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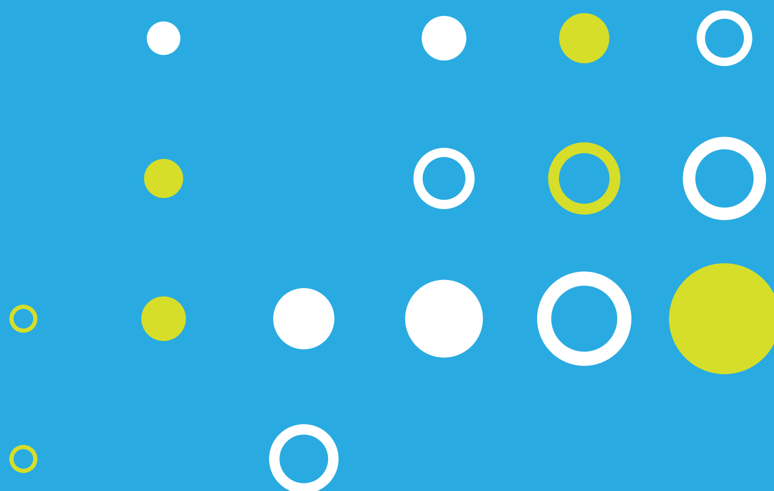
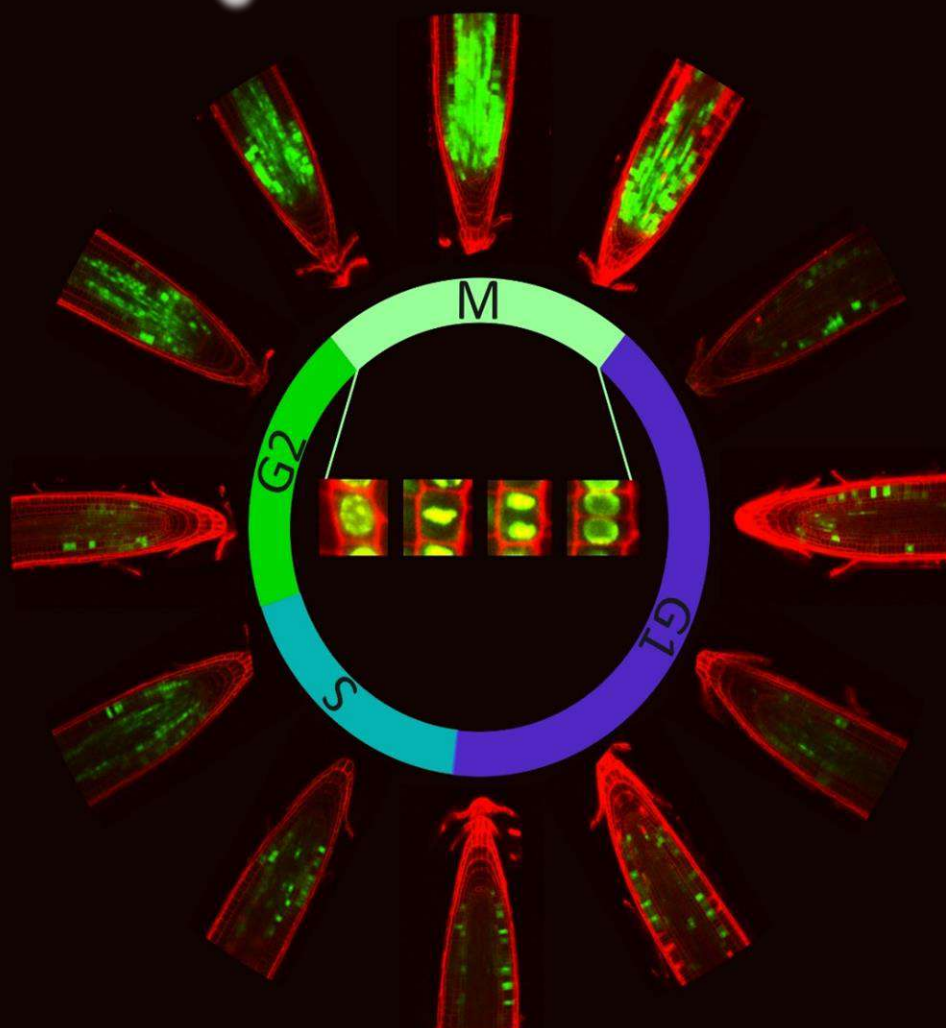


SAIB

Sociedad Argentina de
Investigaciones en Bioquímica
y Biología Molecular



52TH ANNUAL MEETING

ARGENTINE SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY

LII REUNIÓN ANUAL

Sociedad Argentina de Investigación en Bioquímica y Biología Molecular

Pabellón Argentina. Universidad Nacional de Córdoba

November 7-10, 2016

- SAIB -
52th Annual Meeting
Argentine Society for Biochemistry and Molecular Biology

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

November 7th–10th, 2016
Córdoba, República Argentina
Pabellón Argentina
Universidad Nacional de Cordoba

Cover Page:

Confocal microscopy images of Arabidopsis thaliana *root are displayed in the cover. The selected roots are expressing a GFP reporter of a mitotic cyclin (CYCB1;1-GFP, green), also they are counterstained with propidium iodide (IP, red) to display the cell structure. In order to follow the progression through the cell cycle phases, the root cells were synchronized in S phase using HU, and after pictures were taken every 2 hours. This type of experiment was also used to generate RNA samples to analyze the dynamics of different gene expression during the cell cycle. Inside the circle, which shows the cell cycle phases, images of cells expressing a histone fused to the fluorescent protein VENUS and stained with IP, are displayed. Those images allow following the steps of mitosis in vivo inside the root (PL-P56: Identification of cell cycle regulators inplants, by Goldy, C; Ercoli, MF; Vena, R; Palatnik, J, Rodriguez, Ramiro E.)*

Diseño de tapa: Natalia Monjes

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Auditor
INBIOSUR - CONICET
Universidad Nacional del Sur

DELEGATES OF SCIENTIFIC SESSIONS

-Cell Biology-
Laura Morelli
IIBBA – CONICET

-Lipids-
Ana Ves Losada
INIBIOLP - CONICET. Universidad Nacional de La Plata

-Microbiology-
Viviana Rapisarda
INSIBIO - CONICET. Universidad Nacional de Tucumán

-Plant Biochemistry and Molecular Biology-
Jorgelina Ottado
IBR - CONICET. Universidad Nacional de Rosario

-Signal Transduction-
Alejandro Colman Lerner
IFIBYNE–CONICET, Universidad de Buenos Aires

FORMER PRESIDENTS SAIB

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1978-1978	*FEDERICO CUMAR*
1977-1977	*ANTONIO BLANCO*
1976-1976	*HÉCTOR BARRA*
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1970-1970	*RODOLFO BRENNER*
1969-1969	*RANWEL CAPUTTO*
1965-1968	*LUIS F. LELOIR*

Monday, November 7	Tuesday, November 8	Wednesday, November 9	Thursday, November 10
	9:00 - 11:00 **Symposia** *Room A (Sala de las Americas):* **Plants** *Room B(Salon de Grados):* **Lipids**	9:00 - 11:00 **Symposia** *Room A (Sala de las Americas):* **Cell Biology-PABMB-South Cone** *Room B (Salon de Grados):* **Microbiology**	9:00 - 11:00 **Symposia** *Room A (Sala de las Americas):* ***ISN-CAEN*Neuroscience** *Room B (Salon de Grados):* **Signal Transduction-COB**
	11:00-11:30 Coffee break	11:00-11:30 Coffee break	11:00-11:30 Coffee break
	11:30-12:30 **PABMBPlenary Lecture** *Charlie Boone* *Room A (Sala de las Américas)*	11:30-12:30 **"Héctor Torres" Lecture** *Raul Andino* *Room A (Sala de las Américas)*	11:30-12:30 **"Ranwel Caputto" Lecture** *Carlos Dotti* *Room A (Sala de las Américas)*
	12:30 Lunch	12:30 Lunch	12:30 Lunch
14:00 Registration	14:30-16:00 **Oral Communications** *Room B (Salon de Grados):* **Plants (PL-C01 to PL-C06)** *Room C (Salon Rojo):* **Structural Biology (SB-C01 to SB-C03)** **Biotechnology (BT-C01 and BT-C03)** *Room D(Salon Azul):* **Enzymology (EN-C01 and EN-C03)** **Neuroscience (NS-C01-C02)**	14:30-16:30 **Oral Communications** *Room B (Salon de Grados):* **Plant (PL-C07 to PL-C13)** *Room C (Salon Rojo):* **Cell Biology (CB-C01 to CB-C08)** *Room D (Salon Azul):* **Signal Transduction (ST-C01 to ST-C06)**	14:30-16:30 **Oral Communications** *Room B (Salon de Grados):* **Plants (PL-C014 to PL-C21)** *Room C (Salon Rojo):* **Lipids (LI-C01 to LI-C08)** *Room D (Salon Azul):* **Microbiology (MI-C01 to MI-C06)**
	16:10-17:10 **"Alberto Sols" Lecture** *Javier De Las Rivas* *Room A (Sala de las Américas)*	16:45-17:30**TheCOBShortTalk** *Javier Martinez* *om A (Sala de las Américas)*	16:40 Coffee break 16:40-18:40 **Poster Session** *Hall Pabellón Argentina* **BT-P01 to BT-P21** **CB-P47 to CB-P67** **SB-P01 to SB-P03** **ST-P01 to ST-P27** **NS-P01 to NS-P12** **EN-P01 to EN-P17**
18:00 – **UNC Ceremony** **Honoris Causa Prof. Dr. B Alberts** *Room A (Sala de las Américas)* 18:30 - 19:00 **Opening Ceremony**	17:10 Coffee break 17:10 – 19:00 **PosterSession** *Hall Pabellón Argentina* **PL-P01 to PL-P35** **LI-P01 to LI-P36** **MI-P01 to MI-P30**	17:30Coffee break 17:30-19:30 **Poster Session** *Hall Pabellón Argentina* **CB-P01 to CB-P46** **MI-P31 to MI-P51** **PL-P36 to PL-P71**	18:45-19:45**Closing Lecture** *Carolina Vera* *Room A (Sala de las Américas)*
19:00 - 20:15 **Opening Lecture** **IUBMB Jubilee Lecture** *Bruce Alberts* *Room A (Sala de Las Américas)*	19:10-20:00 **PlenaryLecture** *Adriana Grupppi* *oom A (Sala de las Américas)*	19:45**SAIB General Assembly** *Room A (Sala de las Américas)*	19:45-**Closing Ceremony & Awards** *Room A (Sala de las Américas)*
20:30 Cocktail			22:00 Closing Dinner

SAIB 2016

MONDAY, November 7th, 2016

14:00 **REGISTRATION**

18:00- ***UNC CEREMONY-HONORIS CAUSA PROF. BRUCE M. ALBERTS***

SALA DE LAS AMERICAS

18:30-19:00 ***OPENING CEREMONY***

- Jose Luis Bocco

SAIB President

CIBICI, CONICET - Universidad Nacional de Cordoba, Argentina

19:00-20:15 ***OPENING LECTURE"IUBMB JUBILEE LECTURE"***

- Bruce M. Alberts

Department of Biophysics and Biochemistry

University of California San Francisco, USA

"Spreading Science throughout Society:A Challenge for the 21st Century"

Chairperson: Hugo Maccioni

20:30 ***WELCOMECOCKTAIL***

Patio de Las Palmeras Pabellon Argentina

TUESDAY, November 8th, 2016

09:00-11:00 ***SYMPOSIA***

Room A Sala de las Americas

PLANT SYMPOSIUM

Chairpersons:Juan C. Diaz Ricci and Estela Valle

-Regine Kahmann

Max Planck Institute for Terrestrial Microbiology, Dept. Organismic Interactions, Marburg, Germany

"Functional analysis of secreted effector of Ustilagomaydis *essential for host colonization"*

-Pablo Manavella

Instituto de Agrobiotecnología del Litoral (IAL), Centro Científico Tecnológico Santa Fe (CCT),

Santa Fe, Argentina

"Post-transcriptional regulation of the micro RNA pathway"

-John Lunn
Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, Germany

"Trehalose-6-phosphate and sucrose – A tale of two sugars"

-Jose Estevez
Fundación Instituto Leloir and IIBBA-CONICET, Ciudad Autónoma de Buenos Aires Argentina

"ARF5-RSL4 is the molecular link between auxin and ROS-controlled root hair polar growth"

Room B Salon de Grados

LIPID SYMPOSIUM
Chairpersons:Ana Ves LosadaandSusana Pasquare

-Michael A. Welte
Department of Biology, University of Rochester, New York, USA

"Lipid droplets control nuclear functions via protein sequestration"

-Richard Lehner
Department of Cell Biology Universidad de Alberta, Canada

"Carboxylesterases: novel therapeutic targets in nonalcoholic fatty liver disease"

-Nicolás O. Favale
Facultad de Farmacia y Bioquímica - Universidad de Buenos Aires, IQUIFIB - CONICET.
Buenos Aires, Argentina

"The role of sphingolipids metabolism in proliferation, differentiation and tissue organization"

-Natalia Wilke
CIQUIBIC-CONICET,Departamento de Quimica Biológica, Facultad de Ciencias Quimicas,
Universidad Nacional de Córdoba, Argentina

"Sizes of lipid rafts: what have we learnt from artificial lipid membranes?

11:00-11:30 ***COFFEE BREAK***

11:30-12:30 ***PABMB PLENARY LECTURE***

SALA DE LAS AMERICAS

- Charlie Boone

University of Toronto, Donnelly Centre Toronto, Ontario, Canada

"A global genetic interaction network maps a wiring diagram of cellular function"

Chairperson: Alejandro Colman Lerner

12:30 ***LUNCH***

14:30-16:00 ***ORAL COMMUNICATIONS***

Room B** (Salon de Grados)**: Plants (PL-C01 to PL-C06)
Room C** (Salon Rojo Escuela Graduados Medicina)**: Structural Biology (SB-C01 to SB-C03) and Biotechnology (BT-C01 to BT-C03)
***Room D** (Salon Azul Escuela Graduados Medicina):*Enzymology (EN-C01 to EN-C03) and Neuroscience (NS-C01 to NS-C02)

Room B Salon de Grados Pabellon Argentina

Plants (PL-C01 to PL-C06)
Chairpersons: Oscar Ruiz and Mariana Martin

14:30-14:45
PL-C01
MITOCHONDRIAL CONTRIBUTION TO BASAL PLANT DEFENSES VIA PROLINE DEHYDROGENASE (PRODH)
Fabro G, RizziYS, Alvarez ME. CIQUIBIC-CONICET, DQB-FCQ, Univ. Nac.Córdoba. E-mail: gfabro@fcq.unc.edu.ar

14:45-15:00
PL-C02
CHLOROPLAST REDOX STATUS MODULATES GENOMEWIDE STRESS RESPONSES IN SOLANACEOUS PLANTS
PierellaKarlusich JJ[1],Zurbriggen M[1],Shahinnia F[2],Hosseini S[2],Sonnewald S[2],Sonnewald U[2],Hajirezaei MR[2], Carrillo N[1]. [1]Instituto de Biología Molecular y Celular de Rosario (UNR-CONICET) [2]IPKGatersleben, Alemania. E-mail: pierella@ibr-conicet.gov.ar

15:00-15:15
PL-C03
REGULATION OF CENTRAL METABOLISM BY TREHALOSE 6-PHOSPHATE
FigueroaC,[1,2],Feil R[1], Ishihara H[1], Krause U[1], Hoehne M[1], Encke B[1], Stitt M[1], Lunn JE[1][1]MPI of Molecular Plant Physiology, Golm, Germany. [2]IAL, UNL-CONICET, Santa Fe, Argentina. Email: carfigue@fbcb.unl.edu.ar

15:15-15:30
PL-C04
UNRAVELING THE CONTRIBUTION OF NADP-MALIC ENZYME 1 TO ALUMINUM STRESS RESPONSE IN *Arabidopsis*ROOTS
Badia MB, Gerrard Wheeler MC, Andreo CS, Drincovich MF.CEFOBI, FCByF, UNRosario, Argentina E-mail: badia@cefobi-conicet.gov.ar

15:30-15:45

PL-C05

INFLUENCE OF SINAL7 IN VEGETATIVE PARAMETERS IN *Arabidopsis*

Peralta DA, Gomez Casati DF, Busi MV. CEFOBI CONICET Fac Cs Bioquimicas y Farmacéuticas, Universidad Nacional de Rosario E-mail: peralta@cefobi-conicet.gov.ar

15:45-16:00

PL-C06

SCF E3 LIGASE REDOX REGULATION: IMPACT ON HORMONAL SIGNALINGS

Iglesias MJ, Terrile MC, Casalongue CA Instituto de Investigaciones biológicas IIB-CONICET-UNMDP E-mail: majoi84@hotmail.com

Room C Salon Rojo Escuela Graduados Medicina

Structural Biology (SB-C01 to SB-C03) and Biotechnology (BT-C01 to BT-C03)

Chairpersons: Augusto Bellomio and Horacio Heras

14:30-14:45

SB-C01

GENERATION OF NANOBODIES AS A TOOL FOR STRUCTURAL BIOLOGY

AlzogarayVA, GoldbaumFA. Fundación Instituto Leloir. E-mail: valzogaray@leloir.org.ar

14:45-15:00

SB-C02

STRUCTURAL AND FUNCTIONAL STUDIES OF THE NTRX RESPONSE REGULATOR, A DIMERIC ATP BINDING PROTEIN

Fernández I[1], Cornaciu I[2], Carrica MC[1], Uchikawa E[2], Márquez JA[2], Goldbaum FA[1]. [1]Fundación Instituto Leloir, IIBBA (CONICET). [2]EMBL Outstation GrenobleE-mail: ifernandez@leloir.org.ar

15:00-15:15

SB-C03

UNRAVELLING THE LONG-RANGE SIGNALING MECHANISM OF BACTERIO-PHYTOCHROMES

Otero LH[1], Klinke S[1], Rinaldi JJ[1], Velázquez F[2], Mroginski M[2], Hildebrandt P[2], Goldbaum FA[1], Bonomi HR[1]. [1]Fundación Instituto Leloir, Argentina. [2]Technische Universität Berlin, Germany. E-mail: lotero@leloir.org.ar

15:15-15:30

BT-C01

CHARACTERIZATION OF ANTARCTIC MICROBIAL PHOTOLYASES AND RECOMBINANT PRODUCTION

Marizcurrena JJ[1], Morales D[1], Martinez W[2], Castro Sowinski S[1]. [1]Bioquímica y Biología Molecular, Fac Ciencias, UdelaR. [2]Epigenetica e Inestabilidad Genomica, IIBCE. E-mail: j_jmarrena@hotmail.com.

15:30-15:45

BT-C02

BIOCHEMICAL CHARACTERIZATION OF A CELLULOLYTIC COCKTAIL FROM AN ANTARCTIC *Flavobacterium*ISOLATE

Herrera Marrero LM[1], Braña V[1], Franco Fraguas L[2], Castro Sowinski S[1]. [1]Bioquímica y Biología Molecular, Fac Ciencias, UdelaR, Uruguay. [2]Bioquímica, Fac Química, Udelar. E-mail: herreramarrerolorena@gmail.com

15:45-16:00

BT-C03

CADMIUM AND LEAD RESISTANT RHIZOSPHERIC BACTERIA AS CANDIDATES FOR RHIZOREMEDIATION PROCESSESS

Saran A[1], Fernandez L[1], Massot F[2], Merini LJ[1]. [1]EEA-Anguil, INTA-CONICET. [2]Instituto NANOBIOTEC, UBA-CONICET. E-mail: saran.anabel@inta.gob.ar

Room D Salon Azul Escuela Graduados Medicina

Enzymology (EN-C01 to EN-C03) and Neuroscience (NS-C01 to NS-C02)
Chairpersons: Eleonora Campos and Patricia Setton

14:30-14:45
EN-C01
IDENTIFICATION AND CHARACTERIZATION OF A NOVEL STARCH BRANCHING ENZYME FROM *Ostreococcus tauri*
Hedin N, Barchiesi J, Gomez-Casati DF, Busi MV. CEFOBI-CONICET-UNR. Facultad de Ciencias Bioquímicas y Farmacéuticas. Rosario, Argentina. E-mail: hedin@cefobi-conicet.gov.ar

14:45-15:00
EN-C02
KINETIC AND FUNCTIONAL CHARACTERIZATION OF OTDSP, A PHOSPHOGLUCAN PHOSPHATASE FROM *O. tauri*
Carrillo JB, Martín M, GomezCasati DF, Busi MV.CEFOBI-CONICET. Facultad de Ciencias Bioquímicas y Farmacéuticas. Suipacha 531. Rosario, Argentina.E-mail: carrillo@cefobi-conicet.gov.ar

15:00-15:15
EN-C03
ALTERNATIVE CATALYTIC PROPERTIES IN THE GLYCOGEN-SYNTHASE FROM ACTINOBACTERIA
Asencion Diez MD[1], Cereijo AE[1], Alvarez HM[2], Iglesias AA[1]. [1]IAL (UNL-CONICET) [2] Instituto de Biociencias de la Patagonia (UNPSJB-CONICET. E-mail: masencion@fbcb.unl.edu.ar

15:15-15:30
NS-C01
THE VISUAL CYCLE IN THE INNER RETINA OF CHICKEN AND THE ROLE OF RETINAL G-PROTEIN-COUPLED RECEPTOR
Diaz NM[1]; Morera LP[1]; Tempesti TC[2]; GuidoME[1]
[1]CIQUIBIC-CONICET, FCQ, UNC [2]INFIQC-CONICET, FCQ UNC, E-mail: mguido@fcq.unc.edu.ar

15:30-15:45
NS-C02
METABOLIC DYSFUNCTION WORSENS COGNITION AND NEURONAL RESILIENCE IN A RAT MODEL OF EARLY ALZHEIMER
Martino AdamiPV[1]; Galeano P[1]; Wallinger ML[2]; Rabossi A[1]; Radi R[3]; Gevorkian G[4]; Cuello AC[5]; Morelli L[1]
[1]FIL-IIBBA CONICET. [2]FMed, UBA. [3]CEINBIO, UdeLaR. [4]UNAM. [5]McGill UniversityE-mail: pmadami@leloir.org.ar

16:10-17:10 ***"ALBERTO SOLS" LECTURE***

SALA DE LAS AMERICAS

- Javier De Las Rivas
Bioinformatics and Functional Genomics GroupCancer Research Center (CiC-IBMCC)
Consejo Superior de Investigaciones Científicas (CSIC) and Universidad de Salamanca (USAL)
Salamanca, SPAIN

"Human interactomics: build and analyse genome-wide protein networks using proteomics, transcriptomics and bioinformatics"

Chairperson: Viviana Rapisarda

17:10 ***COFFEE BREAK***

17:10-19:00 ***POSTER SESSION***

HALL PABELLON ARGENTINA

LI-P01 to LI-P36MI-P01 to MI-P30

PL-P01 to PL-P35

19:10-20:00 ***PLENARY LECTURE***

SALA DE LAS AMERICAS

- Adriana Gruppi
CIBICI-CONICET, Facultad de Ciencias Químicas
Universidad Nacional de Córdoba, Argentina

"B lymphocytes and plasma cells do more than antibodies"

Chairperson: Jose Luis Bocco

WEDNESDAY, November9th, 2016

09:00-11:00 ***SYMPOSIA***

Room A: *Sala de las Americas*

CELL BIOLOGY SYMPOSIUM-PABMB-SOUTH CONE
Chairpersons:Laura Morelli and Marcelo Rodriguez-Piñon

-Carlos Robello
Instituto Pasteur de Montevideo, Uruguay

"Cell reprogramming of human cells during the early Trypanosomacruzi *infection"*

-Fernando Lopez Diaz
Laboratory of Regulatory Biology, The Salk Institute for Biological Studies, La Jolla, CA USA-Argentina,

"*Genome plasticity, cellular stress and cellular reprograming in human breast cancer"*

-Alejandra Loyola
Laboratory of Epigenetics and Chromatin. Fundación Ciencia & Vida, Chile.

"The processing and maturation of newly synthesized histones"

-Flavio Meirelles
FZEA/Universidade São Pablo, Brazil

"The epigenetic errors arising from the reprogramming process."

Room B: *Salon de Grados*

MICROBIOLOGY SYMPOSIUM
Chairpersons:Carolina Touz and Viviana Rapisarda

-Jose Echenique
CIBICI CONICET Departamento de Bioquímica Clinica, Facultad de Ciencias Quimicas, Universidad Nacional de Cordoba, Cordoba

"Crosstalkbetweensignal transduction systemscontributes topneumococcal pathogenesis"

-Beatriz E. Baca
Centro de investigaciones en Ciencias Microbiológicas, Instituto de Ciencias, Benemérita Universidad Autónoma de Puebla, México

"Structure, functional prediction, and phenotyping studies on genes encoding for proteins involved in cyclic-di-GMP in Azospirillum*."*

-MichaelSeeger
Laboratorio de Microbiología Molecular y Biotecnología Ambiental, Universidad Técnica Federico Santa María, Valparaíso, Chile

*"*Burkholderia xenovorans*LB400 synthesizes a novel non-ribosomal peptide siderophore for iron transport"*

-Pablo Iván Nikel
Systems and Synthetic Biology Programme, Spanish National Center for Biotechnology (CNB-CSIC), 28049 Madrid, Spain

"Unleashing the catalytic potential of environmental bacteria."

11:00-11:30 ***COFFEE BREAK***

11:30-12:30 ***"HÉCTOR TORRES"PLENARY LECTURE***

SALA DE LAS AMERICAS

- Raul Andino
Department of Microbiology and Immunology, UCSF, San Francisco, USA

"Trans-generational antiviral immunity in insects"

Chairperson: Eduardo Ceccarelli

12:30 ***LUNCH***

14:30-16:30 ***ORAL COMMUNICATIONS***

Room B*(Salon de Grados):*Plants (PL-C07 to PL-C14)
Room CSalon Rojo *(Escuela Graduados Medicina):*Cell Biology (CB-C01 to CB-C08)
Room DSalon Azul *(Escuela Graduados Medicina):*Signal Transduction (ST-C01 and ST-C06)

Room B Salon de Grados

Plants (PL-C07 to PL-C14)
Chairpersons:Ariel Goldraij and Georgina Fabro

14:30-14:45
PL-C07
INSIGHT INTO DIVERSIFICATION AND EVOLUTION OF HD-ZIP I TRANSCRIPTION FACTORS IN STREPTOPHYTES
Romani FA, Chan RL, Moreno JE. Instituto de Agrobiotecnología del Litoral (IAL-UNL-CONICET). E-mail: fromani@santafe-conicet.gov.ar

14:45-15:00
PL-C08
AN OPEN READING FRAME PRESENT IN THE 5'UTR OF THE *Arabidopsis*ATHB1 GENE REPRESSED ITS TRANSLATION
Ribone PA, Capella M, Chan RL. Instituto de Agrobiotecnología del Litoral (UNLCONICET). Santa Fe. E-mail: pamela.ribone@santafe-conicet.gov.ar

15:00-15:15
PL-C09
CYTOCHROME C MODULATES PLANT GROWTHRATE AND THE ACTIVITY OF THE GIBBERELLINPATHWAY
Racca S, Welchen E, Gonzalez DH. Instituto de Agrobiotecnologia del Litoral (IAL-UNL-CONICET).Santa Fe, Argentina.E-mail:sofia.racca@hotmail.com

15:15-15:30
PL-C10
TCP15 CONNECTS GIBBERELLIN AND AUXINPATHWAYS DURING STAMEN FILAMENTELONGATION IN *Arabidopsis*
Gastaldi V, Lucero LE,Gonzalez DH. Instituto de Agrobiotecnologia del Litoral (IAL-CONICET-UNL)Santa Fe, Argentina. Email:vgastaldi@santafe-conicet.gov.ar

15:30-15:45
PL-C11
POST-TRANSLATIONAL REGULATION OF MICRO RNA BIOGENESIS
Achkar NP, Manavella PA Instituto de Agrobiotecnología del Litoral (IAL), UNL CONICET Email: natalia.achkar@gmail.com

15:45-16:00
PL-C12
INTEGRATION OF LIGHT AND TEMPERATURE CUES IN PLANT DEVELOPMENT
Legris M[1], Costigliolo Rojas MC[1], Vierstra R[2], Casal J[1] [31]Fundación Instituto Leloir, IBBA–CONICET. [2]Washington University.[3]IFEVA, FAUBACONICET. E-mail:mlegris@leloir.org.ar

16:00-16:15

PL-C13

PAP-SAL1 RETROGRADE PATHWAY IS INVOLVED IN IRON HOMEOSTASIS IN *Arabidopsis thaliana*

Balparda M[1], Estavillo G[2], Gomez-Casati DF[1], Pagani MA[1] [1]CEFOBI (UNR-CONICET), Rosario, Argentina. [2]CSIRO Plant Industry, Canberra, Australia. E-mail: balparda@cefobi-conicet. gov.ar

16:15-16:30

PL-C14

IMPORTANCE OF THE PRECURSOR PRIMARY AND SECONDARY STRUCTURE DURING MICRORNA PROCESSING IN PLANTS

Rojas A, Bresso E, Schapire A, Moro B, Mateos J, Palatnik J. Instituto de Biología Molecular y Celular de Rosario (IBR)
E-mail: arojas@ibr-conicet.gov.ar

Room CSalon Rojo Escuela de Graduados de Medicina

Cell Biology (CB-C01 to CB-C08)

Chairpersons:Gaston Soria and Claudio Fader Kaiser

14:30-14:45

CB-C01

NEURAL STEM CELL DIFFERENTIATION INDUCED BY LIPIDS

Montaner A; Costa M; Banchio C. Instituto de Biología Molecular y Celular de Rosario (IBR)- CONICET, Rosario, Argentina.E-mail: montaner@ibr-conicet.gov.ar

14:45-15:00

CB-C02

SUPPRESSION OF STARD7 PROMOTES ENDOPLASMIC RETICULUM STRESS AND INDUCES ROS PRODUCTION

Flores Martín J; Reyna L; Ridano ME; Panzetta-Dutari GM; Genti-Raimondi S. Dpto. Bioquímica Clínica, Facultad de Ciencias Químicas-UNC. CIBICI-CONICET. Argentina. E-mail: jflores@fcq.unc.edu.ar.

15:00-15:15

CB-C03

STRATEGY TO STUDY PARAMETERS ABLE TO PREDICT LONGEVITY IN MEDFLY POPULATIONS

Bocchicchio P[12], Pujol-Lereis L[1], Rossi F1, Turdera L[2], Pérez M1, Rabossi A[1], Quesada-Allué L[12]. [1]IIBBA-CONICET y F. Inst. Leloir, [2]Depto.Quim.Biol. FCEyN-UBA. E-mail: pbocchicchio@leloir.org.ar

15:15-15:30

CB-C04

LOW NRF2 EXPRESSION DETERMINES LOW ADIPOGENESIS, INFLAMMATION AND HIGH METABOLIC RISK IN BOYS AND RA

Santillan LD, Gimenez MS, Ramirez DC. [1]Lab. of Exp. and Transl. Med. &[2]Lab. Nutr. and Environ. IMIBIO-SL-CONICET-UNSL. E-mail: lucassantillan2011@gmail.com.

15:30-15:45

CB-C05

NATURAL GENETIC VARIATION DETERMINES PROMOTER SHAPE, AFFECTING ROBUSTNESS OF GENE EXPRESSION

Schor I[12]; Degner JF[2]; Harnett D[2]; Cannavo E[2]; Casale FP[3]; Garfield D[2]; Stegle O[3]; Furlong EE[2]. [1]IFIBYNE (CONICET)-DFBMC (FCEN, UBA). [2]GB Unit, EMBL-Heidelberg (Germany); [3]EMBL-EBI (UK). E-mail: ieschor@fbmc.fcen.uba.ar.

15:45-16:00

CB-C06

MITOCHONDRIA-TARGETED CATALASE PREVENTS OXIDATIVE STRESS AND REVERTS ANTIOXIDANT RESPONSE IN DOWN SYNDROME

Helguera PR; Zamponi E; Busciglio J. Instituto de Investigación Médica Mercedes y Martín Ferreyra. E-mail: prhelguera@immf.uncor.edu.

16:00-16:15

CB-C07

MAPPING THE DYNAMICS OF THE GLUCOCORTICOID RECEPTOR AND ITS COREGULATOR NCOA-2 IN THE NUCLEUS

Stortz MD[1] , Presman DM[2], Bruno L, Annibale P[4], Hager GL[2], Gratton E[4], Levi V[56], Pecci A[15]. [1]IFIBYNE-CONICET. [2]NIH, USA. [3]IFIBA-CONICET [4]LFD, UC Irvine, USA. [5]QB, FCEN-UBA. [6]IQUIBICEN-CONICET. E-mail: mstortz@qb.fcen.uba.ar.

16:15-16:30

CB-C08

ANTITUMORAL EFFECTS OF BIOENERGETIC MODULATION IN FELINE MAMMARY CARCINOMA CELLS

Arbe MF[1], Fondello C[1], Agnetti L[1], Tellado M[2], Alvarez G[2], Glikin GC[1], Finocchiaro LM[1], Villaverde MS[1]. [1]UTG, Área de Investigación, IOARH, FMed, UBA. [2]Cátedra de Química Biológica, FVet, UBA. E-mail: florenciaarbe@hotmail.com.

Room DSalon Azul Escuela Graduados Medicina

Signal Transduction (ST-C01 to ST-C06)

Chairpersons: Paula Portela and Veronica Gonzalez-Pardo

14:30-14:45

ST-C01

STRESS GRANULES CONTROL PROTEIN SYNTHESIS AND HAVE A NOVEL LINK TO NEURODEGENERATION

Perez-Pepe M, Katz MJ, Wappner P, Boccaccio GL. Fundación Instituto Leloir - IIBBA Conicet E-mail: maperez@leloir.org.ar

14:45-15:00

ST-C02

ROLE OF THE SCAFFOLD PROTEIN STE5 IN THE INTEGRATION OF CDK AND MAPK SIGNALS: A DYNAMIC VIEW

Repetto MV[1], Bush A[1], Winters MJ[2], Pryciak PM[2], Colman-Lerner AA[1]. [1] IFIBYNE-CONICET and Departamento de Fisiología, Biología Molecular y Celular, FCEN, UBA, Argentina. [2] MGM UMASS Med. School, USA. E-mail: vrepettor@fbmc.fcen.uba.ar

15:00-15:15

ST-C03

PROTEIN KINASE A LOCALIZATION IS CRITICAL FOR SPERM CAPACITATION

Stival V C[1], Ritagliati C[1], Luque GM, Baro Graf C[1], Visconti PE, Buffone MG, Krapf D[1]. [1]Laboratory of Cell Signal Transduction Networks, IBR (CONICET-UNR), Rosario, Argentina E-mail: stival@ibr-conicet.gov.ar

15:15-15:30

ST-C04

ESSENTIAL ROLE OF CFTR IN HUMAN SPERM REGULATION OF MEMBRANE POTENTIAL AND PHI DURING CAPACITATION

Puga Molina LP[1], Pinto NP[1], Krapf DK[2], Buffone MB[1]. [1]Instituto de Biología y Medicina Experimental [2]Instituto de Biología Molecular y Celular de Rosario E-mail: krapf@ibr-conicet.gov.ar

15:45-16:00

ST-C05

TWO-COMPONENT SYSTEMS IN BACTERIA: HOW IS THE SIGNAL UNIDIRECTIONALLY TRANSMITTED?

Imelio J, Trajtenberg F, Mechaly A, Larrieux N, Buschiazzo A. Molecular & Structural Microbiology Lab, Institut Pasteur Montevideo.

16:45-17:30 ***THE COMPANY OF BIOLOGISTS SHORT TALK***

SALA DE LAS AMERICAS

- Javier Martinez
Institute of Molecular Biotechnology– IMBA – and Medical University of Vienna, Austria

"Molecular mechanisms, biology and disease of mammalian tRNAsplicing"

Chairperson: Omar Coso

17:30 ***COFFEE BREAK***

17:30-19:30 ***POSTER SESSION***

CB-P01 to CB-P46 MI-P31 to MI-P51
PL-P36 to PL-P71

19:45 ***SAIB GENERAL BUSSINESS MEETING***

THURSDAY, November 10th, 2016

09:00-11:00 ***SYMPOSIA***

Room A Sala de Las Americas

ISN-CAEN-TRANSLATIONAL NEUROSCIENCE SYMPOSIUM
Chairpersons:Laura Morelli and Mario E. Guido

-Alejandra Alonso
Center for Developmental Neuroscience, College of Staten Island, CUNY, USA

"Mechanism of tau-induced neurodegeneration: Identification of the elements for new putative therapeutic targets"

-Ernesto Bongarzone
Dept. Anatomy & Cell Biology. College of Medicine, University of Illinois, Chicago, USA

"AAV9 gene therapy and hematopoietic transplant prevent neurological decline in Krabbe disease"

-Mauricio Farez
Centro para la Investigación de Enfermedades Neuroinmunológicas (CIEN), FLENI, Buenos

Aires, Argentina

"Melatonin signaling pathways in Multiple Sclerosis"

-Maria Dolores Ledesma Muñoz
Centro de Biología Molecular Severo Ochoa (CBMSO), Madrid-Spain

"Modulating lipids in the brain: towards a therapy for Niemann Pick disease"

Room B Salon de Grados

SIGNAL TRANSDUCTION SYMPOSIUM
Chairpersons:Alejandro Colman-Lerner and Pablo Aguilar

-Hernán García
University California Berkeley, CA, USA

" In the shadow of the fly: molecular mechanisms of shadow enhancers in development"

-Ezequiel Petrillo
IPFL Viena Austria

"A chloroplast retrograde signal regulates nuclear alternative splicing… in the roots!"

-Yoshikazu Ohya
Dept of Integrated Biosciences, University of Tokyo, Japan

"A cell cycle checkpoint to insure the integrity of the cell wall synthesis".

-Peter Pryciak
University of Massachussets,MA,USA

"Role of cyclin docking in CDK substrate choice and multi-site phosphorylation"

11:00-11:30 ***COFFEE BREAK***

11:30-12:30 ***"RANWEL CAPUTTO" LECTURE***

SALA DE LAS AMERICAS

- Carlos Dotti
Centro de Biología Molecular Severo Ochoa (CBMSO), Universidad Autónoma de Madrid, Madrid, Spain.

"Brain cholesterol dysregulation with age: contribution to the cognitive deficits of the old"

Chairperson: Jose Luis Daniotti

12:30 ***LUNCH***

14:30-16:30 ***ORAL COMMUNICATIONS***

Room B*(Salon de Grados):*Plants (PL-C15 to PL-C22)
Room C*(Salon Rojo Escuela Graduados Medicina):*Lipids (LI-C01 to LI-C08)
Room D*(Salon Azul Escuela Graduados Medicina):*Microbiology (MI-C01 to MI-C05)

Room B Salon de Grados

Plants (PL-C15 to PL-C22)

Chairpersons: Elina Welchen and Claudia Spampinato

14:30-14:45

PL-C15

BACTERICIDAL AND CYTOTOXIC ACTIVITIES OFPOLYPHENOL EXTRACTS FROM ANDEAN ANDINDUSTRIAL POTATOES

Lanteri ML[1], Silveyra MX[1],Damiano R[12], Andreu AB[1] [1]Instituto de Investigaciones Biológicas, UNMdP, CONICET .[2]INE "Dr. Juan H. Jara" Mar del Plata. Email:lanteri@gmail.com

14:45-15:00

PL-C16

REGULATION OF THE PLANT MICRO RNAMACHINERY BY A MSS47-MEDIATEDEPIGENETICMECHANISM

Ré DA, Manavella PA. Instituto de Agrobiotecnología del Litoral – UNL-CONICET SantaFe, 3000, Santa Fe, Argentina.E-mail:delfina.a.re@gmail.com

15:00-15:15

PL-C17

PHYTOCHROME B REGULATES SYSTEMICSIGNALING OF DEFENSE RESPONSE IN*Arabidopsis*

Moreno JE, Etchevers L Laboratorio de Biotecnología Vegetal, Instituto de Agrobiotecnología del Litoral (UNL-CONICET) E-mail:javier.moreno@santafe-conicet.gov.ar

15:15-15:30

PL-C18

PLANT NATRIURETIC PEPTIDES IMPROVE PLANTRESISTANCE DURING BIOTIC STRESS

Grandellis C, Ficarra F,Garavaglia BS,Gottig N,OttadoJ Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET-UNR) E-mail:grandellis@ibr-conicet.gov.ar

15:30-15:45

PL-C19

A GLYCINE RICH PROTEIN IS INVOLVED IN *Xanthomonas citri* SUBSP. *citri*-PLANT INTERACTION

VranychC, PiazzaA,OttadoJ,GottigN. Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET-UNR),Ocampo y Esmeralda, RosarioE-mail:vranych@ibr-conicet.gov.ar

15:45-16:00

PL-C20

DESIGN OF A GFP-BASED NON-INVASIVE BIOSENSOR TO DETERMINE NADP$^+$(H) REDOX STATE IN LIVING CELLS

Molinari PE[1], Zurbriggen M[2], Bustos-Sanmamed P[3], Krapp AR[3], Carrillo N[31]FBIOyF UNR, [2]Inst. Synthetic Biol. Heinrich Heine Univ. Dusseldorf Alemania, [3]IBR-CONICET Argentina E-mail: pmolinari@fbioyf.unr.edu.ar

16:00-16:15

PL-C21

HEAT STRESS INDUCES FERROPTOSISLIKECELLDEATH IN PLANTS

DistéfanoA[1], MartinM*[1],Córdoba J[1], Bellido A[1], RoldánJ[1],BartoliC[2],Zabaleta E[1], FiolD[1],StockwellB[3], DixonS[3],PagnussatG[1]*[1]*IIB-CONICET-UNMdP, Mar del Plata, Argentina*[2]*INFIVE-CONICET-UNLPLa Plata Argentina*[3]*DeptBiological Sciences, Columbia University, NY USA*equal contribution of both authors. E-mail: adistefa@mdp.edu.ar*

Room CSalon Rojo Escuela Graduados Medicina

Lipids (LI-C01 to LI-C08)

Chairpersons: Natalia Furland and Javier Valdez-Taubas

14:30-14:45

LI-C01

HIGH-NACL INDUCES SREBP-MEDIATED TRANSCRIPTIONAL REGULATION OF TRIGLYCERIDES

Weber K; Casali CI; Malvicini R; Parra LG; Etcheverry T; Fernandez MCUBA, FFYB, BCM; CONICET-IQUIFIBE-mail: kweber@ffyb.uba.ar

14:45-15:00

LI-C02

THE ETHER-LINKED LIPIDS OF RAT EPIDIDYMIS ARE AFFECTED BY MILD HYPERTHERMIA

Luquez JM; Santiago Valtierra FX; Oresti GM; Aveldaño MI; Furland NEINIBIBB, CONICET-UNS, 8000 Bahía Blanca, ArgentinaE-mail: jluquez@criba.edu.ar

15:00-15:15

LI-C03

ROLE OF GPA3/4 IN GLYCEROLIPID SYNTHESIS, PHAGOCYTOSIS AND CYTOKINE RELEASE IN ACTIVATED MACROPHAGES

Quiroga IY[1]; Pellon-Maison M[1]; Coleman RA[2]; Gonzalez-Baro MR[1][1]*INIBIOLP-UNLP-La Plata, Argentina*[2]*Dept. Nutrition, UNC, USAE-mail: yoseli_quiroga@hotmail.com*

15:15-15:30

LI-C04

A METABOLIC CIRCADIAN CLOCK CONTROLS RHYTHMS IN IMMORTALIZED HUMAN GLIOBLASTOMA T98G CELLS

Wagner PM[1]; Sosa-Alderete L[1]; Gorné L[1]; Gaveglio V[2]; Salvador G[2]; Pasquare S[2]; Guido ME[1]
[1]*CIQUIBIC-CONICET, Dept. Biol Chem. FCQ-UNC, Cordoba, Argentina.* [2]*INIBIBB-CONICET. Bahía Blanca.*
E-mail: pwagner@fcq.unc.edu.ar

15:30-15:45

LI-C05

EXPRESSION OF ELOVL4 AND FA2H WITH SPERMATOGENIC CELL DIFFERENTIATION IN THE RAT TESTIS

Santiago Valtierra FX; Peñalva DA; Luquez JM; Furland NE; Aveldaño MI; Oresti GMINIBIBB, CONICET-UNS, Bahía Blanca, ArgentinaE-mail: gmoresti@criba.edu.ar

15:45-16:00

LI-C06

LOW-DENSITY MEMBRANE FRACTIONS FROM MALE GERM CELLS LACK SPHINGOLIPIDS WITH VERY LONG CHAIN PUFA

Santiago Valtierra FX; Mateos MV; Aveldaño MI; Oresti GMINIBIBB, CONICET-UNS, Bahía Blanca, Argentina E-mail: gmoresti@criba.edu.ar

16:00-16:15

LI-C07

MEMBRANE RESTRUCTURING INDUCED BY THE ENZYMATIC GENERATION OF CERAMIDES WITH VERY LONG CHAIN PUFA

Peñalva DA; Antollini SS; Ambroggio EE; Aveldaño MI; Fanani MLINIBIBB, CONICET-UNS, Bahía Blanca, and CIQUIBIC, UNC-CONICET, Córdoba, ArgentinaE-mail: gmoresti@criba.edu.ar

16:15-16:30

LI-C08

AN EXPANSION OF CYTOCHROME P450 GENES IN TRIATOMINES IS ASSOCIATED WITH PYRETHROID RESISTANCE

Pedrini N; Calderón Fernández GM; Salamanca JE; Dulbecco AB; Moriconi DE; Kumar S; Juárez MP
INIBIOLP (CONICET-UNLP)E-mail: nicopedrini@yahoo.com

Room DSalon Azul Escuela Graduados Medicina

Microbiology (MI-C01 to MI-C06)

Chairpersons: Monica Delgado and Sandra Ruzal

15:00-15:15

MI-C01

THE ROLE OF RESPIRATORY OXIDASES IN THE MECHANISM OF ACTION OF MICROCIN J25

Galván AE[1], Chalón MC[1], Schurig-Briccio L[2], Minahk CJ[1], Gennis R[2], Bellomio A[1]. [1]INSIBIO (CONICET-UNT). Tucumán, Argentina. [2]Deparment of Biochemistry. University of Illinois. E-mail: emilcegalvan@hotmail.com

15:15-15:30

MI-C02

FUNCTIONAL CHARACTERIZATION OF THE CELL DIVISION PROTEIN FtsA OF *Streptococcus pneumoniae*

Yandar NY, Reinoso N, Cortes PR, Echenique J. Dpto. Bioquimica Clinica/CIBICI-CONICET, Fac. Cs. Químicas, UNC. E-mail: nyandar@fcq.unc.edu.ar

15:30-15:45

MI-C03

REGULATION OF THE SUBPOLAR FLAGELLUM SYNTHESIS IN*Bradyrhizobium diazoefficiens*

Dardis C, Mengucci F, Althabegoiti MJ, Lodeiro AR, Quelas JI, Mongiardini EJ. Instituto de Biotecnología y Biología Molecular (IBBM) CCT-La Plata CONICET, UNLP. E-mail: carolinadardis@biol.unlp.edu.ar

15:45-16:00

MI-C04

ENTEROBACTIN: A FENTON-SAFE SIDEROPHORE

Peralta DR, Adler C, Corbalán NS, Paz García EC, Pomares MF, Vincent PA. INSIBIO, CONICET-UNT. Chacabuco 461, T4000ILI – Tucumán, Argentina. E-mail: drperalta@fbqf.unt.edu.ar

16:00-16:15

MI-C05

THE*MAP*LOCUSOF*Brucella suis*IS INVOLVED IN CELL ENVELOPE BIOGENESIS AND VIRULENCE

Bialer MG[1], Ruiz-Ranwez V[1], Estein SM[2], Russo DM[1], Altabe SG[3], Sycz G[1], Zorreguieta A[1]. [1]Fundación Instituto Leloir, IIBBA-CONICET, Bs.As. [2]CIVETAN-CONICET, Tandil. [3]IBR-CONICET, Rosario. E-mail: mbialer@leloir.org.ar

16:15-16:30

MI-C06

CLONING, EXPRESSION AND CHARACTERISATION OF THE HEPATITIS E VIRUS CAPSID PROTEIN OF GENOTYPES 1-4 FOR SERODIAGNOSTIC

Arce L[1], Stellberger T[2], Baiker A[2], Vizoso Pinto MG[1]. [1]INSIBIO (UNT-CONICET). Facultad de Medicina de la Univ. Nac. De Tucumán. Argentina. [2]LGL, Erlangen, Germany

16:40 ***COFFEE BREAK***

16:40-18:40 ***POSTER SESSION***

BT-P01 to BT-P21	**CB-P47 to CB-P67**
EN-P01 to EN-P17	**ST-P01 to LI-P27**
NS-P01 to NS-P12	**SB-P01 to SB-P03**

18:45-19:45 ***CLOSINGLECTURE***

SALA DE LAS AMERICAS

-Carolina Vera

Centro de investigaciones del Mar y la Atmósfera

(CIMA/CONICET-UBA/FCEN) Ciudad Autónoma de Buenos Aires, Argentina

"Risks and challenges associated with Climate Change".

Chairperson: Silvia Moreno

19:45 ***CLOSING CEREMONY AND AWARDS***

22:00 ***CLOSING PARTY***

- ABSTRACTS:

All abstract will be published in:

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www.cricyt.edu.ar/biocell/

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- Lipids: LI-01 to LI-04
- Microbiology: MI-01 to MI-04
- Plant Biochemistry and Molecular Biology: PL-01 to PL-04
- Signal Transduction: ST-01 to ST-04
- Translational Neuroscience: NS-01 to NS-04

- Oral Communications:
- Biotechnology: BT-C01 to BT-C03
- Cell Biology: CB-C01 to CB-08
- Enzymology: EN-C01 and EN-C03
- Lipids: LI-C01 to LI-C08
- Microbiology: MI-C01 to MI-C06
-Neuroscience: NS-C01 to NS-C02
- Plant Biochemistry and Molecular Biology: PL-C01 to PL-C21
- Structural Biology: SB-C01 to SB-C03
- Signal Transduction: ST-C01 to ST-C05

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LECTURES AND SYMPOSIA ABSTRACTS

MONDAY, November 7th, 2016

L-01
SPREADING SCIENCE THROUGHOUT SOCIETY:A CHALLENGE FOR THE 21ST CENTURY

Alberts, BM
Chancellor's Leadership Chair in Science and Education, University of California, San Francisco

From my 5 years in Washington and my 30 years of interacting with students at universities, I am convinced that our nation and the world badly need more people from the scientific community in a wide variety of professions. Not only the problem-solving skills of scientific inquiry, but also the values of science are critical: honesty, generosity, and a respect for all ideas and opinions regardless of their source of origin. The Worldwide Web makes real the dream of being able to connect all those trained as scientists to common sources of knowledge and discourse. But the Unites States can only lead the way if our university science departments greatly enlarge their own view of their mission. Most of all, we need our science faculty members to appreciate the many different types of contributions their students can make after they leave the university; to do this, we need to continually bring back to each of our departments not only those graduates who are making outstanding contributions to the scientific research, but also those who are making a difference in public policy, industry, government, journalism, law, commerce, community colleges, and our public school systems.

The National Academy of Sciences has been working to overcome the many challenges that face us in attempting to achieve the above goal. You can visit us at **www.nas.edu,** where the full text of more than a thousand of our publications are available for free, on-line.

TUESDAY, November 8th, 2016

LECTURES

L-02
A GLOBAL GENETIC INTERACTION NETWORK MAPS A WIRING DIAGRAM OF CELLULAR FUNCTION

Boone, C
Donnelly Centre, University of Toronto, Ontario, Canada

We generated a global genetic interaction network for *Saccharomyces cerevisiae*, constructing over 23 million double mutants, identifying ~550,000 negative and ~350,000 positive genetic interactions. This comprehensive network maps genetic interactions for essential gene pairs, highlighting essential genes as densely connected hubs. Genetic interaction profiles enabled assembly of a hierarchical model of cellfunction, including modules corresponding to protein complexes and pathways, biological processes, and cellular compartments. Negative interactions connected functionally related genes, mapped core bioprocesses, and identified pleiotropic genes, whereas positive interactions often mapped general regulatory connections among gene pairs, rather than shared functionality. The global network illustrates how coherent sets of genetic interactions connect protein complex and pathway modules to map a functional wiring diagram of the cell.

L-03
HUMAN INTERACTOMICS: BUILD AND ANALYSE GENOME-WIDE PROTEIN NETWORKS USING PROTEOMICS, TRANSCRIPTOMICS AND BIOINFORMATICS

De Las Rivas, J
Cento de Investigación del Cáncer, Consejo Superior de Investigaciones Científicas y Universidad de Salamanca (CiC-IBMCC, CSIC/USAL) Salamanca, Spain
http://www.i-m.mx/jdelasrivas/BioinfoFuncGenomicsCiC/ yhttp://www.cicancer.org/

Identification of the interactions between the biomolecular elements that comprise a cellular system is crucial to unravel its architecture and dynamics. Modern genome-wide technologies provide compendiums of the biomolecular entities that configurate a living system, including all the genes encoded, the corresponding derived proteins and the interactions between them. The maps of such interactions constitute the "interactomes", and we have developed bioinformatic tools and resources focused towards the construction and analyses of interactomes from different organisms (apid.dep.usal.es) displayed as complex protein networks. After using several quality controls to determine the confidence of the interactions, we present several studies to build different views of the "human interactome" using either integrated proteomic or transcriptomic data. We also present some specific examples of our current investigations in cancer to show how biomedical research can be better driven and focused using validated networks, because they allow revealing the specific links and associations between human genes and proteins.

L-04
B LYMPHOCYTES AND PLASMA CELLS DO MORE THAN ANTIBODIES

Gruppi, A
Dpto.BioquímicaClínica,CIBICI-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina
B lymphocytes are the only cell type in the organism capable of producing antibodies (Abs), which play an essential role in controlling replication of many pathogens. B cells may have a pathogenic role if they generate antibodies against the self-antigens, called autoantibodies.
B lymphocytes after stimulation, in particular contexts, can differentiate into short- or long-lived plasma cells or memory B cells. The latter cells are part of immunologic memory and responsible for lasting humoral immunity. In addition, B lymphocytes can influence immunity in multiple ways such as antigen presentation to T cells, expression of surface co-stimulatory moleculesand cytokine secretion. Consequently, B cells can act as drivers of innate and adaptive immunity. In this way, we observed that B cells and, particularly, plasma cells can produce cytokines as IL-17 and expresshigh levels of inhibitory molecules PD-L1 and CD39. Through these molecules B cells and plasma cells can regulate T cell immunity. The mechanism of IL-17 production and PD-L1 induction and function of IL-17+ and PD-L1+ B/plasma cells will be discussed.

SYMPOSIA

PL-01
FUNCTIONAL ANALYSIS OF SECRETED EFFECTOR OF *Ustilagomaydis*ESSENTIAL FOR HOST COLONIZATION

Liang L[1], Schipper K[1, 2], Ludwig N[1], Lo Presti L[1], Zechmann B[3], Glatter T[1], Lanver D[1], Reissmann S [1], Kahmann R[1]
[1]Max Planck Institute for Terrestrial Microbiology, Dept. Organismic Interactions, Marburg, Germany [2]Present address: Heinrich Heine University Düsseldorf, Dept. Microbiology, Düsseldorf, Germany[3]Baylor University, Center for Microscopy and Imaging, Waco, Texas 76798, USA
Smut fungi comprise a large group of biotrophic pathogens that infect cereal crops and wild grasses. The best studied member of this group, *Ustilagomaydis*, infects maize and induces characteristic tumor formation and anthocyanin coloring. Interaction with the plant is largely determined by about 300 novel protein effectors that are conventionally secreted and are induced only after plant colonization. A successful colonization requires active effector-mediated suppression of plant defense responses and host tissue reprogramming. Secreted effector proteins can either display their activity in the apoplast or translocate to host cells. Based on a comprehensive RNAseq analysis during the different stages of host colonization we have classified the secretome into discrete effector classes and initiated their functional analysis. Among the early-induced effector genes we have found five genes which each lead to an early arrest phenotype of the respective single gene deletion mutant, abolish virulence completely and elicit massive plant defence responses. By performing Co-IPs of tagged versions of these five effectors from infected maize tissue followed by mass-spectroscopic analysis we have detected four of these proteins in a complex. We will discuss where this complex resides and speculate on its function during infection.

PL-02
POST-TRANSCRIPTIONAL REGULATION OF THE MICRO RNA PATHWAY

Manavella PA.
IAL-CONICET-UNL. Santa Fe-Argentina
MicroRNAs (miRNAs) are small RNA molecules with critical roles during development of multicellular organisms. In plants, these small regulatory molecules are produced from primary miRNA transcripts by a single nuclear enzyme, DICER-LIKE 1 (DCL1). The accurate excision of a miRNA relies on the interaction of DCL1 with its cofactor HYPONASTIC LEAVES1 (HYL1). Once a miRNA is produced it is loaded into an ARGONAUTE (AGO) protein leading the RISC complex to a target mRNA. The miRNAs pathway comprises multiple well-orchestrated steps to ensure the precise and balanced silencing of target genes. In the past years, we have used a large-scale luciferase-based genetic screen, followed by whole-genome sequencing, to identify new co-factors regulating the miRNA production and activity. The analysis of the isolated mutants revealed new and intriguing layers of regulation of the pathway. Among them, we have found that the dephosphorylation of HYL1, by CPL1, is required for full activity of the protein. Such regulation is tightly controlled tissue specifically by RCF3. Lately we also found the biological purpose of the phosphorylated, inactive, reservoir of HYL1 in the cell. In another layer of post-translational regulation, we have identified mutant plants with an impaired AGO1 stability that lead to severe developmental defects caused by an unbalance miRNA-mediated gene silencing.

PL-03
TREHALOSE-6-PHOSPHATE AND SUCROSE – A TALE OF TWO SUGARS

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Trehalose 6-phosphate (Tre6P) is an essential signal metabolite in plants that influences leaf growth and senescence, stomatal function, flowering, inflorescence architecture and embryogenesis. Tre6P closely tracks diurnal and externally imposed fluctuations in the levels of sucrose. We propose that Tre6P functions as both a signal and negative feedback regulator of sucrose levels, helping to maintain intracellular sucrose concentrations within an optimal range. This function can be compared with the insulin-glucagon system for regulating blood glucose levels in animals. In leaves, Tre6P regulates photoassimilate partitioning to sucrose during the day and the

remobilization of transitory starch reserves to sucrose at night, linking both of these to demand for sucrose from sink organs. In meristems and other growing tissues, Tre6P signals the availability of sucrose for growth, influencing developmental decisions and the fate of imported sucrose. The intertwined relationship between sucrose and Tre6P is captured in the sucrose-Tre6P nexus concept. This model helps us to understand how Tre6P exerts such a profound influence on plant growth and development, and provides a framework for engineering Tre6P metabolism for crop improvement.

