoid the later enhanced silencing of the others PPO isoforms in the plant. The construction engineered to silence Pot 32 was cloned in the pZP200HYG vector and introduced into *A. Tumefaciens*. At the moment we are transforming potato plants and expressing recombinant Pot 32 and Pot 33 enzymes in the heterologous system of *E. coli* in order to obtain antibodies to analyze the expression pattern of the transformed plants.treated with the glucan.

PL-P39.

EFFECTS OF THE USE OF ANTIBIOTICS ON *IN VITRO* CULTURE OF SUNFLOWER

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Genetic transformation of plants mediated by *Agrobacterium tumefaciens* requires the use of antibiotics in the regeneration media in order to control bacterial growth without interfering with the regeneration potential of the transformed cells. Carbenicillin, cefotaxime and more recently timentin have been the antibiotics most commonly used for this purpose with varying effects on different plant species. The aim of the work presented here was to evaluate the efficacy of these three drugs for the suppression of strain EHA101 of *A.tumefaciens* and their effects on the *in vitro* culture of sunflower genotype HA89 after the process of agrotransformation.
The results demonstrated that carbenicillin was not efficient at eliminating this strain of *Agrobacterium*, whereas timentin and cefotaxime did prevent bacterial growth. To evaluate the effects of these drugs on the explants, several parameters were analysed: regeneration capacity, weight and height of the shoots, chlorophyll and protein content, root development and the incidence of undesired traits, such as hyperhydricity and premature flowering. All the parameters studied were negatively affected when cefotaxime was present in the culture media whereas the use of timentin had favourable effects on all of them, especially on the regeneration potential. As sunflower is considered a recalcitrant specie due to its poor *in vitro* response, all these results indicate that timentin could be considered as the best antibiotic to be used in future assays with this genotype.

PL-P40.

EXPRESSION AND CHARACTERIZATION OF A POTATO TRANSCRIPTIONAL COACTIVATOR (StMBF1) UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

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MBF is an evolutionary conserved transcriptional coactivator that connects a regulatory factor and TATA element-binding protein (TBP). We have reported the identification of a *Solanum tuberosum* transcriptional coactivator, named *St*MBF1,which is up-regulated during *Fusarium eumartii* attack as well as wounding in potato tubers. *St*MBF1 is phosphorylated under *in vivo* and *in vitro* experimental conditions after treatment of potato cells with hyphal cell wall components. In addition, *St*MBF1 interacts *in vitro* with plant transcription factors of the Hd-Zip family. Since some members of Hd-Zip proteins are inducible by plant growth regulators and stress conditions, we attempted to know the *St*MBF1 expression pattern in potato suspension cells under different physiological conditions. The results indicated that *St*MBF1 accumulates by ABA, SA and heat shock treatments. In order to evaluate the putative interaction between *St*MBF1 and TBP proteins we expressed the potato TBP protein in *E. coli*. Protein-protein interaction is currently being tested by GST-pull down assays. Our results suggest that *St*MBF1 may posses a versatile regulation and it would mediate the coupling of different environmental signals within developmental program.
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PL-P41.

ISOLATION AND PARTIAL CHARACTERIZATION OF AN EXTRACELLULAR CYSTEINE PEPTIDASE FROM *JACARATIA DODECAPHILLA* (Vell.) FRUITS

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Jacaratia docecaphylla (Vell.) is a tree of the Caricaceae family, found in the Parana Subtropical forest of the province of Misiones. The fruit is used in popular medicine as a soft painless laxative. The fruits that contain latex are only edible when very ripe. A buffered, medium ionic strength phosphate extract of the fruit mesocarp analyzed by 11% SDS-PAGE, and stained with Brilliant blue showed 10 proteinaceous bands, plus a non resolved band migrating with the front. Vacuum infiltration of the tissue with water during 5 minutes followed by low-speed centrifugation for recovery of the extracellular washings allowed the selective extraction of a protein that showed a single band in SDS-PAGE with an estimated molecular mass of 34.000 Da, and isoelectric point above 9. Proteolytic activities of the enzyme were activated by thiol protease activators and inhibited by thiol protease inhibitors, indicating the enzyme to be a cysteine protease. The enzyme hydrolyzed denatured natural substrates such as casein, azocasein and azocoll with a high specific activity. It showed a peak of activity in alkaline conditions and at temperatures of 50°C. It had not detectable carbohydrate moiety. Antibiogram assays showed inhibitory action on the growth of hyphae of *Colletotrichum gloeosporioides* and *Penicillium digitatum*, further characterizing the enzyme as an extracellular plant defense cysteine peptidase.

PL-P42.

ENHANCEMENT OF THE NATURAL DISEASE RESISTANCE OF POTATOES BY CHEMICALS

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The mechanism involved in systemically induced resistance (SIR) can be induced nonspecifically in susceptible plants. In response to pathogens the natural defense mechanism of plants includes the production of lignin, phytoalexins and the induction of plant enzymes. The aim of this investigation was to study the induction of SIR mediated by the chemical activator DL-3-aminobutiric acid (BABA) and the fungicide Fosetyl-aluminium (Aliette) in processing potato cultivars with different levels of resistance against *Phytophthora infestans*. For the chemical induction of resistance, foliage of potato cultivars were sprayed with BABA, Aliette or water (as a control) and after three days the foliage was inoculated with *P. infestans*. After several days, the following parameters were evaluated: development of disease symptoms in foliage, protein level of several enzymes, and phenols and phytoalexin content in post-harvest tuber samples. After application of the chemicals the highest level of protection against *P. infestans* in foliage was observed at early stages of the development of the crop (30 days after sprouting). An increase in the resistance against Late Blight was also detected in plant tubers or post-harvest. There was also an increase in protein level of ß1-3 glucanase and aspartic protease, as well as in phenols and phytoalexins content in potato tuber disks post infection. This effect seemed to persist throughout the whole of plant. This treatment may offer the possibility of controlling both foliage and tuber blight and could have a major impact in reducing overwinter survival of *P. infestans* in tubers.

PL-P43.

EXPRESSION OF ASR GENES DURING EMBRYOGENESIS AND CELLULAR LOCALISATION OF TRANSCRIPTS UPON WATER-STRESS IN VEGETATIVE ORGANS

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Asr genes of tomato are a family of putative transcription factors up-regulated during water-stress. Several homologues have been cloned from different plant species, but little is known about these genes. Recently, an Asr from grape has been shown to activate an hexose transporter promoter. In this work, we analyze the pattern of expresion of Asr1 and Asr2 during embryogenesis and investigate the cellular localization of the transcripts in roots and leaves under normal and stress conditions. Both proteins are detected only in late embryogenesis (55-70 days post-anthesis). This expression correlates with the lowest seed water content and the accquisition of dessication tolerance. However, ASR proteins lack any of the known late embryogenesis abundant (LEA) protein motifs. In situ hibrydization assays showed that Asr1 and Asr2 are specifically transcribed in companion cells of the leaf phloem under normal conditions. Upon water-stress, Asr1 and Asr2 presence expands to surrounding parenchima cells. Asr1 mRNA was not detected in roots under both normal and stress conditions. In contrast, Asr2 mRNA is present in root phloem under water-stress treatment. According to our preliminary results, tomato ASR proteins may form heterodimers or homodimers in their native state.

PL-P44.

MOLECULAR CLONING OF A POTATO LEAF CDNA ENCODING AN ASPARTIC PROTEASE

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A number of aspartic proteases (APs) cDNA have been isolated from different plants. The sequence of APs can be divided into three regions: two of them, an amino-terminal region and a carboxy-terminal region show high similarity to each other and to enzymes from mammalian and microbial sources; the third one, a plant specific insert (PSI) is present only in precursors of heterodimeric plant APs. We have cloned a full-length cDNA encoding an potato leaf AP (*St*LAP1). The sequence of the longest clone has 1682 pb, with three regions in common with all plant APs. The open reading frame has a capacity to encode a protein of 497 amino acids which shares 94% sequence identity with AP from tomato. A single band was detected on gel blots of total RNA probed with the *St*LAP1. The size of this mRNA was estimated to be 1.8 Kb which is in good agreement with the lenght of the cDNA cloned. Also, we have analyzed by northern blot the expression of *St*LAP1 in different potato leaf cultivars under stress conditions. These analyses showed that expression levels of *St*LAP1 were highest in leaf after mechanical wounding and in potato cultivars resistant towards *P. infestans* after infection with this pathogen. These results and the results obtained previously about of the accumulation and the antimicrobial activity of the purified potato leaf AP (APH2) suggest that this enzyme may have a role in the defense response of potato.

PL-P45.

STRUCTURAL AND BIOLOGICAL ACTIVITY RELATIONSHIP OF AMARANTH STORAGE PROTEINS

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Amaranth storage proteins, albumins and globulins show conformational differences. Albumins present structural flexibility, with low proportion of disulfide bridges and low denaturation temperature. Globulins, of high molecular weight and stabilized by disulfide bridges, include 11S-globulin, globulin-P and glutelins. Although they share polypeptidic patterns, they present different aggregation states. Glutelins are highly aggregated whereas 11S-globulins behave as non-polymerized molecules. Globulin-P presents an intermediate state of aggregation, and contains a characteristic monomeric polypeptide (P56), which may be a non-processed precursor. The objective of the present work was to correlate the structural characteristics of amaranth storage proteins with their biological activity during germination. Storage proteins (albumins, 11S-globulin, globulin-P and glutelins) were extracted from *Amaranthus hypochondriacus* seeds at different hours after imbibition (h.a.i.) The protein fractions were analyzed by polyacrilamide gel electrophoresis under denaturing and non-denaturing conditions, gel filtration chromatography and immunological techniques. Results showed a faster breakdown on albumins, 11S-globulin and globulin-P. Albumins and globulin-P lost their reactivity against a rabbit anti-globulin-P polyclonal antiserum at 15 (h.a.i.) indicating the disappearance of conformational epitopes. Conversely, 11S-globulin maintains its reactivity for longer periods of hydrolysis. Chromatographic and electrophoretic techniques support these structural differences. Glutelins breakdown was observed after longer periods of germination. The albumins early mobilization may be the consequence of its loose structure. The different behavior of globulin-P in comparison with the other globulins may be associated with the presence of the monomeric P56 polypeptide, which is located on the molecule surface and was shown to be more easily hydrolyzed.

PL-P46.

WHEAT LEAF EXTRACELLULAR PROTEINS ARE OXIDIZED DURING WATER DEFICIT

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Reactive oxygen species accumulate in plant tissues in response to biotic and abiotic stress. Their generation pathway and/or their location are not completely known. Because the apoplast connects the environment with the interior of the cell, this is the site where important defence reactions take place. Previously, in wheat leaf, we showed that water deficit induces the accumulation of H_2O_2 and decreases the extracellular SOD activity. At the same time, the content of apoplastic proteins is reduced while its oxidative state increases. Now, we present evidence that O_2^- and H_2O_2 mainly accumulate in vascular tissues, despite that cell types are different. Thus, the phloem-xylem communication and the continuum xylem-apoplasm support the increment of oxidized apoplastic proteins. Western-blott experiments showed specific oxidation of different apoplastic polypeptides. In addition, proteic and carbonylation profiles obtained from unstressed intercellular washing fluids (IWF) incubated for 12 hs at 32°C were similar to those displayed by drought stressed plants. The most important changes observed were the loss of a 70.5 kDa band whose specific carbonylation was low, and the increment in the oxidative state of three polypeptides; two of mass higher than 92 kDa and one of 33 kDa.

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PL-P47.

***HELIANTHUS ANNUUS* POLLEN PROTEIN AS FLORAL ORIGIN MARKERS IN HONEY**

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In this work we report the development of a novel method for the assessment of floral origin of honey based on the study of pollen proteins. Proteins are a minor honey component, however, they are used as internal standard in the evaluation of adulteration by stable carbon isotope ratio. Honey proteins come from honeybee and also from pollen. Considering that honeybee proteins should be common to all types of honey, we decided to verify the usefulness of pollen proteins as floral origin markers in honey. Previously we have identified *H. annuus* pollen proteins that could only be detected in Sunflower honey. The specific proteins of Sunflower honey were evaluated by means of Western Blot using polyclonal antibodies raised in rabbits by immunization of *H. annuus* pollen extracts. Herein, we were able to identify a double band of approximately 33-36 kDa only in those honey containing different percentages of *H. annuus* pollen. Afterwards, in order to quantify the presence of these pollen proteins in honey, we develop a competitive Enzimoimmunoassay (EIA). For this purpose we purified the 33-36 kDa proteins by a Mono Q-Sepharose column using a FPLC system. These purified proteins were used for the standardization of the EIA procedure. Several honey samples from different botanical origin were assayed and the results showed a significant correlation with the standard melissopalynology procedure. Thus, the analysis of pollen proteins by immunoassays allows us to identify *H. annuus* floral origin of honey.

PL-P48.

DETERMINATION OF TRACE ELEMENTS IN POLLEN OF *Acacia caven* (Mol.) Molina, USING SPECTROMETRY OF ATOMIC ABSORPTION

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The pollen was used by the man like a food supplement, energy booster, aids beauty, etc. In this work the toxic and essential metals of *Acacia caven* (Mol). Molina pollen was investigated. Due to the low levels of concentration of some elements in the samples was necessary to use a methodology of the higher sensitivity. In this aspect, the spectrometry of atomic absorption with flame (AAS) and electrothermic atomization (ETAAS) were the most suitable analytical methods. Additionally, the Spectrometry of Optical Emission was used associated to the plasma connected inductively (ICP-OES) with conventional or ultrasonic nebulization. The pollen of *Acacia caven* (Mol.) Molina contains calcium, phosphorous, silica, magnesium, iron, copper and molybdenum as trace amounts. Conversely, three trace elements that are noticeably absent in food are found in significant quantities in *Acacia caven* pollen. These elements play a role in numerous biological reactions: chrome, zinc and manganese. Finally, pollen is very high in nickel and selenium.

PL-P49.

***Acacia caven* (Mol) Molina POLLEN, AN IMPORTANT FOOD COMPLEMENT**

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The pollen is a natural nutritional supplement with high content in protein and it is used in medicine. Contains an amazing amount of vitamins, essential amino acids, proteins and minerals like potassium, magnesium, calcium, copper, iron, phosphorous, manganese, sulfur and chlorine. In this work the analytical composition of pollen of *Acacia caven* (Mol.) Molina, was determined to know its value as a food complement and to contribute to the denomination of quality in origin of apiculture products from the Cuyo region (Argentine). The humidity was determined in a vacuum oven (45 mm Hg) at 65°C, until constant weight. The ash determination was carried out by burned of the sample in capsule of Pt at 600°C. The total protein determination was made by the method of Kjeldhal-Arnold-Cunning. The carbohydrate determination was carried out by the methods of Dubois and modified Fehling-Causse-Bonnans. The results were expressed in percentage of humidity, ashes, total content of carbohydrates and proteins in dry base of the sample. The results were agreed with the established data in the Article N° 785 of the Argentine Alimentary Codex, for the analytical characteristics of pollen composition as nutritional complement.

PL-P50.

***Arabidopsis thaliana* TRANSGENIC PLANTS EXPRESSING OAT ADC UNDER CONTROL OF RD29A PROMOTER**

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Biotechnological uses of transgenic plants usually relays on constitutive transgene expression. However, sometimes the constitutive expression of transgenes might not be desirable. In such cases, an alternatively technological approach could be use of an inducible promoter. To evaluate this hypothesis, we developed a binary vector encoding the oat–arginine decarboxylase gene (ADC; EC 4.1.1.19), since this enzyme is known to be critical in polyamine metabolism and can be considered the main supply of putrescine in plants subjected to abiotic stresses. In general, the function of the RD29A gene product is still unknown. It responds to different signals produced by osmotic and cold stresses. Dehydration and low temperature induce RD29A gene expression showing a biphasic kinetic in the homologous system. It was suggested that RD29A promoter has at least three kinds of *cis* acting regulatory elements. One of them, denominated ABRE (ABA Responding Element) is induced in ABA-dependent manner. In our laboratory, homozygote transgenic plants of *Arabidopsis thaliana* harboring this binary vector have been obtained, and they didn't show deleterious phenotypes frequently associated with constitutive expression of ADC. The ADC induced activity by ABA spraying was accompanied by changes in free polyamines levels. Potential regulatory roles of the increased polyamines levels on the induction of rd29A will be discussed.

PL-P51.

CHARACTERIZATION AND PREVENTION OF THE PROTEOLYTIC CLEAVAGE OF PLANT PHOSPHO-ENOLPYRUVATE CARBOXYKINASE

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In plants, phosphoenolpyruvate carboxykinase (PEPCK) catalyses a key step in the gluconeogenesis during germination of fat storing seeds, plays an important role in photosynthetic carbon assimilation in some CAM and C_4 plants, as well as in the CO_2–concentrating mechanism of certain algae. Plants PEPCKs have unique, species-specific, N-terminal extensions that are rapidly lost by proteolysis during preparation of cell extracts. Being the site of reversible phosphorylation, proteolytic cleavage of these extensions hinders the study of the regulation of PEPCK activity. The aim of this study was to find proper conditions to extract and purify native PEPCK from endosperm of 5 days old *Ricinus communis* germinating seeds. PEPCK presented a subunit molecular mass of 80 kDa in extracts obtained under denaturating conditions when analyzed by western blot using affinity-purified antibodies against truncated PEPCK from pineapple leaves. Studying the time course state of PEPCK subunit in extracts made at different pH values with and without DTT, we concluded that a cysteine endopeptidase might be responsible for the degradation of PEPCK to a truncated subunit form of 66 kDa. A wide range of protease inhibitors was tested but none was completely useful to prevent proteolysis. A combination of high pH and protease inhibitors was found to be most effective to prevent proteolytic cleavage and maintain activity of PEPCK.

PL-P52.

STUDY OF EXPRESSION OF H6h GENE IN *BRUGMANSIA CANDIDA* HAIRY ROOT CULTURES

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The tropane alkaloids hyoscyamine and scopolamine are valuable drugs employed as antispasmodics and in the treatment of motion sickness. Their production by biotechnology processes is under study in *Brugmansia candida* hairy roots (HR) cultures. Elicitation is a strategy used to increase secondary metabolites including alkaloids. In previous work, we reported that acetate buffer is able to increase hyoscyamine (700%) and scopolamine (200%) in these HR cultures. Hyoscyamine-6-β-hydroxylase (H6H) is the enzyme responsible for converts hyoscyamine into scopolamine. In order to understand the difference levels of alkaloids reached after elicitation in this work, we study the expression of H6h mRNA in HR cultures elicited with acetate buffer. *B. candida* hairy root cultures were obtained from seedlings transformed with *Agrobacterium rhizogenes* LBA9402 according to Giulietti et al. (1993). In elicitation experiments 0, 1.0 and 2.0 mM of acetate buffer (Ac^-) was added to the culture medium. The samples were collected at 0, 6, 12 and 24 h after subculture. Total RNA was obtained with RNeasy Plant Kit (Qiagen). cDNA synthesis was done using Superscript II reverse Transcriptase (Life Technologies). PCR reaction was done on bases of *Hyoscyamus niger h6h* gene specific primer design due to high homology founded for the transcript among Solanaceae family. The results obtained shown the presence of H6hmRNA after 24 h of elicited treatment. Strong expression of h6hgene was detected with 2.0 mM of Ac^- buffer while a weak expression was detected at 1.0 mM of Ac^- buffer.

PL-P53.

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

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Ferredoxin-NADP(H) reductases (FNR) catalyse the reversible electron transfer between two molecules of obligatory one-electron carriers as ferredoxin and flavodoxin (Fd) and a single molecule of NADP(H). Tobacco plants expressing *Anabaena PCC7119* flavodoxin displayed enhanced tolerance to a variety of oxidative stress conditions. Recently, plants overexpressing FNR in chloroplasts were also obtained. In this work, we showed that this tobacco plants overexpressing FNR were more tolerant to excess light or to the reactive oxygen species generated by the herbicide methyl viologen (MV). We also evaluated the tolerance to MV of transgenic plants overexpressing both FNR and the cyanobacterial flavodoxin. These plants were obtained either by cross-fertilization or by transient expression of FNR directed to chloroplasts or cytosol developed by leaf infiltration with *Agrobacterium tumefasciens* carrying the appropriate plasmid construct. Results related to the enhanced tolerance to MV of these double transgenic plants will be discussed.

PL-P54.

ANTIOXIDANT RESPONSES IN APPLES EXPOSED TO SUNBURN INJURY

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We reported previously that fruit development under solar stress was accompanied by an increase in lipid peroxides positively correlated to sunburn damage. In the present study we examined changes in ascorbate (AsA), glutathion (GSH), superoxide dismutase (SOD) and catalase (CAT) in the adaptive development of sun-exposed vs. shaded fruit of apple cultivars "Braeburn" (BR) and "Red Delicious" (RD). Reaching maturity, SOD increased sharply in shaded fruits (1527 U/gFW in BR and 436 U/gFW in RD), and was additionally induced by sun exposure, mainly in BR where it peaked to 8X and then decreased. CAT showed different profiles between both cultivars, increasing in RD and decreasing in BR, and was induced by solar radiation, being lower in BR (15 IU/gFW) vs. RD (36 IU/gFW). AsA content increased early in sun-exposed fruits, being always higher in BR, and declining to harvest (112 to 88 nmole/gFW). GSH content was similar in shaded fruits and the increase by solar exposure was higher in RD (160 nmole/gFW, 2X vs BR). Sun exposure of fruits from early stages would cause an adaptive response related to induction of antioxidant compounds and enzymes in both cultivars. The higher sensitivity of BR fruits may be related to reduction in CAT with high SOD levels, and to a higher solar exposure due to morphological and physiological characteristics.

PL-P55.

REACTIVE OXYGEN SPECIES (ROS) AND SALICYLIC ACID (SA) SIGNAL PROLINE ACCUMULATION IN PLANT:PATHOGEN INCOMPATIBLE INTERACTIONS

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L-proline (Pro) accumulation is a classical response against abiotic stresses (drought, salinity and cold). Although its protective role is not well understood, it is thought to function as compatible osmolyte and ROS scavenger. We recently demonstrated that Pro accumulation is also triggered by biotic injuries. Pro content increases in *A. thaliana* tissues developing incompatible plant:pathogen interactions with avirulent races of *Pseudomonas syringae* pv.*tomato* (*Pst*) activating a Hypersensitive Response (HR). Pro increase in hr correlates with transcriptional activation of Pro biosynthetic (*AtP5CS2*) and catabolic (*ProDH*) genes. *AtP5CS2*:*GUS* and *AtP5CS2*:*LUC* transgenes are induced inside and around HR lesions where Pro also accumulates. To investigate whether *Pst*-induced Pro enhancement requires signaling components of the HR, we evaluated the role of ROS and SA in the activation of this novel response. We quantified Pro levels and *AtP5CS2* expression in: i) SA- and ROS-treated wild type plants, ii) SA-deficient mutants. We analyzed both responses in interactions involving two avirulence genes (*Pst avrRpm1* and *Pst avrRps2*) which have different SA requirements. As other defenses, pro accumulation was more sensitive to SA deficiency when elicited by *Pst avrRps2*. Meanwhile, exogenous ROS activate both Pro enhancement and *AtP5CS2* expression. SA may then potentiate Pro accumulation affecting early steps of the defense cascade, probably involving ROS as intermediate signal.

PL-P56.

CLONING AND CHARACTERIZATION OF FRUCTOSYLTRANSFERASES GENES IN GRAMINEAE

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Abiotic stresses induce complex responses in plants, affecting productivity of agronomically important crops. Acclimation to low temperatures involves biochemical and physiological processes that are associated to cold tolerance. Accumulation of compatible solutes was found during cold acclimation and many Graminean species accumulate fructans (fructose polymers) after being exposed to chilling temperatures. Fructans are produced in the vacuole by the action of fructosyltransferases and might be related to low temperatures tolerance in Gramineae species. The aim of this work was to isolate and characterize genes codifying for fructosyltransferases from Gramineae species: a native one, Bromus pictus and an agronomically interesting species, like wheat. The isolation of these genes will allow expression studies involving transformation of species that lack fructan metabolism and are susceptible to low temperatures and drought. PCR techniques (RT-PCR, 5´ and 3´ RACE) and a genomic library were used to isolate homologue sequences with fructan genes in B. pictus. A cDNA and a genomic clone from B.pictus were isolated showing high similarity with Graminean fructosyltransferases genes. A genomic BAC library of T. monococcum was screened with a fructosyltransferase cDNA. Sixteen positive clones were found and a 4 kb fragment was subcloned and analyzed. Sequence analysis showed high similarity with fructosyl transferases and invertases from Gramineae species, but reveled low similarity with cDNAs that codify fructan enzymes recently isolated from Triticum aestivum.

LI-P1.

INSULIN INCREASED Δ6 DESATURASE ACTIVITY IN LIVER RAT BY PRE- AND POST-TRANSLATIONAL MECHANISMS

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It has been recognized that rat liver microsomal Δ6 desaturase activity is defective in experimental diabetes, fact that may be reverted by means of insulin treatment. In the present study, we used streptozotocin-induced diabetic rats in order to determine the regulatory role of insulin on the activity and gene expression of hepatic Δ6 desaturase. Insulin supplementation in diabetic rats rapidly (2 h) restored the specific activity of the enzyme, whereas the level of mRNA and desaturase protein remained at the same level to that of control diabetic animals. On the other hand, desaturase insulin-induced up-regulation of gene transcriptions resulted in a 17-fold increase in desaturase transcript amounts after 18 h, with a concomitant increase of the specific activity of the enzyme. These results suggest that the insulin-induced Δ6 desaturase activity arises from two responses. First, a preexisting latent desaturase becomes activated around 2 h after insulin administration. This might include the release sequestered enzyme or activation by post-translational modification. Second, the amounts of the desaturase transcript are increased as a result of insulin-induced gene transcription by pre-translational events.

LI-P2.

EFFECTS OF THALLIUM (I) AND THALLIUM (III) ON LIPOSOMES MEMBRANE PHYSICAL PROPERTIES

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The hypothesis that thallium (Tl) interaction with membrane phospholipids could result in the alteration of membrane physical properties was investigated. Working with liposomes composed of brain phosphatidyl choline and phosphatidyl serine, we found that Tl^{+}, Tl^{3+}, and $Tl(OH)_3$ (0.5 to 25 μM): (a) increased membrane surface potential, (b) decreased the fluidity of the anionic regions of the membrane, in association with an increased fluidity in the cationic regions, and (c) promoted the rearrangement of lipids through lateral phase separation. The magnitude of these effects followed the order Tl^{3+}, $Tl(OH)_3 > Tl^{+}$. In addition, Tl^{3+} also decreased the hydration of phospholipid polar headgroups, and induced membrane permeabilization. The present results show that Tl-interacts with membranes inducing major alterations in the rheology of the bilayer, which could be partially responsible for the neurotoxic effects of this metal.

LI-P3.

EFFECT OF AN EXTERNAL ELECTRIC FIELD ON AN ORDERED LIPID LAYER

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Galactocerebroside layers were transferred onto conducting substrates (electrodes) using the Langmuir-Shaeffer method. Then, the covered electrodes were immersed in $NaNO_3$ 20 mM, and different potential values were applied. Using Electrochemical Impedance Spectroscopy (EIS), the effect of the applied potential on the layers structure was analyzed. This technique provides the electrical capacitance of the interface as well as the electrical resistance of the film.
The galactocerebroside film has been investigated in our laboratory previously using epifluorescence microscopy, ellipsometry (Manuscript in preparation, J. Phys. Chem), infrared reflection spectroscopy and other electrochemical techniques (Langmuir 2001,17, 3980-3986, J. Electroanal. Chem. 537 (2002) 67-76, Langmuir 2003, 19 (17), 6876-6880). The experiments indicate that in the aqueous environment the lipid film leave holes onto the electrode, allowing water molecules to penetrate. Besides, a negative potential applied to the film would increase the holes proportion and/or sizes. In the EIS experiments this is evidenced by the membrane resistance decrease at negative potential values. The interfacial capacitance of the covered electrode is lower than that of the bare interface, due to the low permittivity of the lipid layer.

LI-P4.