PL-04
ARF5-RSL4 IS THE MOLECULAR LINK BETWEEN AUXIN AND ROS-CONTROLLED ROOT HAIR POLAR GROWTH

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Polar-growth present in root hairs is sustained by oscillating levels of Reactive Oxygen Species (ROS). These cells endogenously controlled by auxin are able to grow hundred-folds of their original size toward signals important for plant survival. Here, we showed that ROS-production is under the control of the transcription factor RSL4, who in turn is regulated by auxin through the Auxin Responsive Factor 5 (ARF5). In this manner, auxin controls ROS-mediated polar-growth depending on NADPH oxidases (or RBOHs for RESPIRATORY BURST OXIDASE HOMOLOG proteins) and secreted type-III Peroxidases (PER). A novel group of two RBOHs (RBOHH,J) and four PERs (PER1,44,60,73) are then required to modulate $_{apo}$ROS homeostasis. Finally, $_{apo}$ROS as H_2O_2 would be transported by the Plasma membrane Intrinsic Protein PIP2;7 back to the cytoplasm to activate downstream responses. Overall, our findings indicate that auxin regulates $_{apo}$ROS-reliant root hair polar growth through the action of ARF5-mediated RSL4 expression.

LI-01
LIPID DROPLETS CONTROL NUCLEAR FUNCTIONS VIA PROTEIN SEQUESTRATION

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Inside cells, neutral lipids are stored inside lipid droplets (LDs), unique organelles with a neutral lipid core, a phospholipid shell, and dozens if not hundreds of proteins. LDs have well-characterized roles in cellular lipid metabolism; emerging evidence indicates that they also regulate nuclear functions, via the exchange of lipids, transcription factors, and chromatin components. In Drosophila embryos, LDs are associated with large quantities of histones, anchored via the novel protein Jabba. Using Jabba mutants, we found that histone binding to LDs serves two biological functions: it allows eggs to safely store large amounts of histones to support embryogenesis, and it buffers the histone supply, preventing histone overaccumulation in the nucleus. We are now identifying the regions of Jabba that mediate LD- and histone binding. Using live imaging, we found that early on histones are dynamically bound, rapidly exchanging between LDs; this is presumably how histone buffering is achieved. Intriguingly, at a particular time during embryogenesis, histones exchange ceases. We are now examining the molecular basis of this transition and its physiological consequences. Our work may provide a paradigm for how LDs regulate and buffer protein availability in general.

LI-02
CARBOXYLESTERASES: NOVEL THERAPEUTIC TARGETS IN NONALCOHOLIC FATTY LIVER DISEASE

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Aberrant triacylglycerol (TG) metabolism is central in obesity and associated pathologies that include insulin resistance and type 2 diabetes, nonalcoholic fatty liver disease and cardiovascular disease. Fatty liver is the leading cause of abnormal liver functions. We have recently demonstrated that an endoplasmic reticulum-localized carboxylesterases modulate hepatic TG content. Carboxylesterase 1d/Triacylglycerol Hydrolase (Ces1d/TGH, also termed Ces3 in mice or CES1 in humans) participates in the provision of substrates for very-low density lipoprotein (VLDL) assembly. Mice lacking Ces1d/TGH show decreased blood lipid concentration, improved glucose metabolism and are protected from high fat diet-induced atherosclerosis and inflammation. Therefore, one can conclude that Ces1d/TGH plays a pro-atherogenic, pro-diabetic and pro-inflammatory role. Carboxylesterase 1g/Esterase-x (Ces1g/Es-x), which shares 76% amino acid sequence identity with Ces1d/TGH, exhibits different function to Ces1d/TGH. Mice lacking Ces1g/Es-x show hallmarks of metabolic syndrome including insulin resistance, hyperinsulinemia, increased lipogenesis, hepatic and adipose lipid accumulation and hyperlipidemia. Therefore, Ces1g/Es-x is protective against the development of hyperlipidemia, hyperinsulinemia and insulin resistance.

LI-03
THE ROLE OF SPHINGOLIPID METABOLISM IN PROLIFERATION, DIFFERENTIATION AND TISSUE ORGANIZATION

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Sphingolipids (SLs) are a diverse group of lipids which serve a variety of functions in mammalian cell physiology. Sphingosine Kinase (SK) and its product sphingosine-1-Phosphate (S1P) are classically recognized to play critical roles in cell proliferation and survival. We investigate the importance of SK/S1P pathway in cellular fate of MDCK cells subjected to hypertonic medium. In this condition the cells pass through three stages: proliferative, confluent and finally differentiated state. We observed that in proliferative stage the partial inhibition of SK induced a cell cycle arrest in G0. Moreover, we observed an induction to differentiation in a mechanism that did not involved S1P receptor activation. On the other hand, we demonstrated that for the acquirement of the

differentiation phenotype, MDCK cells required a basal S1P synthesis to maintain adherent junction (AJ) formation. In this condition SK-S1P pathway, by modulation the de novo synthesis of SLs, regulated the establishment of AJ. Preservation of epithelial tissue requires an efficient renewal of cells initiated by their extrusion. In this condition SK inhibition and knock-down avoided the correct extrusion. These results showed that SK/S1P pathway has multiple functions on the cellular fate depending on their physiological condition.

LI-04

SIZES OF LIPID RAFTS: WHAT HAVE WE LEARNT FROM ARTIFICIAL LIPID MEMBRANES?

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Lipids are able to arrange in different supramolecular structures (artificial model membranes), and phase coexistence is often observed, with domain sizes distributing in a very wide range, starting from the nanometer (reported in Giant Unilamellar Vesicles and supported films) to the micrometer (observed in a lot of model membranes). Domain growth by coalescence and Ostwald ripening are generally slow (minutes to hours), being the domain size correlated with the size of the capture region. Therefore, domain sizes strongly depend on the amount of domains which, in the case of a nucleation process depends on the oversaturation of the system, on line tension and on the perturbation rate in relation to the membrane dynamics. Here, the influence of each of these factors on the distribution of sizes of the domains in different model membranes will be discussed. The analyzed parameters respond to very general physical rules, and therefore a similar behavior for the rafts in the plasmatic membrane of cells is proposed, but taken into account the obstructed mobility of the molecules and the continuous changes in the system.

WEDNESDAY, November 9th, 2016

LECTURES

L-05

TRANS-GENERATIONAL ANTIVIRAL IMMUNITY IN INSECTS

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Effective antiviral protection in multicellular organisms relies on both cell autonomous and systemic immunity. Systemic immunity mediates the spread of antiviral signals from infection sites to distant uninfected tissues. In arthropods, RNA interference (RNAi) constitutes the main intracellular antiviral response. Whether insects possess a systemic antiviral protection system remains unclear. Here we show that insects have a complex systemic RNAi-based antiviral response mediated by macrophage-like haemocytes. Haemocytes take up dsRNA from infected cells and, through endogenous transposon reverse transcriptases, produce virus-derived complementary DNAs (vDNA). These vDNAs recombine with endogenous retrotransposons and integrate into the insect genome. We discovered a diverse set of endogenous viral elements (EVEs) in insect genomes that are responsible to generate active antiviral sRNAs. EVEs are acquired horizontally and are integrated into piClustersto template *de novo* synthesis of secondary viral siRNAs (vsRNA). In turn, EVE-derived siRNAs are secreted in exosome-like vesicles to protect uninfected tissues. Thus, similar to vertebrates, insects use specialized cells to generate acquired systemic antiviral immunity and immunological memory.These results suggest the exciting possibility that insects have evolved CRISPR-like, trans-generational adaptive antiviral immunity through the acquisition of EVEs that serve as templates for biogenesis of small antiviral RNAs.

L-06

MOLECULAR MECHANISMS, BIOLOGY AND DISEASE OF MAMMALIAN tRNA SPLICING

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Institute of Molecular Biotechnology– IMBA – and Medical University of Vienna.

Transfer RNAs (tRNA) are encoded as precursor molecules that must undergo processing to generate mature tRNAs for protein translation. Processing entails chemical modifications and removal of 5'-leader, 3'-trailer and intronic sequences. Removal of introns during pre-tRNA splicing requires endonuclease andligase activities. We have dissected the pre-tRNA splicing machinery in mammalian cells identifying the 5'-RNA-kinase CLP1, a subunit of the tRNA splicing endonuclease (TSEN) complex; the pentamerichuman tRNA ligase complex, joining tRNA exon halves; and archease, a co-factor that provides multiple turnover to the tRNA ligase. We havealso adventured *in vivo*by generating knockout and knock-in mice and analyzing fibroblasts from patients with mutations in CLP1 and TSEN: We uncovered the function of CLP1 in motor neuron disease and assigned the tRNA ligase complex and archease to the cytoplasmic splicing of the Xbp1-mRNA during the unfolded protein response. We are currently investigating the unexpected role of the tRNA ligase complex in oxidative stress and digging into the yet obscure topic of RNA repair by purifying a novel, 2', 3'-cyclic phosphodiesterase in human cells. Taken together, thesestudies contribute to a renewed interest in the so-called "old" tRNA molecules, with highlights from their synthesis, processing and functions beyond translation.

SYMPOSIA

CB-01
CELL REPROGRAMMING OF HUMAN CELLS DURING THE EARLY *Trypanosoma cruzi*INFECTION

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Trypanosoma cruzi invades almost any type of cell: when parasites enter their host the establishment of the infection depends on their ability to rapidly invade epithelial cells that constitute the first barrier against infections; trypanosomes can invade macrophages, with consequent relevance on immunity, and in chronic disease a significant percentage of patients evolve to cardiomyopathy. We studied the early response of human cells to *Trypanosoma cruzi* infection, in different cells through the analysis of the transcriptome, showing that hundreds of genes are up and down regulated immediately after infection. Surprisingly, each cell type has extremely different responses. Epithelial cells are mostly altered in pathways related to inflammatory and lipid metabolism genes; in cardiomyocytes energy metabolism and protein synthesis related genes are the most affected pathways; in macrophages although, as expected, immune response related genes are the most affected, a more in deep analysis at the level of alternative splicing patterns indicates that the most up regulated genes are related to autoimmune diseases. In summary, human cells are reprogrammed by *T. cruzi*, and early responses are probably exploited by the parasite to establish the initial infection and posterior systemic dissemination.

CB-02
GENOME PLASTICITY, CELLULAR STRESS AND CELLULAR REPROGRAMING IN HUMAN BREAST CANCER

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Genome plasticity can be thought as ageneral principleunderpinning how cells functionally organize, make use of, or modify DNA sequencesto integrate multiple molecular circuits into a unifiedgene expressionoutput affecting cell fatein response to environmental cues.Myresearchfocuses on the molecular mechanisms of gene regulation inhuman mammary cellsand its involvementin both cancer initiation and progression.I will present data showing how cellular stresscan play decisive but alsosubordinate roles in determining cell fatein different stages of the cancer progression timeline.For example, the ubiquitously active Transforming Growth Factor (TGF)-β/Smad pathway overrides the stress response in pre-cancerous and tumor cells contributing to tumorigenesis and drug resistance by affecting both transcription and translation of the p53 coding gene. On the other hand, we have found through a single-cellRNA-sequencing approach that stress can shape abundance and fidelity of RNAs securing diversity within cancer cells populationsand helping to sustain reversible drug tolerance states. I will also discussour most recent approaches to understand how cellular stress can impact on the physical and functional genome organization of normal mammary cells. I will discuss the implications of these findings in our understanding of cancer evolution and potential newer treatment avenues.

CB-03
THE PROCESSING AND MATURATION OF NEWLY SYNTHESIZED HISTONES

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Histone proteins are synthesized in the cytoplasm by free ribosomes, after which they are translocated into the nucleus for its deposition into chromatin. Before nuclear translocation occurs, newly synthesized histones undergo a cascade of events that allow them to acquire their correct folding and to establish post-.translational modifications. In this processing pathway, at least four different protein complexes participates, each one comprised of specific histone interacting proteins, including chaperones. How this processing pathway is regulated and what is the impact into gene expression remains unclear and is the focused of our research. Methylation of lysine 9 on histone H3 (H3K9), a mark that primes the formation of heterochromatin, is the only lysine methylation detected on newly synthesized histone H3. We showed that H3K9 mono- and dimethylation is imposed during translation of histone H3 by the methyltransferase SetDB1. We discuss the importance of these results in the context of heterochromatin establishment and maintenance. Our results enabled us to propose a regulatory means of these marks for controlling cytoplasmic/ nuclear shuttling and the establishment of early modification patterns.

CB-04
CELLULAR REPROGRAMMING AND EPIGENETIC CONSEQUENCES

Meirelles FV[1], Bressan FF[1], Machado LS[1], Lima MA[1], Therrien J[2], Smith LC[2].
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Reprogrammed cells may be generated through nuclear transfer (NT) or forced expression of pluripotency factors (iPS cells) *in vitro*, and may contribute to both increasing animal production technologies and for regenerative medicine. However, the epigenetic process that enables cellular reprogramming is usually incomplete or impaired, resulting in unfavorable outcomes such as unhealthy offspring and poorly reprogrammed iPS cell lines. For instance, imprinted genes, essential for embryo and placental development, are reported to be severely disturbed by reprogramming techniques. We have reported that H19 and SNRPN imprinted genes were disturbed in NT-derived embryos and fetuses. We further investigated the methylation pattern and expression of imprinted genes in iPS lines generated from fetal fibroblasts (bFF). iPS line was differentiated into primordial germ-like cells (PGCs) also resulted in abnormal patterns of methylation in imprinted genes were observed in iPS cells when compared to bFFs; and hypomethylation or even demethylation was observed when iPS cells were induced into PGCs-like cells, probably due to the *in vitro* epigenetic induced errors. Epigenetic errors

are observed in biPS clones as well as cloned offspring, suggesting a direct connection between low success rates of cloning and iPS derivation procedures.Financial support: NSERC, FAPESP (13/13686-8, 11/08376-4, 57877-3/2008, CTC-08.135-2/2013); CNPq (573754/2008-0, 482163/2013-5).

MI-01
CROSS TALK BETWEEN SIGNAL TRANSDUCTION SYSTEMS CONTRIBUTES TO PNEUMOCOCCAL PATHOGENESIS.

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Living cells modulate their gene expression patterns in response to environmental cuesto adapt and to survive. In bacteria, extracellular signals are transduced into the cells mainly by signal transduction mechanisms named as two-component systems (TCS). *Streptococcus pneumoniae,* a significant bacterial human pathogen, induce competence by a quorum-sensing mechanism associated to the ComDE TCS under slight alkaline conditions. We previously described thatacidic stressinduces two types of cellular response in *S. pneumoniae*, either by triggering cell death by autolysis (and releasing the pneumolysincytotoxin), or by inducing a survival mechanism known as acid tolerance response (ATR). In this work, we report an alternative activation pathway of ComE. We performed molecular, biochemical and functional assays to characterize another signal transduction system that activates ComE by crosstalk phosphorylation. We also found that this new signaling pathway regulates autolysis and ATR under acidic stress, and that these mechanisms modulate the intracellular survival of *S. pneumoniae* in pneumocytes. We propose that the convergence of these signal transduction systems represents a key pathway in the global stress response and contributes to the pneumococcal pathogenesis.

MI-02
STRUCTURE, FUNCTIONAL PREDICTION, AND PHENOTYPING STUDIES ON GENES ENCODING PROTEINS INVOLVED IN CYCLIC-DI-GMP IN *Azospirillum.*

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The cyclic-di-GMP (c-di-GMP) encompasses a signaling system that regulates many bacterial behaviors among them, the switch between motile and sessile life-styles in bacteria. Cell c-di-GMP level in bacteria is regulated by opposite enzyme activities of diguanylate cyclase (DGC) and phosphodiesterase (PDE), which are proteins possessing GGDEF and EAL domains, respectively. We analyzed *Azospirillum brasilense* and *Azospirillum lipoferum* genomes, by using bioinformatics and structural approaches to determine how many genes occur in genomes, encoding for predicted proteins involved in turnover of c-di-GMP. Analyzed sequences showed that, *A. brasilense* Sp245, Sp7, and AZ39 encode for 36, 32, and 36 proteins, respectively. *WhileA. lipoferum* B510 and 4B encode for 42 and 41 proteins, respectively. 22 proteins are conserved in all genomes including 10 DGCs, 4 PDEs, and 8 hybrid proteins. As reported in other soil bacteria, *Azospirillum* genomes encode for a large number of predicted proteins involved in c-di-GMP metabolism. Weanalyzed four DGC proteins encoded by *cdgA, cdgB, cdgC*, and *cdgD* genes from *A. brasilense* 245 by mutant construction and comparative analysis of motility and adherence phenotypes against WT, by motility assays, biofilm and exopolysaccharide (EPS) production, and microscopy observations. This work shows that genes studied were functional and participate in motility, chemotaxis, biofilm formation and cell division.

MI-03
*Burkholderia xenovorans*LB400 SYNTHESIZES A NOVEL NON-RIBOSOMAL PEPTIDE SIDEROPHORE FOR IRON TRANSPORT

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B. xenovorans LB400 is a model bacterium to study the metabolism and biotransformation of aromatic compounds. The aim of this study was to characterize a non-ribosomal peptide synthetase containing gene cluster in *B. xenovorans* LB400. LB400 *mba* gene cluster encodes proteins involved in the biosynthesis and transport of a siderophore, and possesses a unique *mba* gene organization. Bioinformatic analysis revealed in the *mba* gene cluster the presence of promoters that are probably regulated by the ferric uptake regulator protein Fur and by the RNA polymerase extracytoplasmic function sigma factor MbaF. RT-PCR analyses showed under iron limitation the expression of six iron-regulated transcriptionalunits. Chrome azurol S assay indicates that strain LB400 synthesized a siderophore. ESI-MS and MALDI-TOF-MS analyses revealed that the siderophore is a non-ribosomal peptide that forms an iron complex of 676 Da. Based on bioinformatic prediction and functional analyses, we propose a novel structure of the LB400 siderophore involved in iron transport, which is closely related to malleobactin-type siderophores from other *Burkolderiales*. *Supported by RIABIN, FONDECYT (1151174 & 1110992) and USM (131562 & 131342) grants.*

MI-04
UNLEASHING THE CATALYTIC POTENTIAL OF ENVIRONMENTAL BACTERIA

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Novel microbial cell platforms are required to expand the scope of basic knowledge and for a number of technical applications, and this quest has been further boosted by the increasing availability of dedicated genetic toolboxes over the last few years. The so-called "model" bacteria, such as *Escherichia coli* or *Bacillussubtilis*, are mostly appropriate hosts in the context of basic research but they are often not entirely suitable for performing some specific applications. Contemporary Synthetic Biology relies on the adoption of a biological *chassis* for plugging-in and -out genetic circuits and for engineering new-to-Nature functionalities. In contrast to several well established hosts, environmental bacteria constitute an almost ideal starting point to design flawless microbial *chassis*, since these microorganisms are pre-endowed with a number of metabolic and stress-endurance traits which are optimal for biotechnological needs. One such example is represented by the ubiquitous soil bacterium *Pseudomonas putida* KT2440, originally isolated from polluted soil, and perhaps the best saprophytic laboratory Pseudomonad that had retained its ability to survive and function in the environment. Against this background, recent developments on the metabolic taming of *P. putida* will be discussed in the context of Synthetic Biology strategies.

THURSDAY, November 10th, 2016

LECTURES

L-07
BRAIN CHOLESTEROLDYSREGULATION WITH AGE: CONTRIBUTION TO THE COGNITIVE DEFICITS OF THE OLD.

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Aging is associated with the occurrence of numerous physiological modifications in various organs including the brain. With advancing age, neurons lose their capability to adapt to and recover from accumulating and potentially damaging alterations such as oxidative stress, DNA damage, mitochondrial impairment, and protein misfolding and aggregation. One of the most evident consequencesof these biochemical changes are deficits in learning and memory. While these are, in general, minimally invalidating, the underlying biochemical alterations constitute an indispensable condition for the occurrence of pathological brain aging. In fact, althoughaging is not sufficient it is a necessary condition for neurodegenerative conditions like non-familial Alzheimer´s disease. Thus, we searched for potential genetic master switches responsible forthe age-associated cognitive loss. In addition to unbiased screenings, we searched for up and down-regulated genes responsible for the levels of synaptic lipids, asthey make the mattresses from whichall membrane receptors signal to survival and performance pathways. We identifiedage-associated changes in the levels of expression of cholesterol and sphingomyelinregulatory genes. In this presentation, I will address the causes and short and long-range consequences of the change in expression of a cholesterol catabolism gene.

L-08
RISKS AND CHALLENGES ASSOCIATED WITH CLIMATE CHANGE

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"Climate Change" is a concept globally used as a reference to the impact of human activities on the global climate, mainly through changes in the atmosphere composition. Decades ago, the possibility of such anthropogenic influence was alerted by the international scientific community. On December 2015, the countries adopted the Paris Agreement, in which they agreed to work to limit global temperature rise. In this context, the lecture will provide a brief review of the scientific basis underlying the climate change at global scales and in particular over South America. Special focus will be made in describing the key climate change signals influencing Argentina that require the implementation of adaptation options. The lecture will also discuss the last inventory of greenhouse gases emissions of the country and the potential mitigation options to reduce them.
The global scientific assessment reports made by the Intergovernmental Panel on Climate Change (IPCC) have a large influence on the government decisions made not only at United Nation levels but also at regional and national levels. The lecture will describe which are the current and future challenging questions that the global scientific community needs to urgently address and communicate, not only related with the climate change but also with the more general framework of the sustainable development.

SYMPOSIA

NS-01
MECHANISM OF TAU-INDUCED NEURODEGENERATION: IDENTIFICATION OF NEW PUTATIVE THERAPEUTIC TARGETS

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Hyperphosphorylated tau is one of the markers of Alzheimer disease and other tauopathies. We have shown that the conformational change on tau induced by hyperphosphorylation results in a gain of toxic function that disrupt the microtubule system, act as a "prion" protein to the normal tau and translocate into the nucleus and ourin vitro preliminary results suggest that tau might be involved in mRNA stability.

We created an inducible pseudophosphorylated tau (Pathological Human Tau, PH-Tau) mouse model to study the effect of conformationally modified tau in vivo. Leaky expression resulted in two levels of PH-Tau; 4% basal and 14% upon induction of the endogenous tau respectively. Unexpectedly, low PH-Tau resulted in cognitive deficits, decrease in the number of synapses and synaptic proteins, and localization to the nucleus. Induction of PH-Tau triggered neuronal death (60% in CA3), astrocytosis, and loss of the processes in CA1. These findings suggest that changes in tau phosphorylation and localization might play a key role in controlling cognitive functions by two different mechanisms depending on the levels of PH-Tau expression, ranging from microtubule stability to regulation of gene expression, affecting the neuronal transcriptome before the appearance of traditional markers.The understanding of these two different mechanisms will provide new therapeutical targets.

NS-02
AAV9 GENE THERAPY AND HEMATOPOIETIC TRANSPLANT PREVENT NEUROLOGICAL DECLINE IN KRABBE DISEASE

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Krabbe disease is a lethal genetic disorder, which causes progressive central and peripheral demyelination, sensory-cognitive deficits, muscle atrophy, and early death.The disease is due to loss-of-function mutations in the gene encoding for the lysosomal enzyme galactosylceramidase (GALC) and the resulting toxic accumulation of the lipid psychosine.The current standard of care for Krabbe patients is Hematopoietic Stem Cell Transplantation (HSCT) from healthy donors, which extends lifespan, but still results in serious debilitating traits.

Here, we developed, optimized, and testedthe use ofadeno-associated virus serotype9 (AAV9) to correct for GALCdeficiency in combination with HSCT.Using the Twitcher mouse model of Krabbe disease, we show that AAV9 gene therapy restored GALC activity in CNS, PNS, and systemic organs, thereby significantly reducing the accumulation of psychosine.Immunohistology demonstrated spreadtransduction of central neurons and astrocytes. When combined with neonatal HSCT, AAV9 gene therapy nearly completely corrected the neurological phenotype, with twitcher mice surviving by over 1000% of their expected lifespan. Histopathologyshowed the reversal of demyelination, neuro-inflammation, and neuropathy. These results reveal the profound benefit of AAV9 gene therapy could have on human Krabbe'spatients when used in conjunction with current therapies.

NS-03
MELATONIN SIGNALING PATHWAYS IN MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is an immune-mediated disease of the central nervous system characterized by the destruction of myelin by autoreactive T cells. $CD4^+$ T cells producing IFN-γ (Th1 cells) or IL-17 (Th17 cells) are considered important contributors to MS immunopathogenesis. $FoxP3^+$ regulatory T cells (Tregs) and IL-10-secreting type 1 regulatory T cells (Tr1) regulate the activity of effector T cells, accordingly, deficits in Tregs and Tr1 cells have been described in MS. Seasonal changes in disease activity have been observed in MS, suggesting that environmental factors influence the disease course. We found that melatonin levels, whose production is modulated by seasonal variations in night length, negatively correlate with MS activity in humans. Treatment with melatonin ameliorates disease in an experimental model of MS, autoimmune experimental encephalitis, and directly interferes with the differentiation of human and mouse T cells. Melatonin induces the expression of the repressor transcription factor Nfil3, blocking the differentiation of pathogenic Th17 cells and boosts the generation of protective Tr1 cells via Erk1/2 and the transactivation of the IL-10 promoter by ROR-α. These results suggest that melatonin is another example of how environmental-driven cues can impact the immune system and have implications for autoimmune disorders such as MS.

NS-04
MODULATING LIPIDS IN THE BRAIN: TOWARDS A THERAPY FOR NIEMANN PICK DISEASE

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Alterations of brain lipid levels contribute to the pathology of an increasing number of neurological diseases including lysosomal storage disorders and neurodegenerative diseases. Understanding the roles that lipids play in neurons and whether we can modulate their levels and counteract the consequences of their alterations are main goals in our laboratory. In recent years we have addressed these questions using mice lacking the acid sphingomyelinase (ASMko),

which mimic Niemann Pick disease type A. This is a genetic lysosomal storage disorder causing neurodegeneration and early death. We have determined that the most abundant sphingolipid, sphingomyelin, accumulates in the lysosomes and in plasma and synaptic membranes of ASMko mice neurons. High sphingomyelin levels lead to unpolarized distribution of molecules, impaired autophagy, calcium imbalance and synaptic alterations. We have characterized the molecular mechanisms leading to these phenotypes that include inefficient endocytosis, lysosomal membrane permeabilization, impairment of the plasma membrane calcium ATPase and low actin polymerization. These read outs have allowed us to evaluate the efficacy of different compounds that can cross the brain blood barrier and diminish sphingomyelin storage and/or its deleterious consequences in the brain opening therapeutic perspectives for a devastating disease.

ST-01
IN THE SHADOW OF THE FLY: MOLECULAR MECHANISMS OF SHADOW ENHANCERS IN DEVELOPMENT

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An abiding mystery in biology is how a single cell develops into a multicellular organism. As this cell divides, its progeny read their DNA to become the cell types such as muscle, liver, and brain cells. We now know that during development, cells not only decide which genes to express; they also decide about when, where, and how to express them. This gene expression control is dictated by DNA sequences called enhancers. Recently, it was discovered that many developmental genes are regulated by additional “shadow” enhancers: both primary and shadow enhancers can independently drive comparable patterns of gene expression. However, it is not clear how enhancer pairs work together and whether they exercise regulatory functions that a single enhancer cannot. I will discuss how we used the fruit fly *Drosophilamelanogaster* to uncover the molecular mechanisms behind the combined action of enhancer pairs by integrating theoretical modeling with novel technology to quantify enhancer activity in single cells of a developing embryo. Our results suggest that the competition of enhancer pairs for the target gene can give rise to different behaviors—they can work additively, subadditively, or superadditively. This work provides a framework to predictively understand developmental programs by identifying the regulatory strategies used by the fruit fly embryo to shape the adult body plan.

ST-02
A CHLOROPLAST RETROGRADE SIGNAL REGULATES NUCLEAR ALTERNATIVE SPLICING… IN THE ROOTS!

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Light is a source of energy and also a regulator of plant physiological adaptations. We have previously shown that light/dark transitions affect alternative splicing of a subset of Arabidopsis genes, preferentially those encoding proteins involved in RNA processing. These effects require functional chloroplasts and are also observed in the roots when the communication with the photosynthetic tissues is not interrupted, suggesting that a signaling molecule travels through the plant. We are now aiming to identify the nature of the light signals that communicate the chloroplast status to the nuclei of leaf and of root cells. Focusing on the determination of the nature of the mobile signal that impacts in the roots, we have found evidence implying sugars as the main candidate to be responsible for the observed effects in the non-photosynthetic tissues, and we are currently dissecting the signaling pathway in the root cells.

ST-03
A CELL CYCLE CHECKPOINT TO INSURE THE INTEGRITY OF THE CELL WALL SYNTHESIS

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Critical events during cell cycle progression are regulated by cell cycle checkpoints to ensure the proper completion of cell division. A cell cycle checkpoint to insure the integrity of the cell wall synthesis in the budding yeast is called the cell wall integrity (CWI) checkpoint (Suzuki et al., 2004; Nat Cell Biol 6:861-). Without a supply of cell wall materials for bud growth, the cell cycle is arrested with duplicated and adjacent SPBs before entry into M phase by downregulating M-phase cyclin *CLB2*. We have identified more than 20 factors involved in this checkpoint. In addition to dynactin complex (Arp1, Nip100 & Jnm1), Las17 complex, HOG MAPK signaling pathway, CWI MAPK signaling pathway, late S-phase transcription factor Hcm1 (Negishi et al., 2016; Mol Cell Biol 36:491-) and M-phases specific transcription factors (Fkh2 & Ndd1) had a critical function in the CWI checkpoint. Examination of double mutants suggested that HOG MAPK functions in the upstream of the dynactin complex and Las17 complex, as well as CWI MAPK pathway. These signaling fed to the downstream negative regulation of the transcriptional machinery. Our study revealed cellular pathways that regulate proliferation in response to cell wall stresses to coordinate the cell cycle and the cell wall synthesis.

ST-04
ROLE OF CYCLIN DOCKING IN CDK SUBSTRATE CHOICE AND MULTI-SITE PHOSPHORYLATION

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Eukaryotic cell division is driven by cyclin-dependent kinases (CDKs). Distinct cyclin-CDK complexes are specialized to drive different cell cycle events, though the key molecular differences are only partly understood. In budding yeast, cell cycle entry is regulated by three G1 cyclins. We found that some CDK substrates contain a novel docking sequence (“LP” motif) that is recognized only by specific G1 cyclins and not by later S- or M-phase cyclins. To probe the molecular basis, we used phylogenetic comparison to identify key cyclin residues that permit docking. Mutation of these residues disrupts efficient, multi-site phosphorylation of substrates and causes a delay in cell cycle entry. To shed light on how cyclins became functionally diversified during evolution, we have tested homologs from numerous fungi. The findings suggest that LP docking existed in an ancestral fungal G1 cyclin, and then diverged among distinct subgroups of yeast cyclins to yield a pattern of both shared and unique targets. Finally, we are studying why some CDK substrates are poorly phosphorylated in M phase. The results imply selective pressure on M-cyclins to reduce potency, which may impose greater reliance on cyclin-specific recognition mechanisms. These studies illuminate how variation in both substrate docking and intrinsic potency of cyclins helps shape the CDK-substrate network.

ORAL COMMUNICATIONS

BIOTECHNOLOGY

BT-C01
CHARACTERIZATION OF ANTARCTIC MICROBIAL PHOTOLYASES AND RECOMBINANT PRODUCTION

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Hymenobacter and *Sphingomonas* are microbial genera widely known for their high resistant to UV radiation. Photolyases are enzymes that repair the DNA lesions (cyclobutane pyrimidine dimers, CPD , and 6,4 photoproducts, 6,4-FP) produced by UV-irradiation (mainly by UVC). These enzymes are flavoproteins that belong to the photolyase/cryptochrome family. The aim of this work was the search for photolyases in two Antarctic UVC-resistant isolates, *Hymenobacter sp*. UV11 and *Sphingomonas sp*. UV9, and their photolyase in silico characterization. Both, UV11 and UV9 genomes were sequenced using Illumina HiSeq 2000 and annotated using RAST. The search in the annotated genomes showed that UV11 produce a CPD-photolyase, whereas UV9 produce two CPD-photolyases and a 6,4 photolyase. Their classification as novel CPD- or 6,4-photolyases was supported by their comparison with references sequences (by BLAST using NCBI and the cryptochrome DataBase) and by the construction of their evolutionary history. Their modeling and structure visualization/comparison were performed using HHpred and Pymol. The activity of the UV11 photolyase CDS was confirmed by recombinant production and activity using comet assays. Results suggest that UV9 and UV11 produce novel CPD- and 6,4-photolyases, with potential biotechnological applications.

BT-C02
BIOCHEMICAL CHARACTERIZATION OF A CELLULOLYTIC COCKTAIL FROM AN ANTARCTIC *Flavobacterium*ISOLATE

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Cellulases are a group of enzymes that hydrolyze the glycosidic bonds of cellulosic materials. They have many potential industrial applications, including pulp and paper and biofuel, among others. Thus, the search for novel cellulolytic enzymes that affect industrial strategies is continuously in progress. The aim of this work was the characterization of a cellulolytic raw preparation produced by a psychrophilic Antarctic isolate, *Flavobacterium sp*. AUG42. Psychrophilic microbes produce cold-adapted enzymes that usually show high catalytic efficiency, kcat/KM, as compared with enzymes produced by mesophilic microbes. AUG42 produces endo- and exo-cellulases at the stationary growth phase, using filter paper (FP), avicel, cellulose and carboxymethyl cellulose (CMC) as carbon source, showing the largest production using FP. The enzymatic preparation exhibited maximal endo- and exo-cellulase activity at 50°C/5.5 and 50°C/4.5 (temp/pH), respectively. In addition, CMC zymography and isoelectric focussing suggested the production of at least 4 cellulases with pI in the range of 4,5-7,5. Currently, the protein bands that showed activity are under analysis for MALDI-TOF MS. Both activities were inhibited by few compounds. These properties may allow their use as biocatalyst for saccharification of industrial wastes and fermentation by *S. cerevisiae* for the production of bioethanol.

BT-C03
CADMIUM AND LEAD RESISTANT RHIZOSPHERIC BACTERIA AS CANDIDATES FOR RHIZOREMEDIATION PROCESSESS

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Although *Helianthus petiolaris* has been identified as a cadmium and lead tolerant plant species, little is known about its rhizospheric microorganisms. Such microorganisms are crucial for enhancing the plant biomass production and tolerance to heavy metals, accelerating the phytoremediation process of contaminated soils. The aim of this work was to isolate and identify the cadmium and lead tolerant rhizospheric bacteria from *H. petiolaris* and assess its plant growth-promoting (PGPR) capabilities. Bacteria isolation was performed by washing the roots with saline and saline/Tween 80 solutions, which were inoculated into sterile GY media supplemented with either 10 mg $L^{-1}Cd^{+2}$ or 100 mg $L^{-1}Pb^{+2}$. After several rounds of enrichment culture in the GY/metal medium and subsequent purification by plate culture, nine different morphotypes were isolated. From biochemical analysis and 16S rRNA sequencing, the lead resistant bacteria were identified as *Bacillus subtilis*, *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Rhodococcus equi*. Identification of cadmium tolerant strains is in progress. In addition plant growth promotion capabilities, such as phosphate solubilization, phytohormones production (AIA), nitrogen fixation and siderophore production, were determined for the strains.

CELL BIOLOGY

CB-C01
NEURAL STEM CELL DIFFERENTIATION INDUCED BY LIPIDS

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Neural stem cells (NSCs) have potential for self-renewal and differentiation into neurons, astrocytes and oligodendrocytes during nervous system development and after brain injuries; however, in the last case, this capacity is limited. We have previously shown that phosphatidylcholine (PtdCho) enhances neuronal differentiation while phosphatidyletanholamine (PtdEtn) promotes glia differentiation. By Time Lapse Microscopy analysis we concluded that these phospholipids affect precursors (undifferentiated post mitotic cells) but not progenitors (dividing cells) behavior. Furthermore, we demonstrated that these lipids neither affect the rate of cell proliferation nor the mode of NSCs division. By immunofluorescence analysis using specific markers, we evidenced that PtdCho acts on unspecified precursors driving neuronal specification and, as a consequence, promoting neurogenesis. Furthermore, under this condition, astrogenesis is reduced due to a decrease in astrocytes precursors. In the case of PtdEtn, it enhances astrocytes specification and differentiation. To get insight into the molecular mechanism, we analyzed the signaling pathways that participate in each differentiation process specifically altered by PtdCho or PtdEtn, and identified that PtdEtn but not PtdCho induces astrocyte differentiation activating the MEK-ERK pathway.

CB-C02
SUPPRESSION OF STARD7 PROMOTES ENDOPLASMIC RETICULUM STRESS AND INDUCES ROS PRODUCTION

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StarD7 transcript encodes an intracellular lipid transport protein, a member of the START domain superfamily, which is involved in many physiological processes. It facilitates the delivery of phosphatidylcholine to the mitochondria and previous results indicated that StarD7 knockdown decreases A*CB*G2 multidrug transporter level, cell migration, proliferation, and phospholipid synthesis. Since lipids and protein transport between organelles are involved in the organization of the different cell compartments, we hypothesized that StarD7 may be involved in maintaining cell homeostasis. We analyzed the effect of StarD7 silencing on endoplasmic reticulum (ER) stress response and on the production of reactive oxygen species (ROS) in HepG2 cell line. StarD7 knockdown generated alterations in mitochondria and ER morphology, initiating an unfolded protein response pathway associated with an increased basal ROS and augmented levels of the hemeoxygenase-1 and catalase enzymes. Also, StarD7 silencing reduced cell viability after H2O2 exposure. Moreover, downregulation of p53 by a degradation mechanism was established in StarD7 siRNA cells. Finally, no changes in autophagy and apoptosis were observed in StarD7 siRNA. Together these results indicate that, beyond its role in lipid transport, StarD7 contributes to maintain cellular redox homeostasis.

CB-C03
STRATEGY TO STUDY PARAMETERS ABLE TO PREDICT LONGEVITY IN MEDFLY POPULATIONS

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The Medfly (*C.capitata*) is the main orchards world pest. Functional Senescence refers to real physiological output, eventually leading to different mortality rate. To predict changes in longevity dependent on genetic microheterogeneity of Medfly lab populations subjected or not to stress we worked out both lethal and non-lethal assays. To establish a parameter reflecting different homeostatic equilibria, we analyzed quantitative changes of 38 Lipids categories, further reduced to significant 25 ones. Thus giving rise to

Principal Components for each sex and body parts. Higher temperature of growing, chill coma induction or oxidative stress induced by Hematoporphyrin were the main factors studied. These and other biochemical and gene expression parameters were correlated with non lethal estimations of behavioral output like mobility and RING (negative geotaxis) experiments. Looking further for longevity predictors we analyzed spontaneous and/or fight-induced supine events during the early adulthood of medflies. We demonstrated a correlation between the rate of supine events and longevity that also roughly correlated with changes in biochemical and gene expression parameters. Most important, supine rate also correlated with other non-lethal assays like RING. Age- and stress-dependent changes in a peculiar immune reaction were also explored

CB-C04
LOW NRF2 EXPRESSION DETERMINES LOW ADIPOGENESIS, INFLAMMATION AND HIGH METABOLIC RISK IN BOYS AND RA

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Excess of energy is metabolized to free fatty acids which should be stored as triglycerides otherwise they cause inflammation. Nrf2 controls the expression of phase II, antioxidant and adipogenic genes. Low Nrf2 expression may determine inflammation and a high metabolic risk in obesity. To test this hypothesis we made a study in children and in an experimental model in rats. We measured clinical and biochemical parameters related to lipid metabolism, oxidative stress and metabolic syndrome in a population of overweight boys (OW, n=22) and normal weight boys (NW, n=27) from San Luis City. Compared to NW, OW boys had insulin resistance, higher atherogenic index, altered plasma lipid profile, increased markers of oxidative stress and inflammatory fatty acids. GPx activity and GSH/GSSG ratio and leukocyte Nrf2 expression were also lower. Nrf-2 expression negatively correlated with metabolic syndrome parameters in OW boys. Experimentally we fed SD rats (n=12) for 16 weeks with a hypercaloric diet and found that some rats were obesity sensitive (OS, n=7) whereas the others were obesity resistant (OR, n=5). Compared to OS and in perirenal adipose tissue, OR rats showed increased oxidative stress (NOX2), inflammation (VCAM), but reduced Nrf2, PPAR-γ and lipogenic enzyme expression. Low Nrf2 expression determines reduced adipogenesis; but increased inflammation and metabolic risk.

CB-C05
NATURAL GENETIC VARIATION DETERMINES PROMOTER SHAPE, AFFECTING ROBUSTNESS OF GENE EXPRESSION

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Promoters act as platforms for converging cis-regulatory cues, which regulate the initiation of gene expression. Metazoan core promoters have characteristic distributions of transcriptional start sites (TSS), a feature called promoter shape. Although fundamental to understanding transcriptional initiation, the genetic determinants and functional differences between promoters with different shapes remain unclear. Here, we measured TSS usage across a panel of 81 *Drosophila* lines, identifying thousands of thousands of promoters where promoter strength and/or shape are modulated by common genetic variants. Our results identify promoter shape as a variable trait, evolvable independently of promoter strength. Broad promoters, typical of housekeeping genes, show an increase in the number of genetic variants affecting transcription. Since these variants have lower impact in promoter strength than those of narrow promoters, broad promoters appear as intrinsically more robust to genetic variation. By using single-cell expression analysis, we show that shape-shift mutations frequently increase expression noise, while other variants within the same promoter can alleviate these effects, often through epistatic interactions. This study uncovers new functional properties of natural promoters, and proposes the minimization of noise as an important factor influencing promoter evolution

CB-C06
MITOCHONDRIA-TARGETED CATALASE PREVENTS OXIDATIVE STRESS AND REVERTS ANTIOXIDANT RESPONSE IN DOWN SYNDROME

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The free radical theory of aging proposes that cumulative damage caused by reactive oxygen species ROS is responsible of the functional decline of living systems, leading to diseases and eventually death. A successful longevity murine model was designed based on these concepts, targeting over expression peroxide degrading enzyme catalase to mitochondria (mCAT), the main center of reactive oxygen species (ROS) production. Our group has been studying aging in Down syndrome (DS). We have described for DS cells chronic oxidative stress associated with mitochondrial dysfunction that ends up compromising cellular functions, associated to transcriptome showing active antioxidant systems Nrf2 pathway, a master regulator of antioxidant response elements (ARE). Here we are presenting the characterization of mCAT expression in DS cells, using a lentiviral system. The strategy was efficient decreasing mitochondrial chronic oxidative damage, restoring mitochondrial membrane potential and recovering the organelle's structure in these cells. We observed that mitochondrial dysfunction affected DS fibroblasts migration capacity and that mCAT rescued this parameter, too. Finally we explored Nrf2 pathway activation in DS fibroblasts, confirmed its upstream regulators, and observed that mCAT activity impacted the cellular stress response diminishing Nrf2 stabilization and nuclear translocation.

CB-C07
MAPPING THE DYNAMICS OF THE GLUCOCORTICOID RECEPTOR AND ITS COREGULATOR NCOA-2 IN THE NUCLEUS

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The distribution of the transcription machinery among different subnuclear domains raises the question on how this organization modulates the transcriptional response. We used imaging and fluorescence correlation spectroscopy (FCS) based approaches to quantitatively explore the dynamical intranuclear organization of the glucocorticoid receptor (GR) in living cells. This ligand-activated transcription factor diffuses within the nucleus and can engage in shorter and longer-lived interactions with chromatin targets or interact with DNA-dependent foci or promyelocytic leukemia (PML) bodies, which accumulate the coregulator NCoA-2. We also analyzed the recruitment and binding properties of GR and its coregulator to glucocorticoid response elements (GREs). The distribution of the receptor among these different nuclear compartments depends on the interaction with NCoA-2 and GR conformation, as assessed with synthetic ligands and receptor mutants with impaired transcriptional abilities. Our results suggest that the partition of the GR in different nuclear reservoirs may represent another step of transcription regulation, since the interaction with these compartments could ultimately influence the ability to bind to the specific transcriptional targets

CB-C08
ANTITUMORAL EFFECTS OF BIOENERGETIC MODULATION IN FELINE MAMMARY CARCINOMA CELLS

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Feline mammary carcinoma (FMC) is a highly aggressive pathology that has been proposed as an interesting model of breast cancer disease. Metabolism has been described as a hallmark of cancer cell. The aim of the present work was to investigate the effects and mechanism of the modulation of metabolism by metformin (MET, an oral anti-diabetic drug), 2-deoxyglucose (2DG, HK inhibitor) or a combination of both drugs, MET/2DG on two established FMC cells lines: AlRB (HER2 (3+) and Ki67<5%) and AlRATN (HER2 (-) and Ki67>15%). Treatments decreased both FMC cells viability in a concentration dependent manner (APH assay, $p<0.05$). AlRB was more sensitive to 2DG than AlRATN (IC50: 3.15 vs 6.32 mM, respectively). The combination of MET/2DG potentiated the individual drugs cytotoxic effects on FMC cells. Only MET/2DG caused an increased in intracellular oxidants (as determined by DCF, $p<0.05$), AO positive vesicles (related to autophagic mechanism, flow cytometry, $p<0.05$) and completely inhibit colony formation. On the contrary only MET significantly altered plasma membrane integrity (by increasing PI uptake, $p<0.05$), presented late apoptotic/necrotic cells (AO/EB staining, $p<0.05$) and increased both glucose consumption and lactate concentration. This results support further studies to investigate the potential use of this metabolic modulation approach in a clinical veterinary setting.

ENZYMOLOGY

EN-C01
IDENTIFICATION AND CHARACTERIZATION OF A NOVEL STARCH BRANCHING ENZYME FROM *Ostreococcus tauri*

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Starch branching enzyme (BE) is a highly conserved protein from plants to algae. This enzyme participates on starch granule assembly by the addition of α-1,6 glucan branches in the α-1,4 polyglucans. This modification determines the fine structure of amylopectin, and thus, the final structure of the starch granule. In this work we describe the function of a starch branching enzyme from the picoalgae *Ostreococcus tauri*. Although previous *in silico* evidence suggested that this protein is a starch debranching enzyme, structure-function studies confirmed that this polypeptide is a BE comprising two in tandem carbohydrate binding domains (from CBM41 and CBM48 families) at the N-terminal end of the protein followed by a C-terminal catalytic domain. The analysis of truncated isoforms shows that the CBMs bind differentially to starch and the distinct starch fractions. Moreover, no catalytic activity was detected in the CD alone or with the truncated forms of the protein. The results suggest that this *O. tauri* protein is a functional BE containing a CBM41 and CBM48 that are essential for enzyme activity and regulation.

EN-C02
KINETIC AND FUNCTIONAL CHARACTERIZATION OF OTDSP, A PHOSPHOGLUCAN PHOSPHATASE FROM *O. tauri*

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Over the past decade, the phosphoglucan phosphatases have surfaced as fundamental proteins that regulate storage carbohydrate metabolism in plants as well as mammals. The functional mechanism of these enzymes is understood but little is known about its evolution in the green lineage and no reports exist concerning green algae. In this sense, we have identified and characterized a novel glucan phosphatase of the ancient picoalga *Ostreococcus tauri*. We verified OtDSP phosphatase activity *in vitro* with pNPP as well as

its natural substrate amylopectin, but with a different kinetic behavior. To further characterize the enzyme and based in OtDSP homology with *A. thaliana* LSF2 we identified amino acidic residues involved in catalysis and binding to substrate that were mutagenized. Wild type OtDSP and its mutants counterparts were studied by means of native-PAGE, size exclusion chromatography and binding assays to polysaccharides. The results obtained suggest OtDSP as a fully functional enzyme *in vivo*.

EN-C03
ALTERNATIVE CATALYTIC PROPERTIES IN THE GLYCOGEN-SYNTHASE FROM ACTINOBACTERIA

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A new pathway (named GlgE) for prokaryotic glycogen metabolism was described in Actinobacteria. In this pathway, the key enzyme GlgE extends the glucan in two glucose units by means of maltose-1P; whilst in the classical GlgAC pathway glycogen-synthase (GlgA, EC 2.4.1.21) elongates the polymer in one unit, using ADP-glucose as the glucosyl donor. Recently it was reported that the mycobacterial GlgA catalyzes maltose-1P synthesis consuming glucose-1P and ADP-glucose as substrates. Thus, we analyzed this reaction in several GlgAs from different sources so far characterized in our lab. Amongst them, GlgAs from Actinobacteria (Streptomyces and Rhodococcus) were active for maltose-1P synthesis. Moreover, both actinobacterial GlgA showed some degree of promiscuity towards sugar-1Ps but not for NDP-sugars. Particularly, rhodococcal GlgA used glucosamine-1P to the same extent than glucose-1P. Actinobacterial GlgAs also catalyzed the synthetic reaction using ADP-Glc and maltose-1P (~50% lower regarding glucose-1P) as substrates. In addition, the maltose-1P forming activity was detected in crude extracts from *Rhodococcus jostii*, thus suggesting a biological significance for this alternative catalytic property. Results support a critical role of maltose-1P and a multifaceted GlgA function (being involved in the classical as well as in the GlgE pathway) for glycogen metabolism in actinobacteria.

LIPIDS

LI-C01
HIGH-NACL INDUCES SREBP-MEDIATED TRANSCRIPTIONAL REGULATION OF TRIGLYCERIDES

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Hypertonicity regulates phospholipids (PLs) and TAG synthesis. These metabolic pathways can be regulated by transcriptional activation of their biosynthetic enzymes. Several transcription factors may be involved in such regulation but sterol response element binding protein (SREBP) is considered the master regulator of lipogenic genes. We showed that MDCK cells subjected to high NaCl induce changes in mRNA expression and cell distribution of SREBP isoforms. These changes were consistent with the increased levels of PLs and TAG in treated cells and with the decrease in lipid synthesis after fatostatin treatment. However, we did not establish which isoform, SREBP1 (S1) and/or SREBP2 (S2), is responsible for the increased lipogenic activity. The present work was aimed to address this. Before the addition of hypertonic medium, MDCK cells were treated with S1-siRNA, S2-siRNA or both. After NaCl treatment lipid synthesis was studied. PLs and 1,2 DAG synthesis were not affected by any siRNA. In contrast, both 1,3 DAG and TAG synthesis were blocked. S1-siRNA decreased DAG and TAG synthesis by 33 and 46 %. S2-siRNA decreased DAG and TAG by 40 and 37%, respectively. Both siRNAs reduced synthesis by 55 %. So, SREBPs are needed to maintain TAG synthesis and its degradation to DAG but PLs synthesis remains constant indicating that SREBP-mediated transcriptional regulation is not involved

LI-C02
THE ETHER-LINKED LIPIDS OF RAT EPIDIDYMIS ARE AFFECTED BY MILD HYPERTHERMIA

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It is widely known that heat stress temporarily suppresses spermatogenesis in the mammalian testis, but the impact on the epididymis is barely known. The aim of this study was to examine the effects of short, repeated once-a-day episodes of hyperthermia (43ºC) on the ether-linked glycerophospholipids (GPL) and triglycerides (TG) of rat epididymis. One-week post-treatment, the expression (mRNA) of alkylglycerone phosphate synthase (AGPS), a key peroxisomal enzyme in the synthesis of these lipids, significantly fell in caput and corpus epididymis. Coincidentally, levels of the plasmalogen precursor, plasmanyletanolamine, decreased. Concurrently, plasmenylethanolamine and plasmenylcholine accumulated in caput, ascribable to injured and motionless cells and sperm collecting in the lumen. Catabolism of such GPL had started in the epididymal epithelium, as suggested by the build-up of ether-linked TG. Between weeks 2 and 6, spermatogenesis restarted in the testis. Although the epididymis was still sperm-free at week 6, its levels of ether-linked GPL and TG were much higher than those of untreated controls, in agreement with the recovery of AGPS expression. Ether-linked TG were formed by de novo synthesis and during GPL breakdown. The presence of spermatozoa in the epididymal lumen apparently plays a regulatory role in the biosynthesis of ether-linked lipids by the epididymal epithelium

LI-C03
ROLE OF GPA3/4 IN GLYCEROLIPID SYNTHESIS, PHAGOCYTOSIS AND CYTOKINE RELEASE IN ACTIVATED MACROPHAGES

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Glycerol-3-phosphate acyltransferase (GPAT) regulates de novo glycerolipid synthesis. GPAT activity is up-regulated during macrophage activation, when PL and TAG accumulation in lipid droplets (LDs) is increased. We studied the role of GPAT3 and GPAT4 during macrophage activation in a shGpat3 macrophage cell line and Gpat3$^{-/-}$and Gpat4$^{-/-}$ mice Bone Marrow Derived Macrophages (BMDM). All the LPS-activated Gpat-silenced macrophages accumulate less LDs, TAG and PL than the Gpat-expressing control cells. We analyzed the incorporation of [^{14}C]-Acetate and [^{14}C]-Oleic acid (OA) into lipids in activated shGpat3 cells, Gpat3$^{-/-}$ and Gpat4$^{-/-}$ BMDM; the incorporation of both substrates decreased in the absence of GPAT3 or 4 and while GPAT3 participates in both PL and TAG synthesis, GPAT4 is mostly involved in TAG synthesis. To investigate the physiological effect of impaired lipid synthesis, we analyzed the phagocytic capacity of shGpat3 cells, Gpat3$^{-/-}$and Gpat4$^{-/-}$ BMDM and it was 45, 22 and 31% lower than in the activated controls. We found that the expression and cytokine release during macrophage activation in these cells was also altered. Taken together, these results prove that GPAT3 and 4 contribute to the increase in total glycerolipid content, phagocytosis and cytokine production during macrophage activation.

LI-C04
A METABOLIC CIRCADIAN CLOCK CONTROLS RHYTHMS IN IMMORTALIZED HUMAN GLIOBLASTOMA T98G CELLS

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Circadian clocks present even in immortalized cell lines temporarily regulate diverse physiological processes and can be synchronized by different ambient signals. The disruption of circadian rhythms may lead to metabolic disorders or higher cancer risk through failures in cell division control. Previous results in immortalized human glioblastoma T98G cells showed that clock genes (*Bmal1, Per1, Rev-Erbα*), some phospholipid (PL) synthesizing enzyme genes and the labelling of ^{32}P-PLs exhibited different temporal profiles depending on the growth condition tested (proliferation: P, partial arrest: A) with metabolic rhythms mainly preserved under P. Here we evaluated redox metabolism (redox state and peroxiredoxin oxidation cycles) and the activities of PL synthesizing enzymes for phosphatidate phosphohydrolase (PAP) and lysophospholipid acyl transferases (LPLAT) in T98G cells under P or A, synchronized with dexamethasone (100 nM) (time 0) and collected at different times for 36 h. Results showed that redox state, peroxiredoxin oxidation cycles and PAP activity exhibited temporal oscillations in both growth conditions tested (P and A) while LPLAT activity seems to be rhythmic under P. Our observations support the idea that a metabolic clock could operate in these tumor cells regardless the molecular clock which was not found to work properly under proliferation.

LI-C05
EXPRESSION OF ELOVL4 AND FA2H WITH SPERMATOGENIC CELL DIFFERENTIATION IN THE RAT TESTIS

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Rat spermatogenic cell membranes contain sphingolipids with nonhydroxy and 2-hydroxy very long chain (C_{24-32}) PUFA. The biosynthesis of such fatty acids requires the expression of very long chain fatty acid elongases (*Elovl4* for > C_{24}) and a fatty acid 2-hydroxylase (*Fa2h*). In this study, mRNA levels of *Elovl4*and *Fa2h* were measured by qPCR in rat testis at different postnatal ages and in cells isolated from the seminiferous epithelium of adults. At early prepuberal ages (P14), *Elovl4* was highly expressed while *Fa2h* mRNA was absent. *Fa2h* started to be detected at P25-30 and increased thereafter, while *Elovl4*mRNA levels decreased. The expression of both genes, but mainly *Fa2h*, was markedly reduced in adult testes that had been depleted of germ cells by mild hyperthermia. In isolated spermatogenic cells, both genes were expressed at lower levels in pachytene spermatocytes than in post-meiotic round and late spermatids. Interestingly, Sertoli cells had high *Elovl4*but lacked *Fa2h* mRNA. The Elovl4 protein was detected in spermatocytes from P21 to adulthood, when the protein was clearly observed in elongated spermatids. The Elovl4 enzyme was functional in germ cells, as these cells, in culture, were able to elongate [^{3}H]20:4 to PUFA longer than C_{24}. Our results underscore the presence of a well-timed, cell-specific regulation of *Elovl4* and *Fa2h* in germ cells as differentiation proceeds.

LI-C06
LOW-DENSITY MEMBRANE FRACTIONS FROM MALE GERM CELLS LACK SPHINGOLIPIDS WITH VERY LONG CHAIN PUFA

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Sphingomyelins (SM) and ceramides (Cer) with very long-chain PUFA (VLCPUFA), in nonhydroxy (n-V) and 2-hydroxy (h-V) forms, are specific components of rat spermatogenic cells. Here we evaluated how differentiation affects their distribution among membrane fractions from such cells. Using a detergent-free procedure, a small light, raft-like low-density (L) fraction and a large heavier (H) fraction, both showing markers typical of cell plasma membranes, were separated from pachytene spermatocytes, round, and late spermatids. MALDI-TOF spectra showed that the L fraction had mostly SM species with saturated fatty acids regardless of

the cell stage, while the H fraction was rich in stage-varying SM and Cer species with VLCPUFA. In this fraction spermatocytes accumulated mostly n-V SM and spermatids h-V SM and h-V Cer species. A third fraction made of intracellular membranes had less SM and more Cer than the H fraction, differentiation also increasing the h-V/n-V ratio in both lipids. The buildup of 2-hydroxy fatty acids correlated with the expression (mRNA) of fatty acid 2-hydroxylase (Fa2h), higher in spermatids than in spermatocytes. The differentiation-dependent rise in h-V Cer in the germ cell H fraction during spermatogenesis is consistent with the eventually uneven distribution that n-V and h-V species of SM and Cer display between the head and the tail of mature spermatozoa.

MICROBIOLOGY

MI-C01
THE ROLE OF RESPIRATORY OXIDASES IN THE MECHANISM OF ACTION OF MICROCIN J25

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The antibacterial peptide microcin J25 (MccJ25) displays an antibiotic activity against*Salmonella*,*Shigella*and*Escherichia coli*. MccJ25 has two cellular targets, the RNA polymerase and the respiratory chain. The terminal oxido-reductases in*E. coli* respiratory system are the cytochromes*bo_3*and*bd*. We studied the effect of MccJ25 in*E. coli*C43 cytochrome mutant strains. The oxygen consumption was diminished by MccJ25 in the wild type strain and in the mutant strain lacking the cytochrome*bo_3*, but did not have any effect in Δ*bd*I strain. In the same way, superoxide production in isolated membranes was increased more than 100 % in the control and Δ*bo_3*strain, whereas in cytochrome*bd*mutant such increment was not observed. Moreover, working with purified cytochromes, MccJ25 inhibited about 25 % the ubiquinol oxidase activity only on cytochrome *bd*I, while under identical experimental conditions*bo_3*oxidase was insensitive to the peptide. These results demonstrate that cytochrome*bd*I plays an important role in the microcin J25 mechanism of action on the respiratory chain of*E. coli*. Our findings would provide a new insight into the application of MccJ25 in food or pharmaceutical industries.