ISOLATION OF A NOVEL START DOMAIN CONTAINING PROTEIN HIGHLY EXPRESSED IN GESTATIONAL TROPHOBLASTIC TUMOUR

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To identify genes whose expression is specifically associated to "gestational trophoblastic diseases" the differential display technique was performed. This strategy resulted in the isolation of an EST named UNCDD1 which identified a clone containing a 3315bp insert from HeLa cell line cDNA library. This cDNA encodes a protein called GTT1/StarD7 of 295 aminoacids that shows 49% of similarity with the phosphatidylcholine transfer protein and has a START domain. Northern blot assays performed with normal, benign and malignant gestational trophoblastic samples revealed a 3.5kb transcript exclusively expressed in the JEG-3 choriocarcinoma cell line. However, semiquantitative RT-PCR analysis carried out with the same samples demonstrated GTT1/StarD7 expression throughout all of them but the expression level was more than 3 times higher in JEG-3 cell line. Semiquantitative RT-PCR assays performed in diverse human tumour cell lines revealed GTT1/StarD7 expression in all of them with the highest levels detected in JEG-3, JAR, HT-29 and HepG2 cells. Finally, an increase of 100% in GTT1/StarD7 expression was observed when dexamethasone was added to JEG-3, HT-29 and HepG2 cell cultures. In conclusion, the high GTT1/StarD7 expression profile in JEG-3 cells, its lipids binding domain and dexametasone regulation suggest that GTT1/StarD7 may play an important role in the phospholipid-mediated signaling of gestational trophoblastic tumours cellular events.

LI-P5.

GANGLIOSIDES, GANGLIOSIDE GLYCOSYL TRANSFERASES AND NEWLY SYNTHESIZED GANGLIOSIDES IN SPHINGOLIPID/CHOLESTEROL-ENRICHED DOMAINS OF GOLGI AND PLASMA MEMBRANES FROM CHO-K1 CELLS

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Glycosphingolipid-enriched microdomains (GEM) are specialized detergent-resistant domains of the plasma membrane (PM) in which some gangliosides concentrate. Although genesis of GEM is consider to occur in the Golgi complex, where the synthesis of gangliosides also occurs, the issue concerning the incorporation of ganglioside species into GEM is poorly understood. Using CHO-K1 cells with different glycolipid composition, we compared the behaviour to cold Triton X-100 solubilization of PM ganglioside species with the same species newly synthesized in Golgi membranes. We also investigated if three ganglioside glycosyltransferases are included or excluded from GEM in Golgi membranes. Results show that a fraction of PM GM3, and most GD3 and GT3 reside in GEM. Immunocytochemical examination of GD3 expressing cells showed GD3 distributed as cold detergent resistant patches in the PM. In Golgi membranes we were unable to find evidence for GEM localization of either ganglioside glycosyltransferases or newly synthesized gangliosides. Since the same ganglioside species appear in PM GEM, it was concluded that *in vivo* nascent GD3, GT3 and GM3 segregate from their synthesizing transferases and then enter GEM. This event could have taken place shortly after synthesis in the Golgi cisternae, along the secretory pathway and/or at the cell surface.
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LI-P6.
CHARACTERIZATION OF ERYTHROCYTE MEMBRANE DOMAINS RESISTENT TO EXTRACTION WITH THE ZWITTERIONIC DETERGENT CHAPS

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The usual approach to study membrane rafts, which are laterally segregated domains enriched in sphingolipids, cholesterol and specific proteins, involves isolation of detergent resistant membrane domains (DRM). In previous works we have obtained erythrocyte DRM using the non-ionic detergent Triton X-100, and characterized their protein pattern and lipid ordering. We showed that DRM could also be obtained from cholesterol depleted erythrocytes (Rivas and Gennaro, *Chem. Phys. Lipids* 122:165-9, 2003). In the present work, we investigate the effects of treating erythrocytes with the zwitterionic detergent CHAPS. We obtained DRM both from intact and from cholesterol-depleted erythrocytes. However, a detergent/lipid rate larger than that used with Triton was needed in order to attain complete hemolysis. The insoluble material was characterized in lipid and protein composition by TLC and SDS-PAGE electrophoresis, and in lipid ordering by spin label EPR spectroscopy. The observed differences between these DRM and those extracted with Triton X-100 reinforce the hypothesis that DRM and rafts should not be considered as identical entities, as DRM characteristics depend on the methodology used in the extraction procedure.
Work supported by Universidad Nacional del Litoral.

LI-P7.
EPR STUDY OF MIXED MEMBRANES OF DMPC AND C8CERAMIDE

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In mixtures of dmPC and C8Cer, calorimetry shows the presence, at 25-30 mole% C8Cer, of a single phase which behaves as a complex. Up to 20 mole% C8Cer and in the gel phase, a part of the membrane is laterally segregated in domains of pure or almost pure dmPC, while C8Cer becomes incorporated in a second phase of composition equal to the complex. EPR experiments show that the rise in C8Cer concentration does not change the mobility of the mixed membrane at neither of the three positions measured at 10°C. When temperature rises to Tm-4°C, the rise in C8Cer concentration produces a change in the mobility of the membrane in the region close to the interface. In the complex samples, the mobility gradient is lost at this temperature, with the interface region as mobile and disordered as the C-12 region. In all samples, in gel phase as well as in fluid phase, the results indicate there is no immobilisation of the terminal part of the chains, which is diagnostic of a non-interdigitated or a partially interdigitaded membrane and rules out the possibility of mixed-interdigitated organisation.

LI-P8.
THE GLUCOCORTICOID-INDUCED FACTOR (FDx) REGULATING FATTY ACID DESATURASE ACTIVITIES PROVED TO BE A LIVER- FABP

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Previous studies from our laboratory demonstrated that either treatment of isolated liver cells with glucocorticoids or the injection of these steroids to rats were able to produce an inhibitory effect on both $\Delta 5$ and $\Delta 6$ fatty acid desaturase activities concomitantly with the stimulation of the $\Delta 9$ desaturase. These modulatory actions were dose-dependent and required specific steroid receptor occupancy, although the 11-β-OH group in the steroid molecule was not an essential requirement for biological activity. The regulatory effects were mediated by a soluble protein (FDx) recovered from the cytosolic fraction of treated cells or injected rats. In this work we reported the isolation and purification of the FDx factor through a protocol that included fractionated saline precipitation, HPLC gel-permeation and ion-exchange chromatography. FDx was purified ca. 6,200 times up to homogeneity as judged by native and SDS-PAGE. Protein sequencing of six olygopeptides obtained after trypsin digestion of native FDx demonstrated a complete homology with rat L-FABP. *In vitro* experiments testing the regulatory capacity of FDx *vs.* purified L-FABP confirmed this finding. Thus, the main contribution of this work was to reveal a novel regulatory function of L-FABP on the hormonal control of the unsaturated fatty acid biosynthesis.

LI-P9.
TOPOGRAPHY OF MYELIN LIPIDS MONOLAYERS WITH AND WITHOUT PLP PROTEIN AND DISTRIBUTION OF GANGLIOSIDE GM1

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Myelin membranes extracted with a mixture of non-polar solvents contains the majority of lipids of the original membrane and proteolipid (PLP) protein (13.6 weight %). Besides, we isolated an extract of the lipid fraction without protein. Monolayer films were spread from both fractions and observed by Epifluorescence and Brewster Angle microscopy (BAM). The lipid mixture shows domain segregation with coexistence of liquid expanded (LE) and liquid ordered (LO) phases that merge at low surface pressure ($\approx$ 5mN/m). By contrast, as shown before (Oliveira R, Maggio B. 2002), the system constituted by the lipids and PLP, exhibit domain coexistence up to collapse on a Tris Ca++ subphase. The distribution of some components in LE and LO phases was analysed in monolayers with and without PLP protein. In particular, ganglioside GM1 that partitions into LE domains in mixtures containing PLP, is also enriched in LE phases in absence of the protein. By contrast, in defined mixtures with LE/LO domains, ganglioside GM1 was found in LO phases. BAM showed a marked reorganization of the LE phase containing the PLP protein during compression up to around 20mN/m, where the domain shape also changes. The domains lacking the protein show only slight variations of the optical thickness with surface pressure increases.

LI-P10.
DOMAIN SEGREGATION IN MYELIN MONOLAYERS INDUCED BY AQUEOUS PHASE CONDITIONS

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Purified myelin spreads as monolayers at the air-water interface. These were visualized by fluorescence and Brewster angle microscopy, showing phase coexistence at low surface pressures (below 20-30 mN/m). Beyond this threshold, the phase coexistence was present or not depending on the aqueous phase composition. Pure water, carbohydrate and glycerol solutions (20%) produced homogeneous monolayers, on the other hand, the presence of salts in Ringer´s and physiological solution leads to heterogeneity. These results show (in agreement with previous work) that surface homogeneity is favored by high water activity or when water activity is lowered by highly hydroxilated solutes. It has been previously suggested that domain segregation in nerve myelin multilayers is coupled to closer apposition among adjacent bilayers on dehydration and a subsequent extrusion of proteins in laterally segregated domains. In the monolayer system, where interaction with any other surface is absent, analogous protein-enriched domain segregation is induced. In our experiments, monolayer dehydration can occur by, a) increasing solute concentration in the subphase b) monolayer compression. Nevertheless, both methods have coupled another processes and lead to opposite results.

LI-P11.
THE UNSAPONIFIABLE MATTER OF VIRGIN OLIVE OIL AFFECTS THE POSTPRANDIAL TRIGLYCERIDE CONCENTRATION IN THE PLASMA OF HEALTHY HUMANS

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The present study was carried out in order to evaluate the postprandial triglyceride changes occurring in the plasma of a group of nine healthy subjects after the ingestion of meals enriched in olive oil containing different amounts of its unsaponifiable fraction: refined olive oil (0%, RO), virgin olive oil (2%, VO) or enriched virgin olive oil (4%, EVO). Fasting (0 h) and postprandial blood samples were collected hourly for seven hours. Mean plasma triglyceride, total, LDL- and HDL-cholesterol, apolipoprotein A (apo A) and B (Apo B) concentrations were measured. Only the triglyceride levels were found to change after the intake of the oils. A rapid increase over fasting values was observed, showing a maximum concentration approximately at 2h. The plasma triglyceride profiles (concentration versus time) were adjusted to polynomical equations (r^2=0.996 for the RO curve, r^2=0.999 for the VO curve and r^2=0.999 for the VO curve), which allowed the calculation of the precise times at which peaks of concentration occurred. Thus, we found that the maximum concentration after RO occurred at 1.4h, at 2.0h after VO and at 1.9h after EVO. In addition, we found another maximum peak at 6 hours after the intake of RO and VO. In contrast, the second peak after RO occurred after the 7th hour, the last time recorded. Consumption of RO showed the smallest peak (109.5 mg/dL) and consumption of EVO the highest (176.6 mg/dL). Therefore, we conclude that the amount of unsaponifiable matter in dietary virgin olive oil affects the postprandial metabolism of triglycerides. Interestingly, we observed that the higher the amount of unsaponifiable fraction, the higher the concentration of TG in plasma and chylomicrons and cholesterol in chylomicrons, suggesting that these lipid components might enhance the secretion of these lipid classes to the blood stream or reduce their clearance.
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LI-P12.
VIRGIN OLIVE OIL TRIGLYCERIDE MOLECULAR SPECIES AS INDEPENDENT DETERMINANTS OF VLDL LIPID COMPOSITION IN ELDERLY PEOPLE

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Two virgin olive oils (VOO1 and VOO2), of the same variety (Olea europaea var. hojiblanca), with a similar composition in minor components but differing in the content of triglyceride molecular species, were tested in order to investigate their effect on the VLDL lipid composition of elderly people. Twenty-one participants, 84.9 (SD 6.4) year-old, were recruited for the study. After overnight fasting, blood was collected and VLDL isolated by ultracentrifugation. The lipid classes, TG molecular species and TG fatty acid composition were determined. VOO1 presented higher amounts of triolein (OOO) ($p<0.01$), whereas VOO2 was significantly enriched in linoleic-acid species, such as dilinoleoyl-oleoyl-glycerol (LLO), linoleoyl-dioleoyl-glycerol (LOO) and linoleoyl-oleoyl-palmitoyl-glycerol (LOP) ($p<0.01$). Consumption of VOO1 caused an increase of total TG in VLDL ($p<0.01$) mainly due to higher amounts of OOO and LOO. When VOO2 was administrated an increase in VLDL cholesteryl-esters ($p<0.01$) and arachidonic acid-rich TG ($p<0.01$) was observed. We conclude that the TG molecular species of dietary oils may be determinant of the lipid composition of VLDL in elderly people independently of the fatty acid composition and, therefore, might have a relevant role in regulating lipoprotein metabolism in these subjects.
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LI-P13.
VACCINE DEVELOPMENT: "MIXED SATURATED AND UNSATURATED-POLYMER LIPIDS FORMULATIONS, BIOPHYSICAL AND BIOLOGICAL EVALUATION"

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Since liposomes entrapped materials and protect them from enzymatic attack until they reach a target site, the potential usefulness of liposomes as carriers and adjuvants for developing vaccines has attracted considerably interest. Our main objective is to characterize structurally, morphologically and citotoxicity *in vitro* the performance of polymerized diacetylenic liposomes and its adjuvant capacity *in vivo*. Formulations contained: 1,2-bis(10,12-tricosadiynoil)-sn-glycero-3-phosphocholine (DIAPC) and dimyristoilphosphatidyl choline (DMPC) were UV polymerized . Determination of the hydrodynamic volume was obtained by Light Scattering. The hydrophobicity index of vesicles was determined with MC540 probe at different temperatures. Citotoxicity was evaluated by hemolysis of red blood cells and peroxidation index with TBA method. Serum proteins interactions were tested by SDS-PAGE electrophoresis. The results obtained showed that there is no significant toxicity of the formulations proposed with diacetylenic lipids. Polymerisation induces hydrophobic defects that does not affect the size of the vesicles and showed preferential interaction with high molecular weight serum proteins.

LI-P14.

INTERACTIONS BETWEEN C-FOS AND C-JUN

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The early expression transcription factors c-Fos and c-Jun can form heterodimmers, but only c-Jun homodimmers complexes called activator protein 1 (AP-1) by a leucin zipper domain. AP-1 binds to DNA by a basic domain and regulates processes like cell proliferation and differentiation. These results have been obtained either working with cellular extracts and co-immunoprecipitation, or measuring interaction *in vitro* between short c-Fos and c-Jun peptides that include the leucine zipper and the binding domains, by crystallographic analysis, resonance energy transfer, shifts and footprint analysis, and column molecular filtration. Recent studies show that c-Fos and c-Jun can form stable Gibbs and Langmuir monolayers in an air-buffer interface, and they generate an increment in the surface lateral pressure similar to the increments generated by membrane proteins. c-Fos and c-Jun can also penetrate differently in phospholipid monolayers depending on polar head group of the phospholipid. *In vivo* studies show that c-Fos regulates phospholipid biosynthesis in association with the endoplasmic reticulum membrane. In a simple system with recombinant complete or deletion mutant proteins, we have used electrophoresis in native gels, purification in molecular exclusion columns, cross-linking analysis, immunoprecipitation, and monolayer techniques to explore c-Fos and c-Jun interactions. We show that c-Fos and c-Jun don't form stable hetero or homodimmers complex in solution, although they have the ability to interact in monolayers.

LI-P15.

IDENTIFICATION OF A *LEISHMANIA MAJOR* Ä9 FATTY ACID DESATURASE BY HETEROLOGOUS EXPRESSION IN *SACCHAROMYCES CEREVISIAE*

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Trypanosomatids have a high proportion of long-chain polyunsaturated fatty acids (22:5, 22:6), linoleic acid (18:2) and α-linolenic acid (18:3). The presence of these molecules suggests that fatty acid desaturation occurs via the "plant pathway". It was confirmed with our previous work, where we isolated and characterized an oleate desaturase from *T. brucei*, which is a key enzyme in polyunsaturated fatty acid biosynthesis. These differences on the degree and type of desaturation between the trypanosomatids and their mammalian hosts, make desaturases a good target for chemotherapeutic drugs. *L. major* Δ9 desaturase gene (des9) was identified, cloned and sequenced. It is the first enzyme in the biosynthesis of unsaturated fatty acids. Des 9 gene encoded a protein with a high degree of similarity to Δ9 desaturases from fungi and contained the three conserved histidine boxes, a C-terminal cytochrome b_5 domain and transmembrane domains characteristic of endoplasmic reticulum membrane-bound Δ9-desaturases. The enzyme was functionally characterized by complementation of an *ole1 Saccharomyces cerevisiae* mutant. Fatty acid analysis of yeast transformants expressing the pLmDes9 showed an elevated level of oleic acid (18:1) compared to palmitoleic acid (16:1). A detailed biochemical characterization is shown.

LI-P16.

ROLE OF L-FABP ON THE INCORPORATION OF SATURATED AND POLYUNSATURATED FATTY ACIDS IN ENDONUCLEAR LIPID POOLS

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Our aim was to study the role of L-FABP on the trafficking of fatty acids from cytosol to nuclear lipid pools where they regulate the expression of certain genes. Nuclei and nuclear matrix (Mx: nuclei without membrane) of rat liver cells were incubated *in vitro* with [1-^{14}C]18:0 and [1-^{14}C]20:n-6, either free or bound to the L-FABP. Incorporation and esterification of 18:0 and 20:4n-6 were carried out in nuclear and endonuclear lipid pools. We observed that the incorporated total radioactivity was not modified by the L-FABP. Firstly, [1-^{14}C]18:0 and [1-^{14}C]20:4n-6 were incorporated into FFA>PL>>TAG (in the presence of ATP and CoA). Then, [1-^{14}C]fatty acid incorporated was decreased in FFA, increased in TAG, and saturated in PL, with the incubation time. In endonuclear pools (Mx) 18:0 and 20:4n-6 bound to L-FABP were incorporated, showing a greater proportion in DAG and PI, respectively. In conclusion, 18:0 and 20:4n-6, exogenous, free or bound to L-FABP,are incorporated into total nuclear and endonuclear lipid pools through an acyl-CoA pathway. L-FABP does not affect nuclear FA uptake, but estimulates the FA esterification, directing the fatty acids into specific lipid fractions.

LI-P17.

HOW DOES APOLIPOPROTEIN A-I INTERACT WITH MEMBRANES?

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In a previous work we investigated by using calorimetric and optical techniques, the interaction of apolipoprotein A-I with model membranes of various lipid composition. The dipolar relaxation of the fluorescent probe LAURDAN allows the calculation of the General Polarization (GP). This function lets infer the lipid phase of the membranes. We observed that inclusion of Sphingomyelin (SM) molecules in bilayers of pure phosphatidilcholine (POPC) at T< 32°C induced dynamical restriction of lipid environment (increasing of GP), but yielded the contrary effect at higher temperatures. This phenomenon correlates with the formation of lipid domains observed by Two Photon Fluorescence Microscopy in the same range of temperatures. The study of lipid membranes containing cholesterol (POPC/SM/CHOL) (similar to lipid rafts) gave higher values of GP, showing a change of the lipid phase towards to a gel state. The addition of the protein to the investigated membranes showed a differential behavior depending of the lipid composition of the membrane and the temperature at which the reaction was performed. In membranes of pure POPC at T> 22°C, and of POPC/COL at T> 37°C, the binding of the protein induces a higher fluidity of the membrane. By the contrary, in SM-containing membranes we measured higher values of GP for both, POPC/SM bilayers (at T> 30°C) and for POPC/COL/SM (at T> 15°C). These results show that the composition and the physical state of the membrane play an important role during the interaction of apoA-I with membranes.

LI-P18.

LIPID DYNAMICS IN DISCOIDAL LIPOPROTEIN COMPLEXES OF APOLIPOPROTEIN A-I

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Fiscoidal high density lipoprotein complexes (dHDL) of apolipoprotein AI (apoAI) are intermediates in HDL metabolism and reverse cholesterol transport. Although dHDL formation "*in vivo*" requires the assistance of cellular proteins, they can be obtained "*in vitro*" by mixing apoAI with vesicles of zwitterionic phospholipids at the gel to liquid-crystalline phase transition temperature (Tt). several models for dHDL were proposed which differ in the arrangement of apoAI helices, but all of them agree that the protein is wrapped around the edge of a phospholipid bilayer. in this work, we used electronic paramagnetic resonance (EPR) to obtain information about how the dynamics of disc lipids is affected by apoAI. different spin-labeled phospholipids probes reporting the mobility at different bilayer regions were used: TEMPO-PC (with label in the polar head) as well as 5- and 16-doxyl PCs (with label in the hydrocarbon chain). results show that above Tt, apoAI discs have a decreased hydrocarbon chain mobility (reported by 5- and 16-doxyl PCs) and an increased mobility of polar head groups (sensed by TEMPO-PC) compared with protein-free bilayers. the opposite effect of apoAI is observed below Tt. these results are typical for proteins inserted or spanning the bilayer and are discussed taking into account the proposed models for dHDL

LI-P19.

EFFECT OF APOLIPOPROTEIN A-I ON OLEIC ACID ESTERIFICATION IN CELL CULTURED FIBROBLASTS

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Apolipoprotein A-I (apoAI) is the main protein of high density lipoproteins (HDL) and plays a key role in several steps of reverse cholesterol transport, as lipid removal from peripheral cells, activation of LCAT, and interaction with cellular proteins (ABCAI, SR-BI). It was proposed that apoAI and HDL trigger different signals in peripheral cells. Some of them result in mobilization toward the plasma membrane of intracellular cholesterol depots, as the one available to esterification by acylCoA-cholesterol acyl transferase. Metabolic events taking place in this process are still matter of debate, involving cell type and metabolic rate, as well as characteristics of cholesterol acceptors. On the other hand, nothing is known about apoAI domains involved in these cellular responses. The ability to decrease cholesterol esterification and exerting cholesterol efflux were measured for apoAI and HDLs in two cell types: a normal cell line of human skin fibroblasts (CCD27) and other transformed with SV40 (WI38). Results show that lipid-free apoAI stimulus decreases cholesterol esterification in CCD27 but not in WI38 cells. The possible implication of a central apoAI domain was investigated by using a synthetic peptide and two apoAI mutants in which the central region is replaced or changed in orientation. Lack of response to apoAI stimulus in transformed cells is discussed as well as the involved apoA-I domains.

LI-P20.

ON THE CENTRAL APOLIPOPROTEIN A-I MEMBRANE-INSERTING DOMAIN

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We have previously reported that apolipoprotein A-I (apoAI) in reconstituted high density lipoproteins (rHDL) interacts with membranes through the insertion of a small central domain located between residues 87 and 112 (J Biol Chem 2001). Recent studies using a synthetic peptide (communicated in SAB 2002), indicated: a) that this domain is responsible for the selectivity of apoAI for cholesterol containing membranes, and for promoting membrane cholesterol desorption; and b) that the membrane-inserted state is oligomeric. This last fact and recent evidences indicating that central regions of both apoAI molecules are close together in a rHDL disc, allowed us to hypothesize that an intermolecular helix bundle forms the active membrane inserting domain. We report here additional information on the membrane-inserted state of this region obtained by fluorescence resonance energy transfer (FRET) and fluorescence quenching measurements. Results indicate that at least one of the four apoAI Trp residues (presumably Trp108) locates at a distance short enough from the vesicle bilayer to give measurable FRET toward a phospholipid acceptor (DPH-PC) located in the vesicles. However, no Trp residue would be present in the membrane-inserted region since no detectable Trp fluorescence quenching was produced by vesicles containing spin-labeled phospholipids with the quencher group either at the polar head (TEMPO-PC) or in the hydrophobic region (5-, 7-, or 12-doxyl PC).

LI-P21.

LIPID METABOLISM IN HEP G2 CULTURED CELLS TREATED WITH HMG-CoA REDUCTASE INHIBITORS

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HMG-CoA reductase inhibitors like statins and monoterpenes diminish cellular cholesterol content. This fact increases the proteolysis of the sterol regulatory element binding protein (SREBP), which bind to sterol regulatory elements contained in promoters of genes involved in the synthesis of LDL receptors and key enzymes of cholesterol and fatty acid metabolism. We investigated the alterations produced by simvastatin (a statin) and geraniol (a natural monoterpene) on lipid metabolism in Hep G2 cells. Cells were treated with 1μM simvastatin (S), 10 μM geraniol (G) or 1μM simvastatin + 10 μM geraniol (SG) for 24 h. Three hours prior to starvation, ^{14}C-acetate (1μCi/ml) was added. Total lipids were extracted and ^{14}C incorporation in non saponifiable lipids, fatty acids, neutral and phospholipids were determined. All treatments incremented ^{14}C incorporation in total lipids and in fatty acids. S and SG groups showed a greater ^{14}C incorporation in all phopholipid fractions, G group did not show and increment in the incorporation in PC. All groups showed a diminished ^{14}C incorporation in cholesterol, S group also in lanosterol and squalene, but G group increased its incorporation in squalene. These results demonstrated that all treatments augmented fatty acid synthesis and diminished cholesterol synthesis. Geraniol is likely to inhibit PC synthesis due to the accumulation of an intermediate metabolite as a consequence of an inhibition of the conversion of squelene into cholesterol.

LI-P22.

RESCUE OF GANGLIOSIDE-DEFICIENT CHO-K1 CELLS AND ACETYLCHOLINE RECEPTOR DOMAINS

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In order to investigate the possible association of the nicotinic acetylcholine receptor (AChR) with lipid microdomains ("rafts") we have exploited the intrinsic GM2 synthase intrinsic deficiency of the CHO-K1/A5 cell line that stably expresses adult muscle - type AChR, to produce a double-transfectant new clone (coined CHO-K1/GM2) rescued from such deficiency by expression of UDP-GalNAc:lactosylceramide/ GM3/GD3 β-1,4-N-acetylgalactosaminyl transferase, a med-Golgi localized enzyme. The resistance to Triton X-100 and other mild detergent (CHAPS, sulfobetain and octyl-POE) solubilization was used as one of the assays for microdomain occurrence. Similar solubilization profiles were observed for both cell lines. Density gradients and Western blots of detergent-treated cells labelled with $[^{125}I]\alpha$-bungarotoxin showed the preferential AChR distribution in high density fractions. In view of the importance of cholesterol in AChR function, we modified its content in both cell lines using cyclodextrins. CHO-K1/A5 cells treated with 15 mM methyl-β-cyclodextrin (MβCD) showed a decrease in $[^{125}I]\alpha$-bungarotoxin label of up to 50%. A similar decrease in label occurred in Triton X-100 soluble fractions of both cell lines treated with 0-15 mM MβCD.

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LI-P23.

StarD7 IS SURFACE ACTIVE AND INTERACTS DIFFERENTIALLY WITH PHOSPHOLIPID MONOLAYERS

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Previously, we described the cloning and characterization of a new gene up-regulated in the choriocarcinoma JEG-3 cell line, denominated GTT1 (**G**estational **T**rophoblastic **T**umor 1). Nucleotide sequence analysis of the cDNA and computer-assisted homology search of the deduced amino acid sequence showed approximately 25% identity and 49% similarity with human, bovine or mouse PCTP. In addition, GTT1 shares a conserved extended central region -from amino acids 66 to 250- with the START domain proteins proposed to bind lipids and interact with membranes. For this reason GTT1 was renamed StarD7 (START domain containing 7). In the present report, we demonstrate that StarD7 protein forms stable Gibbs and Langmuir monolayers at the air-buffer interface showing marked surface activity. The latter is enhanced by penetration into phospholipids films at an initial surface pressure above the protein´s own equilibrium adsorption surface pressure to a lipid-free interface. The protein-phospholipid stabilizing interactions at the interface depend on the lipid, with preference for phosphatidylserine, cholesterol and phosphatidylglycerol, and the increases of lateral surface pressure generated are comparable to those of other membrane-active proteins. The surface activity of StarD7 is strong enough to thermodynamically drive and retain StarD7 at the lipid membrane interface where it may undergo lipid-dependent reorganization as indicated by changes of surface pressure and electrostatics.

LI-P24.

INTESTINAL FATTY ACID BINDING PROTEINS: IMPORTANCE OF αI-HELIX IN FATTY ACID TRANSFER MECHANISM TO PHOSPHOLIPID MEMBRANES

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Intestinal fatty acid binding protein (I-FABP) and liver FABP (L-FABP) are both coexpressed in intestinal entherocytes. These proteins, as well as the whole family, have a well conserved terciary structure consisting of a β-barrel domain with a hydrophobic pocket and a portal domain with two short α-helixes (αI y αII). Fatty acid transfer from I-FABP to membranes occurs by direct collisional interaction with lipid bilayer while L-FABP shows an aqueous diffusion mediated process. In addition we have shown the importance of the portal region in determining the FA transfer mechanism to artificial membranes. Based on previous evidence and considering the existence of an amphipatic helix (αI) in I-FABP, we decided to construct two chimeric proteins by exchanging the αI region between I and L-FABP. We obtained a chimeric protein with the ligand binding domain of L-FABP and the αI-helix of IFABP (αII-βLFABP) and the corresponding reversal chimeric (αIL-βIFABP). Integrity of the binding cavity assays (Kd and Kp determination) showed no drastic differences between the chimeras and wild-type proteins. A fluorescence resonance energy transfer assay was used to monitor the rate and mechanism of transfer of FA to phospholipid membranes. Results showed important modifications in rate and mechanism of transfer for αIL-βIFABP chimeric protein compared to wild-type I-FABP and no drastic change for αII-βLFABP chimera compared to wild-type LFABP.