MI-C02
FUNCTIONAL CHARACTERIZATION OF THE CELL DIVISION PROTEIN FtsA OF *Streptococcus pneumoniae*

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FtsA is a divisome protein that connects the master coordinator of cell division, FtsZ, to the cell membrane for tethering the Z-ring and septal formation. Previous reports have showed that FtsA forms a ring-like structure at the division site of streptococcal cells. In addition, some authors reported that *ftsA* is an essential gene. In this work, we could obtain the *ftsA* mutant by insertion mutagenesis demonstrating that *ftsA* is dispensable for cell viability. However, the *ftsA* mutant displayed fitness and morphological alterations. By fluorescence microscopy, we also found a delocalization of FtsZ-GFP in the *ftsA* mutant, phenotype that is compatible with the known FtsA function. The wild-type shape, cell cycle and FtsZ localization were recovered when the *ftsA* cells were complemented by expression of *gfp-ftsA*. By confocal microscopy, we detected the reported localization of GFP-FtsA at the midcell in the wild-type strain, but we also observed an unexpected localization during cell cycle progression. This pattern was confirmed by expression of FtsA fused to HA (human influenza hemagglutinin tag) and revealed with an anti-HA monoclonal antibody. These results revealed new features of FtsA and confirmed that it is an essential piece of the cell division mechanism of *S. pneumoniae*.

MI-C03
REGULATION OF THE SUBPOLAR FLAGELLUM SYNTHESIS IN*Bradyrhizobium diazoefficiens*

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*Bradyrhizobium diazoefficiens*is the soybean nitrogen-fixing symbiont commonly used in inoculant formulations. This α-proteobacterium uses two independent flagellar systems to swim in liquid and viscous media. In our laboratory, we studied the synthesis and regulation of both flagellar systems, and here we show part of the regulatory cascade of the subpolar flagellum synthesis. Flagellar synthesis occurs in steps, each one controlled by different regulators. This process ensures the appropriate timing of the synthesis of different components. First, a master regulator initiates the signal cascade, then class II regulators control gene expression of the intermediate products and class III/IV regulators activate flagellum filament formation, the last product assembled.We present the characterization of*B. diazoefficiens*mutants in two class II regulatory genes (*flbD*and*fliX*) and two class III regulatory genes (*flaF*and*flbT*), by measuring the transcription levels of the putative targets controlled by them and also the type of flagellins that they produced. Our results suggest that the regulation of the subpolar flagellum synthesis is independent from the lateral flagella and is controlled in a cell-cycle manner. These results fit with the model previously described in*Caulobacter crescentus*but not with*Salmonella*model, as was thought in earlier studies.

MI-C04
ENTEROBACTIN: A FENTON-SAFE SIDEROPHORE

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There is increasing evidence that siderophores may play alternative roles, aside of providing cells with the necessary iron. We previously reported that the catechol siderophore enterobactin, is crucial for*Escherichia coli*colony development in culture conditions that increased the oxidative stress. In this work, we demonstrated that enterobactin confers protection against various sources of oxidative stress such us H_2O_2, paraquat and copper, independently of its ability to facilitate iron uptake. Protection against oxidative stress occurs in the cell cytoplasm through ROS scavenging and requires prior hydrolysis of the enterobactin molecule. Interestingly, both the sensitivity to stressors and the colony development arrest phenotype were enhanced when cells harbored the*entE* mutation along with either*sodA*or*katG*. Confirming the link between enterobactin and oxidative stress, we found that enterobactin transcriptional expression and production was induced by oxidative stressors even in the presence of iron. Furthermore, preliminary data indicates that enterobactin transcription would be regulated by oxidative stress through the global regulator SoxS. These results strongly support the involvement of enterobactin as part of the oxidative stress response of*E. coli*.

MI-C05
THE*map* LOCUSOF*Brucella suis*IS INVOLVED IN CELL ENVELOPE BIOGENESIS AND VIRULENCE

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*Brucella*species exhibit unique surface properties, which make them furtive pathogens and more resistant to several host defense compounds. We have identified a*locus*of*Brucella suis* encoding the MapA and MapB proteins, which are predicted to represent the TAM machinery, recently proposed to participate in the translocation/insertion of autotransporters (ATs) in the outer membrane (OM). In*Brucella*, ATs are involved in bacterial attachment to host components. However, the role of TAM in*B. suis*would not be restricted to AT translocation since theΔ*mapB* phenotypes were not only related to adhesion functions. Indeed, Δ*mapB*showed enhanced sensitivity to lysozyme, Triton X-100 and polymyxin B, indicating that the cell envelope integrity is compromised. This effect was not due to major differences in the LPS structure or to altered total fatty acid composition. Analysis by LC-MS/MS and *WesternBlot* of membrane fractions suggested that the extent of some OM proteins is slightly altered in the*mapB*mutant. Interestingly, the number of bacteria recovered from macrophages during the initial stages of infection was reduced in the mutant and it showed an attenuated phenotype in mice. These results suggest that MapA/MapB assists in the correct insertion of an unknown subset of protein substrates or other OM components, which are important for OM stability and virulence.

MI-C06
CLONING, EXPRESSION & CHARACTERISATION OF THE HEPATITIS E VIRUS CAPSID PROTEIN OF GENOTYPES 1-4 FOR SERODIAGNOSTIC

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The National Institutes of Health classified Hepatitis E as an emerging disease because: Hepatitis E Virus (HEV) is the major cause of acute hepatitis in developing countries, there is an increasing number of HEV infections in industrialized countries associated to live stock and, chronicHEV infections are being reported in immunocompromised patients (HIV, transplant recipients, etc). HEV transmission by blood transfusions has also been recently reported. There are scarce epidemiological data about HEV occurrence and prevalence in Argentina, and diagnostic tests are needed. Open reading frame 2 (ORF2) of HEV genotypes (GT) 1 to 4 were recombinatorially cloned into a customized, N-terminally His-tagged bacterial expression vector and analysed for recombinant protein expression and purification. We optimized expression under native and denaturing conditions. Purified proteins were blotted onto a nitrocellulose membrane (dot-blot) and examined for their antigenic potential by serum profiling experiments. Although the four established GTs of HEV belong to a single serotype, the serum of a patient infected with HEV ORF2 GT3 reacted with more intensity with ORF2 GT3. We provide a prototype of immunoassay for complementary confirmation of HEV seropositivity as detected in screening assays such as ELISA and plan to test a panel of sera from Argentinean blood donors.

NEUROSCIENCE

NS-C01
THE VISUAL CYCLE IN THE INNER RETINA OF CHICKEN AND THE ROLE OF RETINAL G-PROTEIN-COUPLED RECEPTOR

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The vertebrate retina contains typical photoreceptor (PR) cones and rods responsible for vision and intrinsically photosensitive retinal ganglion cells (ipRGCs) involved in the regulation of non-visual tasks. Visual photopigments in visual PRs or melanopsin (Opn4) in ipRGCs utilize retinaldehyde as a chromophore. The retinoid regeneration process denominated as "visual cycle" involves the retinal pigment epithelium (RPE) or Müller glial cells. However, it is unknown how the chromophore is further metabolized in the inner

retina. Nor is it yet clear whether an alternative secondary cycle occurs involving players such as the retinal G-protein-coupled receptor (RGR), an opsin of unidentified inner retinal activity. Here, we investigated the role of RGR in retinoid photoisomerization in Opn4x (+) RGC primary cultures from chicken embryonic retinas. Opn4x (+) RGCs display significant photic responses and photoisomerize exogenous all-trans to 11-cis Ral and other retinoids. RGR was found to be expressed in developing retina and in primary cultures; when its expression was knocked down, the levels of retinals and retinol in cultures exposed to light were significantly higher and those in all-trans retinyl esters lower than in dark controls. The results support a novel role for RGR in ipRGCs to modulate retinaldehyde levels in light, keeping the balance of inner retinal retinoid pools.

NS-C02
METABOLIC DYSFUNCTION WORSENS COGNITION AND NEURONAL RESILIENCE IN A RAT MODEL OF EARLY ALZHEIMER

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Alzheimer's disease (AD) is the leading cause of dementia in older adults and represents a serious medical, social and economic problem. Although diet is a modifiable risk factor for AD, the mechanisms linking peripheral metabolism and cognition remain unclear. To address this question, we have chosen McGill-R-Thy1-APP transgenic rats (Tg(+/-) that mimic presymptomatic AD pathology. Wild-type and Tg(+/-) rats were exposed from 35 days to 6 months of age to a standard diet or a Western diet (WD), high in saturated fat and sugar. Our results of peripheral and hippocampal biochemical analysis show that WD induced a metabolic syndrome and decreased presynaptic bioenergetic parameters. Furthermore, cognitive tests, ELISA multiplex, Western blot, immunohistochemistry and quantitative RT-PCR results indicate that WD worsened cognition, increased hippocampal levels of oligomeric and monomeric Aβ species (38/40/42), promoted deposits of N-terminal pyroglutamate-Aβ in CA1 pyramidal neurons and interneurons, reduced neuronal resilience and increased nitrated proteins in Tg(+/-) rats. Our results support the concept that diet-induced metabolic dysfunction may contribute as a "second hit" to impair cognition in the presence of early Aβ pathology, reinforcing the relevance of optimizing fat and sugar consumption for the prevention of AD, at least in people with genetic risk factors.

PLANT BIOCHEMISTRY AND MOLECULAR BIOLOGY

PL-C01
MITOCHONDRIAL CONTRIBUTION TO BASAL PLANT DEFENSES VIA PROLINE DEHYDROGENASE (PRODH)

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The contribution of mitochondria to defense programs against bacterial pathogens has been mostly studied under cell death-triggering conditions. Conversely, its influence over defense programs that trigger broad-spectrum resistance in the absence of cell death has not been established yet. To assess this issue, we monitored PTI (PAMP-triggered immunity) features in wild type tissues treated with inhibitors of the mitochondrial electron transport chain (mETC), and mutants lacking the mitochondrial proline catabolic enzyme ProDH. We found that non-lethal levels of antimycin A or rotenone producing mild mETC alterations, strongly impair PTI. In addition, the absence of ProDH1 or ProDH2 isoenzymes reproduces most of these PTI defects. Major differences were observed in the generation of reactive oxygen species (ROS) and deposition of callose at the cell wall. Based on these results we used a bacterial flagellin-derived peptide (flg22) to perform a detailed investigation of the requirement of ProDH for ROS generation by the membrane NADPH oxidase homolog D (RBOHD). Surprisingly, the RBOHD function was sensitively dependent on the ProDH activity.

PL-C02
CHLOROPLAST REDOX STATUS MODULATES GENOMEWIDE STRESS RESPONSES IN SOLANACEOUS PLANTS

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Chloroplasts are one of the main sources of reactive oxygen species (ROS) in plants. Flavodoxin (Fld) is a photosynthetic protein with antioxidant properties. Although its gene is absent in the plant genome, its expression in transgenic plants confers increased tolerance to different environmental stresses. These plants are an interesting system to study the role of chloroplast redox status and ROS during plant stresses. Thus, we investigated the transcriptomic responses to Fld expression in two members of the economically important Solanaceae family under different stress assays: 1) Fld expressing potato plants were subjected to drought, resulting in a less wilted phenotype than their wildtype siblings, 2) Fld expressing tobacco plants were infected with the non-host bacterium *Xanthomonas campestris* pv. *vesicatoria*, showing a ~48 hdelay in the development of localized cell death in inoculated leaves. In both cases, Fld modulated many genes involved in hormone-based pathways, signal transduction, transcriptional regulation and stress responses. Interestingly, ~5% of leaf-expressed genes were affected by Fld expression *per se* in both tobacco and potato under normal conditions. The results provide a genome-wide picture illustrating the relevance of chloroplast redox status on biotic and abiotic stress responses and suggest new targets to generate stress tolerance to solanaceous.

PL-C03
REGULATION OF CENTRAL METABOLISM BY TREHALOSE 6-PHOSPHATE

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Trehalose 6-phosphate (Tre6P) is an important signal metabolite linking plant carbon metabolism with growth and development. The Tre6P-sucrose nexus model postulates that Tre6P regulates sucrose levels within an appropriate range and *vice versa*. To test this model, we investigated short-term metabolic responses to ethanol-induced increases in Tre6P levels in *Arabidopsis thaliana* plants expressing the *Escherichia coli* Tre6P synthase. Increased Tre6P levels led to a transient drop in sucrose content, post-translational activation of both nitrate reductase and phosphoenolpyruvate carboxylase, and increased levels of organic and amino acids. Radio ($^{14}CO_2$) and stable-isotope ($^{13}CO_2$) labelling experiments in plants with elevated Tre6P showed no changes in photoassimilate export rates, but increased labelling of organic acids. These results suggest that high Tre6P levels divert carbon away from sucrose by stimulating carbon fluxes into organic acids to provide C-skeletons for amino acid synthesis and simultaneously stimulate nitrate assimilation. These findings are consistent with the Tre6P-sucrose nexus model and implicate Tre6P in regulating interactions between carbon and nitrogen metabolism in plants.

PL-C04
UNRAVELING THE CONTRIBUTION OF NADP-MALIC ENZYME 1 TO ALUMINUM STRESS RESPONSE IN *Arabidopsis*ROOTS

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Malate plays a fundamental role in plant aluminum tolerance since its Al-binding capacity. In this work we analyze the participation of *Arabidopsis thaliana* NADP-malic enzyme 1 (NADP-ME1) in root malate metabolism and Al response. Arabidopsis insertional mutant plants lacking *NADP-ME1* showed enhanced tolerance to Al, evidenced by a lower inhibition of root elongation compared to wild type, in presence of toxic Al concentrations. Additionally, qRT-PCR analysis showed a decreased expression of *NADP-ME1* gene in wild type seedlings after 3 hours of Al treatment. The malate levels in roots and exudates after short Al treatments were similar in *nadp-me1* compared to wt, although a significant increase of intracellular malate was observed after a long exposure to Al only in the knockout line. The H_2O_2 content and the transcript levels of several Al tolerance related genes were analyzed in both plant types exposed to Al, as well as the response to glutamate, amino acid implicated in Al stress signal transduction. The results suggested that NADP-ME1 could be involved in regulating the levels of malate in the cytosol of the root apex and its loss may result in an increase in the content of this organic acid. Furthermore, this isoform may be affecting the signal transduction processes which lead to root growth inhibition, such as the generation of ROS or other signaling molecules.

PL-C05
INFLUENCE OF SINAL7 IN VEGETATIVE PARAMETERS IN *Arabidopsis*

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Seven in absentia like 7 gene (At5g37890, SINAL7) from *Arabidopsis thaliana* encodes a RING finger protein belonging to the SINA superfamily with E3 ubiquitin ligase activity. In previous work we described the biochemistry characteristics of SINAL7 protein and its self-ubiquitination capability *in vitro*. Moreover, previous evidence led us to propose the participation of SINAL7 in a hypothetical signaling pathway together with cytosolic glyceraldehyde-3-phosphate dehydrogenase in Arabidopsis. In this work we face the study of SINAL7 *in vivo* in Arabidopsis knockout and overexpressing SINAL7 plants to find out its influence on plant physiology by the phenotypic analysis of the plants. Results showed that SINAL7 may be participating in the regulation of several vegetative parameters including biomass content, senescence and drought tolerance. In summary, we found that different levels of SINAL7 in Arabidopsis may alter plant biomass. In addition, we describe the possible compensatory effect of SINAL7 counterparts in knockout mutant sinal7 plants. Finally, we show the influence of SINAL7 in the complex process of senescence and in drought tolerance

PL-C06
SCF E3 LIGASE REDOX REGULATION: IMPACT ON HORMONAL SIGNALINGS

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In plants, the ubiquitin-proteasome system action has been associated to the regulation of hormone biosynthesis, transport and perception providing a direct mechanism to modulate intensity and duration of different hormonal signalings. Ubiquitin is covalently attached to substrate proteins through the action of a sequential cascade of three enzymes consisting of E1, E2, and E3. The SCF complex is the best characterized multi-subunit ubiquitin E3 ligase. In SCF complex, SKP1 acts as a bridge between CUL1 and the F-box proteins that mediate substrate recruitment. F-box are able to associate with SKP1 in an interchangeable manner to form diverse SCF complexes with different substrate specificities. Several phytohormone receptors are F-box proteins in SCF complexes regulating auxin, jasmonates and gibberellins signalings. The exchange of F-box substrate adapters from SCF complex must be required during changing cellular conditions. In this work, we present data supporting posttranslational redox regulation of different SFC members and their impact on SCF complex assembly, and hormonal-dependent physiological processes. Supported by ANPCyT, CONICET and UNMdP.

PL-C07
INSIGHT INTO DIVERSIFICATION AND EVOLUTION OF HD-ZIP I TRANSCRIPTION FACTORS IN STREPTOPHYTES

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Streptophytes dispersion and evolution onto land was accompanied by an increment in the number of genes encoding transcription factors (TFs). This rise in TFs diversity suggests that they might have played a fundamental role in the adaptation of plants to an environment with less water availability. Homeodomain-leucine zipper I (HD-Zip I) is a TF family whose members are involved in drought responses. In Arabidopsis, this family has 17 members and it was proposed that they have redundant functions. Until now, HD-Zip I were identified only in the land plant lineage. Here, we report the identification of HD-Zip I genes in aquatic charophytes genomes. We also found a single common ancestor in land plants. Duplication events occurring in specific plant divisions might explain the emergence of current clades of HD-Zip I in angiosperms, likely due to neo-functionalization. We also identified a couple of HD-Zip I gene-loss events related to monocot plants that have moved back to aquatic environment. To better understand the diversification and evolution of HD-Zip I TFs, we are now using the livewort *Marchantiapolymorpha*, which occupies a basal position in the evolution of land plants. Marchantia genome also has a low genetic redundancy in regulatory genes and encodes a unique HD-Zip I that could be key to understand the role of these genes during the transition to land.

PL-C08
AN OPEN READING FRAME PRESENT IN THE 5'UTR OF THE *Arabidopsis* ATHB1 GENE REPRESSED ITS TRANSLATION

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AtHB1 is an Arabidopsis transcription factor (TF) belonging to the HD-Zip I subfamily. It was previously demonstrated that this TF interacts with AtTBP2 to exert its function, acting downstream of PIF1. The AtHB1 gene is expressed in hypocotyls and roots to regulate genes involved in the elongation and synthesis of the cell wall. Here we show that bioinformatic analyses considering AtHB1 homologues in several plant species resulted in the detection of a conserved upstream Open Reading Frame (uORF) in the 5'UTR region of these genes, named CPuORF33. These uORFs exhibit a higher degree of conservation in the aminoacidic sequences than in the nucleotide ones, and they do not overlap with the mORF (main ORF). Generation of different constructs, bearing the native or/and the mutated uORF, used to transform Arabidopsis plants allowed us to demonstrate that the CPuORF33 represses the mORF translation but not transcription, independently on the mORF nature. This regulation occurs in cis, probably through a ribosome stalling mechanism. On the other hand, AtHB1 overexpression is also repressed by small RNAs in Arabidopsis. Altogether, our results reveal a novel mechanism by which the expression of AtHB1 (and that of its homologues in other plant species) is tightly regulated in order to avoid aberrant phenotypes observed when the TF is overexpressed leading to sterile plants.

PL-C09
CYTOCHROME C MODULATES PLANT GROWTHRATE AND THE ACTIVITY OF THE GIBBERELLINPATHWAY

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We studied the effect of reducing the levels of the mitochondrial electron carrier cytochrome c (CYTc) in plants. Double knockdown mutants in both genes encoding CYTc in *Arabidopsis thaliana* show a severe delay in vegetative growth and developmental rate. Additionally, CYTc deficiency causes starch and glucose accumulation indicating that these plants accumulate reserves instead of using them for growth. Treatment of mutants with the gibberellin GA47abolishes the developmental delay, suggesting that it is associated to GA deficiency. Transcriptional analyses show that the expression of several genes involved in GA homeostasis is altered in mutant plants, while the levels of DELLA proteins, repressors of GA signaling, are increased. In addition, plants with increased CYTc levels show accelerated growth and reduced levels of DELLA proteins. We propose that hormone regulation of growth is coupled to the activity of components involved in mitochondrial energy metabolism.

PL-C10
TCP15 CONNECTS GIBBERELLIN AND AUXINPATHWAYS DURING STAMEN FILAMENTELONGATION IN *Arabidopsis*

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The TCP transcription factor family is specific of plants.*Arabidopsis thaliana* has 24 TCP proteins divided into classes Iand II. In this work, we characterized the role of TCP15, amember of class I, in stamen development. Plants that express afusion of TCP15 to the EAR repressor motif develop shorterstamen filaments, while plants that express TCP15 under thecontrol of the 35SCaMV promoter show longer filamentscompared to wild-typeplants. Transcript levels of SAUR63, anauxin response gene implicated in filament elongation, arereduced in TCP15-EARand increased in 35S:TCP15 plants.Filament elongation is also promoted by gibberellins (GA) andmutants in GA biosynthesis genes have short stamen filaments.Inhibition of GA synthesis decreases the expression of SAUR63and overexpression of TCP15 rescues the short stamenphenotype of GA deficient plants. In addition, plants thatoverexpress the transcription factor KNAT1 have shorterfilaments while mutants in the corresponding gene show longerfilaments. KNAT1 is a known repressor of GA synthesis andoverexpression of TCP15 rescues the short stamen phenotypeof 35S:KNAT1 plants. We conclude that TCP15 modulates thecrosstalk between GA levels and auxin responses during stamen development and that KNAT1 is an upstream regulatorof the process.

PL-C11
POST-TRANSLATIONAL REGULATION OF MICRO RNA BIOGENESIS

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Gene regulation is an important mechanism used by plants to face unfavourable environmental conditions, and micro RNAs (miRNAs) are central players in such process. The miRNAs, small 21nucleotide RNAs molecules, post-transcriptionally control gene expression. In order to achieve a balance between gene expression and silencing, both the miRNA biogenesis and action are tightly regulated. In this sense, posttranslational modifications of miRNA biogenesis cofactors are essential for the proper functionality of the pathway. HYPONASTIC LEAVES 1 (HYL1), a miRNA biogenesis cofactor, is post-translationally regulated by two mechanisms. On one hand, HYL1 activity depends on the phosphorylation of its RNA and protein-protein interaction domains. On the other, both its stability and degradation are defined by an undefined mechanism controlled by the environmental light conditions. Here, we report that HYL1 phosphorylation pattern changes depending on the growth conditions. Small RNA blots allowed us to find out that the environmental controlled changes in the HYL1 phosphorylation influence its activity impacting the mature miRNAs production. In a similar way, western blot and confocal microscopy allowed us to identify some particular conditions where the stability of HYL1 is impaired.

PL-C12
INTEGRATION OF LIGHT AND TEMPERATURE CUES IN PLANT DEVELOPMENT

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Plants, as sessile organisms, depend on their capacity to perceive and respond to changes in environment in order to survive and reproduce. Among ambient factors, light and temperature are undoubtedly the most relevant for plant development. For example, increasing light signaling inhibits hypocoyl elongation whereas increasing temperature (below the optimum) promotes hypocoyl elongation. The aim of this work is to analyze the combined effects of these signals on plant morphogenesis. Seedlings growing in nature can be shaded by taller plants, with a consequent reduction in radiation which lowers air and leaf temperature. Also, since chlorophylls absorb mainly in the blue and red portions of the spectrum, light under a canopy is enriched in green and far red wavelengths compared to sunlight. Changes in the red/far red ratio and red light intensity are perceived mainly by phytochrome B (phyB), one of the five members of the phytochrome family in Arabidopsis. To assess the effect of light and temperature changes caused by neighbors on plant morphogenesis transgenic lines of Arabidopsis carrying phyB variants of different stability were grown under a wide range of light conditions and temperatures. Hypocotyl elongation and the subnuclear distribution of phyBGFP were recorded. This data allowed us to develop a model which predicts plant growth in different light and temperature conditions.

PL-C13
PAP-SAL1 RETROGRADE PATHWAY IS INVOLVED IN IRON HOMEOSTASIS IN *Arabidopsis thaliana*

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Nuclear gene expression is regulated by a diversity of retrograde signals that travel from the organelles to the nucleus in a lineal or classical model. One such signal molecule is 3´-phosphoadenisine-5´-phosphate (PAP), the levels of PAP *in vivo* are regulated by a phosphatase enzyme SAL1/FRY1 located in chloroplast and mitochondria. This metabolite inhibits the action of a group of enzymes called exoribonucleases (XRNs), which participate in the regulation of the posttranscriptional gene expression. Transcriptome analysis of *Arabidopsis* mutant plants in PAP-SAL1 pathway revealed that the ferritin genes *AtFer1, AtFer3, and AtFer4* are up regulated in these genetic backgrounds, thus establishing a link between the PAP retrograde signaling pathway and the regulation of Fe homeostasis genes. In this work, we studied Fe homeostasis in *sal1* and *xrn* mutants, showing differences in the expression of genes implicated in Fe uptake and storage, we also observe divergences in the enzyme activities concerned in Fe uptake, comparing with wild type. In Fe deficiency conditions, *sal1* and *xrn* mutants grew well while wild type plants were clearly affected. These mutants presented a miscommunication between the root and the shoot, up-regulating Fe acquisition genes even when the nutritional demands are fulfilled.

PL-C14
IMPORTANCE OF THE PRECURSOR PRIMARY AND SECONDARY STRUCTURE DURING MICRORNA PROCESSING IN PLANTS

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MicroRNAs are 2022 nt small RNAs that play a role as posttranscriptional gene regulators. They recognize target sequences in longer RNAs by base complementarity and guide them to cleavage or translational arrest. MicroRNAs are generated from longer precursors that harbor a fold-back structure with the microRNA located in one of arms. In plants, microRNA precursors are completely processed in the nuclei by DICER-LIKE1 (DCL1). Artificial microRNAs can be generated by modifying the microRNA sequence in an endogenous precursor. Currently, artificial microRNAs are widely use in a broad range of species to inactivate specific target genes. Understanding microRNA precursor processing is then also important to improve artificial microRNA technologies. Here, we performed a random mutagenesis approach in Arabidopsis to identify mutations that improve the processing of the precursor of miR172, a microRNA that regulates flowering time. We found that a single base change increased by several folds the amount of

microRNA generated by the precursor. Furthermore, a systematic analysis revealed the importance of the secondary and also the primary sequence at specific positions of the miR172 precursor. The results provide new insights into the molecular basis of microRNA processing in plants and provide elements to design more efficient artificial microRNAs.

PL-C15
BACTERICIDAL AND CYTOTOXIC ACTIVITIES OFPOLYPHENOL EXTRACTS FROM ANDEAN ANDINDUSTRIAL POTATOES

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Potatoes (*Solanum tuberosum*) are a good source of dietary antioxidant polyphenols. This study investigated the *in vitro* antioxidant, bactericidal and cytotoxic activities of the polyphenols present in tubers of three Andean and one industrial potato varieties. Both phenolic acids content and antioxidant activity were higher in skin extracts than in flesh ones, being chlorogenic acid (CGA) the most abundant phenolic acid. Extracts from Andean Moradita flesh and from industrial Summer side skin showed bactericidal activity against E. coli ATCC 25922. Both extracts have high absolute content of CGA, presence of ferulic acid and absence of pigmentation. In contrast, no bactericidal effects were found against pathogenic *E. coli* O157. Positive control with gentamicin and commercial CGA resulted in inhibition of bacterial growth. We also showed that all extracts exerted a dose dependent cytotoxic effect in SH-SY5Yhuman neuroblastoma cells. Skin extracts were more potent than flesh ones, and commercial CGA treatments compromised SH-SY5Ycell viability. On the whole, results demonstrate that extracts with similar phenolic acid level and/or composition do not exert similar antioxidant and/or biological activity. These findings suggest that the activity of potato extracts is a combination of various bioactive compounds and contribute to the revalorization of potato as a functional food.

PL-C16
REGULATION OF THE PLANT MICRO RNA MACHINERY BY A MSS47-MEDIATED EPIGENETIC MECHANISM

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Micro RNAs (miRNA) are 21 nucleotides molecules essential for post-transcriptional gene silencing. These molecules are generated from a structured RNA precursor and then incorporated into the RNA induced silencing complex (RISC).DICER-LIKE1, HYPONASTIC LEAVES 1 and SERRATE are the main proteins involved in miRNA biogenesis but several new cofactors have being described recently. After processing of the miRNA precursors, ARGONAUTE 1 (AGO1) binds the mature miRNAs and inhibits targeted-mRNA translation or induces its cleavage. Using a forward genetic approach we have isolated novel mutants deficient in miRNA activity, from which several cofactors of the pathway, such as CPL1, RCF3 and the THO/TREX complex, have been recently described. Here we describe MIRNA-SILENCINGSUPPRESSED 47 (mss47), another miRNA deficient mutant identified in our genetic screening. Small RNA blots and RT-qPCR showed that mss47 mutants present altered level of miRNAs and targets' mRNAs. MSS47 codifies a protein with a histone-methyl transferase domain. This protein does not directly regulate the transcripts levels of MIRNA genes or its targets. Our experiments show that MSS47epigenetically regulates the abundance of essential factors of the miRNA pathway. Such depletion in the miRNA machinery impairs the production and activity of miRNAs and, therefore, the gene silencing and plant development.

PL-C17
PHYTOCHROME B REGULATES SYSTEMICSIGNALING OF DEFENSE RESPONSE IN*Arabidopsis*

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Phytochromes are a conserved group of photoreceptors that control multiple aspects of plant architecture and health. Phytochromes (Phy) oscillate between an active and inactive state depending on the red (R, 680 nm) to far red (FR, 730 nm)ratio of the incoming light. The R:FR ratio is translated into information by Phy to let the plant perceive and out compete future competitors through the initiation of the shade avoidance syndrome (SAS). PhyB is the main phytochrome controlling SAS. Interestingly, PhyB inactivation or mutation in Arabidopsisis accompanied by suppression of immune responses leading to enhanced susceptibility to insect damage and fungal infection. Given that PhyB controls the systemic differentiation of stomata and systemic development of the photosynthesis apparatus, we wondered if PhyB might also regulate the systemic induction of the defense response controlled by the phytohormone jasmonate (JA). To address this question we used a set of transgenic lines with tissue-specific expression of PhyB or inducible expression of PhyB. In order to establish the PhyB contribution to the systemic defense response, we performed bioassays based on the infection of the necrotrophic fungi *Botrytis cinerea* and monitored total phenolic contents and the expression of marker genes in leaves. The results will be discussed in the context of the current knowledge of the field.

PL-C18
PLANT NATRIURETIC PEPTIDES IMPROVE PLANTRESISTANCE DURING BIOTIC STRESS

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Plant natriuretic peptides (PNPs) are extracellular, systemically mobile molecules that are involved in the modulation of salt and water homeostasis and AtPNPA from *Arabidopsis thaliana* has been extensively studied. Distinctively, the bacterial pathogen of citrus plants, *Xanthomonas citri* subsp. *Citri* (Xcc) contains a *PNP*-like gene (*XacPNP*). Both peptides, AtPNPA and XacPNP induce similar physiological responses when applied on plant tissue, including stomatal opening and photosynthetic efficiency improvement. *A. thaliana-Pseudomonassyringae* pv. *Tomato* (Pst) patho system and its genetic resources allowed us to analyze the role of XacPNP and AtPNPA in *A. thaliana* during infection. To this aim, *A. thaliana* transgenic lines were generated overexpressing *XacPNP* and *AtPNPA*, and RNA interference lines silencing endogenous *AtPNPA* were also obtained. Overexpressing *PNPs* lines showed enhanced resistance to Pst, while *PNP*-deficient plants were more susceptible. Moreover, pretreatment of *A. thaliana* leaves with XacPNP before Pst infection resulted in increased resistance evidenced by higher remnant chlorophyll, lower pathogen survival and induction of defense associated genes. Our results state a role for PNPs during plant biotic stress improving plant performance under stressful conditions.

PL-C19
A GLYCINE RICH PROTEIN IS INVOLVED IN*Xanthomonas citri* SUBSP. *citri*-PLANTINTERACTION

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The Type III Secretion System (TTSS) is critical for pathogenicity and Hypersensitive Response (HR) induction by phytopathogens as *Xanthomonas citri*s ubsp. *citri* (Xcc): causal agent of citrus canker. HrpE protein is a principal component of TTSS and previously we characterized that this protein induces defense responses and HR in several plants. Moreover, by yeast two hybrid assays we had identified a citrus Glycine Rich Protein (GRP) as a putative protein involved in HrpE recognition. Interestingly, GRPs have a key role in plant defense mechanisms in several plant species. In this work, Bimolecular Fluorescence Complementation assays confirmed HrpE-GRP interaction *in vivo*. Also, we studied the response of HrpE intransgenic *Arabidopsis thaliana* knock outs in a GRP endogenous gene that is the closest homolog to the citrus GRP. These mutant GRP lines showed absence or slight HR in leaves infiltrated with HrpE recombinant protein compared to wild type plants. Furthermore, cell death, callose deposition and the expression of defense genes as PR1 were decreased in these transgenic lines treated with HrpE. These results suggest that GRP is involved in HrpE recognition and signal transduction events triggering plant defenses against Xcc. Our results indicate for the first time a role of plant GRP in citrus canker disease. This study enhances the understanding of Xcc infection process.

PL-C20
DESIGN OF A GFP-BASED NON-INVASIVE BIOSENSOR TO DETERMINE NADP$^+$(H) REDOX STATE IN LIVING CELLS

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One key component of central metabolism in all life forms is NADP$^+$(H). Current methods for NADP$^+$(H) determination in biological samples are hampered by lack of sensitivity and reproducibility, precluding studies of redox changes in living cells. The recent development of redox sensitive green fluorescent proteins (roGFPs) as genetically encoded probes allowed spatiotemporal detection of thiol-containing redox metabolites in vivo. We designed a biosensor to determine the cellular redox state of NADP$^+$(H), by fusing roGFP to the coding region of rice NADP$^+$(H) thioredoxin reductase C (NTRC) via spacer arms of variable lengths (30 and 45 residues). NTRC allows transduction of redox signals from the world of pyridine nucleotides to that of thiol-disulfides. The NTRC-roGFP fusions display reversible ratiometric fluorescence changes in response to the redox potential of NADP$^+$(H). *In vitro* characterization of both fusion proteins indicated that ratiometric values varied with the redox potential of NADP$^+$(H) as predicted by the Nernst equation, permitting its accurate determination by measuring the oxidation state of the probe. As a proof-of-concept test we transformed *Escherichia coli* cells and observed fluorescence changes by confocal microscopy in response to the redox state of the organism. This biosensor can be used to study the NADP$^+$(H) redox poise of cells and organelles in real time.

PL-C21
HEAT STRESS INDUCES FERROPTOSIS LIKE CELL DEATH IN PLANTS

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In plants, regulated cell death plays critical roles during development and is essential for plant-specific responses to abiotic and biotic stresses. However, the molecular mechanisms underlying plant cell death remain unclear. In this work, we examined whether ferroptosis, an iron-dependent, oxidative process recently described to occur in animal cells, could be relevant to cell death in plants. Although ferroptotic cell death was not involved in reproductive or vascular development, it was implicated in heat-shock-induced regulated cell death. Analyses of heat shock treated (HS) Arabidopsis roots using DIC and TEM microscopy showed a specific morphology associated to cell death. Biochemical features that are specific for animal ferroptosis are induced in HS-Arabidopsis roots, such as the iron dependent accumulation of ROS and lipid ROS, and the depletion of glutathione and ascorbic acid. The study of the

expression pattern of several genes related to cell death processes in plants and animals showed that a recently described gene (named KOD), which encodes a short peptide that regulates plant cell death is specifically regulated in HS-Arabidopsis roots. Although additional factors involved in the ferroptosis pathway remain to be identified in plants, many characteristics are conserved between plants and animal cells suggesting that ferroptosis is a conserved form of cell death.

STRUCTURAL BIOLOGY

SB-C01
GENERATION OF NANOBODIES AS A TOOL FOR STRUCTURAL BIOLOGY

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A promising approach to increase crystal formation and to improve diffraction quality is the use of crystallization chaperones. Nanobodies are exquisite chaperones for crystallizing complex biological systems such as membrane proteins. Nanobodies are the smallest and most stable single-domain fragments with the full antigen-binding capacity and that naturally occur in camelids. Here we describe a general methodology for the generation of nanobodies to be used as crystallization chaperones for the structural investigation of soluble and membrane proteins. We have set up a general pipeline to obtain nanobodies against different immunogens, particularly those of clinical relevance. Some of them are membrane proteins resuspended in detergent buffers and lipid vesicles for preserving their native conformation. The strategy includes the use of phage display technology to select the nanobodies, a step that was carried out with different methods to coat the antigens. We employed solid surface of an ELISA plate and nickel coated plate that allows to spatially orient the target protein. As a result, after two rounds of selection, phages that recognize conformational epitopes were obtained. The nanobodies are expressed in the periplasmic space and sixty specific clones were selected for sequencing by binding assays and their ability to avoid aggregation.

SB-C02
STRUCTURAL AND FUNCTIONAL STUDIES OF THE NTRX RESPONSE REGULATOR, A DIMERIC ATP BINDING PROTEIN

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Bacteria need to adapt to environmental changes in order to survive. Among the mechanisms employed for this task are the two-component systems (TCS). They are formed by a histidine kinase (HK) that autophosphorylates upon perception of a stimulus and transfers the phosphoryl group to the second component, the response regulator (RR), which modulates gene transcription. Our group has been interested in a TCS formed by the HK NtrY and the RR NtrX, which has been implicated in the detection of low oxygen tension in *Brucella abortus*. NtrX has a REC, a central AAA+, and a DNA binding domain. Previous studies allowed us to obtain the crystal structure of the full-length protein and to analyze the DNA binding. In this opportunity, we have examined some characteristics related to the AAA+ domain in order to gain insights into how NtrX works. We found that this RR is able to bind ATP but it cannot hydrolyze the nucleotide. Furthermore, we solved the structures obtained by soaking NtrX crystals with ATP and ADP, and describe the binding pocket. Also, we found that NtrX is a dimer in solution and that it does not undergo further oligomerization as a consequence of phosphorylation or nucleotide binding. Finally, we have developed a preliminary design for an in-vivo assay with *Caulobacter crescentus* to perform structure-function studies to identify important residues in NtrX mechanism of action.

SB-C03
UNRAVELLING THE LONG-RANGE SIGNALING MECHANISM OF BACTERIOPHYTOCHROMES

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Light-induced reactions allow organisms to adapt to different environmental factors. Bacteriophytochromes (BphPs) are light-sensing proteins found among photosynthetic and non-photosynthetic bacteria that are reversibly photoconverted between a red-absorbing (Pr) and a far-red-absorbing (Pfr) state. Most BphPs share a common architecture consisting of an N-terminal photosensor core module (PCM), which detects the light signal, and a C-terminal variable output module (OM), responsible for transducing this information into a biological effect. To date, it is still not fully understood how structural changes are propagated from the PCM to the OM during the photoconversion Pr-Pfr in the signal transduction event. Here we present the crystal structures of the full-lengh BphP (PCM + OM) from the plant pathogen Xanthomonas campestris (XccBphP) and of its isolated PCM. In the crystals, the full-lengh protein showed a Pr state while the PCM was found to be in the Pfr state. The quaternary assembly reveals a head-to-head dimer in which the OM contributes to the helical dimer interface. In solution, the full-length version behaves as a dimer while the PCM construct is a monomer. Our structural analysis suggests that the long-range signaling in BphPs may involve a kink and a rotation of the OM position via a helical spine movement during the photoconversion Pr-Pfr.

SIGNAL TRANSDUCTION

ST-C01
STRESS GRANULES CONTROL PROTEIN SYNTHESIS AND HAVE A NOVEL LINK TO NEURODEGENERATION

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Stress granules (SGs) are cytoplasmic supramolecular aggregates that form transiently in eukaryote cells upon acute stress. SGs belong to a growing family of "liquid organelles", which are membraneless and depend on protein aggregation domains for their formation. SGs contain repressed mRNAs, translation initiation factors and several RNA-binding proteins and are related to a number of abnormal protein aggregates present in neurodegeneration. Their significance to cell survival remains elusive. Here we show that SGs are involved in the control of the translational reprogramming upon stress. Using a puromycin-based method to measure translation in single cells we found that SGs form after the shutdown of protein synthesis and inactivation of eIF2alpha. However, the recovery of translation correlates with SG dissolution, thus suggesting that SG disassembly and release of mRNAs might be an important event to reinitiate protein synthesis. In a RNAi–based screen performed in Drosophila cells we identified 21 positive and 16 negative modulators of SG formation involved in mRNA metabolism and translation and linked to neurodegeneration. We confirmed the role of the vertebrate homologs in mammalian cell lines and we are currently investigating their role in the neuronal stress response in the Drosophila brain. We thank the DRSC, HMS, and ANPCyT, CONICET and UBA, Argentina for funding.

ST-C02,
ROLE OF THE SCAFFOLD PROTEIN STE5 IN THE INTEGRATION OF CDK AND MAPK SIGNALS: A DYNAMIC VIEW

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In *S. cerevisiae*, pheromone activates a GPRC coupled to a MAPK cascade pathway that, among other effects, arrests the cell cycle in G1. However, in cells committed to a new round of mitosis, CDK activity blocks pheromone response. Plasma membrane (PM) recruitment of the mating MAPK scaffold, Ste5, is a key step in pheromone signaling. It is this membrane interaction that is inhibited by CDK activity by phosphorylating residues flanking the Ste5 PM binding domain. Using a quantitative microscopy method to measure protein re-localization over time we studied the early dynamics of Ste5 recruitment in single live cells. We found that Ste5 inhibition by CDK requires negative feedback by the mating MAPK. Further analysis suggested that CDK-mediated inhibition and the Fus3-dependent negative feedback operate via the same set of phosphorylation sites in Ste5. Despite the inhibitory potential of both kinases, proper signal inhibition after cell cycle "start" can only be achieved by collaborative activity of CDK and MAPK. MAPK alone cannot displace Ste5 from the membrane but its activity is required to block MAPK signaling at low levels of CDK activity. We argue that this collaborative effect may be especially helpful in late-G1, when the key cyclins are not yet at peak levels, and that the negative feedback of the MAPK acts as a mechanism to impede improper activation of the mating program.

ST-C03
PROTEIN KINASE A LOCALIZATION IS CRITICAL FOR SPERM CAPACITATION

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Capacitation is the process that renders sperm able to fertilize. It is characterized by a shift in the motility pattern (hyperactivation) and acquisition of acrosome responsiveness. Protein Kinase A (PKA) is known as a key player during capacitation events, coordinating downstream events such as membrane hyperpolarization and hyperactivation. PKA is activated early during capacitation due to a rapid increase of intracellular cAMP. However, current evidence supports the hypothesis that proper localization of the enzyme is also crucial for its regulation, either positioning the kinase in close contact with its substrates or alternatively, refraining contact from them. Here, we address the role of PKA anchoring to AKPAs through the usage of a permeable peptide named st-HT31, to study this type of PKA regulation in capacitation and during acrosome reaction (AR) using mouse sperm. Delocalization of PKA blocked both its activity as well as tyrosine phosphorylation. Moreover, this blockade prevented membrane hyperpolarization of sperm. In this regard, st-HT31 also prevented the acrosome reaction and in vitro fertilization. Worth noticing, even though the biological activity of PKA was affected, the chemical activity of PKA was not impaired, as addressed by in vitro activity of PKA in the presence of st-HT31. Our results uncover a new type of PKA regulation during sperm capacitation.

ST-C04
ESSENTIAL ROLE OF CFTR IN HUMAN SPERM REGULATION OF MEMBRANE POTENTIAL AND PHI DURING CAPACITATION

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At ejaculation, mature sperm are not able to fertilize an oocyte. They require to spend a limited period of time in the female reproductive tract and undergo several maturational changes, all grouped under the name of capacitation. From a molecular point of view, one of the first events that occur during capacitation is a HCO3--dependent activation of the atypical soluble adenylyl cyclase ADCY10 which leads to cAMP synthesis and the subsequent activation of PKA. Also, HCO3- triggers alkalinization of the cytoplasm

and membrane hyperpolarization. However, the mechanisms by which HCO3- is transported into the human sperm and modulates intracellular pH and membrane potential is not well established. There is evidence that CFTR activity is involved in the human sperm capacitation but how this channel is integrated in the complex signaling cascades associated with this process remains largely unknown. In the present work we have analyzed the extent to which CFTR regulates of intracellular pH and membrane potential during capacitation. We observed that inhibition of CFTR affects HCO3- entrance resulting in lower PKA activity and that cAMP/PKA-downstream events are essential for the regulation of intracellular pH and membrane potential.

ST-C05
TWO-COMPONENT SYSTEMS IN BACTERIA: HOW IS THE SIGNAL UNIDIRECTIONALLY TRANSMITTED?

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Two-component systems (TCS), the main signaling pathways in prokaryotes, are canonically constituted of a sensor histidine-kinase (HK) and a cognate response regulator (RR). Upon a given environmental stimulus, the HK can autophosphorylate in a conserved His residue using ATP, and then transfer the phosphoryl to a conserved Asp of the RR, who often acts as a transcription factor executing a physiological adaptive response. In the absence of signal, the HK keeps its RR in a dephosphorylated state, acting also as a cognate phosphatase. These pathways have been shown to be highly specific and efficient, with a “His-to-Asp” irreversible phosphoryl pathway. However, more complex TCS termed phosphorelays, rely on shuttle domains that allow phosphate flow to occur bidirectionally (also “Asp-to-His”). By X-ray crystallography and biochemical *in vitro* assays, this work aims to elucidate how these canonical TCS elicit adaptive outputs through a tightly controlled and unidirectional phosphate flow. We have solved the crystal structures of the study model DesK-DesR complex, of *Bacillus subtilis*, in the phosphatase and phosphotransfer states at atomic resolution, using cytoplasmic DesK mutants known to stabilize different functional states. Structural data here presented contribute to understand how signaling occurs unidirectionally, ensuring the connection between stimulus and adaptive response.

POSTERS

BIOTECHNOLOGY

BT-P01
RECOMBINANT EXPRESSION OF SWEET PLANT PROTEIN MNEI IN FOOD-GRADE *Lactococcus lactis*
Garay Novillo JN, Amaranto M, Barra JL. CIQUIBIC-CONICET. Dpto. de Qca. Biológica, Fac. de Cs. Químicas, Universidad Nacional de Córdoba. E-mail: jgaraynovillo@gmail.com
BT-P02
USE OF AFFINITY TAGGED VMA1 INTEIN FOR THE PRODUCTION OF RECOMBINANT PHARMACEUTICAL PROTEINS.
Amaranto M, Correa EME, Garay Novillo JN, Barra JL. Departamento de Química Biológica, CIQUIBIC-CONICET. Facultad de Ciencias Químicas, UNC. E-mail: marilla.amaranto@gmail.com
BT-P03
EXPRESSION OF THE HYBRID BACTERIOCIN ENT35-MCCV IN *Saccharomyces cerevisiae*
Lanza L, Acuña L, Minahk CJ, Bellomio A, Chalón MC. Instituto Superior de Investigaciones Biológicas INSIBIO-CONICET-UNT, Tucumán, Argentina. E-mail: lulanza_522@hotmail.com.ar
BT-P04
ETHANOL FERMENTATION BY ANTARCTIC YEASTS
Braña V[1], Herrera L[2], García-Laviña CX[2], Castro-Sowinski S[2]. [1]Microbiología Molecular, IIBCE, Montevideo. [2]Bioquimica y Biología Molecular, Fac Ciencias, UdelaR. E-mail: victoriabra@gmail.com
BT-P05
ROLE OF XYLR ON XYLOSE METABOLISM IN *Herbaspirillum seropedica* EZ69
Malan AK, Batista S. Molecular Microbiology Unit, BIOGEM Department, IIBCE. Montevideo, Uruguay. E-mail: kmalan@iibce.edu.uy
BT-P06
STRATEGY FOR THE CONSTRUCTION OF *Saccharomyces cerevisiae* STRAINS ABLE TO ASSIMILATE XYLOSE
Fagundez A[1], Malán AK[1], Carbo N[1], Giménez M[1], Catalán AI[1], Guigou M[2], Lareo C[2], Batista S[1]. [1]Molecular Microbiology Unit, IIBCE. Uruguay. [2]Dept. Bioengineering, Faculty of Engineering, Uruguay. E-mail: alefagundez87@gmail.com
BT-P07
TRANSGENIC MAIZE PLANTS EXPRESSING HAHB11: A PROMISING PROOF OF CONCEPT
Raineri J, Otegui ME, Chan RL. Instituto de Agrobiotecnología del Litoral (IAL- UNL-CONICET). E-mail: jesica.raineri@santafe-conicet.gov.ar

BT-P08

MALTOOLIGOSACCHARIDES PRODUCTION FROM GLUTEN FREE STARCHES

Caminata Landriel S[1], Castillo de las Mercedes J[1], Rodríguez Gastón JA[1], Taboga O[2], Ferrarotti SA1, Costa H[1]. [1]Lab. Qca. Biológica, Dto. Cs. Básicas, Univ. Nacional de Luján. [2]Inst. Biotecnología, CICVyA-INTA. E-mail: solcaminata@yahoo.com.ar

BT-P09

DODECENLY SUCCINIC ANHYDRIDE-COLLAGEN MODIFIED HYDROGELS

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BT-P10

ANTIOXIDANT ACTIVITY OF *Larrea divaricata* LOADED IN MUCOADHESIVE POLYMERS AND SILICA COMPOSITES

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BT-P11

CASEINOLYTIC AND MILK-CLOTTING ACTIVITY OF *Solanum tuberosum* ASPARTIC PROTEASES (*ST*APS)

Tito FR, Pepe A, Frey ME, D'Ippólito S, Daleo GR, Guevara MG. IIB. UNMdP-CONICET. Mar del Plata. Argentina.
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BT-P12

VSPS OF *G. lamblia* AS CARRIERS IN CHRONIC ORAL ADMINISTRATION OF PEPTIDE DRUGS

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BT-P13

ACTIVE PACKAGING AGAINST *Escherichia coli* O157:H7 IN MEAT INDUSTRY

Koltan M[1], Paz García EC[1], Sanchez M[2], Pomares MF[1], Blanco Massani M[2], Eisenberg P[2], Vincent PA[1]. [1]INSIBIO, CONICET-UNT. Tucumán, Argentina. [2]INTI-Plásticos. Buenos Aires, Argentina. E-mail: marianokoltan@gmail.com

BT-P14

CLONING AND EXPRESSION OF A ROTAVIRUS VP6-FLIC131 FUSION PROTEIN

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BT-P15

BENEFICIAL RHIZOBACTERIA ENCAPSULATED IN NANOFIBERS FOR POTENTIAL APPLICATION AS SOYBEAN INOCULANTS

De Gregorio P[1], Michavila G[1], Lemes Santos F[2], Bonilha F[2], LM[1], Caram Di Santo M[1], Pomares M[1], Vincent P[1]. [1]INSIBIO, CONICET-UNT, Tucumán, Argentina. [2]Federal University of Rio Grande do Sul, Brazil. E-mail: priscilladegregorio@gmail.com

BT-P16

DESIGN OF A BIOTECHNOLOGICAL TOOL FOR INCREASING PROTEIN EXPRESSION IN PLANTS

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BT-P17

***Pseudomonas stutzeri* AS A PROMISING PLANT GROWTH-PROMOTING BACTERIA FOR SOYBEAN IN SALINE SOILS**

Lami MJ[1], Costa SB[1], Zenoff AM[1], Caram C[1], Esquivel-Cote R[2], Vincent PA[1], Espinosa Urgel M[3], De Cristóbal RE[1]. [1]INSIBIO CONICET-UNT, Arg. [2]Microbiología, Edafología, Col. Postgraduados, Mex. [3]EEZ, Granada España. E-mail: majesuslami@hotmail.com

BT-P18

DEVELOPMENT OF HETEROGENEOUS BIOCATALYST FOR PECTIN HYDROLYSIS OF VEGETABLE RESIDUES

Ramírez Tapias YA[1,2], Lapasset Laumann A[1], Britos CN[1], Trelles JA[1,2]. [1]Laboratorio de Investigaciones en Biotecnología Sustentable, UNQ. [2]CONICET.

BT-P19

BIOSYNTHESIS OF GALACTOSYL-FLOXURIDINE USING IMMOBILIZED *Β*-GALACTOSIDASE FROM *Micrococcus luteus*

Sarquiz A[1,2], Britos CN[1], Rivero CW[1,2], Trelles J.A[1,2]. [1]Laboratorio de Investigaciones en Biotecnología Sustentable, UNQ. [2]CONICET.

BT-P20

ENCAPSULATION *ECHINOCOCCUS GRANULOSUS* ANTIGENS FOR THE DEVELOPMENT OF A NANOVACCINE

Silvarrey C[1], Benavídez U[1], Briancon S[2] and Esteves A[1]. Universidad de la República, Uruguay; [2]Universidad Claude Bernard, Lyon, France.

BT-P21

BIOREMEDIATION STRATEGIES BASED ON A NATIVE STRAIN ISOLATED FROM SITES CONTAMINATED WITH HYDROCARBONS.

Conde Molina D[1], Liporace F[1], Vázque S[2], Merini L[3], Giulietti AM[2], Quevedo C[1].[1.]Biotechnology Research Laboratory, FRD-UTN, [2]. NANOBIOTEC, FFyB,UBA-CONICET,[3]. EEA-INTA Anguil, Argentina.

CELL BIOLOGY

CB-P01

PROTEIN S-ACYLATION IN *Trichomonas vaginalis*

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CB-P02

A NOVEL SIGNAL FOR ENDOCYTOSIS AND POLARITY IN YEAST

Bigliani GY, González Montoro A, Valdez Taubas J. CIQUIBIC, CONICET - Depto de Química Biológica, Fac. Ciencias Quimicas, Univ. Nac. de Córdoba. E-mail: gonzalobigliani@gmail.com

CB-P03

ST3GAL II AND β4GALNT I ARE S-ACYLATED AT N-TERMINAL CYSTEINES INVOLVED IN HOMO-DIMERIZATION

Chumpen Rami SV, Ruggiero FM, Daniotti JL, Valdez Taubas J. Depto. Química. Biológica., CIQUIBIC-CONICET, Fac. Cs. Químicas, Univ. Nac. de Córdoba. E-mail: schumpen@dqb.fcq.unc.edu.ar

CB-P04

IDENTIFICATION OF A PLASMA MEMBRANE FUSION SUPERFAMILY, FUSEXIN, SUFFICIENT TO FUSE GAMETES, ENVELOP

Valansi C[1#], Moi D[2#], Matveev E[1], Graña M[3], Romero H[4], Aguilar PS[2], Podbilewicz B[1]. [1]Dep of Biology, Technion- Israel Institute of Technology, Israel. [2]Lab Biol Cel de Membranas, IIB-UNSAM, Argentina. [3]Institut Pasteur Montevideo, Uruguay. [4]Lab de Org y Evol del Genoma, Facultad de Ciencias, UdeLaR, Uruguay. [#]contributed equally to this work. Email: paguilar@iibintech.com.ar and podbilew@technion.ac.il

CB-P05

CHANGES IN SECRETORY PATHWAY MARKERS IN A PC12 CELL MODEL OF PARKINSON´S DISEASE

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CB-P06

POST-TRANSLATIONAL INCORPORATION OF L-DOPA INTO THE C-TERMINUS OF α-TUBULIN IN LIVING CELLS

Dentesano Y[1], Ditamo Y[1], Hansen C[2], Arce CA[1], Bisig CG[1], [1]C IQUIBIC-CONICET, Depto de Qca Biológica, FCQ, U.N. Córdoba, [2] Inst. Oulton Cba, Argentina. E-mail: ydentesano@fcq.unc.edu.ar

CB-P07

DEVELOPMENT OF SCREENING METHODS TO IDENTIFY TRANSLESION DNA SYNTHESIS INHIBITORS

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CB-P08

KLF6 TUMOR SUPPRESSOR ACTIVITY IS ASSOCIATED TO THE INDUCTION OF CELLULAR SENESCENCE

Sabatino ME, Pansa MF, García AI, Carbajosa S, Villafañez F, Soria GR, Bocco JL. Centro de Investigaciones en Bioquímica Clínica e Inmunología. CIBICI-CONICET. Córdoba, Argentina.E-mail: mesabatino@fcq.unc.edu.ar.

CB-P09

IDENTIFICATION OF TLS INHIBITORS THROUGH THE DEVELOPMENT OF IMAGING-BASED SCREENING PLATFORMS

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CB-P10

KLF6 SUCBELLULAR DISTRIBUTION AS A MARKER OF TUMOR AGGRESSIVENESS IN HUMAN COLON ADENOCARCINOMA

Grupe V[1], Sabatino ME[2], Cordero V[3], Cabalier E[3], Lucca A[1], Cortiñas E[1], Monteverdi L[1], Bocco JL[2]. [1]Fund. Progreso de la Medicina. [2]CIBICI-CONICET-UNC. [3]Hosp. Nacional Clínicas. Córdoba, Argentina. E-mail: verogrupe@hotmail.com.

CB-P11

DEVELOPMENT OF A SCREENING PLATFORM FOR THE IDENTIFICATION OF LETHALITY INDUCERS IN CANCER CELLS

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CB-P12

STAPHYLOCOCCAL α-TOXIN REGULATES C-JUN ONCOPROTEIN ACTIVATION, ITS mRNA LEVEL AND PROTEIN STABILITY

Moyano AJ[2], Racca AC[1], Soria GR[1], Andreoli V[1], Smania AM[2], Panzetta-Duttari G[1], Sola C[1], Bocco JL[1]. [1]CIBICI, Dpto. de Bioquímica Clínica; [2]CIQUIBIC, Dpto. de Química Biológica, Fac. Cs. Químicas, UNC. E-mail: amoyano@fcq.unc.edu.ar

CB-P13

CORRELATION BETWEEN PMCA ACTIVITY AND TUBULIN ON PLATELET FUNCTION IN SPONTANEOUSLY HYPERTENSIVE RAT

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CB-P14

NATURAL ANTISENSE TRANSCRIPTS IN THE REGULATION OF ACSL4 EXPRESSION IN BREAST CANCER CELLS

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CB-P15

INSULIN INDUCES THE EXOCYTIC TRAFFIC OF LRP1 FROM GSV–LIKE STRUCTURAL VESICLES

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CB-P16

CHARACTERIZATION OF HUMAN SIALIDASE NEU3 MEMBRANE ASSOCIATION

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CB-P17

POST-TRANSLATIONAL INCORPORATION OF PHENYLALANINE INTO TUBULIN AS A CAUSE OF NEURONAL DYSFUNCTION

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CB-P18

LRP1 PARTICIPATES IN HEMIN-INDUCED AUTOPHAGY, MODYFING ITS TRAFFICKING IN ERYTHROLEUKEMIA CELLS

Grosso RA, Martín JM, Sánchez MC, Chiabrando GA; Colombo MI; Fader CM. Instituto de Histología y Embriología-CCT-CONICET-Mendoza. E-mail: grossoruben88@gmail.com.

CB-P19

UTEROSOME-LIKE VESICLES PROMPT HUMAN SPERM FERTILIZING CAPACITY

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CB-P20

TEMPORAL REGULATION OF STRESS GRANULES BY CIRCADIAN CLOCKS AND OTHER MECHANISM

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CB-P21

IDENTIFICATION OF KLDHC5 AS AN INTERACTING PROTEIN OF STARD7

Rojas ML[1], Flores-Martín J[1], Panzetta-Dutari GM[1], Bennett EJ[2], Genti-Raimondi S. [1]Dpto Bioq. Clínica, FCQ-UNC. CIBICI-CONICET. [2]Division of Biological Sciences at UCSD, La Jolla, USA. E-mail: mlaurarojas8@gmail.com.

CB-P22

CHLORPYRIFOS INDUCES ENDOPLASMIC RETICULUM STRESS ASSOPCIATED WITH P53 DEGRADATION IN JEG-3 CELLS

Reyna L, Flores-Martín J, Ridano ME, Panzetta-Dutari GM, Genti-Raimondi S. Dpto. Bioquímica Clínica, Facultad de Ciencias Químicas-UNC. CIBICI-CONICET. Argentina. E-mail: lucireyna@fcq.unc.edu.ar.

CB-P23

STARD7 KNOCKDOWN LEADS TO α5β1 INTEGRIN UPREGULATION AND GOLGI FRAGMENTATION IN HTR8/SVNEO CELLS

Cruz Del Puerto MM, Flores-Martín J, Reyna L; Panzetta-Dutari GM, Genti-Raimondi S. Dpto. Bioquímica Clínica, Facultad de Ciencias Químicas-UNC. CIBICI-CONICET. Argentina. E-mail: mdelpuertoi@fcq.unc.edu.ar.

CB-P24

TAMS INDUCE ENDOCRINE RESISTANCE AND STEM CELL-LIKE ENRICHMENT IN BREAST CANCER CELLS

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CB-P25

VITAMIN A DEFICIENCY: ALTERS OXIDATIVE STRESS AND INFLAMMATION GENE EXPRESSION IN MAMMARY GLAND.

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CB-P26

IN VIVO GPAT2 KNOCK-DOWN ACTIVATES APOPTOTIS

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CB-P27

CELLULAR CHANGES ASSOCIATED WITH R-CRT PRO-APOPTOTIC ACTION INDUCED BY BORTEZOMIB IN GLIOMA CELLS

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CB-P28

PERIVITELLIN SYNTHESIS ADAPTS TO REPRODUCTIVE ACTIVITY IN THE SNAIL *Pomacea canaliculata*

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CB-P29

VALIDATION OF REFERENCE GENES FOR REPRODUCTIVE STUDIES IN THE INVASIVE SNAIL *Pomacea canaliculata*

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CB-P30

ROLE OF CHROMATIN STRUCTURE ON SMN2 E7 ALTERNATIVE SPLICING

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CB-P31

4-HYDROXY-3-(3-METHYL-2-BUTENYL)-ACETOPHENONE (4-HMBA) INHIBES PROLIFERATION OF MELANOMA B16F0 CELLS

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CB-P32

FLAVIVIRUS: TOWARDS THE DESIGN OF A MOLECULAR PLATFORM FOR ANTIVIRAL ASSAYS

Vazquez CT[1], Ispizua JI[1], Bandoni Garay DP[1], Lorch MS[1], Contigiani MS[2], Lozano ME[1], Goñi SE[1]. [1]Área de Virosis Emergentes y Zoonóticas, LIGCBM, UNQ, Arg. [2]Lab de Arbovirus, INViV, UNC, Arg.E-mail: tahiscecilia@hotmail.com

CB-P33

NS1: DIFFERENT APPROACHES TO IMMUNE RECOGNITION AND FLAVIVIRUS DIAGNOSIS

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CB-P34

MOLECULAR TOOLS DEVELOPMENT FOR ST. LOUIS ENCEPHALITIS VIRUS PATHOGENESIS STUDY

Ispizua JI[1], Brandoni Garay DP[1], Vázquez CT[1], Albrieu G[2], Díaz LA[2], Contigliani MS[2], Lozano ME[1], Goñi SE[1]. [1]Área de Virosis Emergentes y Zoonóticas, LIGCBM, UNQ, Arg. [2]Lab de Arbovirus, INViV, UNC, Arg.E-mail: Ispizua.juan@hotmail.com

CB-P35

GOLGI BODIES IN THE GOLGI-LACKING PARASITE *Giardia lamblia*

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CB-P36

DEVELOPMENT OF AN ORAL VACCINE AGAINST TUBERCULOSIS BASED ON VIRUS-LIKE PARTICLES

Martina MA[1], Rupil LL[1], Serradell MC[1], García VE[2], Colombo MI[3], Berod L[4], Sparwasser T[4]; Luján HD[1]. [1]UCC, CIDIE-CONICET. [2]IQUIBICEN, UBA-CONICET. [3]IHEM-CONICET, UNCuyo. [4]TWINCORE. E-mail: mmartina@cidie.ucc.edu.ar.

CB-P37

IMMUNOGENIC PROPERTIES OF THE EXTRACELLULAR DOMAIN OF VARIANT SURFACE PROTEINS OF *Giardia lamblia*

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CB-P38

VARIANT-SPECIFIC SURFACE PROTEINS AS MEDIATORS OF ANTIGENIC VARIATION IN *Giardia lamblia*

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CB-P39

VLPS PSEUDOTYPED WITH VARIANT SURFACE PROTEINS OF *Giardia*AS AN EFFECTIVE ORAL INFLUENZA VACCINE

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CB-P40

ROLE OF MVBS FORMATION DURING ANTIGEN CROSS-PRESENTATION BY DENDRITIC CELLS

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CB-P41

CHARACTERIZATION OF VAMP ISOFORMS INVOLVED IN CORTICAL GRANULE EXOCYTOSIS IN MOUSE OOCYTE

Garrido F[12],De Paola M[1], Cappa AI[1], Michaut M[12]. 1IHEM, CONICET-UNCuyo 2FCEN, UNCuyo. E-mail: mmichaut@gmail.com.

CB-P42

ASSESSMENT OF KRÜPEL-LIKE FACTOR 6 FUNCTION IN HUMAN EXTRAVILLOUS TROPHOBLAST CELLS

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CB-P43

FUNCTIONAL CROSS-TALK BETWEEN THE GLUCOCORTICOID AND PROGESTIN RECEPTORS

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CB-P44

N-TERMINAL DOMAIN OF C-FOS AS A NEGATIVE DOMINANT FOR BRAIN CANCER THERAPY

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CB-P45

LIPOSOMAL VEHICULIZATION OF ZN PHTHALOCYANINES AND AMINE DERIVATES IN PDT INACTIVATION OF T98G CELLS

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CB-P46

CYTOPLASMIC FRA1 AND CFOS AS POTENTIAL TARGETS FOR BREAST CANCER THERAPY

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CB-P47

POSSIBLE TREATMENTS AGAINST *Trypanosoma cruzi* THROUGH THE COMBINED USE OF TRYPANOCIDAL DRUGS.

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CB-P48

THE INVOLVEMENT OF SPLICING FACTORS IN THE SUMO CONJUGATION PATHWAY

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CB-P49

SECRETION PROFILE OF THE TCTASV-C PROTEINS IN DIFFERENT *Trypanosoma cruzi* STRAINS

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CB-P50

FORMING EXOSOMES WITHOUT ALL THE FOUNDING PLAYERS

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CB-P51

CHARACTERIZATION OF THE ROLE OF *Saccharomyces cerevisiae* EISOSOMAL MEMBRANE DOMAINS IN AGING

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CB-P52

HUMAN ERYTHROCYTES AS EARLY TARGETS OF THALLIUM TOXICITY

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CB-P53

TL(I) AND TL(III) AFFECT DIFFERENTIALLY PC12 CELL DIFFERENTIATION

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CB-P54

IS THE METABOLISM OF EXTRACELLULAR ATP INVOLVED IN THALLIUM-MEDIATED CITOTOXICITY?

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CB-P55

EARLY RESPONSE OF ANTIOXIDANT ENZYMES TO TL(I)- AND TL(III)-MEDIATED OXIDATIVE STRESS IN PC12 CELLS

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CB-P56

THE TRANSLATION INHIBITOR 4EBP IS REQUIRED FOR ADAPTATION TO HYPOXIA IN *Drosophila*

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CB-P57

miRNA TURNOVER CONTROL BY LINEAR AND CIRCULAR RNA TARGETS

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CB-P58

ANTITUMOR EFFECT OF A CU(II) COMPLEX WITH SACCHARINATE AND GLUTAMINE RELEASED FROM SILICA SPHERES

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CB-P59

ANALYSIS OF NEW ORGANOMETALLIC COMPOUNDS AS POTENTIAL AGENTS AGAINST CHAGAS DISEASE

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CB-P60

BDNF EXPRESSION IN THE TESTES OF RESERPINE-TREATED RATS

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CB-P61

SYK: A SPECIFIC TARGET FOR CELLULAR IMMUNOTHERAPY OF RETINOBLASTOMA

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CB-P62

GALECTIN-3 DEFICIENCY DRIVES LUPUS-LIKE AUTOIMMUNE DISEASE BY PROMOTING SPONTANEOUS GERMINAL CENTERS FORMATION

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CB-P63

TOWARDS NEW THERAPIES AGAINST CANCER: STUDYING PIN1 AS A THERAPEUTIC TARGET IN NEUROBLASTOMA

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CB-P64

RETINOIDS AND HER2 INHIBITORS AFFECT THE BEHAVIOR OF MAMMARY CANCER STEM CELLS

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CB-P65

NORCANTHARIDIN IMPAIRS MAMMARY CANCER STEM CELLS GROWTH AND IN VIVO TUMOR PROGRESSION

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CB-P66

GLUCOSE 6-PHOSPHATE DEHYDROGENASE INHIBITION SENSITIZE MELANOMA CELLS TO METFORMIN TREATMENT

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CB-P67

DIFFERENTIAL EFFECTS OF TWO ORGANOPHOSPHORUS PESTICIDES ON POLYAMINE METABOLISM IN TOAD EMBRYOS

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ENZYMOLOGY

EN-P01

KINETIC AND STRUCTURAL CHARACTERIZATION OF UDP-GLUCOSE PYROPHOSPHORYLASE FROM Euglena gracilis

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EN-P02

CRDSP, A PHOSPHOGLUCAN PHOSPHATASE INVOLVED IN STARCH METABOLISM IN *C. reinharditii*

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EN-P03

OXIDOREDUCTASE ACTIVITY AND IRON-SULFUR CLUSTER BINDING OF GLUTAREDOXINS FROM *Leptospira interrogans*

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EN-P04

HETEROLOGOUS PRODUCTION AND CHARACTERIZATION OF A THERMOSTABLE GH10 FAMILY ENDO-XYLANASE

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EN-P05

MODELING AND CHARACTERIZATION OF β-XYLOSIDASE ECXYL43 ON NATURAL AND ARTIFICIAL SUBSTRATES

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EN-P06

ASSOCIATION BETWEEN ALDOSE REDUCTASE AND TUBULIN: EFFECT OF TYROSINE DERIVATIVES.

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EN-P07

STIMULATION OF ALDOSE REDUCTASE ACTIVITY BY TUBULIN: EFFECT OF PHENOLIC ACID DERIVATIVES

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EN-P08

RIBULOSE 5-PHOSPHATE EPIMERASE ISOENZYMES IN *Trypanosoma cruzi*: STRUCTURE, KINETICS AND LOCALIZATION

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EN-P09

CHARACTERIZATION OF HEME OXYGENASE AND FERREDOXIN-NADP+ REDUCTASE IN *Leptospira biflexa*

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EN-P10

BIOCHEMICAL CHARACTERIZATION OF THE GLYCOGEN STORAGE DISEASE-ASSOCIATED A16P MUTANT OF GLYCOGENIN

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EN-P11

STRUCTURE TO FUNCTION STUDIES ON SUCROSE SYNTHASES FROM *Anabaena variabilis*

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EN-P12

SHIFTING COFACTOR SPECIFICITY OF PEACH GLUCITOL DEHYDROGENASE BY STRUCTURE-GUIDED MUTAGENESIS

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EN-P13

KINETIC AND REGULATORY CHARACTERIZATION OF *Arabidopsis thaliana* PHOSPHOENOLPYRUVATE CARBOXYKINASE

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EN-P14

MECHANISM OF INHIBITION OF EPIGALLOCATECHIN ON THE PLASMA MEMBRANE CA2+-ATPASE

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EN-P15

DIFFERENTIAL INHIBITION OF PLASMA MEMBRANE CA2+-ATPASE BY QUERCETIN AND GOSSYPIN

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EN-P16

BERYLLIUM AND ALUMINUM FLUORIDE COMPLEXES TO STUDY PHOSPHORYLATED STATES IN THE NA,K-ATPASE

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EN-P17

REDOX MODULATION OF HSIRT6, KEY ENZYME OF METABOLISM AND INFLAMMATION

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LIPIDS

LI-P01

OXER1 IS INVOLVED IN ADRENOCORTICAL CELL PROLIFERATION

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LI-P02

ω-6 AND ω-3 FATTY-ACIDS ON EARLY STAGES OF MICE SUBMANDIBULAR GLANDS TUMOR

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LI-P03

ROLE OF PHOSPHATIDYLCHOLINE BIOSYNTHESIS ON NEURONAL DIFFERENTIATION

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LI-P04

BIOCHEMICAL CHARACTERIZATION OF GM1 MICELLES-AMPHOTERICIN B INTERACTION

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LI-P05

NITRO-FATTY ACID MODULATES MACROPHAGE LIPID METABOLISM

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LI-P06

LIPID CHARACTERIZATION OF CORTICAL BRAIN IN A STZ-INDUCED RAT MODEL OF SPORADIC ALZHEIMER'S DISEASE

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LI-P07

IN VITRO AND IN VIVO EVALUATION OF MANDARIN PEEL OIL ON LIPID METABOLISM AND TUMOR GROWTH

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LI-P08

2-ARACHIDONOYL GLYCEROL IN *Caenorhabditis elegans* DAUER DIAPAUSE REGULATION

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LI-P09

EXPLORING THE LINK BETWEEN BRANCHED CHAIN FATTY ACIDS AND ENDOCANNABINOIDS IN*Caenorhabditis elegans*

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LI-P10

SPHINGOSINE 1 PHOSPHATE RECEPTOR 2 REGULATES EPITHELIAL CELLS MONOLAYER INTEGRITY

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LI-P11

SPHINGOMYELIN SYNTHESIS MODULATES E-CADHERIN MATURATION AND RENAL EPITHELIAL CELL DIFFERENTIATION

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LI-P12

THE ROLE OF XBP-1 IN OSMOTIC ACTIVATED-LIPID SYNTHESIS.

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LI-P13

Tl(I) AND Tl(III) INDUCE ALTERATIONS IN LIPID METABOLISM IN DIFFERENTIATED MDCK CELLS

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LI-P14

ETHER-LINKED LIPIDS OF RAT DEVELOPING AND ADULT EPIDIDYMIS

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LI-P15

DISRUPTION OF THE CYTOSKELETON AND ALTERED LIPID METABOLISM IN SERTOLI CELLS

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LI-P16

DECREASED OXLDL UPTAKE AND CHOLESTEROL EFflUX IN THP1 CELLS ELICITED BY CORTISOL

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LI-P17

MOLECULAR CONSEQUENCES OF GPAT2 KNOCK-DOWN IN BREAST CANCER CELLS

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LI-P18

STUDIES ON THE MOLECULAR CLOCK AND THE CIRCADIAN REGULATION OF HEPATIC TUMORAL CELL METABOLISM

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LI-P19

NEUTRAL LIPIDS ARE INDUCED IN THE APPLE SNAIL *Pomacea canaliculata* BY CYPERMETHRIN PESTICIDE

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LI-P20

EMERGING ROLES OF PHOSPHOLIPASES D IN RETINAL PIGMENT EPITHELIUM CELLS EXPOSED TO HIGH GLUCOSE

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LI-P21

PLD1-PKCε PATHWAY PROTECTS FROM LPS-INDUCED CELL DAMAGE IN RETINAL PIGMENT EPITHELIUM CELLS

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LI-P22

UNSATURATED FATTY ACID BIOSYNTHESIS IN*MYCOBACTERIUM SMEGMATIS*

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LI-P23

PHYLOGENETIC ANALYSIS OF FATTY ACID DESATURASES REVEALS CONTRASTING EVOLUTIONARY CLUES IN CILIATES

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LI-P24

ENDOCANNABINOID METABOLISM IN ROD OUTER SEGMENTS DEPENDS ON THE ILLUMINATION STATE OF THE RETINA

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LI-P25

EXPRESSION ANALYSIS OF CYTOCHROME P450 GENES IN PYRETHROID-RESISTANT *TRIATOMA INFESTANS*

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LI-P26

THE CORRECT LOCALIZATION OF PTEN IN EPITHELIAL CELLS DEPENDS ON GLYCOSPHINGOLIPID METABOLISM

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LI-P27

HEPATIC CES3/TGH IS DOWNREGULATED IN THE EARLY STAGES OF LIVER CANCER DEVELOPMENT IN THE RAT

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LI-P28

GLUCOSE METABOLISM IN SKELETAL MUSCLE OF RATS FED A FUNCTIONAL MILK FAT AT HIGH LEVELS

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LI-P29

EFFECT OF CONJUGATED LINOLEIC ACID AND HIGH FAT DIETS ON TRIACYLGLYCEROL METABOLISM IN RAT OFFSPRING

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LI-P30

PHOSPHATIDIC ACID SIGNALING PARTICIPATES IN THE NEURODEGENERATION INDUCED BY α-SYNUCLEIN

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LI-P31

***Halamphora coffeaeformis*: A SOURCE OF LIPIDS FOR BIODIESEL AND VALUE-ADDED CO-PRODUCTS**

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LI-P32

HYDROCARBON ASSIMILATION IN EUROTIALEAN AND HYPOCREALEAN FUNGI: ROLES FOR CYP52 AND CYP53 GENES*Huarte Bonnet C; Ponce JC; Saparrat MCN; Santana M; Pedrini N*
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LI-P33

GANGLIOSIDE SYNTHESIS BY PLASMA MEMBRANE-ASSOCIATED ECTOSIALYLTRANSFERASE IN MACROPHAGES

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LI-P34

LIPID METABOLISM IN CANCER CELLS: ROLE OF FABP5

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LI-P35

CELLULAR FATE OF PHOSPHOLIPIDS (PL) SYNTHESIZED DURING G2/M

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LI-P36

STUDY OF THE INTERACTION OF L-PHE WITH LIPID MEMBRANES AND ITS IMPLICATIONS IN BIOLOGICAL MEMBRANES

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MICROBIOLOGY

MI-P01

PROTEOME TURNOVER ANALYSIS IDENTIFIES PHYTOENE SYNTHASE AS A LON SUBSTRATE IN *Haloferax volcanii*

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MI-P02

IDENTIFICATION OF POTENTIAL TARGETS OF PROTEASE RhoII IN*Haloferax volcanii*

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MI-P03

EXPRESSION OF NOS FROM MARINE MICROORGANISMS IMPROVES GROWTH AND NITROGEN METABOLISM IN*E.coli*

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MI-P04

LOW LEVELS OF POLAR FLAGELLIN EXPRESSION IN MATURE BIOFILMS FROM*Azospirillum brasilense*

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MI-P05

POLYPHOSPHATE ROLE IN*Gluconacetobacter diazotrophicus*ABIOTIC STRESS RESISTANCE AND BIOFILM FORMATION

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MI-P06

BEHAVIOR OF *Bradyrhizobium* SEMIA6144 MEMBRANE DURING ADAPTATION TO WATER DEFICIT

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MI-P07

IMPACT OF ARSENIC IN *Bradyrhizobia* STRAINS AND IN THE SYMBIOTIC INTERACTION WITH PEANUT PLANT

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MI-P08

MESORHIZOBIUM LOTITYPE VI SECRETION SYSTEM, A RELEVANT PLAYER IN THE LOTUS NODULATION

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MI-P09

ANALYSIS OF FUNCTIONAL REDUNDANCY OF RIESKE SUBUNIT IN CYTOCHROME BC OF *Mesorhizobium loti*

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MI-P10

LIPID SIGNALLING IN RESPONSE TO HYDRIC DEFICIT IN*Azospirullum*-INOCULATED BARLEY SEEDLING

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MI-P11

VOLATILE COMPOUNDS FROM*Klebsiella oxytoca*KD70 PROMOTE GROWTH OF ARABIDOPSIS SEEDLINGS

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MI-P12

ROLES OF RHIZOBIAL SURFACE COMPONENTS ON PROTECTION AGAINST ENVIRONMENTAL STRESSES

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MI-P13

PYROSEQUENCING REVEALS CHANGES IN FUNGAL SOIL COMMUNITIES UNDER*Lotus tenuis* MONOCULTURE

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MI-P14

MutS-DEPENDENT REGULATION OF THE ERROR-PRONE Pol IV ACTION: DNA STRUCTURES AS KEY MODULATORS

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MI-P15

A TWO-COMPONENT SYSTEM AFFECTS THE LOCALIZATION OF A DIVISOME PROTEIN IN *Streptococcus pneumoniae*

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MI-P16

AN ALLOSTERIC TRIGGER IN THE REGULATION OF ADP-GLUCOSE PYROPHOSPHORYLASE FROM ACTINOBACTERIA

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MI-P17

STUDY OF EUKARYOTE-LIKE ACETYL-CoA CARBOXYLASES OF ACTINOBACTERIA

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MI-P18

MUTAGENIC-MEDIATED DIFFERENTIATION IN *Bacillus subtilis*AFTER INTERACTION WITH *Setophoma terrestris*

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MI-P19

DNA RECOMBINATION IN*Escherichia coli*AND *Pseudomonas aeruginosa*

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MI-P20

THE RcsB-DEPENDENT MOTILITY BEHAVIOR REQUIRES THE LONG AND SHORT O-ANTIGEN CHAIN LENGTH DETERMINANTS

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MI-P21

IDENTIFICATION AND CHARACTERIZATION OF A NEW ADHESIN IN *Brucella.*

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MI-P22

EXPRESSION ANALYSIS OF THE*sua*GENE IN BIOFILM*Streptococus uberis*STRAINS

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MI-P23

CHARACTERIZATION OF A*Streptococcus uberis*MUTANT STRAIN DEFICIENT IN THE*sua* GENE

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MI-P24

MONOCLONAL ANTIBODIES TO DISTINGUISH BETWEEN SHIGA TOXIN-PRODUCING*E. coli*O157 AND O145 SEROGROUPS

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MI-P25

USE OF A TAT–DEPENDANT SYSTEM TO STUDY PEDIOCIN PA–1 MECHANISM OF ACTION AGAINST*E. coli*

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MI-P26

RNA POLYMERASE IS THE PRIMARY TARGET OF MICROCIN J25 IN*Salmonella enterica* serovar Newport

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MI-P27

THE*prtR*GENE INDIRECTLY ACTIVATES THE BACTERIOCIN PRODUCTION IN*P. fluorescens* SF4C

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MI-P28

NEW HYBRID BACTERIOCIN WITH ACTIVITY AGAIST FOODBORNE PATHOGENS

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MI-P29

TbRRM1, A SR-RELATED PROTEIN, REGULATES TRANSCRIPTION RATES IN*Trypanosoma brucei* PROCYCLIC CELLS

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MI-P30

ISOTRETINOIN INHIBITS ESSENTIAL METABOLITES TRANSPORT AND EXERTS TRYPANOCIDAL ACTIVITY

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MI-P31

CHARACTERIZATION OF MUTATIONS IN THE β-LACTAMASE AmpC FROM*Pseudomonas aeruginosa*CF ISOLATES

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MI-P32

***Proteus mirabilis*SECRETES FACTORS THAT AFFECT*Klebsiella pneumoniae*GROWTH AND SURVIVAL IN URINE**

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MI-P33

COMMUNITY-ASSOCIATED METHICILLIN RESISTANT *S. aureus*HOSPITAL ACQUIRED, ARGENTINA

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MI-P34

SURVIVAL AND GENES EXPRESSION OF MRSA EPIDEMIC CLONES ON AN ENVIRONMENTAL INERT SURFACE

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MI-P35

KTCF20: A KILLER AGENT AGAINST*Candida*

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MI-P36

A NOVEL AND RAPID METHOD FOR THE PURIFICATION OF KILLER TOXINS

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MI-P37

THE KILLER EFFECT OF LACTOFERRIN IN *Giardialamblia* INVOLVES CRITICAL MORPHOLOGICAL DEFECTS

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MI-P38

EFFECT OF DICHLOROACETATE ON *S. cerevisisae* RESISTANCE TO FLUCONAZOLE

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MI-P39

ANALYSIS OF*Giardia Lamblia* PROTEIN-S-ACYLTRANSFERASES USING COMPLEMENTATION ANALYSIS IN YEAST

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MI-P40

GENOMICS AND PROTEOMICS OF BACTERIOCIN-PRODUCING STRAIN*Pseudomonas fluorescens*SF4C

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MI-P41

OMICS APPROACH FOR SAFETY AND INDUSTRIAL POTENTIAL ASSESSMENT OF FOOD ISOLATED ENTEROCOCCI

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MI-P42

ISOLATION AND CHARACTERIZATION OF PATHOGENS CAUSING FOODBORNE DISEASES

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MI-P43

ISOLATION OF POTENTIAL BENEFICIAL BACTERIA WITH PROTEOLYTIC ACTIVITY FROM POULTRY FEED

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MI-P44

GENOME-SCALE METABOLIC MODEL OF *Lb. casei* BL23 REVEALS THE ROLE OF REDOX BALANCE IN FLAVOR FORMATION

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MI-P45

ACCUMULATION OF POLYPHOSPHATE IN LACTIC ACID BACTERIA AND ITS INVOLVEMENT IN STRESS RESISTANCE

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MI-P46

EFFECTS OF LACTIC ACID BACTERIA ON INFLAMMATORY CYTOKINES PRODUCTION BY ARPE-19 CELLS

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MI-P47

S-LAYER PROTEINS OF*Lactobacillus sp.*AS POTENTIAL TREATMENT FOR BACTERIAL AND VIRUS PATHOGENS

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MI-P48

INTRA-STRAIN VARIABILITY IN THE AMINO ACID SEQUENCE OF S-LAYER PROTEINS FROM *Lactobacillus kefiri*

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MI-P49 BIOSYNTHESIS OF 5-HALOGENATED NUCLEOSIDES USING NANOSTABILIZED LACTIC ACID BACTERIA

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MI-P50 ANTIVIRAL COMPOUND BIOSYNTHESIS BY A STABILIZED MULTI-ENZYMATIC SYSTEM

De Benedetti EC[1,2], Rivero CW[1,2], Zinni MA[1], Trelles J.A[1,2]. [1]Laboratorio de Investigaciones en Biotecnología Sustentable, UNQ. [2]CONICET.

MI-P51

NEMATODE M01F1.3 AND C45G3.3 FUNCTIONALLY COMPLEMENT MICROBIAL MUTANTS IN LIPOYLATION PATHWAYS

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NEUROSCIENCE

NS-P01

HIPPOCAMPAL BDNF IN RESERPINE-TREATED ADOLESCENT WISTAR RATS

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NS-P02

ROLE OF C-FOS IN NEURONAL DIFFERENTIATION

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NS-P03

NEUROINFLAMMATORY RESPONSES IN A MOUSE MODEL OF AUTISM SPECTRUM DISORDER (ASD)

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NS-P04

DEFICITS OF HIPPOCAMPAL STRUCTURAL PLASTICITY IN A MOUSE MODEL OF MECP2 DEFICIENCY

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NS-P05

PKD1 REGULATION OF TRK RECEPTORS' TRAFFICKING: EFFECT ON NEURONAL DEVELOPMENT AND FUNCTIONALITY

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NS-P06

IGF-1R AND PI3K-AKT SIGNALLING PATHWAY ARE ESSENTIAL FOR FORMATION OF BRAIN CORTEX

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NS-P07

DIETARY SOY-BASED PROTEIN MODULATES THE OXIDATIVE AND INFLAMMATORY EFFECTS OF CADMIUM IN HIPPOCAMPUS

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NS-P08

STUDY ON VISUAL AND NON-VISUAL OPSINS IN A MODEL OF RETINAL DEGENERATION CAUSED BY LED LIGHTS

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NS-P09

NOVEL PHOTORECEPTORS IN THE AVIAN INNER RETINA: HORIZONTAL CELLS EXPRESSING MELANOPSIN X

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NS-P10

ADULT HIPPOCAMPAL NOTCH ACTIVATION IMPAIRS Aβ CLEARANCE AND COGNITION IN A RAT MODEL OF ALZHEIMER

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NS-P11

EXPERIMENTAL GUILLAIN-BARRE SYNDROME: ROLE OF THE CARRIER PROTEIN KLH

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NS-P12

IMPAIRED AUTOPHAGY FLUX IN MÜLLER GLIAL CELLS EXPOSED TO HYPOXIA: IN VITRO AND IN VIVO MODELS

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PLANT BIOCHEMISTRY AND MOLECULAR BIOLOGY

PL-P01

MOLECULAR ANALYSIS AND SUBCELLULAR LOCALIZATION OF PEPTIDASE SILPEPSIN 2 FROM S*silybum marianum*

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PL-P02

MITOCHONDRIAL PPR-CONTAINING PROTEINS ARE ESSENTIAL TO SUSTAIN EMBRYOGENESIS IN *ARABIDOPSIS THALIANA*

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PL-P03

TOWARDS UNDERSTANDING THE INTERPLAY BETWEEN PRODH AND ROS BURST IN PLANT HYPERSENSITIVE RESPONSE

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PL-P04

USE OF NON-THERMALPLASMA FOR PATHOGENCONTROL AND IMPROVEMENT ON THEBIOCHEMICAL QUALITY OF SEEDS

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PL-P05

POLYAMINES REDUCED GROWTH BY MODULATINGREACTIVE OXYGEN SPECIES AND NITRIC OXIDEFORMATION IN WHEAT

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PL-P06

EFFECT OF MAGNETITE NANOPARTICLES ONALFALFA (*Medicago sativa* l.)PLANTS

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PL-P07

DROUGHT STRESS EFFECTS ON CARBON ANDNITROGEN METABOLISM IN THE PEANUT-RHIZOBIAINTERACTION

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PL-P08

CONTRIBUTION OF TOMATO ELECTROPHILECOMPOUNDS TO THE THERMOTOLERANCE IN *C. elegans*

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PL-P09

CLONING AND SEQUENCING OF A NEW CYSTEINEPEPTIDASE FROM FRUITS OF *Bromelia hieronymi* mez

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PL-P10

ANALYSIS OF METABOLIC INTEGRATORS FROM *Nannochloropsis gaditana*

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PL-P11

FUNCTIONAL CHARACTERIZATION OF PEACH FRUITPPZAT12 AND *Arabidopsis* ATZAT12TRANSCRIPTION FACTORS

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PL-P12

ATTRAF: A NOVEL RNA CHAPERONE INVOLVED INTEMPERATURE STRESS RESPONSES IN Arabidopsis

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PL-P13

CHARACTERIZATION OF STONE HARDENINGDURING THE PEACH FRUIT DEVELOPMENT

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PL-P14

THE TRANSCRIPTION FACTOR ATHB5 IS ANEGATIVE REGULATOR OF LIGNIN ACCUMULATION

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PL-P15

IMPROVEMENT OF STRESS TOLERANCE IN TOBACCO PLANTS BY EXPRESSING CYANOBACTERIAL FLAVODIIRON PROTEINS

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PL-P16

THE SUNFLOWER TRANSCRIPTION FACTORHAHB11 INTERACTS WITH A KINESIN INARABIDOPSIS TRANSGENIC PLANTS

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PL-P17

THE HOMEODOMAIN-LEUCINEZIPPERTRANSCRIPTION FACTOR ATHB23 PLAYS A ROLE INROOT DEVELOPMENT

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PL-P18

PLEIOTROPIC EFFECTS INDUCED BY LIHSP83-SAG1VACCINE ANTIGEN EXPRESSION INTRANSPLASTOMIC PLANTS

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PL-P19

ROLE OF 90KDAHEAT SHOCK PROTEIN IN PLANTIMMUNITY

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PL-P20

***COLLETOTRICHUM ACUTATUM* PRODUCES A LOWMOLECULAR WEIGHT COMPOUND THAT SUPPRESSES-INDUCEDDEFENSE**

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PL-P21

SOFT MECHANICAL STIMULUS INDUCES RESISTANCE AGAINST *BOTRYTIS CINEREA* IN CULTIVATED STRAWBERRY

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PL-P22

ASES INDUCES PHYSIOLOGICAL ANDBIOCHEMICAL CHANGES OF AVOCADO FRUITDURING RIPENING

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PL-P23

HET ELLAGITANNIN ALTERS CELL REDOX STATUSAND ROS ACCUMULATION IN STRAWBERRYPLANTS

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PL-P24

EFFECT OF BRASSINOSTEROIDS TREATMENT INSTRAWBERRY DEFENSE MARKERS

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PL-P25

STRUCTURAL DETERMINANTS INVOLVED IN THEREDOX REGULATION OF THE ARABIDOPSISFUMARASES ENZYMES

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PL-P26

DIFFERENTIAL METABOLIC REARRANGEMENTSAFTER COLD STORAGE OF DIFFERENT PEACHFRUIT VARIETIES

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PL-P27

FIRST ANALYSIS OF THE WHOLE PUTATIVETHIOREDOXIN FAMILY MEMBERS IN *ZEA MAYS*

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PL-P28

FUNCTIONAL CHARACTERIZATION OF A DC1-DOMAINPROTEIN ESSENTIAL FOR EARLYGAMETOPHYTIC DEVELOPMENT

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PL-P29

OPTIMIZATION OF RECOMBINANT MAIZE CDKAPRODUCTION IN *ESCHERICHIA COLI*: ASTATISTICAL APPROACH

Mendez AA[1], Pena LB[1], Garza Aguilar SM[2], Vazquez Ramos JM[2], Gallego SM[1]. [1]Dto Qca Biológica, FFYB, UBA-IQUIFIB, CONICET. [2]Dto Bioquímica, Fac Química, UNAM-CONACYT, México. E-mail: amendez@ffyb.uba.ar

PL-P30

CITRATE METABOLISM IN OILSEEDS

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PL-P31

PHOSPHATE DEFICIENCY IN PLANTS: THE ROLE OFEXTRACELLULAR AND INTRACELLULARRIBONUCLEASES

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PL-P32

THE CYSTEINE DESULFURASE ATNFS1 IS INVOLVEDIN FE-SCLUSTER ASSEMBLY AND IRONMETABOLISM

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PL-P33

TWO MITOCHONDRIAL SCO PROTEINSDIFFERENTIALLY AFFECT SALT STRESSRESPONSES IN *A. THALIANA*

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PL-P34

TCP14 AND TCP15 PARTICIPATE IN TEMPERATURE-DEPENDENT DEVELOPMENTAL RESPONSES IN *ARABIDOPSIS THALIANA*

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PL-P35

SURF-1MODULATES HYPOCOTYL GROWTH BYINFLUENCING AUXIN AND GIBBERELLIN ACTION IN*Arabidopsis*

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PL-P36

OVEREXPRESSION OF STAP-PSIDOMAIN IN *ARABIDOPSIS THALIANA* INCREASES RESISTANCETO *B. CINEREA*

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PL-P37

STUDYING THE CYTOSOLIC GA3PDHASE INAUTOTROPHIC AND HETEROTROPHIC *CHLORELLA*CELLS

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PL-P38
ALTERNATIVE SPLICING REGULATION AND RNAPOLYMERASE II ELONGATION MEDIATED BY LIGHT
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PL-P39
STUDY OF CALMODULINBINDINGPROTEIN IQ67-DOMAINCLASS IV (IQD) IN *ARABIDOPSIS THALIANA*
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PL-P40
LIPID PROFILING OF PEACH LEAVES FROMGENOTYPES WITH CONTRASTING SUSCEPTIBILITYTO *TAPHRINA DEFORMANS*
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PL-P41
REGULATION OF PLANT DEVELOPMENT BY A NON-CODINGRNA TRANSCRIBED BY THE BIDIRECTIONALHAWRKY6 PROMOTER
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PL-P42
MECHANISMS OF LOADING, SELECTION ANDRETENTION OF THE MICRO RNAS STRANDS INPLANTS
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PL-P43
FINDING NEW COMPONENTS OF THE MIRNABIOGENESIS IN *ARABIDOPSIS*
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PL-P44
RELATIONSHIP BETWEEN PII PROTEIN ANDENVIRONMENTAL STRESSES IN *ARABIDOPSIS*
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PL-P45
TEMPERATURE STRESS TOLERANCE IN*ARABIDOPSIS THALIANA*: A ROLE FORALTERNATIVE SPLICING
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PL-P46
CONTROL OF BRASSINOSTEROID SIGNALING BYSHADE AND TEMPERATURE CUES
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PL-P47
NEW GENES FROM *XANTHOMONAS CITRI* SUBSP.*CITRI* INVOLVED IN BACTERIAL EPIPHYTICSURVIVAL
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PL-P48
FRATAXIN OLIGOMERIZATION AND METAL BINDINGPROPERTIES IN PLANTS
Buchensky C, Sanchez M, Capdevila M, Dominguez VeraJ, AtrianS, Pagani MA, BusiMV, Gomez-Casati DF. E-mail: buchesnky@cefobi-conicet.gov.ar
PL-P49
GATA* TRANSCRIPTION FACTORS DURING HEATSTRESS IN *ARABIDOPSIS THALIANA
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PL-P50
CONTROL OF PLANT DEVELOPMENT BY THETRANSCRIPTIONAL COACTIVATOR AN3
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PL-P51
INTERACTIONS BETWEEN GROWTH REGULATINGSYSTEMS IN *ARABIDOPSIS THALIANA*

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PL-P52

BIOCHEMICAL AND MOLECULARCHARACTERIZATION OF TWO *CITRUS RETICULATA*VARIETIES: MURCOTT AND ELLENDALE

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PL-P53

UV-BLIGHT ENHANCES ANTIMICROBIAL ACTIVITYOF POSTHARVEST LEMON PEEL AGAINST*PENICILLIUM DIGITATUM*

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PL-P54

CONTRIBUTION OF *GLUCONACETOBACTER DIAZOTROPHICUS* PAL5 TO PHOSPHORUSNUTRITION IN STRAWBERRY PLANTS

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PL-P55

DIFFERENTIAL SENSITIVITY OF *MENDICAGO SATIVA*, *ZEA MAYS* AND *RAPHANUS SATIVUS* TOOLIVE CAKE AND SOIL

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PL-P56

IDENTIFICATION OF CELL CYCLE REGULATORS INPLANTS

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PL-P57

PROTEOMICS OF THE CHLOROPLAST STROMA OF*LOTUS JAPONICUS* UNDER COLD STRESS

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PL-P58

MULTIPLE MYB TRANSCRIPTIONAL FACTORS AREINVOLVED INTO CONDENSED TANNINBIOSYNTHESIS REGULATION

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PL-P59

REGULATION OF PHOTOSYNTHETIC MALICENZYMES IN C_4 MILLETS

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PL-P60

ANALYSIS OF MITOCHONDRIALALKALINE/NEUTRAL INVERTASE MUTANTS

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PL-P61

EFFECTS OF PHENOL TREATMENT ON CLOCKGENES EXPRESSION IN TOBACCO HAIRY ROOTS

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PL-P62

EFFECT OF ZINC STRESS ON PLANT MISMATCHREPAIR

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PL-P63

METABOLOMIC RESPONSE OF SOYBEAN LEAVES TO *FUSARIUM TUCUMANIAE*

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PL-P64

ENDOGENOUS NO MODULATES SPATIALDISTRIBUTION OF PIN2 DURING GRAVITROPISM IN ARABIDOPSIS

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PL-P65

TIR1 S-NITROSYLATIONIS A REGULATORYCOMPONENT IN TEMPERATURE-DEPENDENT*ARABIDOPSIS* SEEDLING GROWTH

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PL-P66

BDIRCN4 REGULATES MERISTEM FATE IN*BRACHYPODIUM DISTACHYON*

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PL-P67

IDENTIFICATION OF DOWNSTREAM TARGETS OFATERF019 TRANSCRIPTION FACTOR IN DROUGHTSTRESS

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PL-P68

PLD/PA MODULATES PROLINE AND H_2O_2 LEVELS INBARLEY EXPOSED TO SHORT-ANDLONG-TERMCHILLING STRESS

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PL-P69

THE TCP DOMAIN MEDIATES THE ANTAGONISTICACTION OF TCP8 AND TCP23 ON FLOWERING IN*ARABIDOPSIS*

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PL-P70

STUDY OF MITOCHONDRIAL PROTEINS ENGAGED INGROWTH AND DEFENSE IN *ARABIDOSPSIS THALIANA*

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PL-P71

PLANT GAMMA-CARBONICANHYDRASES ANDTHEIR ROLE IN GROWTH AND EMBRYOGENESIS:THE IMPORTANCE OF CA3 PR

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STRUCTURAL BIOLOGY

SB-P01

3D-STRUCTURE PREDICTION AND STUDY ON THE INTERACTION BETWEEN RAT CALTRIN AND MODEL MEMBRANES

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SB-P02

A TACHYLECTIN AND A PORE-FORMING MACPF PROTEINS COMBINED INTO A SNAIL EGG NEUROTOXIN

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SB-P03

LECTINS AS DEFENSES. CHARACTERIZATION OF THE MAJOR EGG PROTEIN OF THE SNAIL P*omacea diffusa*

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SIGNAL TRANSDUCTION

ST-P01

ACSL4 PROMOTER CHARACTERIZATION AND REGULATION BY SHP2 IN BREAST CANCER CELLS

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ST-P02

SEXUAL PHEROMONE MODULATES THE FREQUENCY OF CYTOSOLIC CA 2+ BURSTS IN SACCHAROMYCES CEREVISIAE

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ST-P03

A NOVEL PHOSPHATASE REGULATES STRESS GRANULES DYNAMICS

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ST-P04

IGF 1 MODULATES ZEB1 STABILITY DURING EPITHELIAL MESENCHYMAL TRANSITION (EMT)

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ST-P05

EVOLUTION OF CELL CYCLE MEDIATED REGULATION OF YEAST SEX

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ST-P06

AN IMAGE-PROCESSING PROTOCOL TO QUANTIFY AKT LOCALIZATION IN DIFFERENT SUBCELLULAR COMPARTMENTS

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ST-P07

THE GPCR STE2 MEASURES FRACTION OF OCCUPIED RECEPTORS BY BOTH ACTIVATING AND INHIBITING G PROTEIN

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ST-P08

ANTIGENIC VARIATION IN GIARDIA LAMBLIA: ROLE OF VSPS TRANSMEMBRANE DOMAIN IN SENSING AND SIGNALING

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ST-P09

ANTINEOPLASTIC EFFECT OF ERK 1/2 AND AKT INHIBITION BY VDR AGONISTS IN VGPCR ENDOTHELIAL CELLS

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ST-P10

QUERCETIN DECREASES THE PROLIFERATION OF ENDOTHELIAL CELLS TRANSFORMED BY KAPOSI SARCOMA HERPESVIRUS

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ST-P11,

COMPARATIVE ACTIONS OF 1α,25(OH)2D3-GLYCOSIDES AND SYNTHETIC 1α,25(OH)2D3 DURING MYOGENESIS

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ST-P12

A FLUORIMETRIC POPULATION ASSAY SHOWS THAT MEMBRANE POTENTIAL OF HUMAN SPERM DEPEND ON PKA A

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ST-P13

IN VIVO OLIGOMERIC STRUCTURE AND PARTNERS OF YEAST PKA-R SUBUNIT THROUGH PULL-DOWN PROTEOMICS

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ST-P14,

SEQUENTIAL ERK PHOSPHORYLATION IN TYROSINE AND THREONINE DETERMINES ITS CELLULAR DISTRIBUTION

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ST-P15

ERK-SPECIFIC PHOSPHATASE MKP-3 SPLICE VARIANTS DIFFER IN REGULATION AND SUBCELLULAR LOCALIZATION

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ST-P16

CENTRAL SIGNALING ROLE FOR THE CONSERVED GLYCINE HINGE OF BACTERIAL CHEMORECEPTORS

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ST-P17

PKA ROLE IN TRANSLATIONAL RESPONSE TO HEAT STRESS IN SACCHAROMYCES CEREVISIAE

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ST-P18

TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL REGULATION OF PKA SUBUNITS IN SACCHAROMYCES CEREVISIAE

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ST-P19

CHROMATIN REMODELING REGULATION OF PROTEIN KINASE A TPK1 SUBUNIT IN SACCHAROMYCES CEREVISIAE BY STRESS.

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ST-P20

INVOLVEMENT OF HISTONE METHYLTRANSFERASE-1 IN GIARDIA LAMBLIA DIFFERENTIATION

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ST-P21

TOLL CONTROL: IMPORTINS AS SENTINELS OF NUCLEAR/CYTOPLASMIC SHUTTLE IN GIARDIA LAMBLIA

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ST-P22

INDOMETHACIN MODULATES BMMC MIGRATION THROUGH PROSTAGLANDIN AND/OR PPARϒ AFTER SCIATIC NERVE INJURY

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ST-P23

A SIGNAL TRANSDUCTION PATHWAY RELATED TO VIRULENCE REGULATION IN LEPTOSPIRA

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ST-P24

ADAPTATION MECHANISMS REGULATING THE SECRETORY PATHWAY IN RESPONSE TO A SECRETORY STIMULUS.

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ST-P25

INVOLVEMENT OF BOX2 IN THE REGULATION OF CHKA EXPRESSION IN NEURO-2A CELLS AND IN HUMAN TUMOR CELLS.

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ST-P26

STUDYING THE ROLE OF CPSF6 AS A MUTANT P53 EFFECTOR IN TUMOR AGGRESSIVENESS

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ST-P27

BMP5 INDUCES IN VITRO MIGRATION OF BOVINE OVIDUCTAL EPITHELIAL CELLS

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POSTERS

BIOTECHNOLOGY

BT-P01
RECOMBINANT EXPRESSION OF SWEET PLANT PROTEIN MNEI IN FOOD-GRADE *Lactococcus lactis*

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The world prevalence of metabolic diseases due to the consumption of sugar is increasing dramatically. Sweet proteins are rising as potential replacements for the currently available sweeteners in industrial food and beverage preparations. These natural sweet compounds can be 100,000 times sweeter than sucrose on a molar basis. In this study we analyzed whether the expression of MNEI protein (a single chain derivative of the sweet plant protein Monellin) in food-grade *Lactococcus lactis* could be used for industrial purpose. We initially designed an expression vector containing a strong constitutive promoter followed by a lactococcal signal peptide downstream fused with *L. lactis* codon optimized MNEI coding sequence and a His-tag sequence for later affinity protein purification. In addition a derivative plasmid for cytosolic expression in *L. lactis* was generated by elimination of the signal sequence. The current results exhibited that recombinant MNEI has been successfully secreted and expressed intracellularly in food-grade *L. lactis*. Cytosolic production of MNEI showed to be both effective and reproducible, and large quantities of soluble MNEI were purified. On the other hand, although extracellular purification showed to be minority, further expression in an extracellular protease mutant strain is currently in process. This is the first time that MNEI is expressed in *L. lactis*.

BT-P02
USE OF AFFINITY TAGGED VMA1 INTEIN FOR THE PRODUCTION OF RECOMBINANT PHARMACEUTICAL PROTEINS.

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We analyzed whether the affinity tagged *Saccharomyces cerevisiae* VMA1 intein, fused to a chitin binding domain tag (CBD), can be used to produce recombinant pharmaceutical proteins without the N-Formilmethionine (fMet) terminal residue, using *E. coli* as protein factory. We designed and constructed an expression vector to produce the human growth hormone (HGH) C-terminal fused to the CBD-intein chimeric protein. HGH coding sequence was *E. coli* codon optimized and it was synthesized so as its first amino acid after the natural N-terminal methionine, phenylalanine, is right after cleavage site of VMA1 intein. The CBD permits subsequent protein affinity purification while VMA1 intein undergoes a self-cleavage reaction enabling the elution of HGH having now the phenylalanine residue as the first N-terminal residue. Optimization of growth, induction, extraction and purification conditions allowed us to reproducibly produce the HGH recombinant protein in large quantities and with high purity. These results suggest that the CBD-VMA1 intein, having the coding sequence of the protein of interest cloned right after the intein cleavage site could be used for commercial production of recombinant proteins with impact in biopharmaceutical and/or biotechnological industries.

BT-P03
EXPRESSION OF THE HYBRID BACTERIOCIN ENT35-MCCV IN *Saccharomyces cerevisiae*

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The hybrid bacteriocin Ent35-MccV is a construction made by the fusion of genes encoding Enterocin CRL35 (*munA*) and Microcin V (*cvaC*). Enterocin CRL35 is active against the foodborne pathogen *Listeria monocytogenes*. On the other hand, Microcin V is active specifically against Gram-negative bacteria. These bacteriocins were fused in order to obtain a peptide with broad antimicrobial spectrum. The aim of this work was to express Ent35-MccV in *Saccharomyces cerevisiae*, a GRAS microorganism, and evaluate its activity against Gram-positive and negative pathogen bacteria. To achieve this, munA-cvaC with a leader peptide were cloned into the yeast-*E. coli* shuttle plasmid pCEV-G1. *S. cerevisiae* 23344C was transformed by the lithium acetate method. The expression of the hybrid Ent35-MccV was induced with the addition of galactose. The hybrid bacteriocin Ent35-MccV was obtained from the yeast producing supernatants. The antibiotic activity was assayed against different pathogen bacteria. The chimerical bacteriocin displayed

antimicrobial activity against *E. coli* 0157, *L. monocytogenes* and *Salmonella* gallinarum bovar gallinarum and pullorum, strains that infects chickens. Therefore, the hybrid bacteriocin may find important applications in veterinary to control salmonellosis in poultry.

BT-P04

ETHANOL FERMENTATION BY ANTARCTIC YEASTS

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Cellulosic raw biomass could be used as sugar source to replace starchy materials in fermentation. Researchers have studied the process for simultaneous saccharification/ethanol production from wastes, but so far without success. We aimed to analyze ethanol fermentation on Antarctic hydrolytic enzyme producing yeasts. Eight cellulolytic/esterolytic enzyme producing yeasts were isolated and identified by sequencing a partial fragment of the 26S rDNA gene, as *Rhodotorula mucilaginosa* (six isolates), *Rhodotorula glutinis* (one isolate) and *Cystobasidium slooffiae* (one isolate). They showed cellulose activity in the range of 10-30°C, as revealed by the formation of halos of hydrolysis around the colonies when growing in carboxymethyl cellulose. The ability to produce ethanol of *R. mucilaginosa* AUG6, *R. glutinis* AUG43 and *C. slooffiae* AUG30 was analyzed by GC-FID. Yeasts were grown in minimal medium supplemented with sucrose and ethanol fermentation was anaerobically performed at 30°C. Both, AUG6 and AUG43 produced approximately 1 and 2.5 g/L ethanol, after 72 and 140 h growth. Although the values of ethanol production are not industrially relevant, the results suggest that these yeasts may be targeted for sacharification and ethanologenesis of cellulosic material. Currently, the production of ethanol using different carbon cellulosic materials is in study.

BT-P05

ROLE OF XYLR ON XYLOSE METABOLISM IN *Herbaspirillum seropedica* EZ69

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D-xylose constitutes the greatest proportion of fermentable sugars in hemicellulose. This polymer, included in lignocellulosic residues, is a cheap resource for biotechnological production. However, few microorganisms able to consume xylose have been described, involving in some cases mechanisms of catabolic repression by glucose. *Herbaspirillum seropedicae* is a β-proteobacteria. When xylose is used as carbon source, these bacteria are able to grow and accumulate more than 50% of their dry-weight as PHB, a thermoplastic biopolymer. Strain Z69 expresses Weimberg pathway for xylose catabolism. When this pathway was disrupted by a mutation in fabG gene (xylose dehydrogenase), the mutant strain was still able to grow on xylose at low rate and seemed to express the oxo-reductive path. Regulatory mechanisms for xylose metabolism have not been studied in *H. seropedicae*. A xylR gene was identified in these bacteria, similar to a gene encoding a transcriptional activator of xyl operon, previously characterized in *Escherichia coli*. A defined knock-out mutant strain, Z69xylR, was constructed to study the role of XylR in xylose metabolism. This strain exhibited a reduced growth on xylose, suggesting an active role of XylR in xylose metabolism. Expression of fabG gene in Z69 and Z69xylR is being studied by transcriptional fusion analysis, using reporter genes. Support: PEDECIBA, ANII

BT-P06

STRATEGY FOR THE CONSTRUCTION OF *Saccharomyces cerevisiae* STRAINS ABLE TO ASSIMILATE XYLOSE

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Sets of recombinant *Saccharomyces cerevisiae* strains derived from CCTCC M94055 and CAT-1 were constructed by incorporation of selected genes involved on xylose metabolism. Cassettes of genes organized in tandem and surrounded by two fragments of about 200nts identical to specific regions of GRE3 gene, were synthetized and inserted by homologous recombination in both chromosomes. Genes selected for these constructions are involved in two main pathways described for xylose metabolism. One set contained XR (xylose reductase, modified with increased affinity for NADH) and XDH (xylitol dehydrogenase) genes from *Scheffersomyces stipitis*, modified for host optimization. In other cases, XI (xylose isomerase) gene from *Streptomyces coelycolor*, with a sequence optimized for yeasts was incorporated. In both cases, additional copies of homologous XK (xylulokinase) and TAL1 (transaldolase) genes under the control of strong promoters were also incorporated in those constructions. A copy of Gal2 gene, modified for encoding a transporter with high affinity for xylose was introduced in these strains. The constructions were selected by antibiotic resistance, flanked by lox regions, generating their possible deletion with the Cre/lox system. The strains were used for adaptive evolution protocol and the ability to grow on xylose and the production of ethanol was evaluated. Funded by ANII and PEDECIBA.

BT-P07

TRANSGENIC MAIZE PLANTS EXPRESSING HAHB11: A PROMISING PROOF OF CONCEPT

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HaHB11 is a sunflower transcription factor belonging to the HD-Zip I family. It was previously shown that Arabidopsis plants transformed with this gene had improved biomass and yield as well as an increased tolerance to a variety of abiotic stress factors like drought, waterlogging and submergence. Two genetic constructs in which either the 35S CaMV or the maize ubiquitin promoter direct the expression of HaHB11 (Mora and Giacomelli, unpublished) were carried out. Transgenic maize plants were obtained on the HiII AxB genotype and crossed with B73 (Campi and Gómez, IAL transformation platform*). Here we show a phenotypic analysis of

HaHB11 T1 maize transgenic plants compared with their controls. Plants bearing the UBI promoter had higher transcript levels than those with the 35S CaMV and the differential phenotypes observed were related with these expression levels. HaHB11 plants had higher stems, more leaves and larger total leaf area than controls. Moreover, they were delayed in the onset of the reproductive organs, achieving in some cases, larger ears. The combination of these traits could be contributing to an increased yield. Altogether the results indicate that HaHB11 could be displaying conserved physiological mechanisms between Arabidopsis and maize which supports HaHB11 as a biotechnological tool to improve crops. *Campi M, Gómez M, http://ial.santafe-conicet.gov.ar/

BT-P08

MALTOOLIGOSACCHARIDES PRODUCTION FROM GLUTEN FREE STARCHES

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Cyclodextrin Glucosyltransferase (CGTase) belongs to glycoside hydrolases family 13. Transform starch into oligosaccharides mixtures of cyclodextrins and maltooligosaccharides (MOS), consisting of glucose (G) linked by α-1,4 bounds. These oligosaccharides are used in different industries, in bakery delay retrogradation of the dough, avoiding the aging of the loaves. Celiac disease is the most common and chronic autoimmune enteropathy in which people cannot eat gluten. Gluten is formed by mechanical work on the hydrated protein of wheat, barley, oats and rye flours. In order to verify that starches are greater production of MOS, a CGTase purified from *Paenibacillus sp*. in our laboratory was employed on different gluten free starches. Cassava, corn and potatoes starches provide better production. Also, we develop new biocatalysts by mutations at positions 137, 144, 280 and 329 of the CGTase gene. Singles and double mutants on cassava starch were evaluated. Only mutants 137-280 and 144-329 produce similar MOS yields to the wild type. Double mutants in position 137 have a lower production of maltose (G2) and higher proportion of G4 to G6 than that obtained for wild type. Biotechnological processes to obtain MOS are expensive and involve many enzymes. Using the wild type or mutants, MOS mixtures are obtained with a specific and reproducible composition in successive processes.

BT-P09

DODECENLY SUCCINIC ANHYDRIDE-COLLAGEN MODIFIED HYDROGELS

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Dodecenly succinic anhydride was used to modify collagen hydrogels in order to obtain more hydrophobic surfaces. The base catalyzed reaction was performed under ammonia vapor with DDSA solutions ranging from 3 to 25 g/100 ml in ethanol and hydrogels with a concentration of 5 mg/ml of collagen. Different techniques were used to confirm the modifications. In the IR spectra the band at 2884 cm−1 represents the characteristic vibration due to C−H stretching which increases in DDSA modified gels as well as the band at 1662 cm-1 corresponding to the C=O stretching vibration of a carbonyl group. When DSC analysis was performed over hydrated gels, the denaturation temperature obtained was similar for both modified and pure collagen gels. SEM images demonstrate that DDSA esterification does not significantly alter the morphology of pre-formed collagen gels. However, when DDSA was included in the polymerization process of collagen, a remarkable difference was observed. The DDSA-modified gels were used for cell culture with a pre-osteoblastic cell line MC3T3-E1. No toxicity was observed for the material as the number of total cells per well was not significantly different to unmodified collagen gels, showing their biocompatibility. However, a lower cellular adhesion to the surfaces was found as DDSA concentration increased due to the surface hydrophobicity modification.

BT-P10

ANTIOXIDANT ACTIVITY OF *Larrea divaricata* LOADED IN MUCOADHESIVE POLYMERS AND SILICA COMPOSITES

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Oral diseases therapy has been monopolized for years with clorhexidine, triclosan and essential oils because of their good clinical results. Nevertheless, new alternatives need to be explored to prevent and treat gum disease. Besides, the ongoing knowledge in biomaterials has allowed to a high versatility in clinical approaches in dental field. Biodegradable scaffolds, such as chitosan, have an important role in oral tissue regeneration. Additionally, they are employed as drug delivery systems. Chitosan-silica composites and chitosan-CMC-silica composites were plasticized with glycerol. They were characterized by SEM and FTIR. Both techniques revealed the presence of silica in chitosan polymer matrix. These composites loaded with *L. divaricata* extract, present antioxidant properties. Indeed, DPPH scavenger activity (%) was CMC-SiO_2: 30.8 ± 1.2 and SiO_2: 19.5 ± 2.2 and SOD (adrenaline oxidation % of inhibition) was CMC-SiO_2: 17.1 ± 0.12 and SiO_2: 5.3 ± 2.1. HPLC analysis demonstrated that polar compounds were incorporated to the composites but NDGA, a majority low polar compound found in the extract, was not incorporated in both composites. CMC-SiO_2 composites exerted a higher antioxidant activity than SiO_2 in agreement with higher extract incorporation. Scaffolds loaded with *L. divaricata* have promising antioxidant and anti-inflammatory characteristics suitable for dental care.

BT-P11
CASEINOLYTIC AND MILK-CLOTTING ACTIVITY OF *Solanum tuberosum* ASPARTIC PROTEASES (*ST*APS)

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Increased interest in find suitable calf rennet substitutes for cheese making has led to the screening of new proteases to be used as natural milk coagulants. Several plant proteases obtained from fruits, roots, latex and flowers has shown milk-clotting activity. The aim of this work was to detect and characterize the milk-clotting activity of two *Solanum tuberosum* aspartic proteases, *St*AP1 and *St*AP3. They were previously isolated from potato tubers (*St*AP1) and leaves (*St*AP3). Both enzymes hydrolyzed casein over a broad temperature range (40-60°C). The caseinolytic activity of *St*AP3 was three times higher than the one from *St*AP1 at all temperatures tested. The optimum pH of both enzymes to hydrolyze casein was 8. We also evaluated the milk-clotting activity of *St*AP1 and *St*AP3, being 1.69 and 1.96 milk clotting units mL-1, to *St*AP1 and 3, respectively. These values are similar to the activities found in commercial rennet. These results suggest a new potential use in bioprocesses to *St*APs, particularly as a milk coagulants for cheese making.