LI-P25.

STUDIES OF EFFECTS OF FLUNITRAZEPAM ON THE LAMELLAR-HEXAGONAL II PHASE TRANSITION OF PHOSPHATIDYL ETHANOLAMINE. USE OF MEROCYANINE 540 AS A FLUORESCENT INDICATOR

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Flunitrazepam (FNT) is an extensively used benzodiazepine (BZD) due to its potent anxiolytic and hypnotic action. Previous studies of our group demonstrated that BZD-membrane interaction could be explained by a partition equilibrium model. We showed that BZD partitioning in the membrane increased in quantity and depth as the membrane structural order decreased. Moreover, the localization of BZDs at the phospholipid polar head region could explain the decrease in the size of dpPC vesicles, through a mechanism that involves the increment in the relative volume of this polar head region inducing an increase in the vesicle's surface curvature. In the present work we studied if FNT can affect the lamellar-hexagonal II phase transition of phosphatidyl ethanolamine through a similar mechanism. This study was approached by using the fluorescent dye merocyanine 540 which is sensitive to the molecular packing of membrane lipids. A detailed analysis of the effects of FNT on merocyanine absorption, fluorescence emission and excitation spectra, was performed. The results indicated that merocyanine behaved as a good indicator of this phase transition as was previously described. FNT did not affect the transition temperature but showed a tendency to diminish the dye fluorescence emission intensity which could involve topological changes leading to modifications in the polarity of the region sensed by merocyanine.

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LI-P26.

SOY PROTEIN-WAX BASED EDIBLE BILAYER FILMS

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Among the various edible films and coatings used to maintain or improve food quality, stability and safety, composite hydrocolloid-lipid films are particularly desirable since they have acceptable structural integrity and good barrier properties to water vapour. The first film characteristic is imparted by the hydrocolloids while the second one by the lipids.
The objective of this research was to produce and characterise bilayer films based on soybean proteins and waxes.
Protein films were obtained by casting from a commercial soybean protein isolate dispersion (5% w/v) at pH 10.5, using glycerol as plasticizer (2.5% w/v). Wax layer was applied on the protein film from the molten state. Three types of waxes were used: carnauba, bees and sunflower. Films mechanical, barrier and physical properties were studied and compared with protein control films. All composite films had lower water vapour permeability (WVP) than protein films but the best results were obtained with beeswax. Its WVP value ($8.5\ 10^{-13} g\ m^{-1} s^{-1} Pa^{-1}$) is nearer the ones for synthetic films. Wax presence also reduced film moisture content and water solubility, but not as much as was expected, probably due to the thin thickness of the lipid layer (10-20 µm). Mechanical properties of bilayer films with different waxes were similar; they presented a higher tensile strength than protein films without affecting elongation at break. Colour parameters (L, a and b) were similar for all films, suggesting that no wax crystallization took place. Microstructure study and thermal analysis would prove it.

LI-P27.

MDCK CELLS PROLIFERATION DEPENDS ON SPHINGOSINE KINASE ACTIVITY

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Sphingosine-1-phosphate (S1P) is involved in regulation of cell proliferation. In contrast, ceramide (Cer) and sphingosine (Sph) have a growth -inhibitory and pro-apoptotic effects. In this study we demonstrate, by using an inhibitor of sphingosine kinase, DL-threo-dihydrosphingosine (tDHS), that proliferation of MDCK cells depends on SK activity, since a decrease of total cell number and viability was observed. At 25 µM of tDHS: 25% of the cells present in the control with 72% of viability (vs. 97% of the control) was obtained. To distinguish if cell dead was due to an accumulation of Cer and/or Sph, we studied the incorporation of 3H-palmitic acid in MDCK cells treated with tDHS (25 µM), Fumonisine (50 µM) and tDHS+Fumonisine. In cells treated with tDHS, we observed an increase of radioactive Cer (10% of radioactivity associated to products vs. 6,8% of the control) while a decrease with Fumonisine (4,8% vs. 6,8%) and no change with tDHS+Fumonisine (7,0% vs.6,8%) was obtained. No changes in radioactive Sph was observed with any treatment (control: 18,7%; tDHS: 15,9%; Fumonisine: 17,2%; tDHS+Fumonisine: 16,1%). The presence of Fumonisine plus tDHS lead to an increase in total cell number, respect to the cells treated with tDHS only. Theses data suggest that the inhibition of SK leads to an accumulation of Cer that induce the activation of cell death program.

LI-P28.

PROSTAGLANDIN D_2 INDUCES CCTα TRANSLOCATION FROM THE ENDOPLASMIC RETICULUM TO THE NUCLEUS

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Phosphatidylcholine (PC) is a main lipid of biomembranes with structural and functional importance. In previous works, we have demonstrated that papillary PC synthesis is regulated by the action of endogenous-synthesised prostaglandin D_2 (PGD_2) which modulates nuclear cytidylyltransferase (CCT), which has been reported as the rate-limiting enzyme of PC synthesis. We also found that PC synthesis is modulated by MAPK activation. Two CCT isoforms have been described: CCTβ, associated to endoplasmic reticulum and reported as the responsible for the extra-nuclear PC synthesis, and CCTα which is confined to nuclear compartment but whose function has not been fully understood. In the present work we studied the effect of PGD_2 and MAPK activation on the compartmentalization of CCTα in renal papillary cells by using westernblot analysis. At microsomal compartment, we found two positive immunoreactive bands: 40 and 80 KDa, but no protein was detected in isolated nuclei. When papillary tissue was pre-treated with PGD_2, the presence of CCTα in microsomal membranes diminished with the parallel increase of the 80 KDa band in isolated nuclei, indicating CCTα translocation to this compartment. Such effect seems to be mediated by MAPK activation since it is prevented by U0126. These results suggest that the increased PC synthesis by PGD_2 stimulation could be due to CCT activation by translocation from endoplasmic reticulum to nuclear compartment.

LI-P29.

FLUNITRAZEPAM OVERCOMPENSATES THE EFFECTS OF LIDOCAINE ON THE THERMOTROPIC EQUILIBRIUM OF DIPALMITOYL PHOSPHATIDYL-CHOLINE

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Previously, by analysing erythrocyte morphology, we demonstrated that in the presence of either flunitrazepam (FNT) or lidocaine (LC) the plasma membrane curvature changed but in opposite directions. Moreover, each drug could compensate the effect of the other one in a dose-dependent manner [Chem.Biol.Int. 129(2000)263]. In order to get deeply in the molecular mechanism of this phenomena, in the present work we investigated by HDSC the effect of FNT, LC and FNT+LC on the stability of multilamellar vesicles of dpPC. Only in samples submitted to a second temperature scanning, LC decreased both the enthalpy (ΔH_p) as well as the temperature (T_{mp}) of the pre-transition ($L_{\hat{a}'} \rightarrow P_{\hat{a}'}$) and increased the width of the heat absorption peak, reflecting a decrement in the cooperative of the phase transition. The presence of 59µM FNT, reverted the effect of LC on T_{mp} and tended to increase ΔH_p above the values obtained with the control (dpPC without any drug). FNT alone increased the ΔH of both the pre- and the main transition. The former was reverted by LC. The effects of LC on decreasing T_{mp} and T_{mc} were also observed in the presence of FNT. The relative concentration of each drug in the bilayer, their effects on hydrogen bonds network and water structure at the lipid-water interface.

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LI-P30.
A SPIN LABEL STUDY OF THE INTERACTION OF BIOLOGICALLY ACTIVE TERPENES WITH MODEL MEMBRANES

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Terpenes exert a variety of biological activities. We have investigated the interaction between a series of terpenes (cineol, geraniol, thymol, menthol, and camphor) and phospholipid (in the absence and presence of 25 mole% cholesterol) model membranes by means of spin labeling EPR. EPR spectra of incorporated lipid spin probes (doxyl derivatives of stearic acid carrying the nitroxide moiety at carbons 5, 5-SASL, and 12, 12-SASL) were obtained as a function of terpene concentration. The spectra indicated that the degree of molecular freedom of the probes increased with increasing terpene concentration until a saturation state was obtained. Saturation was ascribed to the achievement of the solubility of the compounds in aqueous solution. It was found that the different molecules affected molecular motion to a different extent, probably due to the contribution of intrinsic differences in chemical structure and to the degree of partitioning in the model membranes. In addition, all terpenes except for camphor, formed micelle-like aggregates at enough concentrations. At these concentrations, in the presence of membranes, the compounds were able to form mixed terpene:phospholipid micelles.
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LI-P31.
EFFECTS OF ISCHEMIA/REPERFUSION AND TRAUMA ON TESTICULAR LIPIDS WITH VERY LONG CHAIN POLYUNSATURATED FATTY ACIDS

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Rat testicular lipids contain, besides molecular species with usual fatty acids (FA), species with very long chain polyunsaturated fatty acids (VLCPUFA). One of these lipids is ceramide (Cer). In search for possible functions of VLCPUFA-containing lipids, we studied them it in situations that in other tissues are known to result in cellular stress-related changes. In testis, ischemia induced by spermatic cord torsion followed by reperfusion, and trauma provoked by puncture, resulted, 1-2 days afterwards, in Cer with an increased proportion of FA other than VLCPUFA. These Cer could be related with the activation of mechanisms responsible for the selective death of germinal cells associated with both damaging conditions. One of the early consequences of testicular injury in both instances was seen in a neutral lipid not related to Cer: the cholesterol esters (CE). In this lipid, 7 days after the experimental harm, the proportion of VLCPUFA decreased while that of normal PUFA (18:2n-6 to 22:5n-6) augmented. The changes in the FA of Cer and CE occurred in the presence of virtually unchanged triglycerides and phospholipids, the major testicular lipids. The latter changed later on in the process, suggesting a relation to the testicular atrophy eventually triggered by the present conditions.

LI-P32.
AMINO ACID CHANGES IN THE INTERMEMBRANE-SPACE DOMAIN OF MITOCHONDRIAL GLYCEROL-3-PHOSPHATE ACYLTRANSFERASE ALTER THE CATALYTIC ACTIVITY IN THE CYTOSOLIC N-TERMINAL DOMAIN

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Mitochondrial glycerol-3-phosphate acyltransferase (GPAT, 828-aa) catalyses the first step in glycerolipid synthesis. It is inserted in the outer membrane by two transmembrane domains, having its active site located in the N-terminal domain, and the C-terminal domain, both facing the cytosol, and a 82-aa loop in the intermembrane space. Even though the loop and active site lay on opposite faces of the membrane, the insertion of a HA epitope (YPYDVPDYA) in the loop (construct HA496) completely inactivated the enzyme. To determine whether the change in the size (91 aa instead of 82) or the polarity of the loop were responsible for the inactivation of the enzyme, two additional loop-mutated GPATs, one with an insertion of 9 neutral aa (loop91) and one with a 42-aa deletion in the loop (loop40), plus the full-length protein with a Flag in the C-terminus (GFlag) were transiently expressed in CHO cells. The level of expression of these constructs was normalized by immunodetection. Loop91 and Loop40 yielded partially active enzymes (8.3 and 2.3% of the GFlag activity), whereas HA496 activity was 'lower than the empty vector. We postulate that the loop is required for activity; it may contribute to the proper folding of the enzyme or it may be involved in supramolecular regulatory interactions.

LI-P33.
CADMIUM AND LIPID METABOLISM IN PITUITARY

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It is known that exposure to 15 ppm of cadmium as CdCl2 in the drinking water by eight weeks decrease the secretion of prolactine and growth hormone. from the anterior pituitary lobe (APL) of adult male Wistar rat.In this work we study the effect of cadmium on dispersed cells of APL on the phospholipase D activity and phosphoinositolbiphosphate (PIP2) synthesis and its relation with the level of serum prolactin secretion.Also we study the histoarchitecture of APL of rats which drunk 15 ppm of cadmium as CdCl2 in the drinking water by eight weeks.The PLD activity was measured by the incorporation of [3H]-myristate into phosphatidylethanol trough the transphophatidylation reaction. PIP2 synthesis was determined by the incorporation of [3H]-myo inositol. The results showed that cadmium cause the next changes in relation to the control (without metal exposition): The activity of Phospholipase D decreased as PIP2 synthesis did it. In APL a significant capillary dilatation and a leucocitary and macrophagic invasion were observed in cadmium treated rats, compared with those of control. All these changes could be related to the low level of prolactine, since the prolactine secretion it is known to be mediated by cellular signals involve phosphoinositide pathways.

LI-P34.

MITOCHONDRIAL LIPID METABOLISMS IS AFFECTED BY VITAMIN A DEFICIENCY IN HEART

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Introduction: We showed that vit A deficiency changes the lipid compositions and decreases Acetyl CoA Carboxylase activity (ACC) in heart. Now, the mitochondrial lipid compositions and fatty acid (FA) β-oxidation is studied. *Methods*: 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. Mitochondrial lipids were quantified after separation by TLC. Fatty acids were determined by GLC. Carnitine Palmitoyl Transferase activity (CPTI) was determined using [^{3}H]-carnitina. Its short-term regulation was study with tetradecylglycidic acid and malonyl-CoA as inhibitors and, the long-term regulation in starved and starved + refeeded rats. *Results*: Mitochondrias of -A rats showed: a decrease of cholesterol and cardiolipin; an increase of sphingomielin, phosphatidic acid and lysophosphatidylcholine; a change in relative % of FA phospholipids: 16:0, 16:1, 18:0, 18:2, 20:4, 22:4; and an increase of FA β-oxidation. *Conclusion*: Vit A deficiency would not affect the long-term regulation of CPTI, but it would interfere with its short-term regulation and also modified the mitochondrial lipid compositions.

LI-P35.