BT-P12
VSPS OF *G. lamblia* AS CARRIERS IN CHRONIC ORAL ADMINISTRATION OF PEPTIDE DRUGS

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Despite the relevance of proteins and peptides as therapeutic agents, very few are available for oral administration. Acid hydrolysis in the stomach due to low pH, luminal degradation in the intestine by different proteases and variability in absorption are the major factors that prevent bioactive peptide administration by the oral route. We here show that different Variant-specific Surface Proteins (VSPs) of the intestinal parasite *Giardia lamblia* can be used as carrier for oral peptide delivery. The extracellular domains of different VSPs (ΔVSPs), which contain multiple CXXC motifs, were produced in an insect cell expression system. Like native *Giardia* VSPs, recombinant ΔVSPs are resistant to acidic pH and proteolytic degradation and adhere to the intestinal mucosa. Chronic administration of IFNα and glucagon, as prototype drugs, in combination with ΔVSPs derived from different *Giardia* assemblages showed that ΔVSPs protect these bioactive peptides from degradation and promote their systemic biological action. These results demonstrate that VSPs are attractive candidates for oral administration of peptides for the treatment of chronic diseases.

BT-P13
ACTIVE PACKAGING AGAINST *ESCHERICHIA COLI* O157: H7 IN MEAT INDUSTRY

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Consumption of minced meat or undercooked hamburger is leading cause of infection with Shiga toxin-producing *Escherichia coli* O157:H7, which can lead to important complications in the human health. Active packaging technology employing polymeric materials as support for antimicrobial agents can improve the food hygienic-sanitary quality. The peptide Microcin J25 (G12Y) [MccJ25(G12Y)] present potential application as feed additive because it is active against *E. coli* O157:H7 and can be degraded by digestive enzymes. The work aims were a) to study the minimum bactericidal concentration (MBC) of MccJ25(G12Y) against *E. coli* O157:H7, b) to evaluate its bactericidal activity in refrigerated conditions, c) to standardize the area/volume for the activation of cellulose film (Fibrous) with MccJ25(G12Y), and d) to study the peptide distribution in the Fibrous after activation. The MBC of MccJ25(G12Y) on *E. coli* O157:H7 was 0.024 mg/ml. In refrigerated conditions, 0.096 mg/ml of microcin presented a bactericidal effect against *E. coli* O157:H7 after 24 hours. An area/volume of 36.9, thirty one times lower to the previously report, was standardized to active Fibrous with MccJ25(G12Y). After activation, an uniform distribution of MccJ25(G12Y) throughout the film was evidenced. The results suggest the potential use of Fibrous treated with MccJ25(G12Y) as a biopreservant in the meat industry.

BT-P14
CLONING AND EXPRESSION OF A ROTAVIRUS VP6-FLIC131 FUSION PROTEIN

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Group A rotavirus is the major etiologic agent of acute gastroenteritis in children. Although, mortality rates have been reduced since the implementation of attenuated vaccines, research on alternative vaccination strategies continues, particularly focused on those based in non-living pathogens. Specifically, our interest is the evaluation of needle-free alternatives able to induce an immune response after mucosal administration. Among rotavirus, VP6 is the most immunogenic and conserved protein. On the other hand, flagellin has been proposed as an important mucosal adjuvant. Thus, fusion ORF containing FliC131, a deletion mutant of *S. enterica* flagellin, and a fragment of VP6 protein, BB (aminoacids 143 to 334), was constructed by SOE-PCR, cloned and expressed in *E. coli*. Protein expression was analyzed by SDS-PAGE and Western blot. Results showed that FliC131-VP6BB was efficiently expressed and recognized by specific antibodies against rotavirus and flagellin, although small amount of FliC131-VP6BB was found in the soluble protein fraction. The biological activity of this fusion protein was assessed on Caco-luc reporter cell showing that it stimulates a dependent pathway activation of TLR5. Protein purification will be performed in order to evaluate its immunogenic potential for mucosal administration to mice in a rotavirus model.

BT-P15
BENEFICIAL RHIZOBACTERIA ENCAPSULATED IN NANOFIBERS FOR POTENTIAL APPLICATION AS SOYBEAN INOCULANTS

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Soybean seed inoculation with plant growth promoting rhizobacteria (PGPR) is an ideal tool to supply high densities of beneficial microorganisms to the soil. The difficulty of maintaining the viability of the microorganisms during seed treatment and storage is a problem. This work aimed to evaluate the stability of two potential PGPR, *Pantoea agglomerans* and *Burkholderia caribensis*, during encapsulation in nanofibers under different conditions, and to determine the impact of seed coating with nanofiber-encapsulated rhizobacteria on bacterial survival during seed storage, on germination and on plant growth parameters. Bacterial nanoencapsulation and subsequently seed coating was carried out by electrospinning. This technique successfully encapsulated *P. agglomerans* and *B. caribensis* because it did not affect their viabilities or beneficial properties. Seed coating with nanofiber-encapsulated rhizobacteria improved *P. agglomerans* and *B. caribensis* survival on seeds stored for 20 days and increased their colonization on plant root. Seed coating with *P. agglomerans* increased germination and length and dry weight of root while seed coating with *B. caribensis* increased leaf number and dry weight of shoot. The technique applied to coat seeds with nanofiber-encapsulated PGPR could be considered a promising eco-friendly approach to improve soybean production using a microbial inoculant.

BT-P16
DESIGN OF A BIOTECHNOLOGICAL TOOL FOR INCREASING PROTEIN EXPRESSION IN PLANTS

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We previously characterized promoter regions of several accessory proteins involved in mitochondrial biogenesis in Arabidopsis. A relative common characteristic of these genes is the presence of an intron in their 5' UTR. We tested one of these introns (I) and a mutated version (IM) as biotechnological tools for increasing expression of different proteins. We analyzed Arabidopsis plants stably transformed with different genetic constructions and observed that the I element is absolutely necessary for gene expression in the context of its own promoter. Furthermore, the IM version produces higher expression levels when compared with I and also acts synergistically with the strong constitutive promoter CaMV35S to increase transcript levels and protein expression. Addition of IM to a construct for expressing an Arabidopsis gene related to oxidative stress increases seed yield above the levels obtained with CaMV35S alone, both under control conditions and after stress situations. The enhancer activity of IM was also observed in monocot plants using transient transformation assays. We propose the use of IM as a genetic tool for increasing the expression of genes and proteins of interest in plants.

BT-P17
PSEUDOMONAS STUTZERI AS A PROMISING PLANT GROWTH-PROMOTING BACTERIA FOR SOYBEAN IN SALINE SOILS

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Bacteria were isolated from roots of a plant, *Sesuvium edmonstone*, that grows well in the Salinas Grandes, province of Santiago del Estero. One of the most salt tolerant isolates was identified as *Pseudomonas stutzeri* after sequencing its 16S rDNA and was named PS19. We characterized biochemical properties of PS19 in regards of its Plant Growth-Promoting ability, studying the auxin and siderophore production, phosphate solubilization, biological nitrogen fixation, desaminase ACC activity and its growth on saline conditions. In addition, we evaluated the PS19 beneficial effect on soybean germination in saline condition assays (100mM NaCl). We used two soybeans seeds varieties, Munasqa and S100, which have different saline susceptibilities. The germination percentage, vigor and growth parameters were evaluated. For both soybean varieties, improved values in the measured parameters were obtained for the inoculated seeds. These results indicate that PS19 is a promising bacterium to enhance growth and to help the plant deal with high soil salinity.

BT-P18
DEVELOPMENT OF HETEROGENEOUS BIOCATALYST FOR PECTIN HYDROLYSIS OF VEGETABLE RESIDUES

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Immobilization technology provides diverse applications in food and environmental industries. As immobilization means associating the biocatalyst with an insoluble matrix, so it can be retained in proper reactor for its reuse under stabilized conditions. Hydrogels allow a low-cost and effective system to develop a heterogeneous biocatalyst with polygalacturonase (PG) activity for pectin degradation derived from agroindustrial residues. Hydrogels are polymeric cross-linked network structures which can confine proteins. In the present work, alginate and agar were used as matrices for entrapment of PG secreted by *Streptomyces halstedii* ATCC 10897. Alginate matrix allowed to develop a new strategy to improve beads stability and enhance mechanical properties by using a mixture of calcium and strontium for ionotropic gelation. This immobilized biocatalyst was able to hydrolyze pectin and release 1.54 mg/mL of D-galacturonic acid after 2h reaction and was stable up to 28 successive reactions. On the other hand, PG stabilized in bacteriological

agar produced 1.88 mg/mL of D-galacturonic acid and was stable for 48 batch reactions. Kinetic parameters Km (0.150 M) and Vmax (2.295 µmol/min) were determined.

BT-P19
BIOSYNTHESIS OF GALACTOSYL-FLOXURIDINE USING IMMOBILIZED *Β*-GALACTOSIDASE FROM *Micrococcus luteus*

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The 5-fluorouracil-2'-deoxyriboside (Floxuridine, FUdR) has been extensively used in colorectal, pancreatic, breast, head, and neck cancer treatment. However, in many cases galactosylation of this compound allows to increase their bioavailability and reduce their cytotoxicity. Nucleoside analogues and their derivatives are principally synthesized by chemical methods that involve arduous steps, which are environmentally unfriendly; so biocatalysis appears as a promising alternative. In this work, seventy bacterial strains have been screened for extracellular β-galactosidase activity and *Micrococcus luteus* ATCC 9341 was selected for subsequent assays. An efficient and simple bioprocess for obtaining *β*-D-galactosyl-floxuridine using immobilized *β*-galactosidase was developed. Under optimized conditions, immobilization yield of 99% was achieved. Additionally, reaction parameters for FUdR galatosylation by *β*-galactosidase of *M. luteus* immobilized in Ca-alginate were optimized, getting yields of 80% at 7 hours of reaction. Besides, the developed biocatalyst was stable for 6 months in storage conditions (4 ºC) and could be reused 18 times without loss of its activity. These results indicate that this immobilized enzyme represents an alternative in the production of a broad spectrum of galactosylated floxuridine derivatives, employing an environmentally friendly methodology.

BT-P20
ENCAPSULATION *Echinococcus granulosus* ANTIGENS FOR THE DEVELOPMENT OF A NANOVACCINE

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Cystic echinococccis caused by the flatworm parasite *Echinococcus granulosus*, is a serious health and economic problem in several countries, including Uruguay, Argentina, Chile, Perú and Brasil. This disease affects both humans and livestock. Diagnosis of the disease is performed using imaging and immunological techniques. However, once the hydatid cyst is detected the most effective treatment is surgery, with the risk of rupture of the cyst and larvae reseeding.

An effective way to eliminate this disease is through the development of vaccines to prevent infection in the definitive host, the dog. This approach is less expensive and more effective than a vaccine designed for intermediate host (sheep and cattle).

It is possible to achieve high levels of complex and reliable protection against metazoan parasites using recombinant antigens. We have selected EgVAL1 and EgVAL2 proteins belonging to the CAP superfamily of proteins. Promising results have been obtained using nematodes proteins of this superfamily as vaccine candidates.

EgVALs were encapsulated in chitosan coated polymeric nanoparticles obtaining the adequate parameters. In addition, the encapsulated antigens were well preserved when submitted to gastrointestinal conditions. Future trials are needed to assess the effectiveness of these antigens in generating a vaccine to be administered orally to dogs.

BT-P21
BIOREMEDIATION STRATEGIES BASED ON A NATIVE STRAIN ISOLATED FROM SITES CONTAMINATED WITH HYDROCARBONS.

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The bacterial strain studied in this work is a member of a bacterial consortium isolated from chronically hydrocarbon-contaminated site in Campana (Bs.As.). This native strain was identified as *Pseudomonas* sp. according to its 16S rRNA gene partial sequence. The ability of this strain to produce biosurfactants was evaluated in Erlenmeyer flasks containing a minimal saline medium (MSM) supplemented with different carbon sources: a mixture of hydrocarbons (HC; $4.5\%_{v/v}$), glycerol (Gly; $2\%_{v/v}$), sunflower oil (SO; $2\%_{v/v}$) and peanut oil (PO; $2\%_{v/v}$). Cultures were performed at 135 rpm and 25 ºC for 4 days. Bacterial growth was measured by cell dry-weight method, and biosurfactant production was estimated by direct measurement of the surface tension (ST). Results showed that bacteria was able to grow on all the carbon sources tested, reaching concentrations of 1.24g/l in HC, 7.69g/l in Gly, 3.98g/l in SO and 9.29g/l in PO. Culture supernatants showed a decrease in ST values when the strain grew on SO and PO (22.5% and 25.5%). No decrease in ST values was observed when HC and Gly were used as carbon source. As results shown, this bacterial strain can produce biosurfactants under certain culture conditions. This potential advantage could be applied in bioremediation strategy of hydrocarbon contaminated sites.

CELL BIOLOGY

CB-P01
PROTEIN S-ACYLATION IN *Trichomonas vaginalis*

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The flagellated protozoan parasite *Trichomonas vaginalis* is the etiologic agent of trichomoniasis, the most common non-viral sexually transmitted infection worldwide. Since T. vaginalis is an obligate extracellular pathogen, adherence to epithelial cells is critical for parasite survival. A better understanding of this process is a prerequisite for the development of therapies to combat infection. In this sense, recent work has shown S-acylation as a key modification that regulates invasion and motility in different protozoan parasites, such as *Plasmodium spp*, *Trypanosoma ssp*, *Giardia ssp* and *T. gondii*. However, up to date there are no reports indicating whether this post-translational modification is a mechanism operating in T. vaginalis. In order to study the extent and function of S-acylation in *T. vaginalis* biology, we undertook a proteomic study to profile the full scope of S-acylated proteins in this parasite and here we report the identification of 504 proteins involved in a variety of biological processes including protein transport, attachment and signaling, among others. Importantly, treatment of parasites with the palmitoylation inhibitor 2-bromopalmitate caused a significant decrease in the adherence to host cells, suggesting that palmitoylation could be modifying proteins that are key players in adherence and concomitant pathogenesis of *Trichomonas vaginalis*.

CB-P02
A NOVEL SIGNAL FOR ENDOCYTOSIS AND POLARITY IN YEAST

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In *Saccharomyces cerevisiae*, polarized distribution of plasma membrane proteins is maintained kinetically due to polarized secretion followed by endocytic recycling. The classical model for endocytosis of transmembrane proteins involves the recognition of cytosolic signals by adaptor proteins that drive the active concentration of cargoes in endocytic vesicles. We previously observed that increasing the volume of residues that constitute the exoplasmic hemi-TransMembrane Domain (TMD) of the yeast SNARE Sso1, which is homogenously distributed in the plasma membrane, results in its polarized localization and this is dependent in endocytosis and recycling. Naturally occurring high-volume exoplasmic hemi-TMDs are also able to confer a polarized distribution to the cytoplasmic domain of Sso1, confirming that the geometry of TMDs represent a novel determinant for endocytosis and polarity. To gain insight into the mechanism involved in the endocytosis of this novel signal, we tested numerous yeast mutants that allowed us to confirm that internalization is clathrin mediated. We tested all known endocytosis adaptors but we were able to identify a specific adaptor for these TMDs. We observed a dependence on the ubiquitin ligase Rsp5. Interestingly, a mutant Sso1 chimera devoid of lysines is still polarized, suggesting that a ubiqutinated adaptor for this novel signal must exist.

CB-P03
ST3GAL II AND β4GALNT I ARE S-ACYLATED AT N-TERMINAL CYSTEINES INVOLVED IN HOMO-DIMERIZATION

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Ganglioside glycosyltransferases (GGTs) are type-II membrane proteins that consist in a short N-terminal cytoplasmic tail, a transmembrane domain (TMD), and a luminally oriented C-terminal domain bearing the catalytic domain. We have previously shown that the N-terminal domain (Ntd) of the GGTs ST3Gal V, ST8Sia I and β4GalNT I are S-acylated at conserved cysteine residues when expressed in CHO-K1 cells.Here, we extended our studies to ST3Gal II, a GT that sialylates glycolipids and glycoproteins, and found that full length ST3Gal II is also S-acylated.S-acylation, commonly known as palmitoylation, is catalyzed by a family of palmitoyltransferases (PATs) that are mostly localized at the Golgi complex but also at the ER and the plasma membrane. Using GT's ER-retention mutants, we found that S-acylation of β4GalNT I and ST3Gal II takes place at different compartments, suggesting that β4GalNT I and ST3Gal II are not substrates of the same PAT and this may be due to the different localization of the conserved cysteines in these GTs (TMD or cytosolic tail, respectively). We also found that cysteines that are target of S-acylation on β4GalNT I and ST3Gal II are involved in the formation of homodimers through disulfide bonds. We show an increase of ST3Gal II dimmers in the presence of the PAT inhibitor 2-bromopalmitate, suggesting that S-acylation may be regulating GTs homo-dimerization

CB-P04
IDENTIFICATION OF A PLASMA MEMBRANE FUSION SUPERFAMILY, FUSEXIN, SUFFICIENT TO FUSE GAMETES, ENVELOP

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Gamete fusion enables sexual reproduction. While proteins mediating cell-cell fusion in tissues have been identified (e.g. C. elegans EFF-1), the machinery catalyzing sperm-egg fusion is still unknown. HAP2 proteins are candidate fertilization fusogens as their

inactivation results in sterility due to failure in gamete fusion in a number of organisms. To determine whether HAP2 is not only necessary for fertilization but is also sufficient for cell-cell fusion we express Arabidopsis HAP2 in cells that normally do not fuse. Heterologous expression in Baby Hamster Kidney cells results in cell-cell fusion, with HAP2 presence required in both fusing cells. This bilateral action can be recapitulated replacing the plant gene with EFF-1 in one of the fusing cells, indicating that HAP2 and EFF-1 can interact and share a similar fusion mechanism. Modeling of HAP2 predicts it is homologous to *C. elegans* EFF-1 somatic fusogen and class II fusion proteins from enveloped viruses (e.g. dengue and Zika viruses). Based on functional and structural conservation of these sexual, viral and somatic cell fusion proteins we name them FUSEXINS: FUSion proteins for sexual reproduction and EXoplasmic merger of plasma membranes. We propose that Fusexins derive from a common ancestor linking the origins and evolution of sexual reproduction, enveloped viruses invasion and somatic cell fusion.

CB-P05
CHANGES IN SECRETORY PATHWAY MARKERS IN A PC12 CELL MODEL OF PARKINSON´S DISEASE

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The secretory pathway must adapt to physiological situations like cell differentiation. There is little known about this adaptation process, as well as the connection between membrane trafficking and neurodegeneration. We aim to elucidate biochemical and morphological changes involving the secretory pathway that occur in Nerve Growth Factor (NGF) differentiated PC12 cells and in these cells treated with an analogue of Dopamine, 6-Hydroxy Dopamine (6-OHDA); a model of Parkinson´s Disease (PD). We found that NGF induces a time-dependent increase in protein levels of ER-Golgi markers, while their mRNA levels do not seem to vary. In contrast, transcript levels of endocytic pathway markers increase. We showed that increase in transport proteins due to 6-OHDA treatment is higher than that caused by NGF and that the Golgi is disrupted. Curiously, we did not detect a significant increase in the levels of α-synuclein, a well known marker of PD. This suggests that, in this model, 6-OHDA toxicity may not be mediated by α-synuclein. Taken together, our data suggest that: a) the regulation of the secretory pathway in differentiating PC12 cells may occur via post-translational modifications and b) the changes in transport factors levels due to 6-OHDA could happen to overcome Golgi fragmentation. We aim to investigate the molecular mechanisms underlying this response in different conditions.

CB-P06
POST-TRANSLATIONAL INCORPORATION OF L-DOPA INTO THE C-TERMINUS OF α-TUBULIN IN LIVING CELLS

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The C-terminal tyrosine of the α-tubulin chain is subjected to its removal and post-translational re-addition (detyrosination/tyrosination cycle). Some molecule analogues, such as L-3,4-dihydroxy phenylalanine (L-Dopa) can be incorporated in place of tyrosine. We found that this L-Dopa incorporation occurs also in living cells. To detect and quantify Dopa-tubulin, we developed a procedure that consists in the determination of the "tyrosination state" of tubulin using specific antibodies against the different isoespecies of tubulin, before and after cells were incubated in the presence of L-Dopa. The presence of Dopa-tubulin (which is not recognized by Tyr- or Glu-antibodies) is assumed to arise from the difference between total tubulin and the sum of Tyr- and Glu-tubulin (subsequently confirmed by HPLC). Post-translational L-Dopa incorporation into tubulin was demonstrated in Neuro 2A cells and others cell lines. Dopa-tubulin had similar capability to form microtubules as Tyr- and Glu-tubulin. Viability, cell morphology and proliferation rate were not modified when 20% of total tubulin in the cells was Dopa-tubulin.

CB-P07
DEVELOPMENT OF SCREENING METHODS TO IDENTIFY TRANSLESION DNA SYNTHESIS INHIBITORS

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Translesion DNA synthesis (TLS) is a DNA damage tolerance process that employs specialized polymerases to bypass DNA damage during replication. Recent evidence indicates that TLS is a key process that promotes the development of resistance to cancer treatments that induce DNA damage. Thus, the inhibition of TLS emerges as a promising strategy for cancer therapy. However, specific chemical inhibitors of TLS are not available. The main goal of our project is to identify specific inhibitors of TLS that can be used as "a proof of concept" in cancer therapy. Our rational is that since TLS polymerases recruitment to sites of DNA damage is a key step for TLS success, we can indirectly monitor TLS efficiency by studying two key markers: 1) The mono-ubiquitylation of PCNA and 2) the accumulation of a TLS polymerase into replication foci. We thus developed two screening methods that allow us to promptly identify inhibitors of these markers through a Western-Blot based platform to follow the mono-ubiquitylation of PCNA and imaging-based assay for the quantification of TLS polymerase foci. In this poster we describe the results of a pilot screening using an open source library of kinase inhibitors from Glaxo Smith Kline and the early validation of the identified hits.

CB-P08
KLF6 TUMOR SUPPRESSOR ACTIVITY IS ASSOCIATED TO THE INDUCTION OF CELLULAR SENESCENCE

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Krüppel-like factor 6 (KLF6) is a transcription factor involved in cell-cycle regulation and differentiation. Indeed, KLF6 is considered a tumor suppressor whose expression is frequently lost in various human cancer types. In previous work, we shown that KLF6 knockdown in normal fibroblast led to cell transformation. As cellular senescence is a crucial anticancer mechanism that prevents growth of cells at risk for neoplastic transformation, we aimed to elucidate the impact of KLF6 on cellular senescence. This cellular process can be elicited through several mechanisms including oncogene activation, DNA damage and oxidative stress. Through H2O2-treatment we induced NIH3T3 fibroblast cells to become senescent, detected by senescence-associated beta galactosidase activity. Then, stable transduction of shRNA for downregulation of KLF6 expression led to a decreased basal index of senescent cells, thereby bypassing H2O2-induced cellular senescence. In addition, ectopic expression of KLF6 in HeLa cervical carcinoma cells was able to induce cellular senescence. This surprising finding, along with the cytostatic function of KLF6 upon oncogenic activation is suggesting that its tumor suppressor activity could be mediated by cellular senescence as an alarm signal in response to certain stimuli producing exacerbated proliferation or cell transformation

CB-P09
IDENTIFICATION OF TLS INHIBITORS THROUGH THE DEVELOPMENT OF IMAGING-BASED SCREENING PLATFORMS

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The identification of compounds and the development of rational strategies to achieve selective cytotoxicity against cancer cells is a current challenge in cancer therapy. A strategy that has yielded promising results is the induction of synthetic lethality (SL), which takes advantage of the mutations of cancer cells to induce cell death. DNA repair pathways are strategic targets to trigger SL due to their central role in the preservation of genomic stability and cell viability. Two key DNA repair mechanisms that protect replication forks integrity are Homologous Recombination (HR) and Translesion DNA synthesis (TLS). Given that different types of tumor cells tend to lose HR, the inhibition of TLS emerges as a promising strategy for cancer therapy. However, specific chemical inhibitors of TLS are not available to date. Our main goal is to identify specific inhibitors of TLS by developing cell-based assays that explore TLS markers. TLS polymerases recruitment to sites of DNA damage is a key step of TLS, which efficiency can be indirectly examined by studying the recruitment of TLS polymerases into replication foci. Here, we show the setup of a screening platform to monitor TLS polymerases recruitment to damage sites, through an imaging-based assay.

CB-P10
KLF6 SUCBELLULAR DISTRIBUTION AS A MARKER OF TUMOR AGGRESSIVENESS IN HUMAN COLON ADENOCARCINOMA

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Colorectal cancer (CRC) is the second most frequent cancer type in our country, representing the 12% of total cancer deaths. However, it is one of the most preventable tumors due to its slow progression and early detection increases complete remission chances up to 90%. Unfortunately, useful molecular biomarkers for CRC early detection are not available. Krüppel-like factor 6 (KLF6) is a tumor suppressor related with several types of cancers though its function in CRC is not deeply known. Our aim was to investigate whether KLF6 expression could be associated with CRC pathogenesis. We establish the frequency of KLF6 suCBellular expression in 37 human CRC by immunohistochemistry and we found that 83% of the cases showed a generalized KLF6 cytoplasmic expression. However, nuclear localization revealed a more gradual pattern, even showing null expression. We correlated these data with CRC pathology grades and we found a positive correlation between KLF6 nuclear expression and tumor stage (r= 0.36) and a negative correlation with tissue differentiation (r= -0.33). In addition, null nuclear expression correlated with low proliferation index ($p<0.05$). In summary, increased KLF6 nuclear localization in CCR could be related with a more advanced and an aggressive tumor behavior, suggesting that KLF6 suCBellular expression might be a plausible marker for early diagnosis and prognosis of CCR.

CB-P11
DEVELOPMENT OF A SCREENING PLATFORM FOR THE IDENTIFICATION OF LETHALITY INDUCERS IN CANCER CELLS

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One of the current challenges in the fight against cancer is the identification of compounds with selective tumor cytotoxicity. A precision strategy of this kind that lead to promising results in recent years is the induction of synthetic lethality, which takes advantage of tumor mutations to induce selective tumor lethality. The homologous recombination (HR) pathway is a strategic target for synthetic lethality because of its important role in double strand break repair, and because HR loss is a key feature of certain types of breast and ovarian cancer (i.e. BRCA1 and BRCA2 deficient cancers). The aim of this work is to identify new synthetic lethality inducers in HR-deficient cells. To achieve this we developed a high-throughput screening platform based on flow cytometry. This platform is based on the generation of HR-proficient and HR-deficient cell lines of isogenic origin that emulate BRCA1 and BRCA2

deficient cancers, and that can be simultaneously exposed to the tested compounds during the screening. We use fluorescent markers to identify each population, assessing cell survival by the ratio of HR-proficient and HR-deficient populations at the end of the experiment. Here we show the development of the platform and the fine-tuning of the setup using drugs that are known to induce synthetic lethality in a pre-screening phase.

CB-P12
STAPHYLOCOCCAL α-TOXIN REGULATES C-JUN ONCOPROTEIN ACTIVATION, ITS mRNA LEVEL AND PROTEIN STABILITY

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c-Jun is a relevant member of the AP-1 transcription complex which participates in a wide range of cellular processes such as proliferation, apoptosis, tumorigenesis, differentiation and survival upon cell stress. However, its role in the context of bacterial infections has not been thoroughly investigated. Staphylococcus aureus is a worldwide health-care concern pathogen which owns an extensive repertory of virulence factors provoking cellular damage. In this sense, we analyzed the role of staphylococcal α-toxin in the activation, expression and protein levels of c-Jun in A549 lung epithelial cells. Staphylococcal α-toxin per se was able to activate c-Jun by inducing phosphorylation of its Ser73 residue. Silencing of the JNK pathway abrogated most of this activation. On the contrary, silencing of ERK exacerbated this response. Cell exposure to α-toxin induced a marked increase in the c-Jun mRNA expression at 120 min. However, the c-Jun protein levels markedly decreased after the same time period as a consequence of proteasomal degradation. Finally, we established that c-Jun contributed to cell survival when cells were challenged with α-toxin. This study constitutes one of the first steps to understand the role of c-Jun in the cellular response to bacterial pore-forming toxins, placing it as a novel component of the complex early machinery to face staphylococcal infections.

CB-P13
CORRELATION BETWEEN PMCA ACTIVITY AND TUBULIN ON PLATELET FUNCTION IN SPONTANEOUSLY HYPERTENSIVE RAT

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In previous work we have shown that tubulin and PMCA form a complex, and the formation of this complex regulates the PMCA activity in normotensive and hypertensive subjects. It has previously been reported that increased calcium intraplatelet can cause hyperactivation of platelets during hypertension. Because of the importance of calcium regulation in platelets we decided to study the regulation of PMCA by tubulin in hypertensive rats. We observed a significant increase in acetylated and detyrosinated tubulin and presence of complex of tubulin and PMCA, suggesting the interaction both proteins. Analysis the PMCA activity revealed that the activity in platelets hypertensive rats is decreased by 50% compared to the control, and this decrease in activity correlates with a higher degree of phosphorylation on tyrosine residues in PMCA. Functional analysis of platelets obtained by measurement of bleeding time indicates that in hypertensive rats the time to form platelet plug is 3 times less than that needed in Wistar rats. In summary would indicate a correlation between the high tubulin content, low PMCA activity and higher activation in platelets hypertensive rats.

CB-P14
NATURAL ANTISENSE TRANSCRIPTS IN THE REGULATION OF ACSL4 EXPRESSION IN BREAST CANCER CELLS

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Acyl-CoA synthetase 4 (ACSL4) is overexpressed in human breast cancer cells and promotes tumor aggressiveness. The mechanisms that regulate its expression are not fully characterized. Natural antisense transcripts (NATs) are endogenous RNAs complementary to mRNA, which regulate their counterparts and play a role in human diseases. We identified a murine ACSL4-NAT, a hormonally-.regulated long non-coding RNA that may influence steroidogenesis through ACSL4 expression regulation. The aim was to evaluate the participation of NATs in regulating human ACSL4 expression in breast cancer cell model. By BLAST algorithm, we compared murine ACSL4-NAT sequence against human ACSL4 mRNA, obtaining a 92% identity and 94% complementary. Using RNA from human breast cancer MCF-7 and MDA-MB-231 cells, sequence-specific RT-PCR experimentally demonstrated the existence of such human ACSL4-NAT. We amplified a fragment of the expected size in both cell lines and observed coordinated expression of ACSL4 NAT and mRNA, with a higher expression of in the more aggressive MDA-MB-231 cells. By 5´and 3´RACE we obtained the complete sequence of this transcript. ACSL4-NAT knockdown via asymmetric shRNA impacted on mRNA and protein levels and cell proliferation. NATs could be part of the mechanism regulating ACSL4 expression and thus contributing to the development of aggressive phenotype in breast cancer cells.

CB-P15
INSULIN INDUCES THE EXOCYTIC TRAFFIC OF LRP1 FROM GSV–LIKE STRUCTURAL VESICLES

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Glucose transporter type 4 (GLUT4) is an insulin-regulated glucose transporter only expressed in adipose and muscle cells. In these cells GLUT4 is stored in specialized structural vesicles termed GSV. LDL receptor-related protein 1 (LRP1) is also detected in GSV.

However, LRP1 is expressed in other cells that not contain GLUT4, such as Muller Glial cells (MGCs). This receptor is intracellularly stored in non well-characterized structural vesicles from which can be sorted to plasma membrane (PM) in the presence of the alpha2-Macroglobulin (a LRP1-related ligand) or insulin (a LRP1-nonrelated ligand) through a putative exocytic route. We hypothesize that LRP1 is stored in GSV-like structural vesicles in all types of cells. Herein, we characterize the structural vesicles storing LRP1 in a MGC line, MIO-M1. By biotin-labeling protein assay we demonstrated that LRP1 translocate to PM after insulin stimulation (100 nM; 30 min), which was inhibited by the expression of a negative mutant of Rab10 GTPase. By confocal microscopy we found the LRP1 colocalization with mCherry-GLUT4-Myc and sortilin, a marker of GSV. Finally, we demonstrate that HA-mLRP4-GFP and mCherry-GLUT4-Myc were trafficked to PM in insulin-stimulated cells. Thus, we conclude that LRP1 is stored in structural vesicles with GSV properties in MIO-M1 cells, from which is trafficked to PM through an exocytic pathway.

CB-P16
CHARACTERIZATION OF HUMAN SIALIDASE NEU3 MEMBRANE ASSOCIATION

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Plasma membrane (PM)-bound sialidase Neu3 is a key enzyme in the catabolism of glycoconjugates. However, there is little information concerning its membrane topology and the mechanism of association with biomembranes. Hence, the aim of this work was to further contribute on our knowledge on this matter. Microscopy analysis showed that Neu3 was mainly localized at the PM and endosomes. 35% of the enzyme was accessible to cell surface biotinylation and two different epitopes were recognized by antibodies only after cell permeabilization. Protease digestion assays on intact cells resulted in the appearance of a protected fragment of Neu3. Together, these results indicated that Neu3 exposes its C-terminus toward the cytosol while other fraction is exposed to the extracellular milieu. By different approaches, we showed that Neu3 interacts with itself via disulfide bridges and with other proteins, most of them facing the cytosol. Binding of Neu3 to the lipid bilayer was independent on electrostatic interactions and 55% of Neu3 had a hydrophobic behavior in TX-114 assay, suggesting a posttranslational modification by lipidation. In fact, we found that Neu3 is S-acylated, representing the first demonstration of a posttranslational modification. These results provide a comprehensive analysis of Neu3 topology and allow us to propose a model of its association with the PM.

CB-P17
POST-TRANSLATIONAL INCORPORATION OF PHENYLALANINE INTO TUBULIN AS A CAUSE OF NEURONAL DYSFUNCTION

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The C-terminus of α-tubulin undergoes post-translational, cyclic tyrosination/detyrosination, and L-Phenylalanine (Phe) can be incorporated in place of tyrosine. Using cultured CAD cell and a newly generated antibody specific to Phe-tubulin, we showed that: (i) Phe incorporation into tubulin is reversible; (ii) such incorporation is not due to de novo synthesis; (iii) the proportion of modified tubulin is significant; (iv) Phe incorporation reduces cell proliferation without affecting cell viability; (v) the rate of neurite retraction induced by FBS addition declines as level of C-terminal Phe incorporation increases; (vi) this inhibitory effect of Phe on neurite retraction is blocked by the co-presence of tyrosine; (vii) microtubule dynamics is reduced when Phe-tubulin level in cells is high as a result of exogenous Phe addition and returns to normal values when Phe is removed; moreover, microtubule dynamics is also reduced when Phe-tubulin is expressed (plasmid transfection). It is known that Phe levels are greatly elevated in blood of phenylketonuria (PKU) patients. The molecular mechanism underlying the brain dysfunction characteristic of PKU is unknown. According to our results, it is conceivable the possibility that Phe incorporation into tubulin is the first event (or among the initial events) in the molecular pathways leading to brain dysfunctions that characterize PKU.

CB-P18
LRP1 PARTICIPATES IN HEMIN-INDUCED AUTOPHAGY, MODYFING ITS TRAFFICKING IN ERYTHROLEUKEMIA CELLS

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Hemin is a natural compound which stimulates hemoglobin synthesis and organelle clearance necessary in erythropoiesis. The transmembrane low density lipoprotein receptor related protein 1 (LRP1), is the scavenger for the hemin-hemopexine complex that allows its internalization. Autophagy is a lysosomal-degradative process leading to degradation of non-necessary organelles. Our aim is to elucidate the possible role of LRP1 in the hemin-induced autophagy. Our preliminary results have shown that Hemin or resveratrol (autophagy inductors) are able to induce LRP1 gene expression and increase the protein levels. Moreover, in hemin-stimulated K562 cells (erythroleukemia cells line), it was observed an increased colocalization of LRP1 structures with late endosomes, lysosomes and also with autophagosomes. This is a new finding in LRP1 trafficking being reported that the receptor is only associated with early endosomes. Interestingly, silencing of LRP1, hamper the hemin-stimulated autophagy in K562 and HeLa cells. Moreover, in cells incubated in presence of hemin, an additional band of was detected by Western blot in a time-dependent manner. The study of the molecular mechanisms that regulate the autophagy induction could provide important data to understand more deeply the mechanisms of proliferation and differentiation of red line cells, especially in hematologic diseases such as leukemia.

CB-P19

UTEROSOME-LIKE VESICLES PROMPT HUMAN SPERM FERTILIZING CAPACITY

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It is known that the uterine environment affects sperm function, but the underlying cellular mechanisms still remain unknown. Extracellular vesicles have been recently identified along the female reproductive tract, including the uterus, where they were called uterosomes. Previous data showed that conditioned medium from endometrial cells stimulate sperm capacitation. Thus, we hypothesize that extracellular vesicles may be mediating this sperm fertilizing capacity induction. In this study, we characterized uterosome-like vesicles (ULV) obtained from the conditioned medium of an endometrial epithelial cell line, and analyzed their interaction with human spermatozoa. We found that the isolated ULV were ~50-200 nm in size, with characteristic cup-shape morphology. In addition, the ULV were positive for CD63 and MFGE8 protein markers, as shown by Western blot and immunogold labelling. Furthermore, after only 15 minutes of in vitro co-incubation, ULV seem to be able to fuse with spermatozoa stimulating capacitation, as determined by the induced acrosome reaction and the protein tyrosine phosphorylation. The present results suggest that the brief transit of human spermatozoa through the uterine environment might be enough to enhance sperm fertilizing capacity through a novel extracellular vesicles mediated mechanism.

CB-P20

TEMPORAL REGULATION OF STRESS GRANULES BY CIRCADIAN CLOCKS AND OTHER MECHANISM

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Stress granules (SGs) are microscopically visible foci formed in response to stress by assemblies of untranslating messenger ribonucleoproteins from mRNAs stalled in translation initiation. Since a number of SG components display circadian rhythms, we had hypothesized that these mRNA granules could be circadianly regulated. Indeed, we have previously shown that SGs induced by oxidative stress display daily changes in their number and size in synchronized NIH3T3 cultures. In order to establish whether the oscillations observed were truly circadian rhythms, we performed similar experiments in clock defective cells. We utilized fibroblasts from knockout or double knockout mice for different clock genes: Bmal1, Per1/2 and Cry1/2 and their corresponding wt controls. In all cases we found significant changes through time in the number, area, and signal intensity of eIF3 immunolabeled SGs in synchronized cultures. However the period, amplitude, and phase of the oscillations were different in each genotype. Since these parameters are altered in cells without a functional circadian clock, we concluded that the described oscillations are modulated by this timing mechanism but also for some other metabolic driver. It has been reported redox rhythms in several systems controlled by mechanisms different from the canonical clock, which could be responsible of the oscillations we observed.

CB-P21

IDENTIFICATION OF KLDHC5 AS AN INTERACTING PROTEIN OF STARD7

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StarD7 belongs to the steroidogenic acute regulatory protein-related lipid transfer domain proteins that transfer phospholipids among intracellular membranes. It facilitates the delivery of phosphatidylcholine to the mitochondria and previous results indicated that StarD7 knockdown decreases ACBG2 multidrug transporter level, cell migration, proliferation, and phospholipid synthesis. The purpose of this study was the identification of candidate proteins that interact with StarD7. Using peptide LC-mass spectrometry, we identified KLDHC5, a substrate-specific adapter of a CBR (BTB-CUL3-RBX1) E3 ubiquitin-protein ligase complex required for mitotic progression and cytokinesis, as a novel protein that interacts with StarD7. The association between the two proteins was confirmed by coimmunoprecipitation and immunofluorescence staining. Cytoplasmic colocalization of both proteins was demonstrated in stable StarD7 HEK293T cells transiently transfected with KLHDC5. Interestingly, HEK293T cells overexpressing StarD7 resulted in an increased number of cells with persistent microtubule bridges between post-mitotic cells as well as multinucleated cells. Taken together these results suggest that StarD7 may participate with KLHDC5 in a protein complex regulating the mitotic division through the localization of lipids required for this process.

CB-P22

CHLORPYRIFOS INDUCES ENDOPLASMIC RETICULUM STRESS ASSOCIATED WITH P53 DEGRADATION IN JEG-3 CELLS

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Chlorpyrifos (CPF) is an organophosphorous pesticide widely used in agricultural, industrial, and household applications. We have previously shown that JEG-3 cells are able to attenuate the oxidative stress induced by CPF through the adaptive activation of the Nrf2/ARE pathway. Considering that there is a relationship between oxidative stress and endoplasmic reticulum stress (ER), herein we investigated whether CPF also induces ER stress in JEG-3 cells. Cells were exposed to 50 µM or 100 µM CPF during 24 h in conditions where cell viability was not altered. Western blot and PCR assays were used to explore the protein and mRNA levels of ER stress biomarkers, respectively. CPF induced an increase of the typical ER stress-related proteins, such as BiP/78 kDa glucose-regulated protein and inositol-requiring enzyme 1α, a sensor for the unfolded protein response, as well as the phosphorylation of eIF-2α and XBP1 splicing. Additionally, CPF led to a decrease in p53 protein expression. The downregulation of p53 protein levels was

blocked when cells were exposed to CPF in the presence of the proteasome inhibitor MG132 indicating that the p53 half-life was decreased by ER stress. Altogether, these findings point out that CPF induces ER stress in JEG-3 cells; however are able to attenuate it by downregulation of the pro-apoptotic p53 protein levels.

CB-P23
STARD7 KNOCKDOWN LEADS TO α5β1 INTEGRIN UPREGULATION AND GOLGI FRAGMENTATION IN HTR8/SVNEO CELLS

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StarD7 belongs to START domain protein family, which is involved in lipid transport, metabolism and signaling. Preliminary results indicate that StarD7 silencing induces a decrease in HTR8/SVneo cell migration measured by wound healing assay. To address the mechanism implicated in this process the levels of α5 and β1 integrin subunits, p-FAK as well as β-catenin proteins were measured in HTR8/SVneo transfected with StarD7 siRNA for 72 h. Surprisingly, a significant increase in the mRNA, and protein levels of α5 integrin in silenced cells were determined by qRT-PCR, western blot and immunofluorescence assays. Also, a clear increase in the transcript level of β1 integrin, as well as in the β1 mature protein was detected. In addition, StarD7 silencing leads to an increase in p-FAK and β-catenin protein levels, whereas a decrease in the level of MMP2 secreted to the culture medium was established in StarD7 siRNA cells. Since it is widely accepted that Golgi apparatus regulates directional cell migration we analyzed its integrity. Immunofluorescence assay usinganti-GM130 revealed that StarD7 knockdown induced Golgi apparatus fragmentation. In summary, our results suggest that StarD7 depletion causes a dysregulation in several molecules as well as in Golgi apparatus, which are involved in maintaining cellular migration.

CB-P24
TAMS INDUCE ENDOCRINE RESISTANCE AND STEM CELL-LIKE ENRICHMENT IN BREAST CANCER CELLS

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It has been observed that tumor associated macrophages (TAMs) cause a marked increase in the proliferative capacity of MCF-7 cells and promote the growth of bigger breast tumors when MCF-7 cells are co-injected with TAMs in immunocompromised mice. We have found that crosstalk between MCF-7 cells and TAMs induce a number of alterations in the breast cancer cells, which can be grouped into two main facts. First, TAMs lead to the acquisition of a stem cell-like phenotype with higher metastatic potential and mesenchymal properties. This phenotype is characterized by the loss of the surface marker CD24. Thus, the normal population of MCF-7 cells that is CD24+ / CD44+ shifts to CD24- /CD44+.Secondly, the interaction between TAMs and MCF-7 cells, which are estrogen-dependent, entails to the development of resistance to estrogen withdrawal or treatments with selective ER modulators (SERMs) as Tamoxifen or ICI in MCF-7 cells.Moreover, the signaling pathways of both the NF-κB and IL-6/STAT3 are required for these responses which include macrophage-mediated phosphorylation of the estrogen receptor ER-α, and activation of proliferative and proinflammatory genes in the breast cancer cells. Indeed, the knockdown of any of these pathways such as STAT3, ER-α or NF-kB in MCF-7 cells, could inhibit proliferation and the endocrine resistance induced by TAMs.

CB-P25
VITAMIN A DEFICIENCY: ALTERS OXIDATIVE STRESS AND INFLAMMATION GENE EXPRESSION IN MAMMARY GLAND.

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The objective of this study is to determine the effect of vitamin A deficiency (VAD) on oxidative stress and inflammation. Wistar female virgin rats were separated at weaning into 6 groups: fed with vitamin A sufficient diet for 3 months (c3m), vitamin A deficient diet for 3 months(d3m), vitamin A deficient diet for 75 days followed by refeeding with vitamin A sufficient for 15 days (r3m), vitamin A sufficient diet for 6 months (c6m), vitamin A deficient diet for 6 months (d6m) and vitamin A deficient diet for 150 days followed by refeeding with vitamin A sufficient for 30 days (r6m). The expressions of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), Nrf-2, iNOS, eNOS, NFκb, TNFα,Cox2 were measured by RT-PCR; and also TBARs and Carbonil levels.TBARS and Carbonil levels and CAT, SOD, GPx, Nrf2, iNOS and eNOS expression did not show differences between groups. The expression of NFκb, TNFα, Cox2 increased in d3m with respect to c3m and r3m ($p<0.05$), except for TNFα r3m that did not reverse. The values in group d6m increased ($p<0.001$) when compared to c6m and r6m, COX2 r6m did not reverse and its expression increased with time deficiency ($p<0.01$). TNFα is correlative with NFκb, (r:0.90, P< 0.0001), also COX2 and TNFα are correlative (r:0.36, P<0,002). VAD does not generate oxidative stress but increased mediators of inflammation.

CB-P26
IN VIVO GPAT2 KNOCK-DOWN ACTIVATES APOPTOTIS

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Glycerol-3-phosphate acyltransferase 2 (GPAT2) is mainly expressed in spermatic cells, differing from the other GPAT family members (GPAT1, 3, and 4) which are abundant in lipogenic tissues. We have recently determined that mouse Gpat2 mRNA is transiently expressed in pachytene spermatocytes. To further understand the role of GPAT2 in spermatogenesis, we performed an in

vivo lentivirus-mediated Gpat2 silencing in mouse testis. Histological analysis showed both a strong meiotic arrest at pachytene stage, a severe decrease in the number of mature sperm cells and depletion in germ cell number. In order to determine if this decrease in cell number is a consequence of increased apoptosis, we performed a qPCR-based array to measure the expression of apoptotic-related genes. Expression analysis revealed significant changes in the group of Gpat2 knockdown (KD) mice since 12 apoptosis-related genes (Atf5, Birc3, Bok, Casp4, Cflar, Dapk1, Fas, Ltbr, Prdx2, Tnfrsf1a, Cd40, Tnfsf12) were significantly upregulated in Gpat2-silencedgerm cells, whereas only 2 genes were downregulated ($p<0{,}001$, Chi-Sq test). Thus, the decrease in cell number observed in Gpat2-silenced seminiferous tubules could be the consequence of the activation of apoptotic pathways. In conclusion, our results indicate that Gpat2 expression in germ cells is necessary for both spermatogenic maturation and germ cell survival.

CB-P27
CELLULAR CHANGES ASSOCIATED WITH R-CRT PRO-APOPTOTIC ACTION INDUCED BY BORTEZOMIB IN GLIOMA CELLS

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A variable efficiency has been reported for the Bortezomib treatment (BT) of different types of cancer. After BT of different human glioma cells, we determined that MO59K cells are resistant to this drug showing increased stress granules (SGs) formation, a phenotype related with drug resistance. These cells showed a modest increase of arginylated calreticulin (R-CRT) that appears confined to SGs. Conversely, the highest susceptibility to BT was shown by HOG cells, which after BT significantly increased R-CRT levels, a modified protein that get enriched at the plasma membrane where it participates of pro-apoptotic signaling. To understand these differences, other parameters were analyzed after BT. Firstly, by Fluo3-AM fluorescence activation, we determined that HOG cells exposed to BT showed a robust mobilization of intracellular calcium that is not shown by MO59K cells. Secondly, by Western blot of different ER stress markers we observed that HOG cells exhibit a stronger ER stress activation than MO59K cells after BT. Moreover, we determined that the susceptibility of MO59K to BT get increased when a cytosolic R-CRT-GFP chimera was overexpressed in these cells. Thus, an efficient BT involve: induction of ER stress associated to intracellular calcium mobilization, concomitantly with increased re-localization of R-CRT at the cell membrane, which induce caspase-3 dependent cell death.

CB-P28
PERIVITELLIN SYNTHESIS ADAPTS TO REPRODUCTIVE ACTIVITY IN THE SNAIL *Pomacea canaliculata*

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The invasive snail *Pomacea canaliculata*, is a serious crop pest and a host of a parasite that causes human meningoencephalitis. Its high fecundity is an important component of its invasive capacity. The albumen gland (AG) is a key reproductive organ that supplies eggs with nutrients and defensive compounds. Among them, the two most abundant egg perivitellins (80% of total protein), PcOvo and PcPV2, are exclusively synthesized and stored in the AG.Our objective was to evaluate the AG perivitellin expression along the reproductive period. To that aim, PcOvo and PcPV2 expression was analyzed in virgin and mated females, after the first oviposition and after a short and a long period of serial oviposition events. Gene expression was determined by qPCR and protein levels were quantified by ELISA. Perivitellin levels and their expression rates were affected by oviposition. After the short oviposition period levels of stored PcOvo and PcPV2 decreased to 46% and 37% respectively, while a compensatory gene overexpression occurred increasing 2.2 and 3.4 times, respectively. After a long oviposition period, perivitellin level further decrease by 79%. The high synthesis of the gland was reflected in females depositing a total egg dry biomass that was nearly twice the AG biomass of virgin females. Our results indicate a remarkable biochemical adaptation explaining the high oviposition output.

CB-P29
VALIDATION OF REFERENCE GENES FOR REPRODUCTIVE STUDIES IN THE INVASIVE SNAIL *Pomacea canaliculata*

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The South American freshwater gastropod *Pomacea canaliculata* is a highly invasive species. It is a serious crop pest, being responsible for great economical losses and ecological damages. It is also a vector of a nematode that causes human meningoencephalitis. Its reproduction, among other aspects, has been extensively studied, but very little research has been conducted regarding gene expression. We performed the validation of reference genes, an essential pre-requisite for accurate qPCR studies that has not been determined for this species before, neither in any other freshwater gastropod. We selected the female albumen gland which plays a central role in reproduction and evaluated the expression stability of 7 candidate reference genes: EF1-a, RPL7, His H3.3, TUBB, 18S RNA, ACTB, and GAPDH. Stability was analyzed under different reproductive activity conditions employing three different approaches: geNorm, NormFinder, and the comparative ΔCt method. NormFinder analysis selects GAPDH and ACTB pair as the best option to be used for normalization, whereas geNorm and the comparative ΔCt method indicate RPL7, GAPDH, and 18S RNA as the most stable ones under the studied conditions. These results will be helpful to continue developing reproductive studies and particularly to perform future evaluation of the factors that may affect fecundity on this conspicuous invasive species by qPCR.

CB-P30
ROLE OF CHROMATIN STRUCTURE ON SMN2 E7 ALTERNATIVE SPLICING

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Spinal muscular atrophy (SMA) is the main genetic disorder responsible for child mortality. SMA is caused by mutations on the SMN1 (survival motor neuron 1) gene causing the loss of function of this protein itself. Humans have a paralog of this gene, named SMN2. Due to a variation in a single nucleotide at the junction of intron 6 to exon 7 (E7), the latter exon is mostly excluded from the messenger RNA being included in just 10% of SMN2 transcripts that therefore code for a fully functional protein. The remaining 90% of SMN2 transcripts result in a truncated protein. At present, one of the most promising therapies for SMA is to restore normal levels of SMN expression by the use of antisense oligonucleotides (ASOs) designed to increase the inclusion of E7 in the SMN2 transcript. One aspect of the ASO therapies assayed is the perdurability of the effect. One possible explanation to it is that ASOs induce changes in chromatin structure, which in turn affect transcriptional elongation and modulate alternative splicing decisions. We are studying if modulating chromatin structure as well as transcriptional elongation have an effect on SMN2 E7 inclusion. The results presented here show that both histone acetylation and a slow elongating RNA polymerase II provoke an increase in exon 7 inclusion, supporting the idea that E7 inclusion can be regulated at the chromatin level.

CB-P31
4-HYDROXY-3-(3-METHYL-2-BUTENYL)-ACETOPHENONE (4-HMBA) INHIBITS PROLIFERATION OF MELANOMA B16F0 CELLS

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4-HMBA is the main secondary metabolite from Senecio nutans, commonly known as chachacoma, a medicinal plant widely used in Andean traditional medicine; this compound exhibits antifungal and tripanocidal activities.We analyzed in vitro the effect of 4-HMBA on proliferation of mouse melanoma cells. B16F0 cells were cultured in presence of ethanol (vehicle) or 5.0-17.5 µg/mL of 4-HMBA dissolved in ethanol. The growing index (GI) ± SE in 3 independent experiments, were assayed from 0 to 72 h. At 72 h of culture, GI of vehicle treatment, was 6.3 ± 0.7 and of 4-HMBA treatments were: 4.9 ± 0.7 (A); 3.2 ± 0.8 (B) and 1.0 ± 0.3 (C) for 7.5; 15 and 17.5 µg/mL respectively. Both (B) and (C) GI were $p \leq 0.001$ vs. vehicle (0 µg/mL 4-HMBA). These results show that 4-HMBA generated a significant inhibition of proliferation in B16 F0 cell in concentration below to 20 µg/mL. Experiments are running out in our lab to evaluate the effect of this compound in B16FO cell experimental melanoma in mice.

CB-P32
FLAVIVIRUS: TOWARDS THE DESIGN OF A MOLECULAR PLATFORM FOR ANTIVIRAL ASSAYS

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Saint Louis Encephalitis Virus (SLEV) is a human pathogen that belongs to the Flavivirus genus of the Flaviviridae family. Flaviviral genome comprises a single molecule of ~11kb plus ssRNA encoding three structural proteins (C, E and prM) and five non-structural (NS 1-5). SLEV's RNA-dependent RNA-polymerase, NS5, has not been described yet.Regardless of its growing sanitary significance, there isn't any kind of broad range antiviral against Flavivirus. Therefore, this work focuses on the initial steps in the development of an in vitro platform for antiviral assays: we'll study thoroughly the potential of NS5 as a therapeutic target. Recombinant expression of full length NS5, structural (E) and catalytic (C) polymerase domain and molecular docking for putative antiviral election will be the first steps. These tools will allow us to design a non radioactive in-vitro assay, complementing the traditional assays for antiviral activities. Lastly, in our laboratory is being developed a reverse genetic systems that constitute a fundamental tool for the complementary study of different aspects of NS5.

CB-P33
NS1: DIFFERENT APPROACHES TO IMMUNE RECOGNITION AND FLAVIVIRUS DIAGNOSIS

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The actual methodology of Flavivirus diagnosis is centered almost exclusively in the detection of early antigens or the determination of the immune response (IgM or IgG) in patients' sera. However, different virus with similar antigenicity, such as the Saint Louis Encephalitis virus (SLEV) and the West Nile virus (WNV), are not quite distinguishable using these techniques. In cases were the immune methods give positive results, a different and more specific technique needs to be performed to confirm the viral agent. Regarding to this situation, a new and easy flaviviral diagnosis method needs to be established.The main aim of this work is to develop the tools capable of identify different flaviviral infections to be used in serological assays. To do this, we chose the NS1 protein of SLEV and WNV and generated two recombinant NS1 versions, one using the whole sequence and another one using only the more antigenic regions (epitopes). Alternatively, we fused the entire sequence of NS1SLEV to a system based on the Z protein of Junín virus. The use of this system allowed us to express enveloped virus-like particles (VLPs) with NS1SLEV attached to its plasma membrane as a surface antigen. These VLPs will be used to inoculate mice and evaluate the humoral response anti-NS1SLEV. Finally, the mice sera and the different NS1 antigens will be analyzed in several enzymoimmunassays (EIAs).

CB-P34
MOLECULAR TOOLS DEVELOPMENT FOR ST. LOUIS ENCEPHALITIS VIRUS PATHOGENESIS STUDY

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St. Louis encephalitis virus (SLEV) belongs, like other human pathogens as Dengue Virus, Zika Virus and West Nile Virus, to the Flaviviridae family of the Flavivirus genus. These viruses share a natural cycle including arthropods (as vectors) and the human being. They possess an 11 kb plus ssRNA genome, capped and flanked by two untranslated terminal repeats, known as 3'UTR and 5'UTR. The translational product is a polyprotein that, once synthesized, is processed by cellular and viral proteins to form the structural (C, E and prM) and Non Structural (NS 1 to 5) proteins. Reverse genetic systems constitute a fundamental tool for the study of these viruses, and being SLEV's replication and transcription mechanisms so poorly characterized, we've begun the construction of a non-infectious clone of SLEV, aiming to broaden the knowledge of its nature. Furthermore, the details of SLEV RNA-dependent RNA polymerase interactomics, NS5, has not been described yet: recombinant expression of a set of variants for an epidemic strain (CbaAr-4005) NS5 will be used to produce a battery of in-vitro tools for the study of its role in viral infection. The integrated and global study of the molecular biology of SLEV will enable the projection of the design, generation and optimization of several assays directly correlated to antiviral therapeutics and pathogenesis comprehension.

CB-P35
GOLGI BODIES IN THE GOLGI-LACKING PARASITE *Giardia lamblia*

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Giardia is a common cause of diarrheal disease. Trophozoites undergo crucial changes to survive outside the intestine by differentiating into infective cysts. Encystation entails the synthesis, transport, secretion, and assembly of cyst wall materials into a protective cyst wall. In our lab, we study the molecular events leading to cyst formation, with emphasis on vesicular transport in this parasite reported to lack a Golgi apparatus. In this work, we identified, characterized, and determined the suCBellular localization of all SNAREs, ARFs, RABs, components of coatomers and many putative endoplasmic reticulum and Golgi molecules encoded in the parasite genome. Our results show that ESVs mold from the ER and that particular components of the secretory pathway localize in novel vesicles with characteristics of Golgi bodies, similar to those found in yeast. Our findings contradict the hypothesis that ESVs are Golgi-like structures and suggest that Giardia requires a small number of SNAREs, ARFs and RABs to accomplish all secretory events that are critical for growth and differentiation. We hypothesize that typical Golgi stack present in more evolved cells is missing in Giardia due to the absence of core glycosylation enzymes while the packing and sorting function of this organelle occurs in these novel Golgi bodies distributed throughout the cytoplasm of this parasitic protist.

CB-P36
DEVELOPMENT OF AN ORAL VACCINE AGAINST TUBERCULOSIS BASED ON VIRUS-LIKE PARTICLES

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An effective vaccine is urgently needed to fight tuberculosis (TB), the leading cause of death from a single infectious agent worldwide. CBG is the only currently available TB vaccine but it is virtually not effective against adult pulmonary TB. Clinical trials suggest that other systemic vaccines have little advantage over CBG and that mucosal vaccines seem to provide better protection. The goal of our work is to produce an oral vaccine for TB by means of the generation of virus-like particles (VLPs) pseudotyped with Variant-specific Surface Proteins (VSPs) from Giardia lamblia and containing Mycobacterium tuberculosis (Mtb) specific antigens. VLPs are highly immunogenic and also safe and non-infectious. VSPs not only are resistant to proteases and low pH but also show adjuvant activity. We were able to express in HEK cells, expressing or not in their surface the extracellular domain of Giardia VSP1267, the Mo-MLV capsid protein GAG fused to ESAT-6, CFP-10 and Ag85B Mtb antigens. These proteins self-assembled into VLPs with or without VSPs on their surface, and were efficiently purified from the cell supernatant. Initial analysis of oral immunization of mice showed that the VLPs are capable of generating humoral and cellular immune responses against Mtb recombinant antigens. Further studies will provide new insights regarding the efficacy of this formulation to avoid Mtb infections.

CB-P37
IMMUNOGENIC PROPERTIES OF THE EXTRACELLULAR DOMAIN OF VARIANT SURFACE PROTEINS OF *Giardia lamblia*

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The human parasite *G. lamblia* is covered by a tight coat comprised Variant-specific Surface Proteins (VSPs). Previous results showed that oral immunization with purified native VSPs, without adjuvants, elicited a protective immune response against challenges with the parasite in gerbils, dogs and cats. To further study the immunogenic properties of VSPs, the extracellular, cysteine-rich domain of three different VSPs was produced in an insect cell expression system, which yields proteins with correct disulfide bonds, in serum free medium, in absence of pyrogen and with high purity. To determine if these ΔVSPs are able to activate receptors of the immune system we used a reporter cell system. When a broad panel of human TLR genes was used for screening, we found that ΔVSP1267 was able to signal though TLR2 and TLR4. Subsequent studies with mouse receptors demonstrated that this activation was dose-

dependent and that it was stronger in mTLR4 than in mTLR2. Similar results were obtained with ΔVSPH7 and ΔVSP9B10. In vitro, these proteins showed activation of macrophages and dendritic cells, inducing up regulation of co-stimulatory molecules. These results indicate that the immunogenic properties of *Giardia* VSPs are related to their ability to activate the innate immune system. Since these molecules have also protective activity, VSPs can be used in the formulation of oral vaccines

CB-P38
VARIANT-SPECIFIC SURFACE PROTEINS AS MEDIATORS OF ANTIGENIC VARIATION IN *Giardia lamblia*

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Giardia lamblia colonizes the upper small intestine of humans and other vertebrates causing giardiasis. Like many pathogenic microorganisms, *Giardia* undergoes antigenic variation (AV), a process that contributes to the production of chronic and/or recurrent infections. The surface of Giardia trophozoites is covered by a dense coat of Variant-specific Surface Proteins (VSPs), which represents the interface between the parasite and the environment. Giardia possesses ~200 VSP genes, but only one VSP is expressed on its surface at a particular time. The stimulus that triggers VSP switching is unknown. VSP are integral membrane proteins comprising a variable extracellular domain and a highly conserved C-terminal region including transmembrane domain and a short cytoplasmic tail. In this work, we show that anti-VSP immunoglobulins (Igs) are not cytotoxic to *Giardia*, as previously reported, but they increase the rate of VSP switching. Besides, we prove that anti-VSP Igs increase the *Giardia* reduced state, which could be linked to the initiation of a still unclear intracellular signal triggered by the presence of the anti-VSP Igs. By using fragmented anti-VSP Igs and point mutations in the conserved C-terminal domain of VSPs, we demonstrate that AV in *Giardia* is a complex mechanism involving both external and cellular processes linked by the particular structure of the VSPs.

CB-P39
VLPS PSEUDOTYPED WITH VARIANT SURFACE PROTEINS OF GIARDIA AS AN EFFECTIVE ORAL INFLUENZA VACCINE

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Oral vaccines possess attractive advantages over parenteral vaccines. However, there are certain drawbacks inherent to this form of immunization because the vaccinal antigen has to resist the passage through the upper gastrointestinal tract. Since *Giardia* Variant-specific Surface Proteins (VSPs) protect this parasite from the harsh environmental conditions of the intestine and have a remarkable intrinsic immunogenicity, we hypothesized that efficient oral vaccines can be generated by combining the benefits of *Giardia* VSPs as protective agents with the advantages of particulate immunogens like Virus-like Particles (VLPs). Here, we generated an Influenza A H5N1 VLPs pseudotyped with the extracellular domain of Giardia VSP1267 and orally immunized mice. When comparing to control VLPs without VSP1267, we found that the vaccines were able to induce the production of HA-specific antibodies in serum, as well as in mucosal fluids such as bronchoalveolar lavage and feces. When the different subtypes of IgG were analyzed in serum, it was found that both IgG1 as IgG2 showed a significant increase. In addition, a high number of IFNγ secreting HA-specific cells were present in spleen and mesenteric lymph nodes. Protection assays with live Influenza virus confirmed the efficiency of this novel oral vaccine, paving the way for the design of oral vaccines to other human pathogens.

CB-P40
ROLE OF MVBS FORMATION DURING ANTIGEN CROSS-PRESENTATION BY DENDRITIC CELLS

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Dendritic cells (DCs) are key players of the immune system by presenting exogenous antigens in association with MHC-I molecules to induce cytotoxic immune responses against tumors and several pathogens in a process known as cross-presentation. The main goal of our study is to investigate the potential role of multivesicular bodies (MVBs) formation during antigen cross-presentation. To accomplish this, we focus our work on the ESCRT machinery, the best characterized proteins that generate intraluminal vesicles in the endocytic network. Here we show that antigen cross presentation is strongly impaired when DCs were treated with the inhibitor of MVBs formation U18666A. By performing immunofluorescence staining and confocal microscopy, we observed a clear recruitment of the MVBs tetrapanin CD63 and the ESCRT protein CHMP4 to the parasitophorous vacuole (PV) of *Toxoplasma gondii* after DC infection. Moreover, in the presence of the inhibitor U18666A the recruitment of CD63 to the PV was reduced. Altogether, these results suggest for the first time that MBVs are efficiently recruited to the *T. gondii*-containing vacuole and that MVBs formation is essential for efficient antigen cross-presentation. Further studies will allow us to understand with better detail the complex interactions that this parasite establishes with the MBVs and the role of the ESCRT machinery in this process.

CB-P41
CHARACTERIZATION OF VAMP ISOFORMS INVOLVED IN CORTICAL GRANULE EXOCYTOSIS IN MOUSE OOCYTE

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Cortical granule exocytosis (CGE) is a secretory process triggered by sperm-oocyte fusion during fertilization. This exocytosis involves the membrane fusion of cortical granule, localized in the oocyte cortex, and oolema. This fusion is regulated by intracellular calcium and is thought to be mediated by the SNARE complex, which is formed by SNAP-25, Syntaxin and VAMP. Syntaxin4 and SNAP-25 have been identified in mouse egg; however it is unknown if VAMP (vesicle-associated membrane protein) participates in CGE. The aim of this work was to identify and characterize the main VAMP isoforms, VAMP1 and VAMP2, involved in exocytosis in other secretory models. RT-PCR assays showed that VAMP1 and VAMP2 are expressed in mouse oocytes. Immunofluorescence analysis showed that VAMP1 had a cortical localization, while VAMP2 had mainly a cytoplasmic distribution. This result suggested that VAMP1 might be involved in CGE. To confirm this hypothesis we performed a functional assay perturbing the function of endogenous VAMP1 and VAMP2 by antibody microinjection. Only VAMP1 antibody was able to inhibit CGE when mouse oocytes were activated parthenogenetically with strontium chloride. These results confirm that VAMP1 is the VAMP isoform involved in CGE. The importance of this work is that we have completed the characterization of SNARE complex during membrane fusion in the CGE of mouse oocytes.

CB-P42
ASSESSMENT OF KRÜPEL-LIKE FACTOR 6 FUNCTION IN HUMAN EXTRAVILLOUS TROPHOBLAST CELLS

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Trophoblast cell differentiation into migratory extravillous trophoblasts (EVT) is critical for human placentation and embryonic development. KLF6 is a zinc-finger transcription factor that has a cellular and signal dependent tumor suppressor activity. klf6 knockout mice die in-utero with a phenotype including impaired placental development, however its role in placenta is partially known. We have demonstrated that KLF6 is required for proper villous trophoblast differentiation and its expression is regulated by hypoxia. In order to evaluate whether it modulates EVT migration we performed wound-healing and western blot analysis in KLF6 silenced HTR8/SVneo cells (human first trimester trophoblasts with an EVT phenotype). Confluent cell monolayers, transfected with a KLF6-specific siRNA (siK) or control siRNA, were "scratched" and cell migration and protein expression were assessed after 4 and 24h. Cell migration tends to be higher in siK cells compared to control, although difference was statistically non-significant. Protein expression levels of β-catenin, connexin-43, α5 and β1 integrin subunits remained essentially unmodified. Present results suggest that downregulation of KLF6 does not alter the EVT differentiation markers analyzed. Further studies are required to determine the possible role of KLF6 in EVT migration

CB-P43
FUNCTIONAL CROSS-TALK BETWEEN THE GLUCOCORTICOID AND PROGESTIN RECEPTORS

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The glucocorticoid and progestin receptors (GR and PR respectively) share many similar structural and functional characteristics. In the mammary gland, the activation of PR is associated with cell proliferation and tumor progression while activated GR is known to favor cell differentiation. The objective of this work was to evaluate whether the activated GR affects the progestin-dependent proliferation of tumoral mammary epithelial cells expressing both receptors. To assess whether PR and GR have the potential to form complexes, a cross-correlation analysis of the intensity fluctuations was performed in living cells by confocal microscopy. In the absence of hormone, a brightness cross-correlation (CBc) distribution centered on zero between eGFPPR and mCherryGR particles was observed, indicating an absence of interaction. However, in the presence of R5020 or Dex mCherryGR and eGFPPR showed a positive CBc value, suggesting that hormonal treatment induces PR/GR complex formation. Moreover, fluorescence correlation spectroscopy (FCS) studies revealed that not only GR activation but also its overexpression decrease the population and the residence time of PR binding to nuclear fixed sites, suggesting that GR would impair PR interactions with its targets. These results correlate with interference of GR on the PR-dependent expression of certain genes involved in cell proliferation

CB-P44
N-TERMINAL DOMAIN OF C-FOS AS A NEGATIVE DOMINANT FOR BRAIN CANCER THERAPY

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Glioblastoma multiforme, a WHO class IV tumor, is the most aggressive central nervous system (CNS) cancer. After diagnosis, the average survival period for these patients is ~1 year with no significant improvements in this survival period observed in the last decades. The development of new therapeutic strategies and the identification of new targets is the principal aim of the field. We have reported a new activity for c-Fos. In addition to its AP1 transcription function it activates phospholipid synthesis in the cytoplasm associated to the endoplasmic reticulum. Since c-Fos is markedly overexpressed in tumors contrasting with lack of detectable expression observed in normal CNS, we propose this cytoplasmic activity of c-Fos as new target for glioblastoma treatment. The aim of the present work is to test N-terminal deletion mutants of c-Fos as possible negative dominants of the lipid activation capacity of c-

Fos. Through transfection and profection methods we identified negative dominants whose overexpression inhibits proliferation of T98G cells (a glioblastoma multiforme cell line) by interfering with the physical association of c-Fos to phosphatidylinositol 4 kinase II α. At present we are testing these negative dominant in an in vivo model of CNS tumors

CB-P45
LIPOSOMAL VEHICULIZATION OF ZN PHTHALOCYANINES AND AMINE DERIVATES IN PDT INACTIVATION OF T98G CELLS

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Photodynamic therapy (PDT) is considered a promising strategy for cancer treatment. Phthalocyanines (Pcs) are good photosensitizers (PS) for PDT because they induce cell death in several cellular models. The lipophilic nature of many PS may be an important factor affecting their preferential accumulation in tissues. However, due to their hydrophobicity, intravenous treatment is greatly hampered. Liposomal preparations are currently used as an effective delivery system in experimental studies and clinical trials. Several reports have shown the advantages of liposomal preparations over other formulations. The PS incorporation into liposomes assures their maintenance in a monomeric state. The aim of this work was to evaluate the properties of two Pcs (ZnPc and TAZnPc) vehiculized into DPPC-cholesterol liposomes for PDT on glioblastoma cells. All the Pcs were innocuous in absence of light at concentrations ≤ 0,5 μM. However, after irradiation, both formulations induce cell death in a concentration and light dose dependent manner. The average size of the resulting liposomes was 112.5 ± 1.35 nm for ZnPc and 228.1 ± 12.3 nm for TAZnPc. Both Pcs show an increase in their photosensitizing activity when they were administrated in DPPC-cholesterol liposomes rather than in DMF. Moreover, we observed a relation between liposomal size and cellular death induction.

CB-P46
CYTOPLASMIC FRA1 AND CFOS AS POTENTIAL TARGETS FOR BREAST CANCER THERAPY

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Breast cancer is the most common type of cancer and the leading cause of cancer death in women worldwide. In less developed countries, most cases are diagnosed at late stages, so development of new therapies to eliminate established tumors is essential. Phospholipids (pl) are the quantitatively most important components of cell membranes and tumor cells require high rates of pl biosynthesis to support membrane biogenesis for their exacerbated growth. Both Fra-1 and c-Fos are overexpressed in breast tumors contrasting with their undetectable levels in the normal tissue and promote pl synthesis by activating the rate limiting enzyme CDP-DAG synthase (CDS) through a physical association with this enzyme. Using in vitro enzymatic reactions we demonstrate that the basic domain of both Fra1 and cFos are involved in the activation of CDS whereas the N-terminal domain of Fra1/cFos physically associates with CDS as evidenced by FRET. We also examined if both N-terminal deletion mutants act as negative dominants to inhibit breast tumor growth by interacting with the enzyme and blocking its activation by their corresponding full length versions. Proliferation of MDA-MB231 cells in culture and in vivo breast tumor growth is significantly inhibited in the presence of either/both deletion mutants in comparison to the respective controls treated with vehicle alone.

CB-P47
POSSIBLE TREATMENTS AGAINST *Trypanosoma cruzi* THROUGH THE COMBINED USE OF TRYPANOCIDAL DRUGS.

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Chagas is an American endemic disease caused by the protozoan *Trypanosoma cruzi*, whose current therapy involves ineffective and unspecific treatments that cause serious side effects. Benznidazole (BNZ) and Nifurtimox are still the only drugs recommended up to date. These drugs require prolonged treatment, have limited efficacy in the chronic phase and against different T. cruzi strains. There is an urgent necessity to discover new therapeutic targets and new anti-chagasic drugs. In this work, we studied the effect of Difluoro-Methyl-ornithine (DFMO or eflornithine), an ornithine decarboxylase inhibitor that decrease the polyamine levels in *Trypanosoma brucei*, the etiologic agent of sleeping sickness and in mammalian cells. While DFMO has not significant effects on *T. cruzi* (TC II) alone, we detected significant trypanocidal action in the BNZ and DFMO combined therapy at concentrations of 100 μM and 5 mM respectively. In contrast, in the same assays, BNZ displayed trypanostatic activity. Furthermore, a reduced number of intracellular amastigotes was observed in the samples treated with both drugs. This result opens the way to reconsider the current dosage of BNZ treatment for Chagas disease.

CB-P48
THE INVOLVEMENT OF SPLICING FACTORS IN THE SUMO CONJUGATION PATHWAY

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SRSF1 belongs to the SR family of proteins, widely characterized as regulators of the splicing process. These proteins share a conserved molecular structure comprising one or two RNA recognition motifs (RRMs) and a serine-arginine rich domain. Some members of this family, and in particular SRSF1, are involved in a wide variety of regulatory functions at different levels of gene expression, being considered multifaceted proteins. Our work is focused on characterizing the E3 SUMO ligase-like activity of SRSF1 that was previously described by our laboratory. In addition to its interaction with enzymes of the SUMO machinery, we identified

different substrates whose SUMOylation is regulated by SRSF1, including proteins involved in different aspects of RNA metabolism and in the cellular response to heat shock. By comparing the SUMOylation-enhancing activity of various members of the SR family, as well as analyzing a variety of deletion mutants, our laboratory postulated the involvement of SRSF1 RMM2 in the E3 ligase-like activity of this protein. By generating a battery of SRSF1 mutants, we have identified a single residue required for the SUMOylation-enhacing activity of this protein and we are currently dissecting the molecular mechanisms underlying this new role of SRSF1 and its regulation, as well as the suCBellular localization where it takes place and its cellular consequences.

CB-P49
SECRETION PROFILE OF THE TCTASV-C PROTEINS IN DIFFERENT *Trypanosoma cruzi* STRAINS

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TcTASV is a *T.cruzi* -specific protein family preferentially expressed in trypomastigotes, the extracellular bloodstream stage of the parasite. In particular, the TcTASV-C subfamily is located at the parasite surface. Mice vaccinated with TcTASV-C are partially protected after a *T. cruzi* lethal challenge. In trypomastigotes of the reference strain (CL Brener; TcVI hybrid lineage) TcTASV-C is secreted into small extracellular vesicles (EVs). Other researchers showed that EVs from different *T. cruzi* strains trigger differential immune responses in the host. Considering that TcTASV-C could be a virulence factor, we here analyze the secretion profile of TcTASV-C in different strains, by ultracentrifugation and western blot. We found that in the highly virulent RA strain (TcVI) TcTASV-C is secreted in similar proportions in small and large EVs. On the other hand, in the low-virulent Sylvio strain (TcI) TcTASV-C is secreted both in the large EVs and the vesicle-free fractions. We also evaluated the reactivity of sera from *T. cruzi* infected hosts against EVs proteins. None of the three species assayed (human, rabbit and mouse) reacted against vesicles, suggesting that secretion of *T. cruzi* antigens into EVs could be a mechanism of immune evasion. In contrast, TcTASV-C vaccinated mice that survived the *T. cruzi* challenge were able to efficiently recognize EV antigens.

CB-P50
FORMING EXOSOMES WITHOUT ALL THE FOUNDING PLAYERS

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Exosomes are vesicles released following multivesicular bodies (MVBs) fusion with the plasma membrane and have been implicated in a broad range of functions. MVBs formation is driven by the endosomal sorting complex required for transport (ESCRT) machinery. Five ESCRT complexes (0, I, II, III and Vps4) are sequentially recruited from the cytoplasm to the surface of the endosomal membrane. Although many of these components are present across all eukaryotic lineages, only a subset of them is present in Giardia lamblia. Using stable transfected parasites expressing HA-tagged proteins, we localized components of the ESCRT II and Vps4 complexes in the cytoplasm and nuclear membrane in trophozoites. Analysis of the interaction between ESCRT components using YTH assays highlighted the existence of intra and extracomplex interactions, suggesting that a conserved form of ESCRT assembly occurs in this parasite. Despite the absence of some ESCRT players and MVBs, Giardia seems to be able to form and secrete exosomes. Microvesicles, isolated from trophozoites grown in serum free medium, were enriched in known exosome markers, such as gActin, g14-3-3 protein and a gSNARE protein. Further analysis interfering with the ESCRT machineries will disclose whether exosome formation in Giardia is an ESCRT-dependent or independent pathway.

CB-P51
CHARACTERIZATION OF THE ROLE OF *Saccharomyces cerevisiae* EISOSOMAL MEMBRANE DOMAINS IN AGING

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Eisosomes are large protein complexes that delimit nanoscale plasma membrane invaginations in *S. cerevisiae*. They are reservoirs of more than 25 proteins including transporters, signaling molecules and proteins of unknown function. Some of these proteins have been reported to be involved in the cellular aging process, considered the greatest risk factor for most age-associated diseases. We are interested in understanding eisosomes´ role in aging using *S. cerevisiae*, an experimental model that has contributed to the identification of many mammalian genes that affect this process. Eisosomes segregate asymmetrically to the older cell and preliminary results show that accumulate with age. Performing yeast aging assays with knockout strains we found that when organization of these domains is lost longevity is significantly enhanced. Mutant strains in genes encoding eisosomal structural proteins where analyzed in different *S. cerevisiae* genetic backgrounds and growing conditions demonstrating that reducing eisosomes´ presence leads to lifespan extension. Finally, we analyzed if conditions that increase the levels of eisosomes in plasma membrane decreases lifespan. Our results strongly suggest that eisosomes play a key role in the aging process and that their proper structuration is essential for fulfilling it.

CB-P52
HUMAN ERYTHROCYTES AS EARLY TARGETS OF THALLIUM TOXICITY

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Thallium (Tl) is a toxic heavy metal that promotes apoptosis in cultured cells, without causing necrosis. Since the highest concentrations of Tl in intoxicated patients were found in blood, where erythrocytes (RCB) constitute the most abundant cell population, we hypothesize that these cells may be early targets of Tl toxicity. In this study we investigated the effects of Tl(I) or Tl(III) exposure (5-180 min) on RCB morphology, integrity, membrane fluidity, and the activation state of cell signals involved in the promotion of eryptosis (RCB apoptosis). No alterations in RCB morphology were observed. Tl(III) promoted RCB osmotic fragility and hemoglobin release, reaching a maximum after 120 min of incubation. In intact cells, Tl(III) decreased the fluidity of the exofacial side and hydrophobic core of RCB membrane, without affecting the fluidity of the cytoplasmic side of the membrane. In addition, Tl(III) promoted the activation of calcium-dependent cytosolic PLA2 (cPLA2), an effect that reached a maximum after 15 min of incubation, and that returned to baseline values at prolonged times. Together, obtained results suggest that Tl(III) –but not Tl(I)– interact with the outer side of RCB membrane, altering their biophysical properties and promoting the activation of signals that lead to hemolysis and/or to the promotion of eryptosis.

CB-P53
TL(I) AND TL(III) AFFECT DIFFERENTIALLY PC12 CELL DIFFERENTIATION

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Thallium (Tl) is a toxic metal that promotes apoptosis in PC12 cells at high micromolar concentrations. In this work we evaluated the effects of sustained administration of a non-toxic concentration of Tl(I) or Tl(III) on PC12 cell differentiation. PC12 cells were incubated for 4 days in the presence of 20 ng/ml NGF with or without 25 μM Tl(I) or Tl(III). Preliminary results show that Tl(I) did not affect the kinetics of cell differentiation. In contrast, Tl(III) accelerated cell differentiation until 72 h, reaching control values at 96 h. Accordingly, neurite elongation was also accelerated in Tl(III)-treated samples respect to controls. Since NGF induces neuronal nitric oxide synthase (nNOS) expression with the subsequent generation of NO that stops cell proliferation and contributes to neurite formation, NO generation was evaluated from nitrite content in the media. Control and Tl(I)-treated cells increased progressively nitrite content to a similar extent. In contrast, Tl(III) induced maximal nitrite release between 24 and 48 h of treatment. This finding may be related to the acceleration of cell differentiation in Tl(III)-treated samples and further experiments are required to elucidate if sub-lethal concentrations of Tl(III) may have a beneficial effect on neuronal differentiation.

CB-P54
IS THE METABOLISM OF EXTRACELLULAR ATP INVOLVED IN THALLIUM-MEDIATED CITOTOXICITY?

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We demonstrated previously that Tl(III) -but no Tl(I)- promotes rapid release of ATP (eATP) from adherent PC12 cells via pannexin channels and exocytosis. Tl(III) also inhibits irreversibly the activity of ectoATPases that act in the micromolar range of eATP concentration. In this work we investigated the effects of Tl on cell viability and if those effects could be related to altered eATP metabolism. Cell incubation in the presence of 100 μM Tl(I) or Tl(III) for 1-72 h caused time-dependent decrease of cell viability evaluated from MTT reduction. This effect was accompanied by decreased amounts of adhered cells, with no viable cells detected in the culture media. To discriminate the causes of Tl-mediated cell death, samples were analyzed by flow cytometry to evaluate the content of hypodiploid cells, and the supernatants were tested for lactate dehydrogenase activity to assess necrosis. While necrosis was negligible, the amount of hypodiploid cells was significantly increased after 72 h of Tl(III) exposure. To investigate if alterations in eATP metabolism may be involved in the early steps of cell response to Tl(III), eATP was removed by adding apyrase to the culture media. The removal of eATP enhanced the noxious effect of Tl on cell viability, suggesting that eATP may play a protective role in Tl-mediated cell death

CB-P55
EARLY RESPONSE OF ANTIOXIDANT ENZYMES TO TL(I)- AND TL(III)-MEDIATED OXIDATIVE STRESS IN PC12 CELLS

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Thallium is a toxic metal that causes oxidative stress in PC12 cells. In this work we evaluated oxygen (ROS)- and nitrogen (RNS)-reactive species production after 6 h of cell exposure to Tl(I) or Tl(III), and how main antioxidant enzymes respond to eliminate them. Both cations increased ROS and RNS contents, returning to baseline values after 6 h of incubation. H2O2 generation via superoxide dismutase (CuZnSOD and MnSOD) was impaired by Tl as well as its degradation by catalase. Glutathione-dependent system was also affected by Tl: while glutathione peroxidase (GPx) expression and activity were increased by Tl(III), glutathione reductase (GR) expression was decreased by both cations. However, GR activity was mildly enhanced by Tl(III). Finally, thioredoxin-dependent system was evaluated. Peroxyredoxin expression was increased by Tl(I) although its activity was increased by both cations. Cytosolic thioredoxin reductase (TrxR1) expression was increased by Tl(I) and partially decreased by Tl(III), while the mitochondrial isoform

(TrxR2) was not affected. However, total TrxR activity was increased by both cations. Obtained results suggest that glutathione- and thioredoxin-dependent antioxidant defense systems are responsible for ROS and RNS detoxification generated upon PC12 cell exposure to Tl(I) or Tl(III).

CB-P56
THE TRANSLATION INHIBITOR 4EBP IS REQUIRED FOR ADAPTATION TO HYPOXIA IN *Drosophila*

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4EBP (eIF4E-binding protein) is an inhibitor of cap-dependent mRNA translation whose activity is regulated by intracellular pathways that respond to energy availability. The transcription factor HIF mediates adaptation to hypoxia through mechanisms that reduce metabolism and oxygen consumption. The aim of this work is to determine if 4EBP is required for fly adaptation to hypoxia in vivo. We found that 4EBP transcription is strongly induced in hypoxia in embryos, larvae and adult flies. Transcriptional induction depends on the transcription factors FOXO and HIF. *Drosophila* 4EBP mutants are viable and fertile in normoxia but fail to survive in hypoxia. In adult flies lipids are equally consumed in wild type or 4EBP mutant flies. While oogenesis is arrested at early stages in wild type flies, in 4EBP mutants it proceeds to post-vitelogenic stages, provoking an energy imbalance that ultimately leads to lethality. We conclude that 4EBP is a central regulator of energy balance essential for adaptation to hypoxia in vivo.

CB-P57
miRNA TURNOVER CONTROL BY LINEAR AND CIRCULAR RNA TARGETS

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MicroRNAs (miRNAs) regulate target mRNAs by silencing them. Reciprocally, however, target mRNAs can also modulate miRNA stability. We have shown that upon highly complementary binding, mRNAs trigger remarkably effective Target RNA-directed miRNA degradation (TDMD) in rodent primary neurons, greatly surpassing TDMD in non-neuronal cells and established cell lines. Although certain viruses employ TDMD to degrade host miRNAs, some of which have antiviral activities, a physiological function of TDMD remains to be identified. While TDMD may be relevant for miRNA regulation in the nervous system, the low natural complementarity between miRNAs and their most commonly described target RNAs questions whether normal endogenous targets physiologically trigger such regulation. Circular RNAs (circRNAs) have been recently shown to be particularly abundant in neurons and, although their function remains enigmatic, some of them seem to interfere with miRNA activity. Here we explore circRNAs that regulate miRNA stability. By manipulating expression of endogenous circRNAs using lentiviral transgenes and CRISPR/Cas9 approaches, we analyse potential circular RNA species that might regulate TDMD in primary neurons.

CB-P58
ANTITUMOR EFFECT OF A CU(II) COMPLEX WITH SACCHARINATE AND GLUTAMINE RELEASED FROM SILICA SPHERES

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Metal complexes show diverse pharmacological effects. In particular, Cu(II) exerts essential functions in bone and in inflammatory processes underlying different illnesses as cancer. Its complexation with organic ligands favors the use of lower doses. Previously, we reported the antitumor effects of a complex of Cu(II) with saccharinate and glutamine (Cu-sac-gln). This complex showed promissory effects since it exerted a stronger cytotoxic action in human osteosarcoma cells (MG-63) than in a normal phenotype murine osteoblasts. The aim of this study was to demonstrate the beneficial effect of controlled release of Cu-sac-gln from a drug delivery system (SiO_2 spheres). Monodisperse SiO_2 spheres (diameter about 1 μm) having micro and mesopores were synthesized according to a reported synthesis and characterized by SEM and N_2 adsorption. Their biocompatibility was demonstrated by the MTT assay in MG-63 cells from 10 to 1000 μg/mL range ($p<0.05$). Moreover, in that concentration range, reactive oxygen species did not show differences with control values ($p<0.05$). Cu-sac-gln released from the spheres was assessed spectrophotometrically. The results showed that the release of Cu(II) complex depends on the initial load and time. On the other hand, the Cu(II) complex released from the SiO_2 reduced osteosarcoma cell viability to 60% ($p<0.05$) improving the outcome obtained by direct exposure.

CB-P59
ANALYSIS OF NEW ORGANOMETALLIC COMPOUNDS AS POTENTIAL AGENTS AGAINST CHAGAS DISEASE

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Chagas' disease, caused by the parasite Trypanosoma cruzi, is an important sanitary problem in Latin America. New Pd, Pt and V compounds were synthesized as potential antichagasic agents showing low IC50 values with excellent SI values. In order to study their mode of action, fluorescent probes were used to determine the cell death induced mechanism. Since nor apoptosis or necrosis type mechanisms were observed by flow citometry at the incubation times and concentrations tested, specific cell viability and vitality markers were employed to prove that the compounds effectively affect cell metabolism and proliferation. Interestingly, increasing concentration and incubation times showed increased cellular metabolic activity, particularly esterase activity. Since the increased metabolic activity could be the result of different cell death mechanisms, parasites were morphologically analyzed. Microscopy images showed that cell morphology is altered in the presence of increasing amounts of the studied compounds. These parasites showed

rounded morphology, loss of flagellum and mobility when increasing compounds concentration. The analysis of global changes in transcriptome and proteome of parasites incubated with each complex to identify affected pathways to understand their mode of action is in progress.

CB-P60
BDNF EXPRESSION IN THE TESTES OF RESERPINE-TREATED RATS

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The neurotrophic factor BDNF affects testicular physiology, although very little is known regarding its action mechanism. Pevious work from our lab indicated that central (hippocampal) BDNF increases by reserpine treatment, a depletor of monoamines that induces behavioral depression. The aim of this study was to investigate the effects of reserpine on testicular BDNF. Fourteen male Wistar rats were treated with reserpine (1.0 mg/kg) or vehicle (0.0 mg/kg) every two days for 8 days. One week after treatment the animals were sacrificed and testes were dissected, weighed and fixed in Bouin solution. Paraffin blocks were then prepared for hematoxylin and eosin staining, followed by immunohistochemistry against BDNF. Testes weight was similar across groups and the morphometry revealed a trend (p=0.08) for bigger seminiferous tubules in reserpine-treated animals. The latter animals exhibited significantly less Leydig cells negative to BDNF (p=0.02). This effect might explain the increase in circulating androgens, which in turn could affect the diameter of the seminiferous tubules. Overall, the data suggest that reserpine may improve testicular functionality.

CB-P61
SYK: A SPECIFIC TARGET FOR CELLULAR IMMUNOTHERAPY OF RETINOBLASTOMA

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Retinoblastoma (RB) is an intraocular cancer that affects small children and presents a high mortality ratio. Cellular immunotherapy offers a promising alternative for the treatment of this type of cancer, due to the ability of immune cells to bypass the hemato-ocular barrier, and their long therapeutic half life. With this in mind, we aimed to develop a cellular immunotherapy for RB, taking into account the production of cytotoxic cells : a) in adequate amount b) with elevated cytoxotic activity c) with specific toxicity against RB. Dendritic cells (DC) and Cytotoxic T Lymphocytes (CTL) were differentiated in vitro from peripheral blood mononuclear cells. Differentiated DCs were transfected with lentivirus for the expression of SYK protein (Spleen Tyrosine Kinase) as a specific surface antigen. Flow cytometry, IF, videomicroscopy and molecular biology were used for the analysis of differentiation, cytotoxicity and specificity. Specific cytotoxicity was tested on the human RB cell line RB-Y79, while human retina cell line hTERT-RPE1 was used as control. Our results show that genetically modified DCs can induce cytotoxic activity on CTL and confer them specificity to RB-Y79. Taking into consideration the failure of traditional therapy against RB, we propose that SYK could be a potential therapeutic target for RB, and cell immunotherapy as an effective way to deal with it.

CB-P62
GALECTIN-3 DEFICIENCY DRIVES LUPUS-LIKE AUTOIMMUNE DISEASE BY PROMOTING SPONTANEOUS GERMINAL CENTERS FORMATION.

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Autoimmune diseases are a major health problem, affecting ~10% of the developed world's population. Since there is no cure for autoimmunity it is extremely important to study the mechanisms that trigger these diseases. One of the hallmarks in the antibody-mediated autoimmune diseases is the spontaneous generation of Germinal Centers (GC).We have recently identified Galectin-3 (Gal-3) as a critical regulator of GC formation and autoantibody production. Mice lacking Gal-3 spontaneously develop a lupus-like disease, characterized by increased numbers of GC B cells, Tfh cells, Antibody-Secreting-Cells, hyperglobulinemia, autoantibody production, and mononuclear infiltration in kidneys.

In the absence of Gal-3, B and T cells exhibitedan activating phenotype that appears to be comprised to GC reaction. In Gal-3 KO micewe observed increased frequencies of CD80, CD86, CXCR4, IL-21R and CD69 positive B cells and increased percentages of CD69 and CD44 positive CD4+T cells,comparably to WT counterparts. Furthermore, we found more proliferating B cells measured by Ki-67 expression in Gal-3 KO mice than in WT mice. Interestingly, Gal-3 KO mice produced excessive quantities of IFN-γ, a cytokine that has long been associated with lupus development. IFN-γ blockade reduced GC B cells and Tfh cell frequencies, and immunoglobulin class switching, demonstrating that IFN-γ overproduction was required to sustain lupus-like disease. These studies demonstrate that absence of Gal-3 profoundly alter the immune homeostasis and suggest that Gal-3/IFN-γ axis is a novel potential pathway for therapeutic strategies in autoimmune diseases.

CB-P63
TOWARDS NEW THERAPIES AGAINST CANCER: STUDYING PIN1 AS A THERAPEUTIC TARGET IN NEUROBLASTOMA

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The development of novel therapies against cancer is a worldwide health priority due to the high incidence of this disease and the lack of effective therapies. Conventional cancer therapies have a very limited ability to distinguish tumor cells from healthy cells. Neuroblastoma (NBL) is a type of childhood cancer that develops from tissues that form the sympathetic nervous system. The ability to interfere exclusively with mechanisms responsible for tumorigenesis can provide the basis for the generation of new therapeutic strategies. Pin1 is an isomerase frequently overexpressed in various tumors and has become an attractive molecule in cancer research.

Our results showed that overexpression of Pin1 in a cellular model of NBL did not significantly impact cell proliferation or differentiation in normal cell culture conditions. However, cells stably overexpressing Pin1 showed a significant anchorage-independent growth when cultured in soft-agar. Notably, this kind of proliferation, which is considered a hallmark of carcinogenesis, is completely reversed in presence of Juglone, a Pin1 inhibitor. Also, wound healing assay revealed that Juglone reduced the migration ability of these cells. We concluded that inhibition of Pin1 altered tumor-associated phenotypes of NBL cells, however more studies are being carried out to validate Pin1 as a possible target of new therapies against NBL.

CB-P64
RETINOIDS AND HER2 INHIBITORS AFFECT THE BEHAVIOR OF MAMMARY CANCER STEM CELLS

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Cancer stem cells (CSC) are resistant to chemotherapy and radiation and are also considered as the metastasis seed. It was described that HER2 receptor would be involved in stem capacity maintenance. So, our objectives were: A) To assess the expression of HER2 receptor both in triple negative mammary cancer 4T1 cells and in its CSC derived population. B) To analyze the modulation of growth potential under retinoids (ATRA), Lapatinib (Lp, Her2 inhibitor) and the combined treatment. C) To evaluate soluble metaloprotease 9 (MMP9) activity on pre-treated CSC. Her2 was selectively expressed in the CSC population, in its active form. While ATRA had no effect, Lp caused a marked growth inhibition in 4T1 monolayers. When the same treatments were applied on CSC (soluble mammospheres culture), ATRA and Lp combination strongly inhibit the formation of these 3D structures, being them small and irregular, with high number of dead cells. Cells derived from pretreated mammospheres were seeded at low density in order to evaluate the clonogenic capacity. While ATRA and Lp reduced clonogenicity, the combined treatment induced a higher effect. Regarding MMP9 activity, the presence of ATRA increases this activity while no differences were found under Lp treatment. The results presented in this work could allow a new therapeutic approach for a subset of tumors lacking effective therapy till nowadays

CB-P65
NORCANTHARIDIN IMPAIRS MAMMARY CANCER STEM CELLS GROWTH AND IN VIVO TUMOR PROGRESSION

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Triple-negative breast cancer (TNCB) is characterized by an abundance of treatment-resistant cancer stem cells (CSC). Norcantharidin (NCTD) is a synthetic demethylated small-molecule analog of the naturally occurring cantharidin isolated from blister beetles. Unlike the conventional chemotherapeutics, NCTD toxicity is higher to cancer cells than normal ones, making this molecule promising for cancer treatment. In this work, using 4T1 and Hs578t triple negative mammary cell lines we propose to: A) Study the effect of NCTD on the in vitro proliferation potential. B) Analyze the effect of NCTD on self-renewal and clonogenic capacity of 4T1 and Hs578t derived CSC. C) Evaluate the effect of NCTD on the in vivo tumor progression of 4T1 cells. We observed that NCTD significantly reduced 4T1 and Hs578t cell proliferation. Related to CSC derived from both mammary cell lines, NCTD impaired the clonogenic capacity as well as the renewal potential. Finally, we performed an in vivo assay, where 4T1 cells were orthotopically inoculated in BALB/c mice, and NCTD was i.p. inoculated twice a week (5 mg/kg). We could determine that NCTD significantly reduced tumor volume in vivo. Our data suggest that NCTD treatment reduces tumor progression both in vitro and in vivo, possibly thorough a direct effect on CSC biology.

CB-P66
GLUCOSE 6-PHOSPHATE DEHYDROGENASE INHIBITION SENSITIZE MELANOMA CELLS TO METFORMIN TREATMENT

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Cancer cells exacerbate not only glycolysis but also TCA cycle and PPP to enhance biosynthetic processes, proliferation and growth. Human malignant melanoma is a frequently life-threatening skin cancer. The aim of the present work was to investigate the cytotoxic effects of metformin (MET, antidiabetic drug), 2-deoxyglucose (2DG, HK inhibitor), dichloroacetic acid (DCA, PDH inhibitor), 6-aminonicotinamide (6-AN, G6PD inhibitor) and methrotrexate (MTX, DHFR inhibitor) on 5 human melanoma cell lines (hM1, hM2, hM4, hM9 and A375). After 24 h of culture, cells were treated with 2DG (0.5-10 mM), MET (1-15 mM), DCA (0.5-50 mM), 6-AN (1-100 uM) and MTX (1-10000 nM). The antitumor effects of bioenergetic modulation were evaluated by APH assay, 5 days after treatments. Each melanoma cell line showed a different profile of sensitivity thus suggesting high metabolic plasticity. Then, we studied the effect of combining the metabolic modulators. We found that independently of the individual response, the combination of 6-AN (50 uM) and MET (5 mM) extremely decreased the viability ($p<0.0001$) of all evaluated melanoma cell lines. Here we reported for the first time that inhibiting G6PD, the rate limiting enzyme of PPP, drastically increased MET cytotoxic effects. This results support further studies to investigate the mechanism involved in this synergistic effect and its biological relevance.

CB-P67
DIFFERENTIAL EFFECTS OF TWO ORGANOPHOSPHORUS PESTICIDES ON POLYAMINE METABOLISM IN TOAD EMBRYOS

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Organophosphorus pesticides like chlorpyrifos (CPF) and azinphosmethyl (AZM) are widely applied in the fruit-producing valley of Río Negro and Neuquén. Amphibian embryos are particularly sensitive to numerous environmental contaminants. Polyamines (PA) are essential for normal cell growth and differentiation, being highly regulated through the balance in the first-step synthesizing enzyme ornithine descarboxylase (ODC) and degradation by polyamino-(APAO), diamino-(DAO) and spermine (SMO) oxidases. Our objective was to evaluate the effects of AZM and CPF on PA oxidation in R. arenarum embryo, measuring oxidase activities through kinetic spectrofluorometry analysis. SMO and DAO activities were increased by 9 mg/L AZM (62% and 69%) in early embryos (3d), while the effect was highly noticeable on APAO (4-fold increase). CPF did not affect oxidases in early stages. At the end of embryo development AZM effect was smoother on DAO (20% increase) but sustained on SMO by 2 mg/L exposure (70% increase). Only a minor decrease (10%) was caused by CPF. According with our previous results, alterations caused by AZM in late embryos may be related to an increase in putrescine driven both by ODC induction and spermidine/ spermine oxidation back to diamine. Conversely, the decrease in PA reported in early embryos exposed to CPF would be only related to the decrease in ODC as oxidases are not affected.

ENZYMOLOGY

EN-P01
KINETIC AND STRUCTURAL CHARACTERIZATION OF UDP-GLUCOSE PYROPHOSPHORYLASE FROM *Euglena gracilis*

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Euglena gracilis is a fresh water protist able to grow photosynthetically or heterotrophically using a diverse array of organic compounds as the sole source of carbon and energy for growth. *E. gracilis* is a suitable source for the generation of several compounds used for the production of cosmeceuticals, nutraceuticals, food, paramylon and wax esters. When grown aerobically it produces an insoluble β-1,3-glucan as storage polymer (paramylon). In protozoa, synthesis of structural and reserve oligo and polysaccharides occurs via UDP-glucose, a key metabolite of the carbohydrate pathways present in most organisms, generated in a reaction catalyzed by UDP-glucose pyrophosphorylase (EC 2.7.7.9; UDP-Glc PPase).We performed *de novo* synthesis and molecular cloning of the gene coding for UDP-Glc PPase from *E. gracilis*. This enzyme was expressed in *Escherichia coli* cells. The purified enzyme was kinetically characterized, catalyzing UDP-glucose synthesis with a Vmax of 3350 U/mg, and affinity for substrates of 0.24 mM and 0.17 mM for glucose-1P and UTP respectively. We determined a molecular mass of 55 kDa by size exclusion cromatography corresponding with a monomeric structure. Also, results show that the enzyme activity is affected by oxidation with diamide and H2O2, being recovered by adding dithiothreitiol, suggesting a possible redox regulation of the enzyme activity in *E. gracilis.*

EN-P02
CRDSP, A PHOSPHOGLUCAN PHOSPHATASE INVOLVED IN STARCH METABOLISM IN *C. reinharditii*

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Phosphatases are enzymes that remove phosphate groups from a wide variety of substrates such us proteins, glucans, nucleic acids and lipids. It has recently become clear that dephosphorylation of glucans is essential for normal starch degradation in plants as well as glycogen metabolism in mammals. To understand the evolution of catalysis and regulation of these enzymes we decided to investigate their existence in unicellular green algae. *C. reinharditii* transcriptome BLAST searches revealed one locus encoding a protein with high sequence similarity to *A. thaliana* starch excess 4 enzyme. An exhaustive *in silico* analysis led us to conclude that the putative protein could be a chloroplastic enzyme with similar structural features to its plant counterpart. To further characterize this finding the cDNA was cloned to express and purify the recombinant protein in *E. coli* cells. We verified phosphatase activity of the recombinant enzyme *in vitro* as well as its ability to bind polysaccharides

EN-P03
OXIDOREDUCTASE ACTIVITY AND IRON-SULFUR CLUSTER BINDING OF GLUTAREDOXINS FROM *Leptospira interrogans*

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Glutaredoxins are small and ubiquitous GSH-dependent oxidoreductases. The classification based on the active site motif and catalytic cycle include two groups; monothiol (1CGrx) and dithiol (2CGrx) Grx. In general, 2CGrxs are able to reduce protein disulfide, while the 1CGrxs are involved in iron homeostasis. The *Leptospira interrogans* genome project presents encoding genes for putative 1CGrx and 2CGrx. In this work, we present functional and structural properties of the recombinants proteins. *Lin*2CGrx (but not *Lin*1CGrx)

was able to reduce *in vitroLin*MsrB (methionine sulfoxide reductase B) dehydroascorbate and HEDS (2-Hydroxyethyl disulfide).On the other hand, gel filtration chromatography and UV-Vis spectroscopy experiments indicated that *Lin*1CGrx isolated from the recombinant *E. coli* cells is a Fe-S cluster binding protein. This holo-protein has homodimer structure and the absorption spectra revealed that the Fe-S cluster had two characteristic peaks at 320 and 420 nm. In addition, the yeast functional complementation experiments demonstrated that *Lin*1CGrx can rescue the phenotype of mutants lacking Grx5, which are partially deficient in growth in SD and YPD media and also highly sensitive to oxidative stress. These results suggest that *L. interrogans* Grxs have important functions in redox and iron metabolism.
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EN-P04
HETEROLOGOUS PRODUCTION AND CHARACTERIZATION OF A THERMOSTABLE GH10 FAMILY ENDO-XYLANASE

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Xylanases show a wide range of biotechnological applications in the feed, pulp and paper industries and production of second generation bioethanol. Development of novel enzymes with enhanced properties for harsh processes requires the bioprospecting and/or engineering of enzymes to be stable and active in acidic or alkaline conditions, high temperatures, oxidative conditions and tolerant to high salinity and/or organic solvents. In this work we present the heterologous expression and characterization of a novel endo-xylanase of glycoside hydrolase family 10 from the white-rot basidiomycete Pycnoporus sanguineus. The coding sequence of mature endo-xylanase GH10ps fused to an N-terminal 6xHis-tag, was cloned in continuous reading frame to the signal sequence of *Saccahromyces cerevisiae* factor, under the transcriptional control of the methanol-inducible promoter AOX1 in the vector pPIC9, for expression in *Pichia pastoris* and secretion to the culture medium. Recombinant GH10ps presented thermostable endo-beta-1,4 xylanase activity, with a half-life of 3 hs at 70°C and a stability higher than 48 h at 60°C. Not only was it capable of releasing xilooligosaccahrides and xilose from agricultural waste biomass, but it also acted synergically with commercial cellulases in cellulose saccharification.

EN-P05
MODELING AND CHARACTERIZATION OF β-XYLOSIDASE ECXYL43 ON NATURAL AND ARTIFICIAL SUBSTRATES

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Beta-xylosidases hydrolyze short xylooligosaccharides to xylose. They are critical for the effective saccharification of biomass in bioprocesses such as production of cellulosic bioethanol. In this work, the structure of recombinant beta-xylosidase EcXyl43 from *Enterobacter sp*. was modeled and its catalytic efficiency on artificial and natural substrates was evaluated. The homology model predicted by I-TASSER server suggested a structure composed by an N-terminal conserved catalytic domain linked to an ancillary C-terminal domain of undefined function through a long loop. A tetrameric quaternary structure was predicted and further visualized by PAGE. The purified enzyme hydrolyzed artificial substrates pNPX and pNPA, corresponding to beta-xylosidase and arabinofuranosidase activities. It was also active on xylobiose (X2) and xilooligosacharides from xylan. Its addition to commercial xylanases increased the release of xylose and arabinose from pre-treated wheat straw. EcXyl43 exhibited high efficiency and thermostability under optimal conditions (40°C-pH 6.5), with a half-life of above 72 hours. The exposition to higher temperatures and saccharification products affected its stability and catalytic efficiency, respectively. Thus, EcXyl43 presents beta-xylosidase and arabinofuranosidase activities and can be used for (hemi) cellulose bioconversion.

EN-P06
ASSOCIATION BETWEEN ALDOSE REDUCTASE AND TUBULIN: EFFECT OF TYROSINE DERIVATIVES.

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In our laboratory we found that tyrosine inhibits the association between aldose reductase (AR) and tubulin (TUB). causing a decrease in activation of AR by TUB. AR activation is associated with diabetic cataract formation, but the tyrosine is able to prevent this onset both *in vivo* and in *ex -vivo* models. Our interest was to determine whether tyrosine derived compounds can have the same effect as tyrosine on the AR/TUB association. Tyrosine derivatives that have the same basic structure of the molecule with substituents on the carboxyl, amino and phenolic group of tyrosine were selected for this study. The first study was the determination of the diabetic cataract lenses *ex-vivo* in mice. Of the seven tested compounds, five decreased, to a greater or lesser degree, the formation of diabetic cataract in lenses treated *ex-vivo*. Nearly all the derivatives inhibited the AR/TUB association complex. 3-iodo-tyrosine proved to be the most effective of the tested compounds that, at concentrations as low as 50 µM, prevented diabetic cataract lenses in *ex-vivo* treated mice and also interacted directly with AR. These results show the first evidence that tyrosine derivatives are capable of regulating the AR/TUB association and thereby prevent diabetic cataract.

EN-P07
STIMULATION OF ALDOSE REDUCTASE ACTIVITY BY TUBULIN: EFFECT OF PHENOLIC ACID DERIVATIVES

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The main pathogenic pathway of diabetes is activation of the enzyme aldose reductase (AR). In our laboratory we describe a new regulatory mechanism of AR activity by tubulin interaction. The tubulin increased 7 times the AR activity. This activation induces changes in microtubule dynamics and inhibition of Na+,K+-ATPase. We also demonstrate that 3-nitro-L-tyrosine (3-NTyr) and tyrosine (Tyr) are able to prevent tubulin/AR association and, in a model of diabetic rats, the administration of 3-NTyr prevents the development of diabetic cataracts. The most important family of inhibitor compounds of AR is the one of carboxylic acids. Although structurally Tyr and 3-NTyr are different they share two characteristics: the presence of an aromatic ring and a carboxylic substituent. The objective of the present study was to evaluate different phenolic acid derivatives in their ability to prevent the formation of tubulin/AR complex and enzyme activation. The drugs tested were mandelic, phenylacetic, lactic and vanilic acid derivates. All drugs tested were able to prevent tubulin/AR complex formation, enzyme activation and tubulin polymerization in cells cultured in high glucose. These results allow us to propose derivatives of phenolic acids as regulators of AR activity and possible preventers of the formation of diabetic cataract.

EN-P08
RIBULOSE 5-PHOSPHATE EPIMERASE ISOENZYMES IN *Trypanosoma cruzi*: STRUCTURE, KINETICS AND LOCALIZATION

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The enzyme of the pentose phosphate pathway (PPP) ribulose 5-phosphate epimerase (RPE) is encoded by two genes in the genome of *Trypanosoma cruzi* CL Brener clone: TcRPE1 and TcRPE2. Whereas recombinant TcRPE2 shows a typical hyperbolic substrate curve, TcRPE1 shows a slightly cooperative response. In addition, both isoenzymes differ considerably in their affinity for Ru5P: TcRPE1 being 670-fold more efficient than TcRPE2. Regarding the subcellular localization, whereas TcRPE1 is a cytosolic enzyme, TcRPE2 is mainly localized in glycosomes, being -to our knowledge- the first PPP isoenzyme that adopts exclusively that location. Both TcRPEs constitute single domains exhibiting the classical α/β TIM-barrel fold, as expected for enzymes with RPE activity. Studies on the quaternary arrangement of these enzymes revealed that both isoenzymes exhibit a mixture of various oligomeric species made up of an even number of molecules. This multiplicity has not been reported for any other RPE studied and it might bear implications for the regulation of TcRPE activity. Although further investigation will be necessary to unravel the physiological significance of these findings, this trait -together with the sub-cellular localization of TcRPE2- differentiates TcRPEs from human RPE, suggesting potential opportunities for intervention with novel anti-trypanosomal drugs.

EN-P09
CHARACTERIZATION OF HEME OXYGENASE AND FERREDOXIN-NADP+ REDUCTASE IN *Leptospira biflexa*

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Heme oxygenase (HO) catalyzes the degradation of heme to biliverdin, iron and carbon monoxide employing oxygen and reducing equivalents. HO is important for heme-iron utilization in bacteria and contributes to virulence in *Leptospira interrogans*. We found that a plastidic type, high efficiently ferredoxin-NADP+ reductase (FNR) provides the electrons for the functioning of HO in *L. interrogans* without the requirement of ferredoxin. Interestingly, phylogenetic analyses revealed that HOs and FNRs are grouped differentially among pathogenic and saprophytic Leptospira species, providing evidence of some function specialization. We have studied the electron transfer between HO and FNR in the saprophyte *Leptospira biflexa*. Structural and functional differences between the enzymes from the saprophyte and its close related pathogen can provide relevant information. LepBiHO and LepBiFNR were cloned, overexpressed and purified. The structural and enzymatic properties of both enzymes were studied. We found that LepBiHO catalyzed the NADPH/LepBiFNR dependent oxidation of heme without the need of ferredoxin with high catalytic efficiency. However, the reaction ended at an intermediate stage of the normal heme degradation pathway, with no production of biliverdin. Our findings contribute to the understanding of the heme degradation pathway in Leptospira, a key target for therapeutic applications.

EN-P10
BIOCHEMICAL CHARACTERIZATION OF THE GLYCOGEN STORAGE DISEASE-ASSOCIATED A16P MUTANT OF GLYCOGENIN

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Glycogen storage diseases (GSD) are inherited metabolic disorders of glycogen metabolism.GSD type 15 is a rare genetic disorder reported only in a few patients to date. It is caused by mutations in the GYG1 gene, which encodes glycogenin-1, one of the isoforms of human glycogenin, the enzyme that initiates glycogen biosynthesis. One of the recently described cases corresponds to a patient that was homozygous for an N-terminal missense variant (c.46G>C, p.Ala16Pro). The patient exhibited skeletal myopathy with storage of polyglucosan in muscle fibers and no cardiac involvement.Ala16 is conserved in many members of glycogenin family, thus it was of interest to analyze the effect of its substitution on the catalytic properties of the protein. Since human and rabbit glycogenin amino acid sequences are 93% identical, we have introduced Ala16Pro mutation into rabbit enzyme and expressed the mutantin *E. coli*.In this

work, we present the analysis of the effect of the mutation on the autoglucosylation, transglucosylation and UDP-glucose hydrolytic activities of the protein and on its substrate binding affinity. Our results suggest that even though Ala16 is not directly involved in UDP-glucose binding, its replacement by Pro would promote a conformational change that would cause a reduction in the substrate binding affinity and, consequently, the loss of the enzyme activity.

EN-P11

STRUCTURE TO FUNCTION STUDIES ON SUCROSE SYNTHASES FROM *Anabaena variabilis*

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Sucrose synthase (SucSase, EC 2.4.1.13) catalyzes the reversible conversion of NDP-glucose and fructose to sucrose and NDP. Plant SucSase has been extensively studied but little is known about the cyanobacterial enzyme. *Anabaena variabilis* (a filamentous heterocyst-forming cyanobacterium) has two genes encoding putative SucSases (susA and susB). In this work we show that recombinant SucSase A is active, whereas SucSase B has negligible catalytic activity. Homology modeling studies using the crystal structure of *Nitrosomonas europaea* SucSase as template showed that SucSase B lacks Pro132 (replaced by Ser) and Phe162 (replaced by Ala), which are important for the interaction between A:D and A:B subunits of the tetramer, respectively. Using gel filtration chromatography we determined that SucSase A is a tetramer and SucSase B is a dimer, suggesting that the quaternary structure is important for the catalytic activity. We hypothesize that, after gene duplication, SucSase B evolved to play a new (regulatory?) role, as it has been described for the plant ADP-glucose pyrophosphorylase large subunit. Alternatively, SucSase B might catalyze a yet unidentified reaction, different from sucrose synthesis/degradation. Experiments to test these hypotheses are currently underway.

EN-P12

SHIFTING COFACTOR SPECIFICITY OF PEACH GLUCITOL DEHYDROGENASE BY STRUCTURE-GUIDED MUTAGENESIS

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Dehydrogenases use nicotinamide adenine dinucleotides as cofactors. The structural domain for NAD(P)+ binding is composed of three β-strands separated by two α-helices. Based on homology modelling studies we hypothesised that Asp216 (located at the end of the second β-strand) determines the marked preference of peach (*Prunus persica*) glucitol dehydrogenase for NAD+. Site-saturation mutagenesis of Asp216 to Ala increased (35-fold) the catalytic efficiency for NADP+ while it decreased (9-fold) the one for NAD+. Interestingly, the triple mutant D216A/V217R/D218S (designed by comparison with a NADP+-dependent dehydrogenase) showed a catalytic efficiency for NADP+ 530-fold higher than the wild type. Moreover, the triple mutant exhibited a catalytic efficiency for NADP+ 2-fold higher than for NAD+. Our results show that both strategies produced enzymes that preferably catalyze glucitol oxidation in a NADP+ dependent manner. These techniques could be useful to produce novel specificities in enzymes with broad applications in biotechnological processes

EN-P13

KINETIC AND REGULATORY CHARACTERIZATION OF *Arabidopsis thaliana* PHOSPHOENOL-PYRUVATE CARBOXYKINASE

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Phosphoenolpyruvate carboxykinase (PEPCKase, EC 4.1.1.49) is a cytosolic enzyme that catalyzes the reversible ADP-dependent carboxylation of PEP to oxaloacetate. Apart from PEPCKase role in CO2 concentrating mechanisms in C4 and CAM species, its products are localized in the interface between sugars, amino and organic acids and lipids metabolisms. However, the knowledge of its regulatory mechanisms remains obscure, mainly due to the rapid proteolytic cleavage of a N–terminal peptide during extraction and purification. It is not known how and if PEPCKase regulation is affected by this process. In this work, the first recombinant production and characterization of a non proteolyzed form of PEPCKase1 from Arabidopsis is reported. The enzyme has a homotetrameric conformation with maximum activity in the range pH 7–8. Divalent cations, such as Mg+2 and Mn+2, were essential for catalysis: increased Mn2+ concentrations produced a 3–fold increment in Vmax and slight changes in Km values for both PEP and ADP. Different metabolites modified PEPCKase activity, being ATP and Glc–6P the main inhibitors. Interestingly, different oxidative compounds, particularly physiological concentrations of H2O2, inactivated the enzyme. As a whole, these results indicate that PEPCKase might be subject to a complex, multi–level regulation to coordinate the many faceted functions it plays in plant metabolism.