EFFECT OF HYPOTHYROIDISM ON GENES RELATED TO CHOLESTEROL METABOLISM IN LIVER AND HEART OF MOUSE

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It is known that hypothyroidism condition produces several effects on lipid metabolism. In the present work we studied biochemical parameters of lipids and the expression of SREBP-2, HMG CoA Reductase and LDL Receptor genes. Male adult Balb/c mice received 10 mg of Metimazol (Danantizol)/100 ml tap water by 30 days (HT group). Control mice received tap water without the drug. Mice were killed by decapitation and blood and tissues were collected. We measured total cholesterol (CT) in liver and heart homogenates by Zak´s method. 1 µg of RNA, extracted by TRIZOL method, was transcribed to cDNA at 42°C using random hexamers as primers and MMLV-RT (Moloney Murine Leukemia Virus). Aliquots of 2 µl of cDNA were used in the amplifications by PCR using specific primers. Beta actin was used as an internal control. In HT mice, CT of liver and heart increased in relation to control. In liver, gene expression of SREBP-2 did not show any changes while HMG CoA Reductase and LDL Receptor decreased. The same situation was observed in heart.
With the dosis of Metimazol used, cholesterol metabolism is affected in both organs.

LI-P36.

OSMOTIC RESPONSE OF LACTIC BACTERIA GROWN AT DIFFERENT WATER ACTIVITIES

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The preservation of lactic bacteria for biotechnological purposes by freezing or desiccation, produces a severe dehydration of the cells causing irreversible damages at membrane level. The damage affect the water and ionic permeability. As rehydration involves a reincorporation of water to the cell, increasing their volume, the permeability properties of the membrane can be measure by means of the osmotic response of the bacteria. In this work we have measure the osmotic response of bacteria grown in different water activities and correlated its response with the changes in the lipid composition and the probable presence of specific transport molecules. The water permeability measured by changes in light scattering was determined in a stopped-flow spectrometry. The results indicate that bacteria grown at low water activities are less permeable to water due to the increase in saturated fatty acids. In addition, it was observed that part of the water flux may be taking place through specific transport systems that are inhibit by $HgCl_2$

LI-P37.

VOLTAMPEROMETRIC BEHAVIOUR OF MONOLAYERS OF MIXTURES OF PHOSPHATIDYL CHOLINE AND PHOSPHATIDYL ETHANOLAMINE ADSORBED ON MERCURY

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Monolayers of phospholipids adsorbed on mercury electrodes show high mechanical stability, high resistance to electric fields and may be well characterized through their capacity and voltammetric responses. Previous works with pure PC and pure PE show specific peaks that can be related with the type of molecular interactions between the lipids in the monolayer. The objective of this work is to characterize the electrochemical behaviour of monolayers of mixed phospholipids. We have applied the same methodology with the purpose of investigating the response of monolayers composed of different ratios of PC and PE. The results show that at 1:3 PE:PC ratio the voltammograms are similar to those of pure PC. However at the 1:1 ratio the peaks of PC are drastically reduced. On the other hand the voltammograms of 3:1 PE:PC show the peaks of PE slightly modified. Results are interpreted in terms of the specific interactions between groups of the polar heads giving place to the dynamical response of the monolayer structure.

CA-P1.

CHARACTERIZATION OF THE REACTION KINETICS OF CATIONS WITH THE Na^+/K^+-ATPase MEASURING THEIR EFFECT ON Rb^+ OCCLUSION

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The active transport of Na^+ and K^+ across cell membranes is mediated by the Na^+/K^+ pump, which is an intrinsic membrane transport system, identical with the Na^+/K^+-ATPase. There is evidence that during the pump cycle 2 K^+ become bound to the Na^+/K^+-ATPase in such a way that their release is slow. This type of binding is called occlusion because it is believed that their access to the bulk of the solvent is restricted by the protein structure. We here show that indirect information on the reactions between the enzyme and ligands like Na^+, K^+, Rb^+, NH_4^+, Li^+, Tl^+ or Mg^{2+} can be obtained from their effects on the kinetics of Rb^+ occlusion. To measure the time course of $^{86}Rb^+$ occlusion each cation was either equilibrated with the enzyme before adding 20 μM $^{86}Rb^+$ (condition A) or added to the enzyme together with $^{86}Rb^+$ (condition B). When the cation was K^+, Rb^+, NH_4^+ or Tl^+ the rate of $^{86}Rb^+$ occlusion was much slower for condition A than for condition B. This result was expected since K^+, Rb^+ and Tl^+ are known to become occluded themselves. When Na^+, Mg^{2+} or Li^+ were tested, no significant difference between the time courses for both conditions were observed. Compared to those obtained for K^+ and its congeners results for Na^+, Mg^{2+} and Li^+, strongly suggest that neither cation forms occluded states under these conditions.

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CA-P2.

EFFECT OF LOCAL ANESTHETICS ON THE CALCIUM ACTIVE TRANSPORT IN SARCOPLASMIC RETICULUM MEMBRANES FROM MASTICATORY MUSCLES

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The Ca-ATPase is a membrane intrinsic protein responsible for active calcium transport from myoplasm to the sarcoplasmic reticulum (SR) lumen, leading to muscle relaxation. Local anesthetics (LA) inhibit the calcium transport in white fast muscle (WFM) cells. The aim of this work was to compare the inhibitory effect of lidocaine (L), bupivacaine (B), tetracaine (T), procaine (Pr) and benzocaine (Bz) on the ATP-dependent calcium uptake in SR membranes isolated from WFM and the masticatory muscles (MM) masseter and medial pterygoid. SR membranes from rabbit MBR and MM were isolated by differential centrifugation. The ATP-dependent calcium uptake was determined at 37ºC in the presence of 50 mM MOPS-Tris (pH 7.2), 0.1 mM Ca-EGTA, 3 mM ATP, 3 mM $MgCl_2$ and 100 mM KCl, using radioisotopic techniques. The half-maximal LA concentration that inhibited the calcium uptake (Ki) was significantly lower in MM for the amide LA. Significant differences in Ki values in MM and WFM for ester LA were not observed. The potency of the LA was: T > L > Pr > B > Bz. A high correlation between the potency of the LA and the partition coefficient octanol/water (taken as an index of hydrophobicity) was found for MM (r = 0.95), masseter muscle (r = 0.84) and medial pterygoid (r = 0.87). The results suggest that the affinity of LA for the Ca-ATPase depends on the muscle tissue type, the type of LA (amide or ester) and the hydrofobicity of the drug.

CA-P3.

INFLUENCE OF CHARGED AMINO ACIDS IN THE αM4 TRANSMEMBRANE DOMAIN ON SURFACE TARGETING OF ACETYLCHOLINE RECEPTOR

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The αM4 domain of the acetylcholine receptor (AChR) is flanked by two basic amino acids (His^{408} and Arg^{429}) located at the two extremes of the transmembrane segment, at the level of the phospholipid polar head region. A series of single- and double αM4 mutants ($H^{408}A$, $R^{429}A$, $H^{408}A/R^{429}A$, $R^{429}E$ and $H^{408}A/R^{429}E$) were produced and co-expressed with wt subunits in an heterologous expression system (CHO-K1 cells). Surface $[I^{125}]$α-BTX binding of wt cells was 60% of the total, whereas $H^{408}A$ and $R^{429}A$ exhibited values of 40% and 5% cell-surface expression, respectively. Mutants with reversed amino acid charge (e.g. $R^{429}E$) did not express measurable levels of AChR at the cell surface and also diminished their total AChR levels. Cell-surface and total AChR levels in the double-mutant $H^{408}A/R^{429}A$ were 20% and 80%, respectively, whereas in $H^{408}A/R^{429}E$ no AChR was detected at the surface. Basic residues in the M1 segment have been implicated in AChR retention at the ER (Wang *et al*., 2002). Our study demonstrates that in addition to such sites in M1, His^{408} and Arg^{429} in αM4 also appear to be involved in cell-surface targeting of the AChR.

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CA-P4.

POST GENOMIC ANALYSIS OF PUTATIVE AMINO ACID TRANSPORTERS IN TRYPANOSOMES

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Trypansoma cruzi cells use amino acids not only as a carbon source (i.e. proline), but also in different metabolic pathways. Phosphorilated arginine acts as an energy reservoir involved in the renewal of ATP. It was also established that proline, aspartate and glutamate participate in the differentiation process. However, the molecular structure of trypanosomes amino acid transporters remains unknown. Using the available data from the *T. cruzi* genome project, we identified and characterized *in silico* different putative amino acid transporter (PAT) groups, containing paralogous sequences. More than 10,000 sequences were used to iterative assembling of contigs and 50-100 PAT open reading frames (ORFs) were detected and characterized. The genome organization of these ORFs suggests that PAT genes are arranged in tandem repeat clusters. Al last, we also identified the sequence orthologous in other trypanosomatids such as *Leishmania spp*. and *Trypanosoma brucei*. The amino acids transport may be considered as the first step in their metabolic pathways, wherefore the identification of transporter genes could be important for metabolic research and drug design.

CA-P5.

D170N SUBSTITUTION ACTIVATES THE PMCA WITHOUT FULLY DISENGAGING THE C-TERMINAL AUTOINHIBITORY DOMAIN FROM THE CATALYTIC CORE

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At low Ca^{2+} the interaction of a C-terminal inhibitory domain with the catalytic core keeps the Plasma Membrane Calcium ATPase (PMCA) in a basal state of low activity. Binding of Ca-Calmodulin to the autoinhibitory domain release the active site and activates the enzyme. We have previously reported that substitution of aspartic 170 by asparagine results in PMCA mutant with high Vmax and high Ca^{2+} affinity in the absence of calmodulin (SAB 2001) suggesting that the mutation disrupts the interaction between the autoinhitory region and the catalytic core. The exposure of the C-terminal inhibitory domain was assessed by measuring the apparent affinity for calmodulin and the sensibility of the C-terminal segment to proteolysis. We found that the concentration of calmodulin for half maximal stimulation was 12.9 nM for D170N and 16.4 nM for WT. On the other hand both the D170N and WT proteins were cut by chymotrypsin at a similar rate and calmodulin produced a similar increase in the rate of proteolysis. These results suggest that in D170N the autoinhibitory region is still in a closed conformation. Thus the activation induced by D170N may be bypassing the need for the complete disengagement of the autoinhibitory region from the catalytic core.

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CA-P6.

SOME PROPERTIES OF A SOLUBLE CYTOSOLIC FACTOR REQUIRED FOR Mg.ATP STIMULATION OF Na^{+}/Ca^{2+} EXCHANGE IN SQUID NERVE

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Squid giant axons subjected to prolonged dialysis and isolated nerve membrane vesicles (Loligo pealei) need a soluble cytosolic protein (SCP, Mr around 13 kDa), for MgATP stimulation of the Na/Ca exchanger. In this work we optimised the purification of that protein to have enough material for its characterization. We used two species of squid: (i) Loligo pealei and (ii) Illex argentinus. The starting material was the 400.000 g supernatant of the optic ganglia. The activity was recovered in a 30 kDa filtrated and was retained in 10 kDa filter. Proteins isolated from both squids promoted Mg.ATP stimulation of Na/Ca exchange in nerve vesicles from Loligo pealei. The activity remained in the flow-through of an anionic resin (1x8-400 Dowex) and was retained in a cationic resin (50x 8-400 Dowex). The 13 kDa protein from both species was phosphorylated by [32-P]-γ-ATP when they were incubated in the presence of active membrane nerve vesicles in conditions for Mg.ATP stimulation of Na/Ca exchange (1 uM Ca^{2+}, 3 mM Mg^{2+} and 0.2 mM vanadate). The protein from Loligo was isolated and sequenced by mass spectrometry. Peptide analysis indicates it is a *de novo* protein.

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CA-P7.

IDENTIFICATION OF RESIDUES IN THE M1 DOMAIN OF THE NICOTINIC RECEPTOR THAT CONTRIBUTE TO CHANNEL GATING

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The nicotinic receptor (AChR) is a pentamer of homologous subunits with an $\alpha_2\beta\epsilon\delta$ composition in adult muscle. Each subunit contains four transmembrane domains (M1-M4). We have combined site directed mutagenesis and construction of chimeric subunits with expression in cells and single-channel recordings to study the contribution of M1 to AChR activation. This segment shows highly conserved residues (positions 1', 2' and 14') or differentially conserved residues between α and non-α subunits (positions 3', 4', 13' and 15´). Our results showed that positions 3' and 14' slightly affect single channel kinetics but positions 1', 2', 4' and 13' are not involved in channel gating. In contrast, mutations at position 15´ significantly increase the rate of channel opening and decrease the closing rate. When the entire αM1 domain is replaced by that of the neuronal α7 AChR profound kinetic changes are observed. The duration of channel openings increase 10-fold, openings appear in clusters at low ACh concentrations, and the dissociation constant is reduced about 3-fold. Our results demonstrate that M1 segment contributes to channel gating and that the degree of conservation of residues is not strictly correlated with their functional roles.

CA-P8.

CONFORMATIONAL CHANGES OCURRING DURING THE ACTIVATION OF THE PMCA BY CALMODULIN. INTRAMOLECULAR FRET IN A BFP-PMCA-GFP FUSION CONSTRUCT

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A BFP-PMCA-GFP fusion protein suitable for intramolecular FRET measurements has been constructed by adding the blue (BFP) and green (GFP) fluorescent proteins at the N and C terminus of the plasma membrane Ca^{2+} transporter (PMCA). The recombinant protein purified from *S. cerevisiae* was a functional PMCA. When the purified protein was suspended in a media containing 500 μM EGTA and excited at 387 nm (BFP excitation maxima) the BFP-PMCA-GFP exhibited a major emission peak at 409 nm corresponding to BFP and a minor one at 509 nm due to GFP emission by FRET (Ida/Ia 0.2). Ca^{2+} did not significantly affect FRET. The removal of the fluorescent proteins from the PMCA by trypsin cleavage lead to a progressive disappearance of the peak at 509 nm (Ida/Ia 0.16). A similar drop in FRET was observed after addition of Ca-CaM. Adding enough EGTA to reduce the Ca^{2+} to less than 0.01 μM recovered Ida/Ia to a value close to that observed in the absence of CaM. These results are indicative of a conformational change occurring upon calmodulin binding and activation of the PMCA and suggest that the C terminal autoinhibitory region is moving apart or reorienting with respect to the N terminus which is part of the catalytic core of the enzyme.

CA-P9.