EN-P14

MECHANISM OF INHIBITION OF EPIGALLOCATECHIN ON THE PLASMA MEMBRANE CA2+-ATPASE

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Epigallocatechin 3-gallate (EGCg) is the major polyphenol component and primarily responsible for the green tea effects. EGCg beneficial effects include mainly antioxidant, anti-inflammatory and neuroprotective effects. EGCg has been reported to inhibit the activity of the Na,K-ATPase, a P-type ATPase that is primarily responsible for maintaining the osmotic balance within animal cells. In order to investigate whether EGCg inhibits other members of the P-type ATPase we evaluated the effect of EGCg on the Plasma

membrane Ca2+-ATPase (PMCA). PMCA transports Ca2+ actively to the extracellular medium coupled to the ATP hydrolysis maintaining the cellular homeostasis. We performed measurements of the ATPase activity and the concentration of phosphorylated intermediates on purified preparations of PMCA obtained from human red blood cells and fluorescence measurements of Ca2+ in HEK293T cells using Fluo-4 as an intracellular dye. Results show that EGCg is a potent inhibitor of PMCA with a K0.5 of 0.032 ± 0.003 μM and the inhibition would occur by a decrease of the rate of the E1P to E2P transition of phosphorylated intermediate of the PMCA catalytic cycle. Further, measurements of Ca2+ flux in HEK293T cells show that EGCg lowered Ca2+ removal rate compared to control, suggesting that the Ca2+ removal systems could be impaired, including PMCA.

EN-P15
DIFFERENTIAL INHIBITION OF PLASMA MEMBRANE CA2+-ATPASE BY QUERCETIN AND GOSSYPIN

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Flavonoids are commonly found in fruit and vegetables and they are also part of human diet. These compounds are also believed to have cancer chemoprotective properties by triggering apoptosis via the Ca2+-dependent mitochondrial pathway which can be activated through an increase of cytosolic [Ca2+] ([Ca2+]c). The aim of this work is to characterize the inhibition of PMCA by quercetin and gossypin, two flavonoids with known anticarcinogenic activity. The differences in the mechanism of inhibition will be analyzed considering these compound's molecular structures and their possible interaction with the pump. We evaluated the [Ca2+]c in HEK293T cells and also studied the Ca2+-ATPase activity in purified PMCA obtained from human erythrocytes. The results showed that (1) PMCA activity was inhibited by quercetin and gossypin with a K0.5 of 0.34 ± 0.01 and 5.1 ± 1 μM, respectively; (2) PMCA phosphorylation showed that quercetin led to the accumulation of phosphoenzyme while gossypin induced a decrease; (3) quercetin produced a larger calmodulin PMCA inhibition than gossypin; (4) in HEK293T cells, quercetin and gossypin lowered Ca2+ removal rate. These results show that quercetin and gossypin inhibit PMCA by different mechanisms, possibly due to their distinct molecular structures, giving rise to the idea that PMCA could be a novel target for flavonoids

EN-P16
BERYLLIUM AND ALUMINUM FLUORIDE COMPLEXES TO STUDY PHOSPHORYLATED STATES IN THE NA,K-ATPASE

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During the transport of Na and K driven by the hydrolysis of ATP, Na,K-ATPase undergoes reactions of phosphorylation-dephosphorylation and conformational changes that allow the transient occlusion of the transported cations. Fluoride complexes with metals such as Mg, Al and Be have been employed for structural analysis since they bind to the phosphorylation site and imitate different sub-states of the phosphoryl intermediate, E2P. Using enzyme partially purified and the fluorescent probes eosin and RH421, which respectively report on E1 and E2P-like states, we performed equilibrium and transient-state experiments. We observed a decrease in eosin fluorescence signal due to Pi, BeF and AlF addition, suggesting the formation of E2P or E2P-like states. Fluorescence changes analyzed with the RH421 probe are parallel to those observed with eosin for the case of Pi but not for BeF indicating a different enzyme conformation in these conditions. Unlike for E2P, the stability of E2BeF and E2AlF is sufficient to prevent the enzyme to return to the E1 state upon Na addition. Regarding the ability of E2P and E2P-like states to bind and occlude cations, it is observed that K (Rb) occlusion is decreased by both Pi and BeF. Results with BeF suggest a change of the equilibria between states with bound and occluded Rb. With grants from CONICET, ANPCYT y UBACYT.

EN17
REDOX MODULATION OF hSIRT6, KEY ENZYME OF METABOLISM AND INFLAMMATION

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Sirtuins are NAD^+ -dependent deacetylases reported as key factors in metabolism regulation, inflammation and stress response. Human sirtuin 6 (hSIRT6) is known as histone 3 dacetylase despite of its low activity *in vitro*. Its deacetylase activity increases in the presence of fatty acids and possess long chain fatty acid deacylase activity. Studies on regulation of hSIRT6 activity are scarce. Our aim was to investigate the potential redox regulation of hSIRT6, characterizing structural and functional changes under oxidant conditions. Recombinant hSIRT6 was expressed and purified from *E. coli*. Deacetylase activity was measured using a coupled-enzyme assay (plus nicotinamidase and glutamate dehydrogenase under non-limiting conditions) following NADPH consumption at 340 nm. A synthetic acetylated histone 3 peptide (H3K9-Ac) was used as a substrate. The basal deacetylase activity increased 3-fold in the presence of 50 uM oleic acid. Total protein thiols were reduced after hSIRT6 treatment with H_2O_2, however, deacetylase activity was not significantly altered, while treatment with an excess of peroxynitrite, not only reduced protein thiol content but also rendered nitrated tyrosines and a significant loss of activity. Identification of the cysteine residues modified, loss of zinc, intra and/or intermolecular disulfides, as well as secondary/tertiary structural changes are underway.

LIPIDS

LI-P01
OXER1 IS INVOLVED IN ADRENOCORTICAL CELL PROLIFERATION

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Lipoxygenase-dependent products of arachidonic acid (AA) metabolism act through a membrane receptor named OXER1. These compounds are produced in steroidogenic cells and are required for the activation of steroid production. Human adrenocortical H295R cells express OXER1. It is involved in the PKA and PKC-dependent stimulation of steroidogenesis. Here we examined the role of OXER1 in the proliferation of H295R cells. We used a cell line characterized by the stable overexpression of OXER1 (H295R-O). The proliferation rate in these cells was higher than in control cells, and, when the endogen production of lipoxygenated products was inhibited, the H295R-O cells behaved like control cells (crystal violet assay, in arbitrary units: H295R-empty 0.028 ± 0.004; H295R-O 0.06 ± 0.01; P < 0.01; H295R-O + inhibitor 0.038 ± 0.003). In addition, H295R cells treated with 8Br-cAMP 1 mM decreased the proliferation rate (control 147 ± 5; 8Br-cAMP 104 ± 3; P < 0.001), while angiotensin II did not produce any significant changes between 0.01 and 1 uM. Therefore, the activation of PKA and PKC kinases might not be involved in the activation of the proliferative process. These results support the hypothesis that lipoxygenase products acting through the activation of OXER1 are involved also in adrenal cell growth. Future experiments should focus on signal transduction pathways triggered by OXER1 activation.

LI-P02
ω-6 AND ω-3 FATTY-ACIDS ON EARLY STAGES OF MICE SUBMANDIBULAR GLANDS TUMOR

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The aim of this work was to assess the impact of diets enriched in PUFAs ω-3 and ω-6 on the lipid profile of submandibular glands (SMG) cell membrane and their effect on cell cycle regulation and apoptosis, evaluated by TP53 and Ki-67 expression in 9,10-dimethyl-1,2-benzanthracene (DMBA) induced tumor development in SMG in a murine model.To generate tumorigenic changes, SMG mice of the experimental group were injected with 50µl of 0.5% of DMBA. Both control (no DMBA) and experimental groups of BALB/c mice were fed with: chia oil (ChO),rich in ω-3 fatty acid or corn oil (CO), rich in ω-6/ω-3 fatty acid; or safflower (SO) oil, rich in ω-6 fatty acid. The PUFAs tumor cell profile was analyzed by gas chromatography and eicosanoids by HPLC. Apoptosis was measured by TUNEL and TP53/Ki-67 were inmmunomarked. Arachidonic acid increased significantly in animals treated with DMBA and fed with SO and CO diet. EPA and ALA increased significantly in animals fed with ChO in control and treated with DMBA. Eicosanoids showed significant variations between animals treated with DMBA and their respective controls. Results demonstrated novel differential effects of ω-3 and ω-6 PUFAs on the regulation of early tumorigenesis events in murine SMG injected with DMBA. This knowledge may help to develop chemoprotective treatments, therapeutic agents and health promotion and prevention activities in humans

LI-P03
ROLE OF PHOSPHATIDYLCHOLINE BIOSYNTHESIS ON NEURONAL DIFFERENTIATION

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Neuronal differentiation implies an increase in membrane phospholipids in order to face the demand for neurites outgrowth. As phosphatidylcholine (PtdCho) is the major component of mammalian membranes, its biosynthesis plays a key role during neuronal differentiation. Interestingly, increased PtdCho biosynthesis not only provides structural components for neurite developing, but also plays an active role in regulating neuronal differentiation in Neuro-2a cells and in embryonic neural stem cells (NSCs). In Neuro-2a cells, retinoic acid (RA) and lisophosphatidylcholine (LPtdCho) promote neuronal differentiation by activating the MAPKK pathway. They also promote an increase in PtdCho synthesis by adapting the expression and activity of choline kinase alpha (CKα) and CTP-phosphocholine cytidylyltransferase alpha (CCTα). In addition, it has been reported that in PC12 cells CCTβ isoform regulates neurite branching.The aim of this study is to analyze the effect that inhibition of CKα CCTα and CCTβ caused on Neuro-2a and NSCs differentiation. We demonstrated that, before the induction with RA and LPtdCho, the selective inhibition of these enzymes by siRNA and by pharmacological approaches impaired neuronal differentiation of Neuro-2a cells. Moreover, pharmacological inhibition of CKα and CCTα in NSCs caused a reduction in neurites length.

LI-P04
BIOCHEMICAL CHARACTERIZATION OF GM1 MICELLES-AMPHOTERICIN B INTERACTION

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Objectives: Perform a thorough characterization of the GM1 micelle-Amphotericin B (AmB) interaction.
Methods: UV-Vis spectrometry, SEC, DLS and in-vitro cultures.

Results: The micelle formation and the drug loading occur spontaneously, although influenced by the physicochemical conditions, pH and temperature. The chromatographic profile of GM1-AmB complexes at different molar ratios shows the existence of two populations. The differential absorbance of GM1, monomeric and aggregate AmB, allowed us to discriminate the presence of all of

them in both fractions. Thus, we noted that at higher proportion of AmB in the complex, increases the larger population which is composed mainly of aggregated AmB. The physical behavior of these micelles shows that both GM1-AmB complexes were stable in solution for at least 30 days. However upon freeze-thawing or lyophilization-solubilization cycles, only the smallest population, enriched in monomeric AmB, showed a complete solubilization. In vitro, GM1-AmB micelles were significantly less toxic on cultured cells than other commercial micellar formulations as Fungizone, but had a similar behavior to liposomal formulations as Ambisome. Regarding the antifungal activity of the new formulation, it was very similar to that of other formulations. Conclusion: GM1-AmB complexes have the potential to improve the antifungal therapeutic efficiency of AmB.

LI-P05
NITRO-FATTY ACID MODULATES MACROPHAGE LIPID METABOLISM

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Alteration of lipids metabolism and oxidative balance leads to lipoprotein modification (oxidation or acetylation) and plaque formation. Thus, inflammation and foam cell formation are important components in the initiation and progression of atherosclerosis. We have demonstrated that activated macrophages form bioactive lipids mediators named nitro-fatty acid (NO2-FA), which exhibit important anti-inflammatory and cytoprotective actions in experimental model of cardiovascular disease. In this work we evaluated the action of NO2-FA to modulate lipid uptake and foam cell formation on RAW264.7 macrophage cell line. NO2-OA increased membrane expression of scavenger receptors LRP-1 and CD36 in a dose dependent manner. This up-regulation is transient and reaches a maximum at 8h, which was not affected by treatment with cycloheximide. These results allow us to speculate that modulation of LRP-1 and CD36 expression by nitrolipids affect protein stability. Uptake of oxidized LDL particles in RAW264.7 macrophages treated with NO2-OA result in an increased lipid accumulation as reveled by Oil Red O staining of lipids droplets. However, we observed that NO2-OA improved the autophagic flux by inducing LC3II accumulation. This result may explain, in part, the beneficial action ascribed to NO2-FAs in atherosclerosis by balancing the intracellular lipid content and foam cell formation.

LI-P06
LIPID CHARACTERIZATION OF CORTICAL BRAIN IN A STZ-INDUCED RAT MODEL OF SPORADIC ALZHEIMER'S DISEASE

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Sporadic Alzheimer's disease (sAD) is the most common form of dementia among older people. This study aimed to examine the lipid classes and fatty acid composition from brain cortex and the cognitive function of a rat model of sAD generated by the intracerebroventricular (icv) injection of a sub-diabetogenic dose of streptozotocin (STZ) to explore possible lipid profile alterations that could correlate with this neuropathology and validate the model for future gene therapy assays. Three-month-old male rats were icvSTZ injected with 1 and 3 mg/Kg or vehicle. On experimental day (ED) 14, cognitive performance was evaluated by Barnes maze and Novel Object Recognition test. Rats were sacrificed on ED 24. Lipid analyses were performed by thin-layer and capillary gas chromatography. The high STZ dose induced significant cognitive deficits ($P \leq 0.01$) and in the brain cortex it displayed low levels of n-3 long chain polyunsaturated fatty acids (mainly 22:6n-3) and reduced unsaturation and peroxidability indexes. Free cholesterol content was not modified but levels of triglycerides and cholesterol esters showed a slight increase. The low STZ dose did not induce significant changes. These results demonstrated that icvSTZ administration of 3 mg/Kg produced alterations in cognitive and lipid profiles that resembled sAD, thus we propose this animal model to study sAD potential therapies.

LI-P07
IN VITRO AND IN VIVO EVALUATION OF MANDARIN PEEL OIL ON LIPID METABOLISM AND TUMOR GROWTH

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The aim of this work was to study the effects of mandarin (Citrus reticulata) peel oil (MPO) on lipid metabolism and cell proliferation. HepG2 and A549 human tumor culture cells were used for *in vitro* assays, and A549 cells were implanted in nude mice for *in vivo* assays. Mice were fed with a control or experimental (2.1 and 6.3 µl MPO/mouse/day) diet for 21 days. Systemic toxicity evaluation, tumor growth measurement, and histological analyses were performed. Radioactive lipid precursors, thin-layer chromatography, flow cytometry, and MTT and TUNEL assays were the methodology employed for *in vitro* and/or *in vivo* assays. Treated mice did not show significant differences in quantity of food intake, body and organ weight, serum activity of aspartate and alanine aminotransferases, and albumin content compared with controls. In tumor cells MPO: 1, decreased ^{14}C-acetate incorporation into cholesterol, phospholipids, and triglycerides; 2, inhibited cell proliferation and cell cycle progression with an arrest at the G_0/G_1 interphase (IC50= 116 µl MPO/L); 3, significantly diminished tumor growth (49%) with 6.3 µl MPO/mouse/day; and 4, increased apoptosis (0.5-1.7% in *in vivo* assaysand 9-30% in *in vitro* assays). Based on these results, we conclude that MPO has a great potential as a natural non-toxic hypolipogenic and chemotherapeutic agent.

LI-P08

2-ARACHIDONOYL GLYCEROL IN *Caenorhabditis elegans* DAUER DIAPAUSE REGULATION

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Polyunsaturated fatty acids (PUFAs) and branched-chain fatty acids (BCFAs) are important membrane components and precursors of signaling molecules. To investigate the role that PUFAs and BCFAs play in *C. elegans* dauer diapause, we suppressed the biosynthesis of these fatty acids by using RNA interference against *let-767*, a major 3-ketoacyl-CoA reductase, in dauer constitutive TGF-β mutants at 20°C. We found that *let-767* RNAi enhances the formation of dauers in *daf-7*(e1372) animals and this arrest can be overcome by feeding the animals with 2-arachydonoylglycerol (2-AG), an important endocannabinoid (eCB). In order to focus on the PUFA-depletion effect we constructed a *daf-7/fat-3* double mutant and found that this strain forms a higher percentage of dauer when compared with *daf-7*. Moreover, this strain can also overcome dauer arrest by 2-AG supplementation. We also show, using the HPLC-MS/MS technique, that this eCB is indeed synthesized by wild type worms, but not by the double mutant*daf7/fat-3*. These results indicate that 2-AG represents a signal that coordinates the status of long-chain fatty acid synthesis with metabolic changes that ultimately determine the decision between diapause and reproduction in *C. elegans*.

LI-P09

EXPLORING THE LINK BETWEEN BRANCHED CHAIN FATTY ACIDS AND ENDOCANNABINOIDS IN *Caenorhabditis elegans*

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We have previously described an experimental model in which reduction of *de novo* synthesis of long chain fatty acids by RNA-interference (RNAi) of *let-767* induces the entry of *C. elegans daf-7(e1372)* mutant strain into dauer state. More importantly, administration of exogenous endocannabinoids (ECs) in these conditions can induce the nematode to resume its reproductive cycle. Gas Chromatography–Mass Spectrometry analysis of the interfered worms shows a sharp reduction in the relative abundance of esterified branched chain fatty acids (BCFAs), and this lower level is maintained in samples treated with the EC 2-arachidonoyl-glycerol (2-AG), which reverses the dauer arrest. BCFAs are central in the regulation of TOR signaling pathway in the worm, a pathway connected with the processes of intestinal cell apical polarization, autophagy and fat accumulation, among others. We set out to explore the impact of *let-767* RNAi on these processes in the *daf-7(e1372)*background, and whether 2-AG treatment is capable of reversing any of these effects, in order to establish a link between EC treatment and the signals that determine reproductive growth in the worm.

LI-P10

SPHINGOSINE 1 PHOSPHATE RECEPTOR 2 REGULATES EPITHELIAL CELLS MONOLAYER INTEGRITY

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Sphingosine 1-Phophate (S1P) is a sphingolipid mediator involved in cellular fate that acts both extracellularly as ligand for cell surface receptors (S1PR), and intracellularly as second messenger. We have demonstrated that hypertonicity induces epithelial cell differentiation which depends on changes in sphingolipid metabolism. Epithelial cell differentiation needs a specific intracellular organization as well as the correct monolayer formation. This process requires an adequate removing of apoptotic cells followed by a rapid closes of empty spaces, thus preserving tissue permeability and integrity. This physiological process is known as cell extrusion and is related to S1P cellular effects. We found that in differentiated epithelial cultures cells, extrusion was dependent on S1P synthesis, the activation of S1PR2/Rho pathway and plasma membrane (PM) S1PR2 localization. To evaluate the importance of S1PR2 localization we obtained cells that overexpress S1PR2 with PM localization. In this condition, confluent cells (and non-differentiated) were extruded from the monolayer. Interestingly, the extrusion was independent of cell apoptosis and the cells were extruded as a dome, with lost of cell-matrix adhesion and the preservation cell-cell adhesion. These results show that SK/S1P pathway has multiple functions that not only dependent on the levels of S1P.

LI-P11

SPHINGOMYELIN SYNTHESIS MODULATES E-CADHERIN MATURATION AND RENAL EPITHELIAL CELL DIFFERENTIATION

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We have demonstrated that sphingomyelin (SM) biosynthesis is essential for hypertonicity-induced MDCK cell differentiation. Under inhibition of SM synthesis, MDCK cells instead to differentiate switch to mesenchymal phenotype thus performing an epithelial to mesenchymal transition (EMT). To study the sphingolipid metabolic pathway involved in such process, confluent MDCK cells were subjected to hypertonicity and concomitantly sphingomyelin synthase (SMS) was inhibited by pharmacological and knockdown strategies. Both strategies showed alteration of polarized phenotype with acquisition of mesenchymal phenotype. EMT can be initiated by the alteration of E-cadherin/beta-catenin/alpha-catenin complex. We observed that the inhibition of SM synthesis did not modify the total E-cadherin level. However, we observed an alteration in E-cadherin maturation. Particularly, D609 an SMS inhibitor and SMS1-siRNA induced an accumulation of the precursor of E-cadherin (with higher molecular weight) with a decrease of mature E-cadherin level. These results suggest that SM synthesis is involved in E-cadherin maturation and adherent junctions' formation.

LI-P12
THE ROLE OF XBP-1 IN OSMOTIC ACTIVATED-LIPID SYNTHESIS.

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It is known that hypertonicity induces an abrupt synthesis of several osmoprotective proteins such as urea transporters, COX2, AQP2, AQP3, among others, and organic osmolytes. It is also known that a massive protein synthesis could cause endoplasmic reticulum (ER) stress. Previously, we showed that hypertonicity activates the expression of ER stress markers in MDCK cells subjected to high NaCl concentrations, XBP-1 and CHOP, among them. The active form of XBP-1 is a transcription factor that activates the expression of lipogenic genes which, in turn, activate membrane biogenesis and ER stress alleviation. As hypertonicity significantly increases lipid synthesis in renal cells, in the present work we evaluated whether XBP-1 is involved in such response. To do that, prior to hypertonicity treatment, MDCK cultures were treated with XBP1siRNA. After 24 h of hypertonic treatment, the synthesis of lipids and the expression of key lipogenic enzymes were assayed. NaCl treatment, significantly increased the synthesis of both phospholipids (PL) and triglycerides (TAG); XBP1 silencing reduced the levels 1,2 DAG and TAG formed. This finding was consistent with the decrease in the levels of Lipin2 and DGAT2 mRNA. Interestingly, PL synthesis was not affected. These results clearly evidence a major role of XBP1 in the regulation of lipid synthesis in renal epithelial cells.*Both last authors.

LI-P13
Tl(I) AND Tl(III) INDUCE ALTERATIONS IN LIPID METABOLISM IN DIFFERENTIATED MDCK CELLS

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Thallium (Tl) is a toxic heavy metal that contaminates the environment and affects human health. Tl intoxication affects several organs and tissues, being the kidney a main target of Tl toxicity. However, the molecular mechanisms are still poorly understood. Tl has two oxidation states, the monovalent (Tl(I)) and trivalent (Tl(III)) cations. Since most heavy metals disturb cell lipid homeostasis, in the present work we studied if Tl may affect lipid metabolism in differentiated renal epithelial (MDCK) cells. Confluent MDCK cells were differentiated in hypertonic medium for 72 h and further incubated for 48 h in the absence or presence of Tl(I) or Tl(III) (10 or 100 µM). After incubation, cells were collected, counted and lipids were extracted. Chloroformic extracts were resolved by TLC; phospholipids (PLs), cholesterol (Cho) and triacylglycerides (TG) contents were analyzed. Both Tl(I) and Tl(III) significantly increased PLs and Cho. Accordingly, microscopy images showed morphological alterations in cells. Together, results could suggest an expansion of membranes. Also, Tl(I) and Tl(III) significantly increased TG content along with an increased LD´s size and number. Also, Tl(I) and Tl(III) increased endogenous lipids biosynthesis. Obtained results indicate that Tl-mediated damage would involve severe alterations in lipid metabolism. *Both have to be considered as last authors.

LI-P14
ETHER-LINKED LIPIDS OF RAT DEVELOPING AND ADULT EPIDIDYMIS

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In mammalian tissues, glycerophospholipids (GPL) and tri-glycerides (TG) occur in three sublcasses, depending on wheth-er the chain at the sn-1 position of the glycerol backbone is a fatty acid, a fatty alcohol, or a fatty aldehyde. This study fo-cused on the ether-linked (EL) subclasses of GPL and TG, fol-lowing their changes during postnatal development in rat epidi-dymis and, in the adult, their distribution among epididymal caput, corpus and cauda regions. At postnatal day 30, in a still scarcely differentiated epithelium lacking spermatozoa, the epididymis already contained plasmanyl- and plasmenyl-ethanolamine (Pls-Et) with 22:4n-9 (DTA), the DTA-containing plasmenyl-choline (Pls-Cho) increasing with development and the presence of sperm. In the adult tissue, the DTA-Pls-Et con-centration per mg protein was highest in the corpus and higher than that of DTA-Pls-Cho in the three epididymal regions, sug-gesting a precursor-product relationship. The latter subclass is in turn the one to increase the most in rat spermatozoa as they mature. The epididymal EL-TG were even richer in DTA than the GPL, the 1-alkyl- predominating over the 1-alk-enyl- sub-class at all ages, their concentration being highest in the corpus. These results correlated well with the expression (mRNA) of a key peroxisomal enzyme involved in the biosyn-thesis of these lipids, alkylglycerone phosphate synthase

LI-P15
DISRUPTION OF THE CYTOSKELETON AND ALTERED LIPID METABOLISM IN SERTOLI CELLS

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Previous work demonstrated that exposures to mild hyperthermia results in altered lipid metabolism in cultured Sertoli cells (SC), as evidenced by accumulation of lipid droplets (LD), and that these changes concur with the disruption of SC cytoskeleton. To further investigate the relationship between cytoskeleton disruption and lipid perturbations, in this study SC cultures were exposed at a constant temperature to nocodazole (NCZ), an antineoplastic agent known to interfere with the polymerization of microtubules. As previously did hyperthermia, the cytoskeletal disarrangement induced by exposure to NCZ was accompanied by a significant alteration of the mitochondrial potential, an increase in triacylglycerol levels, a considerable accumulation of LD, and a functional cell impairment manifested in reduced expression of the SC-specific protein transferrin. As also seen after hyperthermia, the effects of NCZ on all these alterations were reverted after ending the exposures. The time-course of the changes suggest that the cytoskeletal disruption could be the primary cause of the SC mitochondrial alterations, which in turn may respond for the lipid metabolic

alterations, suggestive of intracellular recycling of membrane lipid components. The cytoskeleton is thus functionally relevant in maintaining the viability and survival of SC during metabolically adverse or stressful situations

LI-P16
DECREASED OXLDL UPTAKE AND CHOLESTEROL EFflUX IN THP1 CELLS ELICITED BY CORTISOL

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Data about glucocorticoids role in the development of atherosclerosis are controversial showing different effects in human than in experimental animal models. Atherosclerosis is the result of a chronic inflammatory response to an injured endothelium where an uncontrolled uptake of OxLDL (oxidized low density lipoprotein) by macrophages triggers the development of foam cells, the main component of fatty streaks in atherosclerotic plaque. There are few data about the direct effect of glucocorticoids in macrophages of atherosclerotic plaque. The aim of the study was to elucidate the role of glucocorticoids in the development of foam cells in atherosclerosis initiation. For this purpose we used THP1 cells differentiated to macrophages with phorbol esters and incubated with OxLDL alone or with cortisol or cortisone. Ours results showed that cortisol and cortisone decreased significantly the inflammation promoted by OxLDL, and also diminished the expression of genes involved in influx and efflux of cholesterol resulting in a reduced lipid accumulation. Our results indicate a direct effect of glucocorticoids on macrophages braking atherosclerosis initiation, reducing pro-inflammatory markers and OxLDL uptake and cholesterol re-esterification, but also inhibiting cholesterol output. These effects appear to be mediated, at least in part, by 11bHSD1 activity.

LI-P17
MOLECULAR CONSEQUENCES OF GPAT2 KNOCK-DOWN IN BREAST CANCER CELLS

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GPAT2, a glycerol-3P-acyltransferase isoform, is mainly expressed in pachytene spermatocytes and also highly expressed in certain tumor cells and tissues. We showed that GPAT2 expression promotes the tumorigenic phenotype of MDA-MB-231 cells, as its expression correlates to higher proliferation rate, lower apoptosis, and increased tumorigenic behavior, among others. To determine which genes and molecular pathways could be modified by GPAT2 in MDA-MB-231 cells we performed a transcriptomic analysis using an Agilent SurePrint G3 Human Gene Expression 8x60K v2 Microarray of GPAT2 silenced and control MDA cells. After filtering off for a FC>2 and p value<0.01, we found 616 differentially expressed genes (DEG; 326 up- and 290 down-regulated). Their functional enrichment analysis showed several molecular pathways and cellular processes affected by GPAT2 silencing. We focused on cancer-related pathways, particularly on WNT/Ca^{2+}, which is controversially considered as a tumor inductor or a tumor suppressor pathway, depending on the tissue and other conditions. In our model, several genes belonging to this pathway were significantly down-regulated upon silencing, ascribing to the Wnt/Ca2+ signaling the role of inductor rather than tumor suppressor. Further studies are needed to connect GPAT2 and WNT/Ca^{2+}, and to establish the impact of GPAT2 silencing on other cancer related genes.

LI-P18
STUDIES ON THE MOLECULAR CLOCK AND THE CIRCADIAN REGULATION OF HEPATIC TUMORAL CELL METABOLISM

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The circadian system comprising oscillators present in organs, tissues and even in individual cells temporally controls the body physiology. At the molecular level the “clock genes” (CGs) regulate their own expression and that of others “clock controlled genes” (CCGs). The clock liver controls the metabolism under a circadian base depending on feeding times; however, it is still unknown how liver works under a malignant growth. The aim of this project was to investigate the molecular clock work and the regulation of genes involved in the lipid metabolism in cultures of the human hepatoma cell line HepG2. We performed this study under two proliferative states, partial arrest and proliferation, maintaining cells with 0 or 5% of serum respectively after synchronization with dexamethasone (100 nM). We analyzed the expression of CGs (*Bmal, Per, Cry*), CCGs (*Rev-erb*) and the main enzymes involved in the glycerophospholipids (GPLs) biosynthesis (*Chk, Pcyt*) by qPCR. All genes assessed except *Chkα* showed oscillations under proliferation while no differences were observed in arrested cells except for *Pcyt2*. When we studied the endogenous content of GPLs in proliferation, we observed significant variations in the global and individual GPL levels at different times. These studies suggest that an active circadian control of metabolism takes place in proliferating HepG2 cells

LI-P19
NEUTRAL LIPIDS ARE INDUCED IN THE APPLE SNAIL *Pomacea canaliculata* BY CYPERMETHRIN PESTICIDE

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Lipids are involved in important cellular processes, they are essential for energy metabolism and their fate in organisms is regulated by environmental conditions. Lipid homeostasis could be altered in aquatic organisms exposed to stressors caused by anthropogenic

activities. Agricultural practices require the use of pesticides that also affect non- target organisms. The aim of this study was to determine whether the pesticide cypermethrin (CYP) alters lipid homeostasis. For this purpose, the freshwater snail *Pomacea canaliculata* was chosen as a bioassay model. Adult snails were exposed to sublethal concentrations of CYP and lipids from digestive gland (DG) were studied. Neutral lipids (NL) were stained with BODIPY 493/503 and nuclei with DAPI. Lipids were also characterized and quantified. In DG, NL were organized and stored as lipid droplets (LD) located mainly in cytosol. Besides, in nuclei there was a small LD population. Snail exposure to CYP presented cellular phenotype modification in DG due to an increase in TAG content and number and size of cytosolic LD. In conclusion, lipid metabolism in DG of *P. canaliculata* was altered by CYP at molecular and cellular levels. This is the first description of nuclear LD in mollusks. Histological analysis of neutral lipids could be proposed as a tool in environmental risk assessment since it is a relatively rapid and sensitive method.

LI-P20
EMERGING ROLES OF PHOSPHOLIPASES D IN RETINAL PIGMENT EPITHELIUM CELLS EXPOSED TO HIGH GLUCOSE

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Diabetic retinopathy (DR) is a serious complication of diabetes that can lead to blindness. The aim of this work was to study the role of classical phospholipases D (PLD1 and PLD2) in retinal pigment epithelium (RPE) cells exposed to an in vitro DR model. ARPE-19 cells were exposed to high glucose (16.5 and 33 mM, HG) or to normal glucose (5.5mM, NG) concentrations for 4 and 72 h. After sustained HG (72h) treatment, RPE cell viability was reduced by 30% with respect to NG and reactive oxygen species (ROS) generation was increased 5 times with respect to NG, in accordance with a decreased peroxiredoxin (PRX) expression. Western blot and immunocytochemistry assays showed activation of the extracellular signal-regulated kinase (ERK) and nuclear factor-κB (NF-κB) translocation to the nucleus after 4 h HG exposure. After 4 h of incubation, PLD activity was increased by 80% in cells exposed to HG. The pre-incubation with selective inhibitors showed that ERK and NF-κB activation was dependent on PLD1 and PLD2 activities. Moreover, PLD1, PLD2, ERK and cyclooxygenase-2 (COX-2) inhibition partially restored the reduced cell viability induced by HG. Our results show that subsequent activation of PLDs, ERK and COX-2 mediates RPE cell damage during HG condition and point to the use of classical PLDs as new therapeutic targets for DR treatment.

LI-P21
PLD1-PKCε PATHWAY PROTECTS FROM LPS-INDUCED CELL DAMAGE IN RETINAL PIGMENT EPITHELIUM CELLS

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Inflammation is a key factor in the pathogenesis of several retinal diseases. In view of the essential role of the retinal pigment epithelium (RPE) in visual function, elucidating the molecular mechanisms elicited by inflammation in this tissue could provide new insights for the treatment of retinal diseases. The aim of the present work was to study protein kinase C (PKC) signaling and its modulation by phospholipases D (PLD) in ARPE-19 RPE cells exposed to lipopolysaccharide (LPS). Western blot, immunocytochemistry and confocal microscopy assays showed that LPS induces activation of PKCα and ε. The pre-incubation with selective PLD inhibitors (EVJ and APV) demonstrated that PKCα activation depends on both classical PLDs (PLD1 and PLD2) while PKCε activation depends only on PLD1. The inhibition of PKCα and β with Go6976 did not modify the reduced cell viability induced by LPS. On the contrary, the inhibition of PKCα, β and ε with Ro31-8220 potentiated the decrease in cell viability triggered by LPS, suggesting that PKCε mediates cell survival. Moreover, inhibition of PKCε reduced Bcl-2 expression and Akt activation and increased cleaved Caspase-3, both in cells treated or not with LPS. Our results demonstrate that PLD1 through PKCε regulation protects RPE from LPS-induced damage.

LI-P22
UNSATURATED FATTY ACID BIOSYNTHESIS IN *Mycobacterium smegmatis*

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The *Mycobacterium smegmatis* genome encodes several putative fatty acid desaturases, one of which (MSMEG_1886) is highly homologous to the only fatty acid desaturase present in*Mycobacterium tuberculosis*. We previously showed that a*Mycobacterium smegmatis* strain in which MSMEG_1886 was deleted was able to grow in the absence of exogenous unsaturated fatty acids albeit displaying a long lag phase and colony morphology changes. Due to its growth features and considering that over-expression of none of the remaining putative desaturases was able to correct this phenotype casting doubts on their true function, we searched the genome for other alternative pathways for unsaturated fatty acid synthesis. An operon (MSMEG_1741-3) homologous to the *Pseudomonas aeuriginosa des*BCT operon (having phospholipid acyl desaturase activity) was detected suggesting that it may be responsible for the growth of the ΔSMEG_1886. Here we report that deletion of MSMEG_1741-3 in a ΔSMEG_1886 background made the double mutant dependant on the addition of oleic acid for growth. Simultaneously desaturation of exogenously added 14C stearic acid was lost reinforcing our hypothesis. Interestingly, a ΔMSMEG_1741 ΔMSMEG_1886 double mutant strain also prevented the long lag phase observed in this single mutant, suggesting that loss of the putative repressor relieved the circuit leading to acyl lipid desaturation.

LI-P23
PHYLOGENETIC ANALYSIS OF FATTY ACID DESATURASES REVEALS CONTRASTING EVOLUTIONARY CLUES IN CILIATES

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The Integral Membrane Diiron Enzymes (IMDE) are a class of binuclear non-heme iron proteins widely distributed among prokaryotes and eukaryotes. They are characterized by a conserved tripartite motif consisting of eight to ten histidines. Their key function is the activation of the dioxygen moiety to serve as efficient catalysts for reactions of hydroxylation, desaturation or reduction. To date most studies on IMDE were carried out in metazoan, phototrophic or parasitic organisms, whereas genome-wide analysis in heterotrophic free living protozoa, such as the Ciliophora phylum, has not been undertaken. Our genome wide approach retrieved 91 putative sequences of the IMDE type with large differences in number among the ciliates: 7 in *I. multifiliis*, 13 in *O. trifallax*, 18 in *S. lemnae*, 24 in *T. thermophila* and 30 in *P. tetraurelia*. Interestingly, the analysis of the FA desaturases family showed that the spirotrichs *O. frifallax* and *S. lemnae* have no genes that encode for any of these proteins, suggesting that their lipids derive from algae like *C. reinhardtii*, which is used as food in their cultures. Noteworthy the lipid content of *O. frifallax*measured by GC–MS revealed a quantitative composition in saturated and unsaturated fatty acids that was significantly different from the algae, thus indicating re-routing, modification and regulation of precursors not previously describe

LI-P24
ENDOCANNABINOID METABOLISM IN ROD OUTER SEGMENTS DEPENDS ON THE ILLUMINATION STATE OF THE RETINA

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The aim of the present research was to determine how the synthesis and hydrolysis of endocannabinoid 2-arachidonoylglycerol (2-AG) are modulated by the illumination state of the retina. To this end, lysophosphatidate phosphohydrolase (LPAPase) and monoacylglycerol lipase (MAGL) activities were evaluated in purified rod outer segments (ROS) obtained from dark-adapted retinas or light-adapted retinas. Retinas were dissected from the eyes after dark or light adaptation. Dark-adapted bovine ROS (DROS) and bleached ROS (BLROS) were purified by a discontinuous gradient of sucrose from retinas whose optic cup was maintained under dim red light or exposed to light (288 candelas/m^3, 3000 luxes) for 30 min. LPAPase and MAGL activities were evaluated using [3H]oleoyl-lysophosphatidic acid and [^{3}H]Glycerol-MAG as substrates, respectively. When [^{3}H]-LPA was used, [^{3}H]-MAG and [^{3}H]-oleoyl were produced and both were stimulated by 150 % in BLROS. However, MAGL activity determined by [^{3}H]-Glycerol production was not modified in BLROS. These results suggest a distinct effect exerted by light on endocannabinoid 2-AG metabolism, thus indicating a potential role of this lipid in phototransduction.

LI-P25
EXPRESSION ANALYSIS OF CYTOCHROME P450 GENES IN PYRETHROID-RESISTANT *Triatoma infestans*

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The blood-sucking bug *Triatoma infestans* (Hemiptera, Reduviidae) is the most widespread and relevant vector of Chagas disease in the southern region of South America. Although chemical insecticides have been a successful vector control strategy, a growing number of deltamethrin resistance foci are being documented. Among genes involved in detoxification processes in insects, cytochrome P450 monooxygenases (CYP) are often associated with relevant roles in metabolic resistance. Using the *Rhodnius prolixus*genome information available, we characterized 33 CYP genes belonging to clans 2, 3, 4, and mitochondrial in the integument of *T. infestans*. Five genes belonging to clan 3 and four genes from clan 4 were up-regulated in deltamethrin-resistant fifth-instar nymphs compared with deltamethrin-susceptible specimen, as measured by qPCR. These results suggest that induction of specific CYP genes might be responsible, at least in part, for the high levels of deltamethrin resistance detected in*T. infestans*.

LI-P26
THE CORRECT LOCALIZATION OF PTEN IN EPITHELIAL CELLS DEPENDS ON GLYCOSPHINGOLIPID METABOLISM

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We have previously demonstrated that MDCK cells develop a polarized phenotype when they are subjected to hypertonic medium, and we found that glycosphingolipid synthesis is essential in this process. Polarized cells show two separated membrane domains, apical and basolateral, with different protein and lipid composition. The localization of PTEN, a key enzyme in the metabolism of the polyphosphoinositides, is important in the polarity of epithelial cells. In this study we analyzed the distribution of PTEN in MDCK cells in different experimental conditions. We found that non-differentiated cells cultured under isotonicity showed cytoplasmic localization of PTEN, but when the cells were subjected to hypertonicity developed a mature membrane with apical accumulation of the apical marker gp135 and PTEN concentrated in this membrane domain with a dot – like distribution. Interestingly, when the cells were incubated under hypertonicity in the presence of D-PDMP, an inhibitor of glucosylceramide synthase, cells did not develop the polarized phenotype and PTEN was drastically misslocalized. We previously showed that hypertonicity induces an increase in glucosylceramide synthesis. These results suggest that glycosphingolipids are necessary for the correct localization of PTEN and indicate an important crosstalk between sphingolipid and polyphosphoinositides metabolism.

LI-P27

HEPATIC CES3/TGH IS DOWNREGULATED IN THE EARLY STAGES OF LIVER CANCER DEVELOPMENT IN THE RAT

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Cancer development is associated with metabolic changes. We have previously established a model of hepatic preneoplasia in which adult rats were subjected to a 2-phase model of hepatocarcinogenesis (initiated-promoted, IP) for 6 weeks until they develop altered hepatic foci (AHF). Objectives: to study lipid metabolism and the role of carboxylesterase 3/triacylglycerol hydrolase (Ces3/Tgh) in the early stages of liver cancer development. Results: we found that a whole metabolic shift occurs in order to favor cancer development. IP animals presented with increased plasma lipids due to increased VLDL secretion as well as increased liver lipid accretion due to stimulated transacetylase activity rather than lipogenesis, compared to control rats. Ces3/Tgh presented with a perilobular distribution surrounding lipid droplets in normal livers. It is downregulated both at the protein and mRNA level in liver homogenates and is almost undetectable inside the AHF with no changes in the surrounding tissue. Ces3/Tgh expression is regulated by ω-3 fatty acids, thus, supplementation of diet with fish oil, allowed the restoration of Ces3/Tgh expression inside the foci and, more interestingly, led to the decrease in number and volume of the AHF. Conclusions: these studies show a preventive role of Ces3/Tgh in liver cancer development.

LI-P28

GLUCOSE METABOLISM IN SKELETAL MUSCLE OF RATS FED A FUNCTIONAL MILK FAT AT HIGH LEVELS

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Rumenic acid (RA) could improve the glucose (Glc) uptake and utilization preventing insulin resistance. As dairy products are the major source of RA, its content in milk fat has been increased altering the diet of dairy cows. Since, skeletal muscle is the major tissue in Glc utilization our aim was to investigate parameters related to Glc metabolism in gastrocnemius muscle (GM) of rats fed high fat diets containing a functional milk fat. Male Wistar rats were fed (60-d) with high fat diets containing 7% or 30% of soybean oil (S7 and S30) and 30% of milk fat (M30), functional milk fat (F30) or standardized milk fat (E30). Serum insulin levels, Glc and triacylglycerol (TAG) in serum and GM, and key metabolites levels and enzymes activities from Glc metabolism in GM were assessed. Statistical differences ($p<0.05$) were tested by T test (S7 vs.S30) and ANOVA. Compared to S7 diet, S30 increased serum insulin and Glc and TAG content in GM in parallel with high citrate levels and phosphofructokinase-1 (PFK1) inhibition. This could suggest a lower Glc utilization. In contrast, compared with S30 PFK1 activity increased in all milk fats diets but the flux through PFK1 was higher in F30. Pyruvate levels were lower in F30 and E30 suggesting a higher metabolization rate. Thus, the Glc metabolism in GM of rats fed high fat diets depends on the type of fat and the involved mechanisms are different.

LI-P29

EFFECT OF CONJUGATED LINOLEIC ACID AND HIGH FAT DIETS ON TRIACYLGLYCEROL METABOLISM IN RAT OFFSPRING

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The aim was to investigate the potential beneficial effects of maternal CLA consumption at high fat (HF) diet on Triacylglycerol (TAG) metabolism in adult male rat offspring. Female Wistar rats were fed diets containing recommended (7%) or HF (20%) levels supplemented or not with CLA (C7, C20 and CLA20) during 4 weeks before and throughout pregnancy and lactation. Male offspring of C7, C20 and CLA20 rats were fed with the same diets of their mothers (C7/C7, C20/C20 and CLA20/CLA20 groups), while other group of the offspring of CLA20 were fed C20 diet (CLA20/C20) for 9 weeks. Serum (S) and hepatic (H) TAG levels, H-TAG secretion rate (TAG-SR), adipose tissue (AT) and muscle (M) LPL activity and lipogenic (FAS, ACC, G6PDH) and oxidative (CPT1) activity and expression were measured. Comparing with C7/C7, C20/C20 rats showed a higher H-TAG (57%) associated with a lower TAG-SR (-29%). In addition, S-TAG (-17%), AT-LPL activity (-37%), H-lipogenic enzyme activities and expressions were reduced. CLA20/C20 rats tended to normalize S-TAG and H-TAG levels associated with a normal TAG-SR. Adult offspring consuming CLA at HF levels (CLA20/CLA20) restored the S-TAG and H-TAG levels in parallel to normal TAG-SR and improved AT-LPL activity. To conclude, maternal CLA consumption metabolically may contribute to prevent or attenuate the different TAG alterations of adult offspring at HF levels.

LI-P30

PHOSPHATIDIC ACID SIGNALING PARTICIPATES IN THE NEURODEGENERATION INDUCED BY α-SYNUCLEIN

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Pathological accumulation of α-synuclein (α-syn) is a hallmark of Parkinson's disease. Even though the physiological function of this protein is still unknown, it is well accepted that its aggregation prompts degeneration and death in dopaminergic neurons. One intriguing characteristic of α-syn is its lipid binding affinity. We have previously reported that overexpression of α-syn triggers an

increase in neutral lipid and fatty acid content (SAIB 2014-2015) in dopaminergic neurons. In this work, we investigated the state of phosphatidic acid (PA) signaling in human neurons overexpressing α-syn. Specifically, we studied the state of phospholipase D (PLD) pathway that catalyzes PA generation by phosphatidylcholine hydrolysis. We detected diminished PLD1 expression and ERK phosphorylation in α-syn neurons. Overexpression of α-syn inhibited ERK nuclear localization and the expression of the neuronal marker neurofilament (NF). PLD1 pharmacological inhibition (EVJ) demonstrated that both ERK nuclear localization and NF expression were dependent on this pathway. Enhancers of α-syn toxicity such as copper overload and 6-hydroxydopamine also displayed differential regulation of PLD1 expression. Our results demonstrate that α-syn accumulation promotes neurodegeneration through the inhibition of PLD1 pathway, thus affecting ERK signaling and NF expression. Sponsored by FONCyT-CONICET-UNS

LI-P31

Halamphora coffeaeformis: A SOURCE OF LIPIDS FOR BIODIESEL AND VALUE-ADDED CO-PRODUCTS

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Recent studies have indicated that the sustainable production of biodiesel from microalgae requires the use of a biorefinery approach. In the present work, *Halamphora coffeaeformis*, a marine benthic diatom native from Bahía Blanca Estuary, was cultured in order to evaluate the simultaneous production of triacylglycerols (TAG) for biodiesel and value-added co-products. For this end, the species was grown in a raceway pond in f/2 medium without the addition of vitamins. Total lipid content was 25.2% (dry weight (dw)) and neutral lipids represented the 86%. Coincidently, TAG content was 17% (dw). Palmitoleic (26%), eicosapentanoic (23.6%) and palmitic (16.9%) fatty acids were the main components of the neutral lipid fraction, suggesting that *H. coffeaeformis* oil meets biodiesel standards. In addition, phytosterols were present at 0.22% (dw) while proteins represented 18.5% (dw). Finally, as the cell wall of *H. coffeaeformis* contains mainly silica, its amount was analyzed in the residue obtained after the lipid extraction. Results revealed that *H. coffeaeformis* contains 25% (dw) of silica. These findings provide baseline information and are the starting point for future scaling-up of this species under a biorefinery approach.

LI-P032

HYDROCARBON ASSIMILATION IN EUROTIALEAN AND HYPOCREALEAN FUNGI: ROLES FOR CYP52 AND CYP53 GENES

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Several filamentous fungi are able to concomitantly assimilate both aliphatic and polycyclic aromatic hydrocarbons. Cytochrome P450 monooxygenases catalyze the first oxidation reaction for both types of substrate. Among the cytochrome P450 (CYP) genes, the family CYP52 has been regarded as the first hydroxylation step in alkane-assimilation processes, while genes belonging to the family CYP53 have been linked with oxidation of aromatic hydrocarbons. We performed a comparative analysis of CYP genes belonging to CYP52 and CYP53 families in *Aspergillus niger*, *Penicillium chrysogenum*,*Beauveria bassiana* and *Metarhizium anisopliae*. These species were able to assimilate both types of hydrocarbons, exhibiting a species-dependent modification in pH during this process. By modeling the molecular docking to the active site, we showed that both types of substrates are energetically favored for their binding to enzymes codified by fungal genes belonging to both CYP52 and CYP53 families. Gene expression analyses revealed that CYP53 members are highly induced by phenanthrene, but no induction was observed with *n*-alkanes. These findings suggest that the set of P450 enzymes involved is dependent on phylogeny, and reveal overlapping role but distinct substrate and expression specificities.

LI-P33

GANGLIOSIDE SYNTHESIS BY PLASMA MEMBRANE-ASSOCIATED ECTOSIALYLTRANSFERASE IN MACROPHAGES

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A number of enzymes of ganglioside metabolism have been shown to be associated with the PM. In particular, it was observed in epithelial and melanoma cells that ecto-ST8Sia-I synthetize GD3 at the PM (cis-catalytic activity) and displayed enzymatic activity in PM of neighboring cells (trans-catalytic activity). Here, by biochemical and fluorescent microscopy approaches we extended these investigations to MØ and found that endogenous ecto-ST8Sia-I is present in Golgi complex as well as in PM. In addition, ecto-ST8Sia-I displayed cis-catalytic activity both in LPS-stimulated MØ and unstimulated conditions. Interestingly, LPS stimulation also reduced the ST8Sia-I levels at the PM. Besides, co-treatment of LPS with a NOs inhibitor recovered the ST8Sia-I levels, suggesting that NO formation is involved in the expression/localization of this enzyme at the PM. The enzyme levels correlated with a reduction of GD3 and GM1 and with an increment of GD1a. Moreover, the NO levels from LPS-stimulated MØ were higher in cells previously treated than in cells untreated with an inhibitor of glycolipid synthesis. The data further support the presence and activity of ecto-sialyltransferases at the PM. The variations of ecto-ST8Sia-I and gangliosides in stimulated macrophages provide a promissory information to further explore the role of this and others ganglioside metabolism-related enzymes at the PM.

LI-P34
LIPID METABOLISM IN CANCER CELLS: ROLE OF FABP5

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Cancer cells show a metabolic reprograming towards macromolecular synthesis, required for cellular growth. Fatty acid-binding proteins (FABPs) are intracellular proteins that bind hydrophobic ligands with high affinity. It has been proposed that FABPs could transport theirs ligands to the different compartments within the cell. FABP5 is overexpressed in several types of cancers, but its function in tumor metabolism is still unknown. We found that glucose and exogenous fatty acids alter FABP5 expression in A549 human lung adenocarcinoma cell. In addition, chemical inhibition of de novo fatty acid synthesis decreased FABP5 expression and cellular proliferation. Moreover, the siRNA-mediated reduction of FABP5 in A549 cells impaired fatty acid uptake and proliferation compared to non targeting control siRNA-transfected cells. The distribution of exogenous fatty acids among different lipid species was, however, unchanged. Thus, our results suggest that FABP5 expression is modulated by nutritional factors / metabolic conditions and it is essential for fatty acid uptake and proliferation in human lung cancer cells.

LI-P35
CELLULAR FATE OF PHOSPHOLIPIDS (PL) SYNTHESIZED DURING G2/M

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Cell division ends with the reassemble of the nuclear envelope and separation of daughter cells, process that requires dynamic changes in the distribution and composition of membrane PL. We previously found that lysoPL levels decrease drastically from G2/M to G1 phase, while de novo phosphatidylcholine synthesis increases, suggesting an enhanced membrane production concomitant with a decrease in its turnover. In addition, fatty acid synthesis and incorporation into membranes increases upon cell division. Thus, our aim is to study the cellular fate of synthesized PL during G2/M. We synchronized HeLa cells to study lipid synthesis during G2/M. Metabolic labeling with 14C-acetate and separation of subcellular fractions with a sucrose gradient allowed us to determine the distribution of incorporated label into lipids during cell division. We also used the choline analog propargylcholine (p-Cho) to assess the fate of newly synthesized PL in G2/M. The resulting labeled PL molecules were visualized via a reaction with an azide-Alexa488. Incorporation of 14C-acetate and p-Cho labeling showed that lipids synthesized during G2/M localized in a region compatible with the nucleus and endoplasmic reticulum. These studies will contribute to the knowledge of the metabolic requirements during the cell cycle and could have implications for the treatment and diagnosis of hyperproliferative diseases.

LI-P36
STUDY OF THE INTERACTION OF L-PHE WITH LIPID MEMBRANES AND ITS IMPLICATIONS IN BIOLOGICAL MEMBRANES

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L-phenylalanine (Phe) has shown to be capable of forming amyloid-like nanofibrillar structures under pathologically relevant concentrations associated with a diverse group of diseases such as Alzheimer's, type II diabetes and prion disorders. Moreover, L-phe was able to produce damage to thylakoid membranes and induce solute leakage and membrane fusion under stress conditions. These effects have been associated with lipid-amino acid interactions. In order to understand more about this process the effect of the interaction of L-Phe on different biomimetic systems such as phosphocholine monolayers and liposomes was studied. The data obtained show that the amino acid would be inserted into membrane increasing the distance between the lipid molecules, reducing the organization of dipoles in the membrane and creating a lower total dipole potential. This effect seems to depend on pH and the presence of defect in the membranes.

MICROBIOLOGY

MI-P01
PROTEOME TURNOVER ANALYSIS IDENTIFIES PHYTOENE SYNTHASE AS A LON SUBSTRATE IN *Haloferax volcanii*

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In bacteria and eukaryotes energy-dependent Lon proteases (soluble enzymes, LonA) are involved in protein quality control and in the degradation of specific proteins controlling diverse cellular functions. The archaeal version of Lon (LonB) is unusually membrane-associated and its physiological role and natural substrates remain unknown. We have demonstrated that LonB is an essential protease in the archaeon *Haloferax volcanii* and suboptimal levels of this enzyme affect growth rate, cell shape, lipid composition and cell pigmentation. In this study we examined the proteome turnover of a *H. volcanii* conditional LonB mutant under suboptimal (- trp) and physiological (+ trp) protease levels to discover its natural substrates. To this end, heavy isotope metabolic labeling coupled to tandem mass spectrometry was applied. The overall protein synthesis and degradation rates as well as the turnover of several specific proteins showed significant differences (p-value ≤ 0.05) in response to changes in Lon expression. Based on these results we identified several potential LonB substrates including phytoene synthase, an enzyme that controls a rate-limiting step in the carotenoid biosynthesis

pathway. This is the first study that examines the proteome turnover in an archaeon as well as the first report of a natural target of the archaeal membrane-bound Lon protease.

MI-P02
IDENTIFICATION OF POTENTIAL TARGETS OF PROTEASE RhoII IN*Haloferax volcanii*

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Rhomboids are membrane serine proteases present in the three Domains of Life. These enzymes are implicated in gene regulation and cellular signalling, however, nothing is known about their targets in Archaea. In*Haloferax volcanii*a deletion strain of*rhoII*(MIG1) showed reduced motility, increased sensitivity to novobiocin, impaired recovery after UV irradiation, and a defect in glycosylation of the S-layer. To examine the impact of *rhoII* deletion on*H. volcanii*proteome, a differential proteomics approach was used to compare membrane and cytosol fractions of exponential and stationary phase cultures of MIG1 and the parental strain (H26). About 34% of the proteome was detected including 241 proteins that showed differential concentration between MIG1 and H26 (membrane-associated/secreted and soluble proteins). The former may be potential substrates of RhoII. Data analysis suggests halocyanin as a potential target as well as several differential proteins related to the phenotypes observed in MIG1 such as PibD (motility), DNA gyrase A (novobiocin sensitivity), RecA/B (UV repair) and glycosyltransferases (Agl12, AglE, HVO_2989). These results show that deletion of RhoII has a global effect on*H. volcanii*proteome and predict potential substrates which, after experimental validation, could provide insight on the role of rhomboids in Archaea. Funded by UNMdP, CONICET and ANPCYT.

MI-P03
EXPRESSION OF NOS FROM MARINE MICROORGANISMS IMPROVES GROWTH AND NITROGEN METABOLISM IN*E.coli*

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Nitric oxide synthase (NOS) catalyzes the biosynthesis of nitric oxide (NO), a signal molecule in diverse biological processes, through the oxidation of L-arginine. We have characterized the first NOS enzyme from the plant kingdom, belonging to the alga*Ostreococcus tauri*(OtNOS). Later, we identified a NOS in the cyanobacteria*Synechococcus*PCC7335 (SyNOS), with an extra N-terminal globin domain. In this work, we studied the SyNOS expression in *Synechococcus* and analyzed the impact of the heterologous SyNOS expression in*E. coli*. *Synechococcus* is able to produce NO and express SyNOS in normal culture conditions. The NO production is inhibited by the addition of the NOS inhibitor L-NAME.*E. coli*expressing either SyNOS or OtNOS showed an increased growth rate and viability, compared to bacteria transformed with the empty vector (EV). Furthermore, the recombinant bacteria are able to grow in media containing L-Arg as unique nitrogen source. Bacteria expressing SyNOS display higher growth rate, as well as levels of nitrites and nitrates, compared to bacteria expressing OtNOS and EV. The expression of the*hmp*is indicative of nitrosative stress.*hmp*is induced in bacteria expressing OtNOS but not SyNOS, suggesting that SyNOS is able to detoxify NO. The mechanism by which SyNOS detoxifies NO and displays higher growing rates is under investigation.

MI-P04
LOW LEVELS OF POLAR FLAGELLIN EXPRESSION IN MATURE BIOFILMS FROM*Azospirillum brasilense*

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*Azospirillum brasilense*is a PGPR. Biofilm formation on root surface is essential for successful colonization and to observe a beneficial effect on plant–*Azospirillum*association. The biofilm matrix is primarily composed of exopolysaccharides, extracellular DNA, and proteins, which are required for cell-cell and cell-surface interactions, and development of mature *Azospirilla*biofilms. Two cell-surface structures, the polar flagellum and TAD pili, have shown to be critical for initial attachment and biofilm formation. The aim of this work was to show the presence of polar flagella during*Azospirilla* development in biofilm production, as an approach to define the role of flagella in biofilm matrix construction. We used the quantitative western blot (WB) analysis and confocal laser scanning microscopy (CLSM). WB analysis of planktonic and biofilm grown cells indicated a decrease in polar flagellin expression profiles in either cells after 3 or 4 days of growth. Mature biofilm were analyzed by confocal microscopy with anti-flagellin antibody FITC tagged and DAPI stain. Under these conditions it was observed that, the flagella were localized at basal layers. However, once biofilm formation has been initiated, flagellin expression begins to decrease when biofilm was mature.

MI-P05
POLYPHOSPHATE ROLE IN*Gluconacetobacter diazotrophicus*ABIOTIC STRESS RESISTANCE AND BIOFILM FORMATION

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*Gluconacetobacter diazotrophicus*is a plant growth promoting bacterium able to colonize several plant species of economic importance. Its beneficial effects on plants are nitrogen-fixation, action against pathogens and mineral nutrient solubilization. The use of*G. diazotrophicus*as a biofertilizer requires a study of its physiological properties, including how this bacterium responds to the extracellular environment. We have previously shown that media phosphate (Pi) concentration modulate polyphosphate (polyP) levels in*Escherichia coli*cells affecting several cellular features. The aim of this work was to study the possible physiological changes in*G. diazotrophicus*Pal5 due to variations in growth media Pi concentration that may modulate intracellular polyP levels. Cells grown in

high Pi reached higher OD values than those grown in sufficient Pi. In high Pi media, the cells accumulate high polyP levels and degraded it in stationary phase. This feature was accompanied with the improvement in survival, resistance to different stress agents (NaCl, H_2O_2, Cu(II)salts and copper-related fungicides) and biofilm formation in abiotic and biotic surfaces. Our findings on the relationship between media Pi concentration, resistance, biofilm formation and polyP levels contribute to elucidate the importance of Pi or its metabolites as signals for survival and colonization in this bacterium.