A NEW INSIGHT INTO THE ATP HYDROLYSIS CYCLE OF THE PLASMA MEMBRANE CALCIUM PUMP (PMCA)

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We have developed a microscale method to quantify phosphorylated intermediates of PMCA, isolating it by SDS-PAGE (Echarte *et al*. 2001). Applying this technique, we have shown that *E*P steady-state level at 4ºC depends on [ATP] concentration in a complex way. We also studied the effect of Mg^{2+} measuring *E*P with 1 mM or 8 µM Mg^{2+}, and observed that *E*P dependence on [ATP] can be described by a third degree polynomial quotient. These results suggest the participation of ATP in 3 steps of the reaction cycle, as proposed by Rega and Garrahan (1986).
We have measured the Ca^{2+}-ATPase activity (v), under the same conditions that we achieved phosphorylation experiments, and calculated *E*P turnover (kp*E*P) as the ratio v/*E*P. We observed that when [Mg^{2+}] is saturating (1 mM), kp*E*P increases hyperbolically with [ATP]. When [Mg^{2+}] is limiting (8 µM), kp*E*P as a function of [ATP] follows a complex behavior that cannot be explained by the accepted mechanism for ATP hydrolysis. We measured the rate of *E*P desphosphorylation in the presence of both 8 µM and 1 mM Mg^{2+}, and with 0.010 or 1 mM ATP. According to these results we proposed a model for the reaction cycle, which considers that ATP accelerates the conformational transition $E_1P \rightarrow E_2P$, through a new reaction intermediate E_1P.ATP. This model also explains the behavior of the pump at 25ºC.

CA-P10.

RELATIONSHIP BETWEEN THE PRESENCE OF α7 NICOTINIC RECEPTOR AND APOPTOSIS OF HUMAN LYMPHOCYTES

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Acetylcholine (ach) is well known as a neurotransmitter in both the central and peripheral nervous systems. Ach receptors (achr) have been recently identified in extraneuronal cells such as those of the immune system. By using rt-pcr we have detected mrna of **α**7 nicotinic receptor (achr) in peripheral human lymphocytes after incubation with different concentrations of nicotine (10-100 ì m). To determine the role of **α**7 in lymphocytes, we measured the degree of apoptosis in the presence of different nicotinic ligands. The degree of apoptosis was determined by dna cleavage forming a ladder pattern and by evaluating nuclear condensation with dapi staining. Lymphocytes pretreated with nicotine (10-100 ì m) were less susceptible to cortisol-induced apoptosis compared to untreated cells. The inhibition of apoptosis was concentration-dependent, reaching a value of 50% at 100 µm nicotine. Correlation between the presence of **α**7 mrna and protection from apoptosis was always observed. Furthermore, apoptosis protection was also observed in the presence of the antagonist **α**-bungarotoxin. This effect might be due either to the ability of both, the antagonist and nicotine, to induce upregulation or to desensitize and block achrs. Given that lymphocytes secrete ach, our results suggest that these cells contain functional **α**7 receptors.
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CA-P11.

INHIBITION OF MUSCLE ACETYLCHOLINE RECEPTOR BY THE ANTIEPILEPTIC DRUG LAMOTRIGINE

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Some forms of autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) have been found to be associated with genes coding for the $\alpha_4\beta_2$ neuronal acetylcholine receptor (AChR). Lamotrigine (LTG) is a triazine compound chemically unrelated to other antiepileptic drugs. Its major mechanism of action is blocking the voltage-dependent sodium-channel conductance. Here we characterized the effect of LTG on the *muscle* AChR heterologously expressed in CHOK1/A5 cells by recording the single-channel activity of the receptor using the patch-clamp technique. LTG was found to alter AChR channel kinetics. Exposure to the drug 1) diminished the channel amplitude; 2) caused shortening of the channel open state; 3) increased burst duration, 4) increased the duration and area of the briefest component of the channel closed state. In the concentration range tested (50-150 µM), the effects did not depend on the dose. We propose that LTG blocks the AChR allowing it to reopen quickly, through a mechanism that is compatible with that of open channel blockers.
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CA-P12.

MODULATION OF NICOTINIC ACETYLCHOLINE RECEPTOR SINGLE-CHANNEL BEHAVIOR UPON MODIFICATION OF MEMBRANE CHOLESTEROL

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The ability of the nicotinic acetylcholine receptor (achr) to conduct cations across the membrane and undergo conformational changes is highly dependent on the presence of cholesterol (chol). We studied the effect of membrane chol enrichment or depletion on single-channel kinetics of adult muscle-type achr in cho-k1/a5 cells treated with methyl- **β**-cyclodextrin (cdx) and chol-cdx, respectively. Chol depletion caused a lengthening of the achr channel mean open time, whereas its enrichment diminished this kinetic parameter. These changes were accompanied by significant changes in the open probability of the channel, whereas the burst duration and the number of events per burst remained unchanged. No modifications of channel amplitude were observed. Under hyperpolarizing conditions, the effect of chol depletion was no longer observed. A plausible explanation of these results is that regulation is exerted on endogenous chol at specific sites presumably located at the achr-lipid interface.
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CA-P13.
DESENSITIZATION OF ACETYLCHOLINE RECEPTOR BY THE LOCAL ANESTHETIC PROADIFEN

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The nicotinic receptor (AChR) of adult muscle is composed of five subunits in the order $\alpha_2\beta\varepsilon\delta$. Non competitive inhibitors (NCI) decrease the probability of channel opening by different mechanisms. The binding site for proadifen has been located at the extracellular end of the M2 domain. To elucidate the mechanism of action of proadifen on the AChR we made single-channel and macroscopic current recordings. Single-channel recordings reveal a decrease in the frequency of openings events without significant changes in the mean open time of the channel. This observation is compatible with an increase in desensitization rate from the open or closed state. At high ACh concentrations clusters of openings in the presence of proadifen were similar to those of the control, suggesting that proadifen is affecting the closed state. We also studied macroscopic currents activated by rapid application of ACh. Proadifen decreases the peak current without changing the decay rate due to desensitization. Preincubation with proadifen is necessary for its maximal pharmacological effect. Our results reveal that proadifen desensitizes AChRs that are in the closed state and neither affects open AChRs nor acts as an open-channel blocker.

CA-P14.
IDENTIFICATION OF KEY FUNCTIONAL DOMAINS IN CYS-LOOP RECEPTORS

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The AChR, 5-HT_3 and $GABA_A$ receptors are members of the cys-loop superfamily of ligand-gated ion channels that mediate rapid synaptic transmission throughout the nervous system. Neurotransmitters interact with a ligand-binding site in these channels triggering a conformational change in the protein that results in the opening of an ion channel. The structure of an homopentameric soluble protein (AChBP) from *Lymnaea stagnalis*, which is secreted by snail glial cells into cholinergic synapses, has been recently described at a high resolution. AChBP binds agonists and competitive antagonists of the AChR and its sequence is 20-24% identical to aligned sequences of the amino-terminal, extracellular halves of AChR subunits. To ascertain whether AChBP exhibits the potential to function as the extracellular region of a LGIC, and to identify extracellular domains involved in coupling the recognition of agonist to the opening of the channel, we constructed a chimeric AChBP-5HT_3 subunit and expressed it in mammalian cells. The chimeric receptor shows high expression and the same pharmacological profile as that of the soluble AChBP, indicating that the overall structure of the binding domain is conserved. However, results from macroscopic current recordings show that channel opening is not efficient in this receptor. Exchanging the cys-loop of AChBP by that of 5-HT_3 leads to the lack of expression, suggesting that this domain is essential for appropriate protein folding. The replacement of domains in AChBP until gating is achieved is our current strategy to identify residues involved in channel activation.

CA-P15.
AChR DISTRIBUTION AFTER CERAMIDE TREATMENT OF CHO-K1/A5 CELLS

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With the aim to study the mechanisms involved in the regulation of acetylcholine receptor (AChR) targeting to the plasma membrane, we studied the effect promoted by short (C6)- or long brain ceramides on AChR expression, metabolism, and cellular distribution. C6-Cer reduced by 30-80% the amount of $[^{125}I]\alpha$-BTX AChR detected at the cell surface of CHO-K1/A5 cells. This decrease was a dose- and time-dependent effect, augmenting also the ligand affinity for $[^{125}I]$ α-BTX. The half-life of surface AChR was 5.7 and 8.3 h for control and treated cells, respectively. C6-Cer produced intracellular accumulation of AChR (72±15% vs. 49±3 in control cells) without apparent cell damage. Fluorescence microscopy using $Alexa^{594}\alpha$-BTX labeling showed that brain-Cer and C6-cer decreased the amount of AChR localized at the plasmalemma. Whereas C6-Cer induced intracellular AChR accumulation in a vesicle-like compartment, brain Cer increased the amount of intracellular AChR with a more homogeneous distribution.

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CA-P16.
MUTATION OF CONSERVED RESIDUES IN THE M2 DOMAIN YIELDS A $\alpha 9\alpha 10$ NICOTINIC RECEPTOR WITH A GAIN OF FUNCTION

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Nicotinic acetylcholine receptors (nAChRs) form part of a gene superfamily, which includes $GABA_A$, $GABA_c$, serotonin type 3 and glycine receptors. The putative channel-forming M2 domains of these receptors contain two highly conserved residues: a leucine (L9´) and a valine (V13´), which are postulated to form a constricting hydrophobic girdle in the middle of the ion pathway. The aim of the present work was to study the role of these residues in the $\alpha 9\alpha 10$ nAChR function. cDNAs for rat $\alpha 9$ and $\alpha 10$ subunits, where the amino acids L9´ or V13´ were mutated to threonine (T), were expressed in Xenopus laevis oocytes and agonist-evoked currents were measured under two-electrode voltage-clamp. When compared to wild type receptors, ACh-evoked currents through $\alpha 9\alpha 10$(L9´T) and $\alpha 9\alpha 10$(V13´T) nAChRs exhibited much lower desensitization kinetics and an increase in their apparent affinity for this agonist. Choline, a weak partial agonist of the wild type receptor, behaved as a full agonist of the mutant receptors. Furthermore, nicotine, muscarine and ICS-205 930, antagonists of the wild type receptor, elicited ionic currents in oocytes expressing these mutants. A constitutive activation of a fraction of mutant receptors was observed, even in the absence of the agonist. Our results suggest that the sites where these two residues are located are structurally critical for opening-closing transitions of the $\alpha 9\alpha 10$ nAChR.

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CA-P17.

MECHANISM BY WHICH p-CHLOROMERCURIPHENYL SULPHONIC ACID BLOCKS ATP STIMULATION OF MAMMALIAN HEART Na^+/Ca^{2+} EXCHANGE

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The Na^+/Ca^{2+} exchanger of mammalian heart (NCX1) is activated by ATP through an increase of the phosphatidylinositol-4,5-biphosphate (PtdIns-4,5-P_2) bound to the exchanger. (Asteggiano *et al*., Eur. J. Biochem., 268: 437-442, 2001). A preliminary report (V. Posada, *et. al*., SAB-2002) indicated that p-chloromercuriphenyl sulphonic acid (PCMBS) blocks ATP effect on the NCX1. This result could be due to a direct effect of PCMBS on the PtdIns-4,5-P_2 binding to NCX1, on the synthesis of phosphoinositides or both. In order to test these possibilities we studied the effect of PCMBS on *the novo* synthesis of PtdIns-4-P and PtdIns-4,5-P_2 in plasma membrane vesicles of bovine heart using [32P]-γ- ATP as substrate. The results showed that 0.1 mM PCMBS (15 min preincubation) under conditions that blocked ATP stimulation (1 uM Ca^{2+}, 3 mM Mg^{2+}, 0.3 mM Vanadate), significantly reduced the net production of PtdIns-4-P and abolished the net production of PtdIns-4,5-P_2. An important additional observation was that exogenous PtdIns-4,5-P_2 stimulated the Na^+-gradient-dependent Ca^{2+} uptake in PCMBS pretreated vesicles. This PtdIns-4,5-P_2 stimulation was similar to that seen with ATP in vesicles without PCMBS. The results suggest that the effect of PCMBS on the ATP activation of NCX1 follows an impairment in the pathway/s involved in PtdIns-4-P and PtdIns-4,5-P_2 production.

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CA-P18.

PHOSPHOARGININE UP REGULATION OF Na^+/Ca^{2+} EXCHANGE IN SQUID OPTIC NERVE VESCICLES

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Phosphoarginine (PA) is a phosphagen present in the cytosol of invertebrate cells. We had demonstrated that millimolar [PA] stimulates Na^+/Ca^{2+} exchange in squid axon following a metabolic pathway different from that of ATP [DiPolo, R. and Beaugé, L., Biochim. Biophys. Acta, 1422: 57-71, 1999]. In order to further characterize this PA stimulation we used vesicles of squid optic nerve performing, in parallel, transport and phosphorylation of membrane proteins. The results show that: (i) PA stimulates a Na^+-gradient-dependent Ca^{2+} uptake, even in absence of the cytosolic protein required for ATP stimulation of the Na^+/Ca^{2+} exchanger; (ii) the apparent K_m for PA is in the millimolar range (about 4 mM), similar to that observed in dialyzed squid axons (7 mM); (iii) the effects produced by PA and ATP are additives; (iv) the pattern of [^{32}P]PA phosphorylation, analysed in SDS-PAGE, was different from that see with [^{32}P]ATP. Two phosphorylated bands, that were exclusives of PA, were sequenced. The results showed that they correspond to the NF 60 and NF 70 kDa subunits of the neurofilament protein.

Supported by FONCYT-Argentina (PICT99 05-05158) and Agencia Córdoba Ciencia (181/01).

CA-P19.

MUTATIONS IN THE CHRNA7 GENE IN A FAMILY WITH JUVENILE MYOCLONIC EPILEPSY

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Juvenile Myoclonic Epilepsy (JME) is an idiopathic generalized epilepsy that affects up to 26% of all individuals with idiopathic generalized epilepsy. Approximately a third of JME patients have a positive family history of epilepsy. Strong evidence for linkage was found to polymorphic loci encompassing the region containing the gene that encodes the neuronal AChR α7 subunit, which maps to 15q14. Elmslie *et al*. (1997) suggested that this major locus (EJM2) contributes to genetic susceptibility to JME in the majority of the families studied, but no precise localization was achieved. We studied a family with clinically diagnosed JME, and found a mutation at position 269 of the neuronal α7 AChR subunit. This corresponds to the M2 transmembrane segment, lining the walls of the AChR channel. The mutation consisted of the replacement of codon TTA by the codon ACA, resulting in the substitution Leu^{269}Thr.

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CA-P20.

EFFECT OF THE ANTHELMINTIC AGENT LEVAMISOLE ON MAMMALIAN NICOTINIC RECEPTORS

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Levamisole is an anthelmintic agent that exerts its therapeutic effects by acting as a full agonist of nicotinic receptors (AChR) of muscle nematodes. We explore at the single-channel and macroscopic-current levels the action of levamisole on mammalian muscle AChR. Levamisole is capable of activating mammalian AChRs. However, single-channel openings do not appear in clearly identifiable clusters at high concentrations. In addition, levamisole activation is not enough to elicit macroscopic currents. Both findings suggest that levamisole acts as a weak agonist of mammalian AChRs. In the presence of levamisole the channel mean open time decreases as a function of concentration (7-fold at 100 μM), indicating an additional open-channel blockade (Kd 140 μM at –70mV). Sequence alignment of mammalian and nematode AChR shows several non conserved residues which might be candidates for the differential activation by levamisole. The replacement of the conserved glycine at position 153 in the α subunit by its homologous in the parasite (αG153E) allows levamisole to produce identifiable clusters, thus making mammalian AChRs more sensitive to levamisole activation. We show that levamisole is a weak agonist and an open-channel blocker of mamalian AChRs and that the glutamic acid at position 153 is important for the behavior of the anthelmintic on the parasite AChR.

CA-P21.

AMINO ACID TRANSPORT ALTERATION IN *SACCHAROMYCES CEREVISIAE* MUTANTS DERIVED FROM A L-THREONINE AUXOTROPH (*thr1*) STRAIN

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In the yeast *S.cerevisiae* mutations in five amino acid permease genes (*AGP1*, *DIP5*, *GNP1*, *MUP3* and *LET2*) have been shown to decrease L-threonine transport activity. In addition disruption of the *SSY1* gene which encodes an amino acid sensor localized on the plasma membrane, reduces L-threonine transport activity. Considering this multiplicity of potential threonine transporters we decided to study: a) which permease operates as the primary, high affinity threonine permease in wild type strains and b) physiological conditions where one or more of the other permeases transport L-threonine. To this end a L-threonine auxotroph bearing a *thr1* gene was mutagenized with EMS and plated on minimal medium containing ammonium ion as nitrogen source and 0.3 mM L-threonine. This concentration of L-threonine is near the growth-limiting threshold. Small-colony mutants were selected as potential mutants with L-threonine transport defects. Studies with these mutants, including direct transport measurements, inhibitor resistance and genetic complementation indicated that: 1) There are at least three phenotypic categories among the four mutants 2) Individual mutants respond differently to growth medium composition 3) In minimal medium, higher L-threonine concentration reduces growth rate in the parent and all but one mutant [T2] 4) One mutant [T4] produces very high L-threonine transport activity which decays rapidly in L-threonine-less medium and 5) All mutants except T2 show enhanced L-leucine uptake.

CA-P22.

APPARENT ACTIVATION OF Ca^{2+}-ATPase ACTIVITY BY DILUTION

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Ca^{2+} pumps (PMCAs) are widely distributed among different tissues. Generically the pump is a single polypeptide chain of 127,000 to 137,000 daltons. PMCA is a calmodulin-regulated P-type ATPase that is encoded by a multigene family. To characterize the mechanism of PMCA under different conditions, it is necessary to measure Ca^{2+}-ATPase activity, Ca^{2+} transport and the partial reactions like, phospho and dephosphorylation and occlusion of Ca^{2+}.
We have found that specific activity of Ca^{2+}-ATPase of erythrocyte membranes, measured at concentrations below 50 μg/ml of protein, increases steeply up to 3-5 times at concentrations of 1 μg/ml of total membrane protein. Dilution of the protein did not modify the activation by ATP, Ca^{2+} or Ca^{2+}-calmodulin. This apparent activation of the enzyme is not affected by ionic strength and is independent of the method of Pi measurement (colorimetric or determination of release of γ^{32}P-ATP). The apparent activation on the enzyme is mimicked by 0.0025-0.01% of detergent $C_{12}E_{10}$ and is enhanced when membranes were slowly frozen instead of frozen by immersion in liquid nitrogen. This behavior is also observed in microsomal preparations of h4b PMCA expressed in insect Sf9 cells. These results seem to be accounted for by two reasons: a) dilution reveals sites hidden by high lipid-protein concentrations or b) dilution reveals the action of a modulator like FXYD proteins in Na,K-ATPase.

BG-P1.

INHIBITION OF THE RENIN-ANGIOTENSIN SYSTEM IMPROVES MITOCHONDRIAL FUNCTION

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It has been shown that nitric oxide (NO) can act as a signal that modulate the number of mitochondria in different cell types. In a recent work we found that long term inhibition (9 months)of the renin-angotensin system (RAS) attenuates structural and functional changes in mitochondria, and the decrease in the number of mitochondria that occurs with aging. Here we investigated whether short term inhibition of RAS with enalapril, could modify kidney mitochondrial function, and the role of NO in such effect. Thirty-two 3 mo-old male Wistar rats were divided into 4 groups that received enalapril (E,10mg/kg.day), enalapril+Lname (E+L,1mg/kg.day), L-Name(L-N), or water without additions (Control), during 14 days. Blood pressure was significantly lower (96±5 vs. 113 ± 1 mm Hg,p<0.05), and total mitochondrial protein per gram of wet tissue significantly higher in E vs. Control. Mitochodrial membrane potential was significantly higher in Enal and E+L vs. L-N and Control (154±10, 149±11, 114±7 and 117±5mV, respectively). Mitochondrial H_2O_2 production was lower in E, E+L-N and L-N vs. Control (28.7 ± 5.1, 31.2 ± 4.0, 44.8 ± 10.2, and 93.6 ± 13.5 nmol H_2O_2/min.mg protein, respectively). Mitochondrial uncoupling protein-2 and eNOS protein were significantly higher in E vs. E+L-N, L-N and Control. In conclusion, short-term inhibition of the RAS with E modifies mitochondrial function in rat kidney, independently of the NOS activity. The present results are consistent with an interaction between E and NO, in the regulation of the number of mitochondria.

BG-P2.

FLAVAN-3-OL AND PROCYANIDINS AS INHIBITORS OF ANGIOTENSIN CONVERTING ENZYME (ACE)

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Flavonoids have been mainly claimed to act as free radical scavengers. We have observed that after regular consumption of a flavan-3-ol and procyanidin rich-diet during 14 days, there was a significant decrease in systolic and mean blood pressure in a group of young people. The aim of the present work was to determine if this effect on blood pressure could be ascribed to an inhibition of the angiotensin converting enzyme (ACE). We determined if purified (-)-epicatechin (EC) and its related oligomers (dimers to hexamers) have an inhibitory effect on ACE activity. Determination of IC_{50} using N-hippuryl-L-histidyl-L-leucine (HHL) as substrate, showed that tetramers and hexamers present the lowest values of IC_{50} (12 and 10 μM, respectively). The same inhibition was confirmed using other synthetic substrate (FAPGG). A group of flavonoids belonging to other families, quercetin, miricetin, kaempherol and rutin, showed lower or negligible inhibitory effect. EC, dimers and hexamers presented a competitive inhibition respect to the HHL and FAPGG on ACE activity. Lower Ki values were, 4 and 12 μM for hexamer, for HHL and FAPGG. ACE inhibitory activity was confirmed in different systems, such as rat organ membranes. Procyanidins-rich beverages as red wine and tea showed inhibitory effect on ACE activity depending the polyphenols content. These effects of flavan-3-ols and procyanidins on ACE could contribute to explain the health beneficial effects observed in populations that consume high amounts of foods rich in flavonoids.

BG-P3.

CHARACTERIZATION OF RAT LIVER AND KIDNEY MITOCHONDRIA NITRIC OXIDE SYNTHASE

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Mitochondrial nitric oxide synthase (mtNOS) catalyses NO production in the mitochondrial matrix. The aim of this work was to study the factors that have influence on mtNOS biochemical activity. The highest activity in liver and kidney submitochondrial membranes was obtained at pH 7.4, with a NO production of 1.3±0.2 and 0.59±0.02 nmol/min.mg protein, respectively. Thus, the mitochondrial matrix pH offers an optimal environment for NO formation. At the same pH, NOS activity in the cytosol was 51% lower than in mitochondrial fraction. Values for the apparent K_m (μM) and V_{max} (nmol/min.mg protein) for O_2 were 40 and 0.51 for liver, 37 and 0.42 for kidney. The apparent K_m (μM) and V_{max} (nmol/min.mg protein) for L-arginine were 70 and 1.7 for liver; 4 and 0.62 for kidney. Enzymatic activity was modulated by Ca^{2+} concentration. Given the apparent K_m values for L-arginine and NADPH, the regulation of activity by these substrates seems unlikely under physiological conditions. NO production was influenced by the metabolic state of intact mitochondria: the rates at state 3 were 40-50% lower than those at state 4 suggesting a novel kind of regulation for this enzyme. Addition of Ca^{2+} to the reaction medium produced a 200% increase in O_2 consumption and a 40% increase in mtNOS activity. Characterization of mtNOS activity provides understanding of the regulation of mitochondrial function by NO.

BG-P4.

FERRITIN RADICALS GENERATED BY IRON UPTAKE

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Ferritin-radicals produced during aerobic iron (II) uptake by ferritin was studied by electronic paramagnetic resonance (EPR) in the presence of the trapping agents N-tert-butyl-α-phenyl nitrone (PBN) or 5,5-dimethyl-1-pyrroline N-oxide (DMPO). The spectrum in the presence of PBN was a superposition of two types of signals, an anisotropic signal from a slowly tumbling adduct ($2a^N_{zz}$ = 68.1 G) and an isotropic signal from a rapidly tumbling adduct (a_N=15.9 G and a_H=3.75 G). In the presence of DMPO, the spectrum obtained showed the dominant triplet pattern expected for a slowly tumbling nitroxide ($a^H\alpha$=22.3±1.1 G). In order to identify the detected radicals, chemical modifications were carried out on the native protein. By blocking thiol groups did not affect significantly the signal, however, by blocking tyrosine residues the signal was decreased in approximately 50%. Treatment with a strong oxidant (peroxynitrite) produced a significant decrease in tryptophan fluorescence associated to the same EPR signal, suggesting a role for these chemical species of similar nature in both processes. These results showed evidence of the presence of, at least, two radicals during iron uptake by ferritin: a tyrosyl-centered radical, that could act as an intermediate by transferring the radical character to another specie, tentatively identify as a tryptophan-centered radical.

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NT-P1.

DYNAMIC CHARACTERIZATION OF THE GLOBIN LIKE FAMILY

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The equilibrium dynamics of proteins in the folded state is directly related to their structures. In that sense, we can attempt to identify the common essential feature of equilibrium dynamics for a given protein fold.

In spite of the large number of biological functions that are achieved by a small number of protein structural families, a common dynamics behavior associated to each protein fold might be expected. Thus, a typical dynamic fluctuation behavior could be identified as an invariant of the evolution of each family.

In the present work we propose to explore the global dynamics of structurally similar proteins but sequential and functionally different. We seek the identification of common collective coordinates that were conserved during the evolution. The finding of invariants of evolution can be a first step in the development of simulation models for protein evolution.

We have studied the vibrational dynamics of the globin-like family by normal modes analysis (NMA). The low frequency normal modes describe collective movements that are closely related to the protein biological function. Representative structures of the globin family were considered.

NT-P2.

TOLERANCE OF *Escherichia coli* CELLS TRANSFORMED WITH CHLOROPLAST 2-CYS PEROXIREDOXIN TO OXIDATIVE STRESS

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2-Cys peroxiredoxin (2-Cys Prx) is a ubiquitous family of peroxidases that are devoid of the prosthetic heme group. Two highly conserved cysteines play an important role in modulating the concentration of hydrogen peroxide, thereby control signal transduction and impart tolerance to oxidative stress. To characterize the contribution of 2-Cys Prx to chloroplast metabolism, we cloned and expressed the mature form from rapeseed leaves in pET 22b(+) and subsequently studied the functional characteristics. Next, we analyzed, as a functional assay for undertaking studies of directed evolution, whether this recombinant protein bestows the capacity to tolerate oxidative stress in heterologous systems. Therefore, *E.coli* cells were transformed with the abovementioned plasmid and subsequently grew in media containing a variety of oxidants. Relative to cells transformed with the vector bearing chloroplast fructose-1,6-bisphosphatase (control), counterparts carrying the plasmid that codes for 2-Cys Prx tolerated higher concentrations of hydroperoxides as well as chemical oxidants. At variance, both strains elicited similar response to chemicals that deprive the intracellular concentrations of thiols, diamide and diethylmaleate. These results not only suggested that chloroplast 2-Cys Prx functions in the defense system against oxidation in *E.coli* cells, likely as electron donor to hydrogen peroxide, but also provided a protocol to estimate the functional capacity of chloroplast 2-Cys Prx.

NT-P3.
APPLICABILITY OF FLUORESCENCE FROM THE MAILLARD REACTION AS INDICATOR OF BIOMOLECULE DAMAGE

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The purpose of this work was to analyse the characteristics of fluorescence development from sugar-carbonyl amine reactions (Maillard reaction) in foods, biological and model systems, their manifestations in living organisms, like seeds, and their relationship to human diseases. In the area of physiology, fluorescence has continued to be used since 1985, when advanced glycosylated end-products (AGEs) were detected in human beings suffering diabetes and other diseases as a consequence of the reaction of glucose with long-lived proteins. In living organisms, the excessive non-enzymatic glycosylation of proteins produces major biological effects, like crosslinking of the modified proteins and trapping of soluble proteins by glycosylated extracellular proteins, decreasing proteolysis and promoting human health deterioration. The characteristic fluorescence of AGEs (ex 370/ em 440 nm) has been used as an indicator of the level of AGE-modified proteins since 1985 in research work. However, the chemical structure of many AGE fluorophores is still unknown, and only some fluorescent products were described. The utility of fluorescence measurement as index to follow the degree of reaction was analyzed in systems of variable composition under different environmental conditions to clarify if fluorescent products can be considered early markers of the reaction. The conditions under which chromophores, AGEs and fluorophores are formed are discussed.

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