MI-P06
BEHAVIOR OF *Bradyrhizobium* SEMIA6144 MEMBRANE DURING ADAPTATION TO WATER DEFICIT

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The membrane lipid bilayer is one of the primary cellular targets affected by variations in medium osmolarity, which cause changes in fluidity that may exert negative effects. We evaluate the behavior of the membrane of*Bradyrhizobium*SEMIA6144 during adaptation to polyethylene glycol (PEG). *B*. SEMIA6144 was exposed to growth and PEG shock. A fluidizing effect on the membrane was observed 10 min after shock; however, the bacteria were able to restore optimal fluidity. This rapid response (1 h) was mediated by an increase in lysophosphatidylethanolamine from phosphatidylethanolamine and an increase in saturated fatty acids, resulting in greater membrane rigidity. When the cells were exposed to shock for 5 h and 24 h, the amount of lysophosphatidylethanolamine decreased while phosphatidylethanolamine increased. This phospholipid turnover was associated with elevated phospholipase A activity after 1 h of shock. Membrane fluidity of cells grown with PEG was also equal to that of the control; however, the response was an increase in phosphatidylcholine at the expense of phosphatidylethanolamine, and an increase in levels of unsaturated fatty acids.We conclude that during PEG shock, the membrane composition of *B*. SEMIA6144 is rapidly remodeled to counter fluidizing effect; then, this response is dynamically modified by process that involves phospholipid and acyl chain turnover.

MI-P07
IMPACT OF ARSENIC IN *Bradyrhizobia* STRAINS AND IN THE SYMBIOTIC INTERACTION WITH PEANUT PLANT

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Arsenic (As) contamination constitutes a serious problem in the environment. The aim of this work was to evaluate As effects on the production of reactive oxygen species, the occurrence of oxidative damage and the activity of the antioxidant system in peanut microsymbionts and in the establishment of peanut-bradyrhizobia symbiotic interaction.*Bradyrhizobium*sp. viability test allowed to classify*B*. sp. SEMIA6144 as sensitive to As, whereas*B*. sp. C-145,*B*. sp. NOD31 and*B*. sp. NLH25 as tolerant. Then, the oxidative and antioxidant defense response were analyzed in an As-sensitive and As-tolerant strain (*B*. sp. SEMIA6144 and*B*. sp. C-145, respectively). The sensitive strain showed oxidative stress symptoms as evidenced by the increased lipid peroxidation, induced by H_2O_2content increment. Moreover, SOD and GPX activity increased by As treatment however, CAT, GR and GST activities were reduced. In the tolerant strain H_2O_2content was enhanced but not enough to induce oxidative stress. GST, CAT and GPX activities were unchanged whereas SOD and GR activities were reduced in presence of As. Inoculated peanut plants exposed to As showed a decreased ROS accumulation and increased GSH content and GST activity in roots. Thus, GSH and related enzymes play an important role in As detoxification of free living rhizobia and in the first step of the symbiotic interaction established with peanut

MI-P08
*Mesorhizobium loti*TYPE VI SECRETION SYSTEM, A RELEVANT PLAYER IN THE LOTUS NODULATION

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Endophytic bacteria have evolved several strategies in order to establish and maintain their interactions with plants. Among these, secretion systems display great versatility by selectively translocating proteins from the symbiont into the host. In the present work we characterize the Type Six Secretion System (T6SS) in the nitrogen-fixing bacteria*M. loti*and its relation with nodule formation. Promoter analysis of the T6SS cluster showed maximum activity in the late logarithmic and stationary growth phase, which correlates with the immunodetection of cytoplasmic Hemolysin Coregulated Protein (Hcp), a hallmark protein of the T6SS with both structural and effector function. Secretion of Hcp could not be detected *in vitro*. A deletion of the*mlr2342*gene which codes for the structural and essential T6SS element ImpG produced a strain with an impaired nodulation phenotype in comparison with the wild type. Additionally we have detected Hcp self interaction by pull down assays with purification tags, this validation enables further identification of secretion proteins which are known to interact with the Hcp hexameric structure. We have previously developed a computational method based on phylogenetic profiling in order to detect T3SS secreted proteins which we have modified and validated for the prediction of T6SS secreted proteins with a Area Under roc Curve (AUC) value of 0.93.

MI-P09
ANALYSIS OF FUNCTIONAL REDUNDANCY OF RIESKE SUBUNIT IN CYTOCHROME BC OF *Mesorhizobium loti*

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Nodule development in rhizobia-legume symbiosis leads to changes in both plant and bacteria as result of the interaction. Bacterial respiratory chain is modified allowing nitrogenase activity, being the cytochrome bc complex a key component of this chain. Its Rieske Fe/S subunit has a signal for TAT secretion system.*M. loti*has two Rieske subunits:*mll2707*and*mlr0970*. In this work we proposed to analyze if there are functional redundance for cytochrome bc. By fusion of promoter region to β-gal we only detected activity of 2707. However, under microoxid conditions, 0970 activity was also observed. Gene expression was determined by qPCR, both in*M. loti*wt and a TAT deficient strain (*tatC*). 2707 mRNA levels exceeded those of 0970. In microoxid conditions this relation was inverted, detecting higher levels of 0970 in both strains. In order to test protein stability, 2707 and 0970 were fused to a flag and constitutively expressed for*Western*detection. High levels of 2707 were observed in wt, both at high and low oxygen, and inside the nodule. However, it was absent in*tatC*, suggesting that a functional TAT system is required for the stability of the protein. 0970 was detected in*tatC*only at low oxygen. We have constructed mutants of*M. loti*in 2707, 0970 and double mutant. Their role in nodulation will be determined by inoculation of these strains in*Lotus tenuis*plants.

MI-P10
LIPID SIGNALLING IN RESPONSE TO HYDRIC DEFICIT IN*Azospirillum*-INOCULATED BARLEY SEEDLING

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Water is one of the main resources that limit world agricultural production. A variety of strategies has been used to improve the drought tolerance of crops. Plants modulate rapidly a series of physiological responses in order to economize water use, such as: stomata close, osmotic adjustment by accumulation of compatibles solutes and changes in foliage structure and root system characteristics. The associative rhizobacterium *Azospirillumbrasilense*has been extensively investigated. It is a well-known for its ability to promote root growth, as well as its capacity to mitigate salt, water and osmotic stress in different species of plants. The aim of this study was to investigate the response to drought and test the effect of*Az*39 to mitigate drought stress in tissues of barley. Hydric deficit treatment was imposed by reducing the amount of irrigation to 20 % of its field capacity. Several days after the hydric deficit, a significant decrease in biomass, water content and height was observed. Modification in chlorophyll content, proline and phospholipase D activity were also observed. To assess the capacity of*Az*39 to mitigate drought stress, different concentrations of*Az*39 on germination seedling barley were assayed. Changes in the physiological and biochemical parameters suggest that barley seedling inoculated with*Az*39 may be better prepared to prosper under drought stress.

MI-P11
VOLATILE COMPOUNDS FROM*Klebsiella oxytoca*KD70 PROMOTE GROWTH OF *Arabidopsis* SEEDLINGS

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Plant growth-promoting bacteria (PGPB) can enhance plant growth by direct or indirect mechanisms including release of volatile compounds (VCs), which constitutes an important long-distance signaling between bacteria and plants.In this study we evaluated the ability of a sugarcane endophytic bacterium,*Klebsiella oxytoca*Kd70, to promote growth of *Arabidopsis thaliana*seedlings by production of VCs. Disinfected seeds of*A. thaliana*sown on MS agar plates were exposed to agar plugs (5 mm^2), attached to the Petri dish lid, containing Kd70 bacteria grown on a tryptone-glucose saline medium. Parafilm-sealed dishes containing plant seedlings and bacteria were incubated under optimal plant growth conditions and the length of primary roots, number of secondary roots and total leaf area were determined at 4, 7, 10 and 15 days of growth.Our results show that seedlings exposed to bacterial VCs showed longer primary roots (4 days), greater number of secondary roots (10 days) and foliar development (15 days) than control plants grown without bacteria. These findings indicate that VCs produced by Kd70 stimulate plant growth and could be used as biofertilizers

MI-P12
ROLES OF RHIZOBIAL SURFACE COMPONENTS ON PROTECTION AGAINST ENVIRONMENTAL STRESSES

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Bacterial surface components and extracellular compounds, particularly flagella, lipopolysaccharides (LPSs), and exopolysaccharides (EPSs), in combination with environmental signals, play crucial roles in bacterial autoaggregation, biofilm development, survival, and host colonization. *Ensifer meliloti*, a model alphaproteobacteria, produces two symbiosis-promoting EPSs: succinoglycan (EPS I) and galactoglucan (EPS II). Studies of the *E. meliloti*/alfalfa symbiosis model system have revealed numerous biological functions of EPSs, including host specificity, participation in early stages of host plant infection, signaling molecule during plant development, and protection from environmental stresses. We evaluated functions of EPSs, LPSs and flagella, in bacterial resistance to heavy metals and metalloids, which are known to affect various biological processes. The results show that mutations in these *Rhizobial* surface components affect the normal development of resistance to *E. meliloti*. Specifically the synthesis of EPS II would be as a mechanism of resistance to exposure to toxic metals, probably through different effects such as trapping the metal outside the cells and/or formation of biofilm. Our observations demonstrate that surface components play major roles in rhizobacterial survival.

MI-P13
PYROSEQUENCING REVEALS CHANGES IN FUNGAL SOIL COMMUNITIES UNDER *Lotus tenuis* MONOCULTURE

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Flooding Pampa is the main region for the cattle production in Argentina. The soils from this area are mainly characterized chemical and physical limitations.*Lotus tenuis*is a naturalized forage legume in the Flooding Pampa able to grow under restrictive conditions, and its use is an alternative to increase the yields in beef production. "Promotion" is an agricultural practice consisting in removing competition from weeds and resident grass using herbicides, so the species of interest grows and fully develops, becoming the plant community into a*L. tenuis*monoculture grassland. Fungal communities could be affected by the change in the cover grass composition. Through sequencing by 454 FLX Roche technology of the internal transcribed spacer ITS1-ITS2, we studied the structure of fungal communities in different soils from flooding pampa, affected by this agronomical practice. Our results suggest that 6-7 years of application of this practice could modify the structure of fungal community evidenced by statistical differences between the structures of fungal community in soils affected by*L. tenuis*promotion compared with soils under natural grass cover. In addition, indicators reveal the presence of some fungi as the most representative taxa in this soil. Chemical variables like conductivity and Sodium are more related to the distribution of different fungi in this environment.

MI-P14
MutS-DEPENDENT REGULATION OF THE ERROR-PRONE Pol IV ACTION: DNA STRUCTURES AS KEY MODULATORS

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Translesion DNA polymerases (Pol) function in the bypass of template lesions to relieve stalled replication forks. Effective activity of these Pols requires association with ring-shaped processivity factors, which dictate their access to sites of DNA synthesis. We showed for the first time that the repair protein MutS regulates the access of the mutagenic Pol IV to replication sites. Our previous biochemical data revealed that MutS inhibits the Pol IV interaction with β clamp by competing for binding to a hydrophobic cleft on the ring. Here, we examined the effect of different DNA substrates (single-stranded, homoduplex, heteroduplex, primed and GT-primed DNAs) on the affinity of Pol IV– and MutS–β clamp interactions. Neither DNA substrates had a significant effect on the strength of the Pol IV–β clamp association. The presence of the GT-primed DNA enhanced the MutS–β clamp interaction and modified the MutS oligomeric state bound to β clamp. We also demonstrated that DNA structures modulate the effectiveness of MutS as a competitor for the Pol IV-β clamp interaction. MutS exerted a more strict control over Pol IV binding to β clamp in the presence of primed-DNAs whereas the single-stranded DNA abolished the capability of MutS for limiting the Pol IV-β clamp interaction. Thus, this work reveals that complex protein-DNA interactions are involved in this noncanonical function of MutS

MI-P15
A TWO-COMPONENT SYSTEM AFFECTS THE LOCALIZATION OF A DIVISOME PROTEIN IN *Streptococcus pneumoniae*

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In bacteria, cell division is carried out by more than 15 proteins which assemble at midcell and constitute a complex named divisome. It has been described that signal transduction systems known as two-component systems (TCS), are involved in the coordination of cell wall biosynthesis and cell division mechanism. To determine the putative contribution of pneumococcal TCS in the localization of divisome proteins, we performed a screening of 12 histidine kinase (hk) mutants of *S. pneumoniae* and we determined the localization of FtsZ, FtsA, EzrA and StkP. For this purpose, we cloned and fused the *ftsZ*, *ftsA*, *ezrA* and *stkP* genes with the *gfp* gene in a replicative vector for *S. pneumoniae*. According to previous reports, the FtsZ-GFP, FtsA-GFP, EzrA-GFP and StkP-GFP fusion proteins showed normal localization in the wild-type strain. In particular, we analyzed an hk mutant, here referred as hk-cd mutant, which showed a typical localization patterns of divisome proteins as did in the wild-type strain, with the exception of the StkP-GFP protein fusion. Approximately a 22% of the hk-cd cells showed an elongated morphology and an altered StkP-GFP localization with multiple septum. These results suggested that this TCS is involved in the cell division mechanism and contribute to the localization of StkP, one of the main coordinators of the cell division mechanism of *S. pneumoniae*.

MI-P16
AN ALLOSTERIC TRIGGER IN THE REGULATION OF ADP-GLUCOSE PYROPHOSPHORYLASE FROM ACTINOBACTERIA

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ADP-glucose pyrophosphorylase (EC 2.7.7.27, ADPGlcPPase) is an allosteric enzyme regulated by metabolites from the main carbon route in the organism. Previously, a motive present in all ADPGlcPPase containing Q and W specific residues was proposed as an allosteric trigger. We found that the ADPGlcPPase from actinobacteria is regulated by numerous effectors, although little is known about molecular aspects regarding the allosterism in this enzyme. Thus, we produced mutants in the allosteric trigger from the*Streptomyces coelicolor*(Q62A and W99A) and*Rhodococcus jostii*(Q61A and W98A) ADPGlcPPases. As an example, the Q62A mutant lost 12- and 19-fold activation for PEP and fructose-6P respectively, compared to the wild type enzyme. Importantly, this

mutant has 23-fold less activation for glucose-6P, the main activator in the actinobacterial ADPGlcPPase. As well, the W99A mutant decreased 28-fold its activation by glucose-6P, compared to the wild type enzyme and behaved similarly to the Q62A mutant regarding PEP and fructose-6P activation. Results confirm the importance of the allosteric trigger in the regulation of this enzyme and could be helpful to elucidate a regulatory/allosteric model for the multi-regulated actinobacterial ADPGlcPPase.

MI-P17
STUDY OF EUKARYOTE-LIKE ACETYL-CoA CARBOXYLASES OF ACTINOBACTERIA

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Acetyl-CoA carboxylases (ACCs) catalyze the biotin dependent carboxylation of acetyl-CoA to give malonyl-CoA. These enzymes have a crucial role in fatty acid metabolism and in *Actinobacteria* these enzymes also play a key role in polyketides biosynthesis. ACCs are composed of three domains, which can be located in separate protein subunits as in most bacteria or be part of a multi-domain protein as in eukaryotes. To date there are no reports of the existence or characterization of eukaryotic-like ACC from bacteria. However there is evidence of the presence of genes encoding these multidomain proteins in *Actinobacteria*. Bioinformatic analysis allowed us to identifyingsace_4237gene of *Saccharopolysporaerythraea*as putative multidomain eukaryotic-like ACC. In this work we have cloned this gene and performed protein expression experiments in*E. coli*. We were successful in complementing an*accB*thermosensitive mutant strain of*E. coli*validating its activity*in vivo*. Also we were able to purify the recombinant protein and performed*in vitro*assays to validate the ACC activity. Kinetic study of SACE4237 recombinant protein will be presented. In addition we are doing genetic studies to validate its physiological role on growth, production and accumulation in polyketide antibiotics of*Sac. erythraea*by building conditional mutants or overexpressing the protein under study.

MI-P18
MUTAGENIC-MEDIATED DIFFERENTIATION IN *Bacillus subtilis*AFTER INTERACTION WITH *Setophoma terrestris*

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Microbial interactions are ubiquitous in nature. Microorganisms grow and survive in the environment either in mutualistic relationships or as antagonists. We have previously isolated a strain of*Bacillus subtilis*(*Bs*) from soil under continuous onion culture where co-exist with the fungal pathogen*Setophoma terrestris*(*St*). To understand the mechanisms that govern the interaction between*Bs*and*St*we performed*in vitro*co-cultures in Petri dishes. After 15 days, we recovered the bacteria (*Bs-int*) and analyzed its morphology, biofilm formation, performed whole genome sequence analysis and compared with*Bs*not exposed to*St*(*Bs-ctr*).*Bs-int*exhibited changes in morphology and apparent increase in biofilm formation relative to *Bs-ctr*. Electron microscopy of*Bs-int*revealed a high number of longer and non-sporulating cells. When re-inoculated onto a fresh medium,*Bs-int*maintained the structured colony morphology suggesting a phase variation after interaction with*St*. Comparative genome analysis revealed for*Bs-int*a mutation in the*comA*gene, coding for a regulator involved in the ComPQXA quorum sensing (QS) system, and mutations in genes of uncharacterized non-ribosomal peptides (NRP) synthetases. NRP mediate competitive interactions and they may be controlled by QS. Our results suggest a role of these factors in the mutagenic-mediated cell differentiation experienced by Bs

MI-P19
DNA RECOMBINATION IN*Escherichia coli*AND *Pseudomonas aeruginosa*

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RecA is a central factor in the recombination between DNA molecules containing perfect homology (homologous, HO) or very low divergence (homeologous, HE). Recombination of sequences containing low divergence is inhibited by the action of the mismatch repair proteins MutS and MutL. We have built a genetic system to determine HO and HE recombination in Gram-negative bacteria. Using this system, we determined the recombination rate in the wild-type (WT) and mutant strains (*recA*and*mutS*) of*Pseudomonasaeruginosa*and*Escherichiacoli*. In both bacteria, HO recombination rates were approximately 60-200-fold higher than HE recombination rates. The absence of MutS did not affect HO recombination, but raised HE recombination to values close to that of HO recombination, indicating that MutS controls the exchange between divergent sequences. In the*E. coli*RecA-deficient strain, both HO and HE recombination rates were 60-fold lower than that obtained for the WT strain. The*recA*deletion mutant of *P. aeruginosa*showed that HO and HE recombination rates decreased 15 and 3-fold respectively, compared with the WT strain. These results suggested the existence in*P. aeruginosa*, of an additional recombination system independent of RecA. At present, we are analyzing the molecular structure of the recombinant products with the aim to elucidate this alternative mechanism.

MI-P20
THE RcsB-DEPENDENT MOTILITY BEHAVIOR REQUIRES THE LONG AND SHORT O-ANTIGEN CHAIN LENGTH DETERMINANTS

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The RcsCDB phosphorelay is an adaptive response system composed of three proteins: the sensor RcsC, the response regulator RcsB, and the histidin-containing phosphotransfer RcsD, which also can serve as a sensor. This system modulates the flagella and colanic acid biosynthesis operons and many virulence genes expression. In previous studies we demonstrated that*wzz$_{st}$*gene is required for the

enhanced swarming behavior displayed by the*rcsB*mutant. Our goal was investigate the relation between the O-antigen (Oag) length and the bacteria motility, when the flagella biosynthesis is under or not the control of RcsCDB. Here, we determined the swimming motility of wild type strain;*rcsB*,wzz_{st},wzz_{fepE},$pbgE_2$and $pbgE_3$mutants andwzz_{st}*rcsB*andwzz_{fepE}*rcsB*,$pbgE_2$*rcsB* and$pbgE_3$*rcsB*double mutants. It is important to remark that in *S*. Typhimurium the Oag follows a trimodal length distribution where Wzz_{fepE}controls the very long region, $Wzz_{st}$the long and $PbgE_2$and $PbgE_3$the short part. We also studied the*flhC*gene expressions in the above backgrounds in order to correlate the motility phenotype and the flagella expression. Finally, we analyzed the number and correct assembly of flagella in each analyzed strains, by electron microscopy. Our results demonstrate that only the long and short Oag chain length determinants are involves in the motility behavior controlled by RcsCDB system.

MI-P21

IDENTIFICATION AND CHARACTERIZATION OF A NEW ADHESIN IN *Brucella.*

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Adhesion to host cells is the initial and a crucial step in the virulence process of many pathogens and, therefore, is vital in order to understand the bacterial pathogenic cycle. Our group has identified a unique gene cluster present in all *Brucella*species important in the infectious process, which participates in the adhesion to cells. This region codes for four open reading frames: Bab1-2009, Bab1-2010, Bab1-2011 and Bab1-2012. So far, we had demonstrated that Bab1_2009 is a powerful adhesin. The protein encoded by Bab1_2012 has a domain with homology to bacterial immunoglobulin-like domain, similar to the one present in Bab1_2009 as well as in other bacterial adhesins. For this reason, we hypothesized that this gene might also be involved in the adhesion to cells. In the present work, we characterized the role of Bab1_2012 through antibiotic protection assays in HeLa and MDCK cells showing that the deletion of the gene affects intracellular replication in the initial stages. Additionally, we tested adhesion to cells with the mutant and over-expressing strains and found that over-expression of Bab1_2012 increased the number of bacteria adhered per cell. Subcellular localization studies demonstrated that Bab1_2012 localizes in the outer membrane. Our results strongly suggest that Bab1_2012 is an adhesin that participates in the adhesion to non-phagocytic cell.

MI-P22

EXPRESSION ANALYSIS OF THE*sua*GENE IN BIOFILM*Streptococus uberis*STRAINS

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Bovine mastitis is a disease that causes great economic losses. *Streptococcus uberis*is the main environmental pathogen. It has several virulence factors and the ability to form biofilms has been linked to numerous genes, including the ability to attach to a host's cell surface through the adhesion molecule SUAM, which contributes to infection persistence. Previously, we determined that the*S. uberis*strains isolated from the central dairy region yielded*sua*gene and were biofilm producers. The aim of the present work was to investigate the expression of*sua* gene in an*S. uberis*strain at different times. Additionally, biofilms were imaged by scanning electron microscopy. qRT-PCR was normalized to*gapdh*gene. Changes in transcriptional expression between planktonic and biofilm cells at 24, 48 and 72 hs were calculated by the comparative threshold cycle 2-ΔΔCT method and plotted as the fold change in mRNA expression levels. The results indicated that the biofilm producer strain shown a significant increase in*sua*expression (>2.5 fold) at 48 hs and a decrease at 72 hs. Microscopy images showed the integration of the structure and composition of the biofilm as multicellular aggregates and potential waterways that flow through the substrate, suggesting a change in the cell organization at 72 hs. The results contribute to a better understanding of biofilm in*S. uberis*strains.

MI-P23

CHARACTERIZATION OF A*Streptococcus uberis*MUTANT STRAIN DEFICIENT IN THE*sua* GENE

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Improving the quality of milk is a priority considering the increase of the export capacity of the sector, mainly in Mercosur. Agents causing of bovine mastitis have been classified in contagious and environmental pathogens.*Streptococcus uberis* is considered one of the most prevalent environmental pathogen. The aim of this study was to characterize pheno and genotypically a*sua*mutant strain of*S. uberis*and evaluate their behavior*in vivo*. Previously, we obtained*S. uberis*mutant strains deficient in*sua*gene using conditional replication vectors. Microtiter plate assay revealed that the mutant strain was categorized as strongly positive biofilm producer and showed adhesion capability in bovine mammary epithelial cell line (MAC-T). Intraperitoneal inoculation of the wild-type strain in Balb/c mice showed that 10^9CFU/ml was able to induce death and the strain could be cultured from kidneys, liver, spleen and heart. However, 10^8CFU/ml did not cause death but it could be cultured from the organs, except heard. While the mutant strain (10^8 and 10^9CFU/ml), did not cause death but it could be cultured from all organs. Genotyping by PFGE showed one different band in the mutant strain. The results obtained show new insights regarding to*sua*gene. Knowledge about specific virulence factors exploited by this pathogen is an important key to developing novel control strategies.

MI-P24
MONOCLONAL ANTIBODIES TO DISTINGUISH BETWEEN SHIGA TOXIN-PRODUCING*E. coli*O157 AND O145 SEROGROUPS

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Shiga-toxin producing *Escherichia coli* (STEC) strains are causal agents of bloody diarrhea (BD) and hemolytic-uremic syndrome (HUS). HUS is caused by particular strains of STEC in Argentina, being *E. coli* O157 the predominant serogroup (70%), followed by *E. coli* O145 (9%). Here, we have developed specific monoclonal antibodies against *E. coli* O157 and O145 serogroups using a combined strategy of adjuvated-bacterial immunizations, followed by immunizations with soluble engineered O-polysaccharide-protein conjugates. We obtained 18,8% and 13,3% of positive and specific hybridoma populations to O157 and O145 O-polysaccharide-protein conjugates, respectively. These O-polysaccharide-protein conjugates also allowed the screening of monoclonal antibody (mAb)-producing hybridoma populations and their cloning without interference of other LPS antigenic portions. We selected three specific hybridoma clones for O157 (1E10, 3F10 and 10G2) and three for O145 (2H6, 4C8 and 4E6). All the mAbs showed specific binding to the sought O-polysaccharide section of the LPS by *Western blot*. Whole cell ELISA of heat-killed bacteria also showed specific binding to O157 and O145 serogroups, but no binding to other STEC or to a non-toxic *E. coli* strain was detected. These mAbs are of considerable value for the development of detection and diagnosis assays against STEC.

MI-P25
USE OF A TAT–DEPENDANT SYSTEM TO STUDY PEDIOCIN PA–1 MECHANISM OF ACTION AGAINST*E. coli*

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The bacteriocin Pediocin PA–1 belongs to a group of peptides active on Gram–positive bacteria membrane. They bind to a specific receptor, the mannose phosphotransferase system (man–PTS) that is believed to participate in the formation of a pore. In standard conditions these bacteriocins are not active against Gram–negative bacteria when added to culture medium. Previously in our laboratory, we demonstrated that the intracellular expression of the fusion *etpm–pedA* induces loss of membrane integrity on*E. coli*when anchored to inner membrane, independently of its specific receptor. The assays were performed on*E. coli*because its lack of the receptor. In order to prove that pore formation mechanism is not dependant of*E. coli*mannose transporters Ecol1 y Ecol2, in this work we constructed the fusion *tat–pedA* under the tight control of P_{BAD}promoter. The Tat pathway is capable of exporting heterologous proteins into the periplasm. When Pediocin PA–1 was exported to the periplasm, it was not able to kill*E. coli*. This results confirms that it does not exist a specific receptor for Pediocin PA–1 in*E. coli*, and allow us to conclude that Ecol transporters may not be involved in EtpM–Pediocina PA–1 mechanism of action, supporting our previously developed theory: man–PTS would act simply as an anchor and it would not be involved in pore formation mechanisms.

MI-P26
RNA POLYMERASE IS THE PRIMARY TARGET OF MICROCIN J25 IN*Salmonella enterica* SEROVAR NEWPORT

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The main target of the peptide antibiotic microcin J25 (MccJ25) in*Escherichia coli*is the RNA polymerase. It has been suggested that the antibiotic might have a different mode of action on*Salmonella*Newport. In this study, we determined the frequency of selection of spontaneous resistance to MccJ25 in *S.*Newport. One hundred and fifty MccJ25-insensitive colonies were picked up. As in*E. coli*, mutations conferring resistance resided in*fhuA, tonB, exbB, exbD*, and*sbmA*genes. In only one of the mutants, transformation with a plasmid harboring the*E. coli* *rpoB*and*rpoC*genes fully reversed the MccJ25 resistance phenotype. This result indicated that this mutant possess a change in the RNA polymerase. The fact that the mutation confers complete resistance to high concentrations of MccJ25 suggests that inhibition of the polymerase alone accounts for its antibacterial activity on *S*. Newport. The mutation frequency of resistance to MccJ25 in*S.*Newport was 2 x 10^6(number of mutants per total number of viable bacteria).The high propensity of MccJ25 for selection of resistance *in vitro* could limit interest for development as a future drug candidate, especially for monotherapy. However, if derivatives of MccJ25 with improved features can be developed, these could be a source of novel drug leads.

MI-P27
THE*prtR*GENE INDIRECTLY ACTIVATES THE BACTERIOCIN PRODUCTION IN*Pseudomonas fluorescens* SF4C

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Bacteriocins are proteins produced by bacteria that can kill closely related species.*P. fluorescens*SF4c is a plant-growth promoting bacterium that synthesizes bacteriocins (phage-like pyocins), which inhibit the growth of phytopathogenic strains belonging to the genera*Pseudomonas*and*Xanthomonas*. The complete pyocin cluster was identified in the genome of the strain SF4c, finding structural genes of R and F-type pyocins, as well as, regulatory and lytic genes. The*prtR*gene is one of these putative regulatory genes and its ortholog in*P. aeruginosa*encodes a repressor protein. The aim of this work was to study the function of*prtR*in strain SF4c. The amino acid sequence of PrtR from strain SF4c is characteristic of repressor proteins. PrtR has two Pfam domains: a helix-turn-helix DNA binding domain of transcription regulators in the N-terminal end and a peptidase-S24 domain with a C1 repressor in the C-terminal end. A mutant*prtR*was constructed by gene replacement via homologous recombination and called*P. fluorescens*SF4c-prtR. Surprisingly, this mutant showed a deficiency in bacteriocin production compared with the wild-type strain. These results indicate

thatprtRactivates the pyocin production in strain SF4c, probably, by indirect modulation. PrtR could repress the activity of a second repressor, still unknown, leading to activation of pyocin synthesis as final result.

MI-P28
NEW HYBRID BACTERIOCIN WITH ACTIVITY AGAIST FOODBORNE PATHOGENS

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Bacteriocins are bacterial antimicrobial peptides of ribosomal synthesis active on phylogenetically related organisms.In previous studies we produced a new hybrid peptide named PedA1-GGG-ColV whit broader antimicrobial spectrum.The aim of this work was to evaluate antimicrobial activity against different pathogenic strains and determinate the sensitivity of peptide to proteolytic enzymes present in the stomach and intestinal contents.To evaluate the antimicrobial activity, we spread a standardized amount of bacteria uniformly on the surface of a LB agar plate to obtain a bacterial lawn.Then 10 ml of a solution of each bacteriocin were spotted on plates.To determine the sensitivity of the hybrid bacteriocin to proteolytic enzymes,10 µl of peptide were spotted onto LB plates and 10 µl of proteolytic enzymes solution was placed 1 cm away from the cultured medium drops.The plate was covered with 4 ml of 0.6% agar previously inoculated with the indicator strain. After overnight incubation at 37°C, the growth inhibition zones around the drops of hybrid media were recorded. We interpreted the shapes of growth inhibition zones around these hybrid as suggesting that digestive enzymes diffuse and decreasing the efficiency of indicator strain growth inhibition.PedA1-GGG-ColV displayed an antibiotic effect on most of the tested strains and result inactivated by digestive enzymes in vitro.

MI-P29
TbRRM1, A SR-RELATED PROTEIN, REGULATES TRANSCRIPTION RATES IN*Trypanosoma brucei* PROCYCLIC CELLS

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TbRRM1 is an essential RNA binding protein from*T. brucei* which belongs to the SR-related protein family. Previous studies from our lab indicated that TbRRM1 ablation by RNAi in*T. brucei*procyclic cells leads to cell-cycle block, abnormal cell elongation compatible with the nozzle phenotype and cell death by an apoptosis-like mechanism. Recently, TbRRM1 was shown to be associated to numerous mRNAs and to core histones, which suggests a dual role in both transcriptional and post-transcriptional regulation.In order to evaluate if TbRRM1 is involved in gene transcription regulation, TbRRM1 depleted parasites were subjected to Run-On assays. We found a global decrease of RNA synthesis after RNAi induction. Moreover, transcription analyses of neighboring genes showed that TbRRM1 is required for transcription elongation. In addition, we demonstrated that*TbRRM1*silencing led to heterochromatin formation as seen by formaldehyde-assisted isolation of regulatory elements (FAIRE) technique. Finally, we showed by Chromatin immunoprecipitation (ChIP) assays that TbRRM1 binds to the chromatin, preferentially to gene bodies. Altogether, our results strongly suggest that TbRRM1 participates in the regulation of gene expression keeping an open chromatin state, facilitating thereby the normal transcription rate.

MI-P30
ISOTRETINOIN INHIBITS ESSENTIAL METABOLITES TRANSPORT AND EXERTS TRYPANOCIDAL ACTIVITY

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Trypanosomacruzi is the etiological agent of Chagas disease, a major health problem in Latin America. Polyamines are polycationic compounds that play a critical role in regulation of cell growth and differentiation. In the specific case of *T. cruzi*, which is auxotroph for polyamines, the transport systems are the only way to obtain such molecules. Previous works reported that retinol acetate inhibits *Leishmania* growth and decreases its intracellular polyamine concentration. This work describes a combined strategy of drug repositioning by virtual screening followed by *in vitro* assays to find drugs able to inhibit the only polyamines transporter, TcPAT12. Seven retinoids used in medicine were obtained by similarity screening of 3,000 FDA approved drugs. Using molecular docking techniques isotretinoin, a well-known and safe drug used for acne treatment, bound to substrate recognition residues of TcPAT12 and was chosen for further *in vitro* studies. Isotretionoin inhibited the polyamine transport, and also all tested amino acid transporters from the same protein family (TcAAAP), with calculated IC50 values in the range of 4.6–10.3 µM. In addition, this drug showed a high trypanocidal effect on trypomastigotes, with an IC50 in the nanomolar range. These results suggest that isotretinoin is a promising trypanocidal drug, being a multitarget inhibitor of essential metabolites transporters.

MI-P31
CHARACTERIZATION OF MUTATIONS IN THE β-LACTAMASE AmpC FROM*Pseudomonas aeruginosa*CF ISOLATES

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Mutation-dependent overproduction of intrinsic β-lactamase AmpC is considered the main cause of penicillin and cephalosporin resistance in*Pseudomonas aeruginosa*(PA). We previously sequenced the genomes of 14 mutator isolates of PA from a cystic fibrosis patient, spanning 20 years of infection history. Results revealed uncharacterized mutations in the *ampC*gene which led to 4 new allele variants.*In silico*analysis showed that most of the mutations were located in the omega loop, which is part of the AmpC active site and

a hot spot for mutations able to extend the substrate specificity of β-lactamases. Whereas earliest isolates harbor unaltered*ampC* alleles, those obtained later accumulate 2 to 4 mutations and constitute the different allelic variants. Of note, in those late isolates carrying*ampC*alleles with 4 mutations, we found high resistance level to the β-lactam cefepime. In order to explore the impact of these mutations on β-lactam MICs, we designed a strategy to express the 4*ampC*variants in an*ampC*-deficient background of PA. The*ΔampC*mutant was obtained by allelic replacement using the pKNG101 suicide vector. The*ampC* alleles were amplified by PCR and cloned into the pMBLe vector which allowed us to tightly control the expression of the gene. MICs to different antibiotics, including carbapenems, are being performed to determine if they lead to extended-spectrum AmpCs.

MI-P32
*Proteus mirabilis*SECRETES FACTORS THAT AFFECT*Klebsiella pneumoniae*GROWTH AND SURVIVAL IN URINE

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K. pneumoniae(Kp) and*P. mirabilis*(Pm) are gram-negative bacteria that cause urinary tract infections in individuals with long-term indwelling catheters. Previous studies showed that Pm outcompeted Kp in both Kp-Pm mixed biofilms and planktonic cultures in artificial urine medium (AUM). It is known that Pm produces a urease that hydrolyses urea to $CO_2$and NH_3increasing the urine pH. We aim to study the factors involved in this competitive interaction between Kp and Pm. The ability of each strain to grow in single and mixed cultures was evaluated by counting colony forming units. The pH and NH_3concentration was also monitored. In single cultures both Kp and Pm did grow, while in mixed cultures Kp viability decreased 10^5-fold. A gradual increase of pH and $NH_3$was observed in mixed cultures, with maximal values of 9.3 and 135 mM, respectively. Cell-free supernatants from mixed cultures were able to drastically affect Kp growth (10^5-fold decrease). Evaporation of $NH_3$from the supernatant abolished its detrimental effect over Kp. When evaporated supernatants were adjusted to pH 9.3 a 10-fold decrease on Kp viability was found. However, Kp viable cell counts did not decrease in AUM buffered to pH 9.3. These results suggest that ammonia produced by Pm in AUM, and not the alkalinization by itself, is the major agent responsible for the competitive interaction between Kp and Pm.

MI-P33
COMMUNITY-ASSOCIATED METHICILLIN RESISTANT *Staphylococcus aureus*HOSPITAL ACQUIRED, ARGENTINA

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Methicillin-resistant*S. aureus*(MRSA) burden is increasingly worldwide in hospitals [healthcare-associated (HA)-MRSA] and in the communities [community-associated (CA)-MRSA]. The definition of healthcare-onset CA-MRSA infection is based on time from hospital admission to culture, but the knowledge about the actual time and origin of MRSA acquisition is limited. We aimed to determine prevalence on admission, acquisition rates and molecular epidemiology of MRSA in Córdoba hospitals, Argentina. A prospective multicenter study was conducted (October-November 2014) in 8 Córdoba hospitals. Colonization samples were obtained from nasal, pharyngeal and skin swabs from patients at hospital admission and discharge. MRSA were studied by SCC *mec*and *spa*-typing, PVL, PFGE and MLST. A total of 1419 patients were screened, 58 (4,1%) were colonized by MRSA [44 (3%) CA-MRSA and 8 (1%) HA-MRSA], this proportion differed significantly between pediatric (6%) and adult (3%) patients (p<0.0001). The most frequent genotypes were associated with PVL$^+$CAMRSA epidemic clones: ST5-IVa-t311 (27%) and ST30-IVc-t019(19%) followed by HAMRSA clones C-ST100-IVNv (15%) and A-ST5-I (7%). Overall MRSA acquisition rate was 2.3/1000 patient days at risk and all these MRSA were CA-MRSA genotypes. These results demonstrate the acquisition and transmission of CA-MRSA strains within hospitals in our country

MI-P34
SURVIVAL AND GENES EXPRESSION OF MRSA EPIDEMIC CLONES ON AN ENVIRONMENTAL INERT SURFACE

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Methicillin-resistant*Staphylococcus aureus*(MRSA) causes both hospital (HA) and community-acquired (CA) infections. In order to investigate the relationship between the epidemiological success of epidemic MRSA clones and their capacity to persist on an environmental inert surface, the survival and genes expression during the persistence on melamine surface were analyzed. Volumes of 100 µL of bacterial suspensions (10^{11}–10^{12}UFC/ml in saline solution, SS) of isolates representatives the epidemic clones (CAMRSA: ST5-IV, ST30-IV and USA300-ST8-IV, HAMRSA: ST5-I, ST5-IVnv, ST239-IIIA and ST5-II) were spotted onto a melamine surface. Dried MRSA were collected with SS at 24h; 10; 25 and 40 days to perform the colony counts and for RNA preparation. The genes expression (*spaA, fnbpA, fnbpB, hla, psmA1A2, IsdD, agrA, RNAIII*) was analyzed by qRT-PCR. All MRSA clones showed a similar survival tendency, decreasing progressively towards 40 day, being higher (p<0,05) in ST5-I and C-ST5-IVnv clones than the others. The genes expression levels by each clone at 10 day, were higher than those in its initial condition 24h (p<0,001).*isdD*transcription was increased about 40- and 35-fold in ST5-I and C-ST5-IVnv clones, respectively. These results suggest that*isdD*could be involved in the persistence onto inert surfaces in the hospital setting of both epidemic MRSA clones

MI-P35
KTCF20: A KILLER AGENT AGAINST*Candida*

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Killer yeasts are able to produce proteins or glycoproteins with antimicrobial activity known as killer toxins, and they can be used as biocontrol agents against pathogens yeasts. In this study we evaluated the biocontrol potential of killer toxins over clinical isolates of pathogenic strains of*Candida*spp. A set of 15 killer yeasts was used to perform a qualitative inhibition assay over lawns of 6*Candida*isolates in YPD agar pH 4.5. Growth inhibition in*W. anomalus*Cf20 cell-free supernatant (CFS, 2×10^4aU/ml), was studied at different temperatures and NaCl concentrations. Heat-inactivated CFS (100 ºC, 15 min) was used as control. OD_{600} and cell viability were measured to calculate inhibition. Fluorescence microscopy of pathogenic strains was performed after incubation for 16 h at 20 ºC in CFS+NaCl 1% and live/dead staining using SYTO9 and PI as fluorescent probes.*W. anomalus*Cf20 was selected as the most active killer strain, while*C. albicans*78 and*C. tropicalis*FBUNT3 were the most sensitive strains. CFS produced 74 and 80% inhibition of both strains, respectively, at 20 ºC and NaCl 1%. Fluorescence microscopy and viability studies revealed that CFS did not killed sensitive cells.CFS was able to highly inhibit the growth of the studied strains with fungistatic effects. Higher concentrations should be tested to evaluate if a fungicidal effect is achieved.

MI-P36
A NOVEL AND RAPID METHOD FOR THE PURIFICATION OF KILLER TOXINS

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Killer toxins (KTs) are fungal antimicrobial proteins of growing interest in food, health and pharmaceutical areas. Their application requires previous purification and characterization; however, traditional purification methods are extensive and costly. In this study we describe a rapid and economic method for purification of killer toxin KTCf20, relying on its affinity to sensitive yeast cells. A suspension of the sensitive strain*Pichiaguilliermondii*Cd6 was produced in YPD at 30 °C, 180 rpm for 48 h. Cells were further washed and autoclaved to obtain a dead cell suspension (PDCS) of OD60040. Different interaction conditions between PDCS and a cell-free supernatant (CFS) containing KTCf20 were studied: PDCS:CFS ratio, adsorption time and NaCl concentration, desorption time and temperature. Purification of KTCf20 was performed from 30 ml of CFS and 100 ml of PDCS. Purified was concentrated by ultrafiltration (3 kDa) and analyzed by SDS-PAGE. Optimal adsorption conditions were: PDCS:CFS 3:1; 10 min; 20 °C. Optimal desorption conditions were: NaCl 1 M; 5 min, 20 °C. Purification was completed in 3.5 h with yield 11.6%. Only one band was visualized in SDS-PAGE gel, corresponding to KTCf20. This novel purification method proved to be faster and less costly than traditional ones. However, it should be studied on other KTs in order to validate its effectiveness.

MI-P37
THE KILLER EFFECT OF LACTOFERRIN IN *Giardialamblia* INVOLVES CRITICAL MORPHOLOGICAL DEFECTS

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Lactoferrin (LF) is an 80 KDa iron-binding glycoprotein that plays a significant role in the innate immune system and is considered to be an important microbicide molecule. LF is effective in the treatment of giardiasis, an intestinal infection caused by the protozoan parasite*G. lamblia*. However, the molecular mechanisms by which LF exerts its effect on this parasite are not known. Most of the microbicidal activity of human or bovine LF (hLF or bLF) has been localized at the N-terminal region of the mature LF (lactoferricin: LFcin). LFcin is produced by pepsin cleavage of the native protein*in vitro*and likely*in vivo*. In this work, we analyze the participation of the endocytic machinery of*G. lamblia*in the internalization of LF and LFcin and their effects in cell homeostasis. To this end, we have developed several tools including the stable expression of dominant negative mutants, knock-down strains, specific antibodies, and the use of confocal and electron microscopy. Our results showed that LF and LFcin are internalized by receptor-mediated endocytosis and produces morphological changes in the lysosomal-peripheral vacuoles, altering their content and acidifying the cytoplasm of the cell. Our findings will contribute to disclose the fine mechanism in which LF and LFcin might function as an antigiardial molecule.

MI-P38
EFFECT OF DICHLOROACETATE ON *Saccharomyces cerevisisae* RESISTANCE TO FLUCONAZOLE

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Researches with yeast as a model system have provided information concerning a variety of cellular metabolic processes. Metabolic similarities between mammalian cells and yeast make possible the use of these latter for elucidating the effect of drugs in similar situations. Compound dichloroacetate (DCA) has been used to treat congenital lactic acidosis. Considering the capacity of acidification of yeast in fermentation conditions, this work attempts to analyze: 1) What effect exerts the DCA on the ability of *S. cerevisiae* for acidification? and 2) What result has the phenotype obtained on antifungal fluconazole resistance, by growing yeast in the presence of DCA? Taking into account these objectives, we determined, in rich medium (1% yeast extract, 1% peptone and 2% glucose), the effect of DCA on the external acidification of yeast. Subsequently, the antifungal resistance was tested. The results show that the DCA (3.0 mg / ml) decreases external acidification produced by yeast and sensitizes the cell to growth inhibition by fluconazole. Increased sensitivity of yeast against crystal violet compound is also observed.

MI-P39
ANALYSIS OF*Giardia Lamblia* PROTEIN-S-ACYLTRANSFERASES USING COMPLEMENTATION ANALYSIS IN YEAST

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Protein S-acylation or palmitoylation, is a post-translational modification that consist in the addition of long-chain lipids on cysteine residues through a thioesther bond. This modification regulates processes of great biological importance such us signal transduction and synaptic plasticity and it is also crucial for the infection of host cells by parasites. A family of enzymes named Palmitoyl transferases (PATs) is responsible for this modification. Information regarding these enzymes is still scarce. PATs are very divergent even between orthologues from related organisms and this precludes the identification of orthologues based on sequence homology. *Giardia lamblia* has 9 putative PATs and substrates have been identified for only one. Yeast PATs are well characterized in terms of substrate specificity. We take advantage of the phenotypes produced by lack of individual yeast PATs to carry-out complementation analyses using *G. lamblia* putative PATs. Using this approach, we identified gla_6733 as a putative Akr1 orthologue and gla_8711 and gla_9529 as putative Swf1 orthologues, while gla_2116, gla_6733, gla_8619 y gla_16928 are able to fulfill the role of Pfa3 in agreement with Pfa3 substrates being more promiscuous. We are currently confirming these results by analyzing the palmitoylation of endogenous *G. lamblia* substrates in cells silenced for their respective PATs.

MI-P40
GENOMICS AND PROTEOMICS OF BACTERIOCIN-PRODUCING STRAIN*Pseudomonas fluorescens*SF4C

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Bacteriocins are narrow-spectrum antimicrobial with high potential for application as biocontrol agents. Characterization of new bacteriocins with different action spectra becomes increasingly relevant. In this context, we perform a genome mining study to predict bacteriocin genes in the draft genome of *P. fluorescens*SF4c. This *in silico* analysis revealed the presence of a complete tailocin (phage-like bacteriocins) cluster with structural genes for R (contractile) and F (flexible) -type tailocins. Moreover, two genes corresponding to S-type pyocins (soluble proteins) with their cognate immunity proteins were also identified in the SF4c genome. In order to analyze if the predicted bacteriocins are produced by SF4c a proteomics study was performed. Extracts of supernatants of SF4c cultures induced with mitomycin C were digested with trypsin and analyzed by nano-HPLC coupled to mass spectrometer with Orbitrap technology (Mass Spectrometry Service of CEQUIBIEM-UBA). The spectra were analyzed against SF4c proteome obtained from draft genome. Data analysis showed that all bacteriocin predicted from genome were identified in the SF4c extract, including the 27 structural proteins of R and F tailocins and the 2 pyocins S with their respective immunity proteins. These results show that*P. fluorescens*SF4c is able to produce various bacteriocins with different structures and properties.

MI-P41
OMICS APPROACH FOR SAFETY AND INDUSTRIAL POTENTIAL ASSESSMENT OF FOOD ISOLATED ENTEROCOCCI

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The use of*Enterococcus*in food industry is under debate, they contribute during the ripening process of cheese but can be potentially hazardous to human health. In this work, the genomes of four cheese isolated*Enterococcus*strains were analyzed and relevant findings verified*in vitro*. Phylogenomic studies indicated that strains IQ110, GM70 and GM75 are*E. faecium*species whereas IQ23 (previously described as*E. faecium*), is actually*E. durans*. Regarding their technological properties, genes related to bacteriocins synthesis and bile salt, citrate, casein, peptide and aminoacid degradation pathways were found. The volatile profiles of the strains were evaluated using solid-phase microextraction and GC-MS spectrometry, a correlation with the encoded pathways was found. In contrast, the presence of virulence and antibiotic resistance genes were also identified*in silico*. Then, we challenged*G. mellonella* larvae and found that while IQ23, IQ110, and GM75 were as lethal as*E. faecalis*JH2-2 isolated from urinary infection, GM70 was harmless. Finally, a differential pattern of resistance to ciprofloxacin, nitrofurantoin, chloramphenicol, and rifampicin were observed among strains. In conclusion, bioinformatic as well as biochemical approaches should be conducted to better understand the technological or pathogenic behavior of *Enterococcus*strains independently of their isolation source.

MI-P42
ISOLATION AND CHARACTERIZATION OF PATHOGENS CAUSING FOODBORNE DISEASES

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Foodborne Diseases (FBD) are caused by ingesting food or water polluted with specific etiological agents as bacteria, and characterized by gastroenteritis. In acute case are presented as hemolytic whole diseases producing the host's death. In America the FBD affects about 77 million people, where more than 12,000 die per year. Even when, was observed that the 95% of demises are due to*E. coli*and*Salmonella*, the number of people affected cannot be accurately determined because many of this are not notified. We initiate an epidemiological study in our region to determine the pathogen causing FBD. We isolated and characterized strains from stool cultures samples, during 2013-2016 periods, from Header Hospitals of Tucumán and Santiago del Estero. Here, 1543 strains

were isolated from pediatric patients. We observed that during the 2015/2016 summer period more cases of FBD were recorded. The suspicious strains were then analyzed by biochemical and serology tests to classify the pathogens. We observed that 10% of it belonged to*Salmonella*sp, while the remaining 90% were identified as*Shigella*, many of these were presented as antibiotic multi-resistant strains. Our data suggest that*Shigella* is the pathogen prevalent in the NOA and that in the Tucuman´s population the gastroenteritis cases are more frequently caused by the*Salmonella*as pathogens.

MI-P43
ISOLATION OF POTENTIAL BENEFICIAL BACTERIA WITH PROTEOLYTIC ACTIVITY FROM POULTRY FEED

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Many bacteria possess proteases and peptidases active on proteins and peptides present in wheat, soybeans and other grains, so they could contribute to the production of aminoacids and small peptides in the gut of poultry, fed with cereals and seeds. With the aim of obtain proteolytic strains to be used as poultry feed supplements, different bacteria were isolated from meat meal, soybean meal and feed ready to eat provided by poultry farms. Colonies were obtained from selective media, and morphological characteristics, Gram stain, spore formation, catalase reaction and the response to several biochemical tests were assessed. Then a soybean protein isolate (SPI) was prepared and partially purified to evaluate the proteolytic activity of the isolates. SPI was used as the only source of protein in a modified culture medium. Considering all the results obtained we concluded that 26% of the isolated bacteria from feed and soybean meal belong to the genus*Lactobacillus*while in meat meal this genus was not present. The genus*Enterococcus*was at the same proportion, 19 %, in all the samples analyzed. The genus*Bacillus*represented only 4 % of strains isolated from meals. Proteolytic activity was detected in 67% of strains of *Lactobacillus*, 78% of*Enterococci*and in all the*Bacillus* isolated. Further studies will be used for the selection of new supplements for poultry breeding.

MI-P44
GENOME-SCALE METABOLIC MODEL OF *Lactobacillus casei* BL23 REVEALS THE ROLE OF REDOX BALANCE IN FLAVOR FORMATION

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Lactobacillus casei BL23 is a lactic acid bacterial strain, which is widely used in the dairy industries and is considered an archetypal *Lb. casei* strain. A genome-scale metabolic reconstruction for *Lb. casei* including 851 genes, 920 metabolic reactions, and 792 metabolites, was reconstructed using both manual genome annotation and an automatic SEED model. Validation was performed simulating cell growth on 20 reported carbon sources. More than 300 genes were reannotated in this model comparing our model with recently published *Lb. casei* LC2W model (Xu et al., 2015). The model was used to identify several vitamins and amino acids that are essential for the microorganism's growth. The model was used to gain system level insights in flavor forming pathways from amino acid metabolism. Among the objective functions evaluated, the redox balance best explained the distribution of fluxes through flavor forming pathways in stationary phase for this bacterium.

MI-P45
ACCUMULATION OF POLYPHOSPHATE IN LACTIC ACID BACTERIA AND ITS INVOLVEMENT IN STRESS RESISTANCE

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Polyphosphate (polyP), an ubiquitous linear polymer of hundreds of orthophosphate residues, plays an important role in the response to nutritional stringencies and against oxidative, osmotic and acid stresses. Lactic acid bacteria (LAB) are a heterogeneous group of bacteria useful to produce fermented foods that are normal inhabitants of the oral cavity and the digestive tract in humans. The presence of polyP in these bacteria was previously demonstrated; however, there is scarce information about its role in LAB physiology. We have previously reported that stationary*Escherichia coli*cells grown in >37 mM phosphate media presented high viability and elevated tolerance to exogenous H_2O_2, when compare to cells grown in sufficient phosphate media. Here, we determined intracellular polyP levels of three lactobacilli strains grown in media with different phosphate concentrations. Among them,*Lactobacillus paraplantarum*CRL 1905 was selected for studies since it maintained high polyP levels up to 96 h when cells were grown in 60 mM phosphate medium. It was observed that cells grown for 48 h and 96 h in high phosphate medium were more viable than those grown in sufficient phosphate condition. Since polyP is involved in stress responses in other bacteria, we tested the resistance of*L. paraplantarum*CRL 1905 cells grown in low or high phosphate media to several exogenous stress agents.

MI-P46
EFFECTS OF LACTIC ACID BACTERIA ON INFLAMMATORY CYTOKINES PRODUCTION BY ARPE-19 CELLS

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The anti-inflammatory capacity of probiotic lactic acid bacteria was extensively studied as therapy for inflammatory bowel diseases, but only two studies have been performed in ocular inflammatory disorders. The aim of this work was to investigate the capacity of 4 strains of*Lactobacillus plantarum*(CRL 1081, CRL759, CRL 725, CRL 92) to modulate cytokines production of LPS-challenged ARPE-19 cells (human retinal pigment epithelial cell line). First, bacteria were grown in MRS medium, showing that exponential

growth phase was the optimal for co-culture with cells. Arpe-19 cells were co-incubated with bacterial strains at 10 and 20 MOI in DMEM medium for 6 hours at 37ºC. Then, the cells were stimulated with different LPS concentration (10, 50 and 100 µg/ml) for 24 hours. IL-6, IL-8, IL-1β, TNF-α, IL-10 and IL-12p70 were detected in culture supernatants using flow cytometric bead array technique. ARPE-19 cells treated with 10µg/ml LPS produced IL-6 (141.4 pg/ml) and IL-8 (335 pg/ml). Two strains exerted an anti-inflammatory effect:*L. plantarum*CRL 725 at 20 MOI reduced the production of IL-6 and IL-8 by 25% and*L. plantarum*CRL 759 at 10 MOI totally reverted the secretion of both cytokines induced by LPS in ARPE-19 cells. No IL-1β, IL-10, TNF-α and IL-12p70 secretion was detected. Experimental assays to determine the mechanisms responsible for this effect are under development.

MI-P47

S-LAYER PROTEINS OF*Lactobacillus sp.*AS POTENTIAL TREATMENT FOR BACTERIAL AND VIRUS PATHOGENS

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Surface layer (S-layer) proteins constitute in some species of lactobacilli their outermost cell envelope and have been considered to play a role in the probiotic properties of the strains. Here, we investigated the antibacterial and antiviral ability of S-layer proteins purified from*Lactobacillus acidophilus* ATCC4356,*Lactobacillus brevis*ATCC14869,*Lactobacillus helveticus*ATCC12046 and*Lactobacillus kefiri*JCM5818. Capacity to protect A549 cells (human lung adenocarcinoma cell line) against*Pseudomonas aeruginosa*adhesion and HSV-1 multiplication was evaluated. We found that S-layers alter the *Pseudomonas*pattern of adhesion to A549 cells, from a diffuse adhesion in control cells to a localized or aggregative form in pretreated cells. These results were accompanied by a reduction in the number of bacterial adherence, as evidenced by flow cytometry and plate count. Also, we examined their effect on*Ps. aeruginosa*biofilm formation. Likewise, the standard crystal violet staining procedure shown that these proteins impede biofilm formation without causing bactericidal effects on planktonic bacteria. The antiviral assay revealed that S-layers of*L. acidophilus, L. brevis*and*L. helveticus*have an interesting antiviral effect against HSV-1 at subtoxic concentrations. The results provide knowledge of important application of S-layer as treatment for bacterial and virus pathogens.

MI-P48

INTRA-STRAIN VARIABILITY IN THE AMINO ACID SEQUENCE OF S-LAYER PROTEINS FROM *Lactobacillus kefiri*

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Bacterial S-layers are proteinaceous crystalline arrays composed of self-assembling glycoprotein subunits called S-layer proteins (SLPs). Previous mass spectrometry analysis showed a great structural heterogeneity among SLP from aggregative and non-aggregative strains of*Lactobacillus kefiri*, a potentially probiotic microorganism. Some of them share several fragments with the SLP from phylogenetically related lactobacilli. Based on this information, specific primers located outside and inside the SLP-genes were designed in order to amplify genomic DNA. Ten strains were selected for sequencing of the complete genes. The glycosylation site SSASSASSA was found in all*L. kefiri*SLPs, whereas cysteine is absent. The total length of the proteins varies from 492 to 576 amino acids, and all SLPs have a high calculated pI (9.37-9.60). N-terminal region is relatively conserved and shows a high percentage of positively charged amino acids. Major differences among strains are found in the C-terminal region. Different groups could be distinguished regarding the amino acid sequences and the similarities observed in mass spectra. Interestingly, SLPs of the aggregative strains CIDCA 8321, 8348 and 83115 are 100% homologue, although were isolated from different kefir grains. This knowledge will contribute to the development of products of biotechnological interest from potentially probiotic bacteria.

MI-P49

BIOSYNTHESIS OF 5-HALOGENATED NUCLEOSIDES USING NANOSTABILIZED LACTIC ACID BACTERIA

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Nucleoside analogues biosynthesis has become important because pharmaceutical industries require newenvironmentally friendlyprocesses. These compounds are used as antiviral and antitumor agents. In this work, biocatalytic systems based on immobilized *Lactobacillus animalis* ATCC 35046, were evaluated to obtain 5-halogenated nucleosides. Microorganisms were immobilized by entrapment in a bionanocomposite, using $SrCl_2$ and $CaCl_2$ as crosslinking solutions to prepare gel beads. Biocatalytic activity of stabilized *L. animalis* was evaluated using 6 mM of thymidine and 2 mM of different pyrimidine bases (5-fluoruracil, 5-bromouracil, 5-chlorouracil and 5-iodouracil) in 1 mL of 25 mM Tris-Cl buffer at 30 °C and 200 rpm. Immobilized biocatalysts were able to biosynthesized four nucleoside analogues with proved pharmaceutical activity. Productivity values were 65 mg/L h for 5-fluoro 2´-deoxyriboside, 50 mg/L h for 5-bromo 2´-deoxyriboside, 80 mg/L h for 5-iodo 2´-deoxyriboside and 25 mg/L h for 5-chloro 2´-deoxyriboside. Moreover, different green parameters such as E-factor and mass economy were analyzed and physicochemical properties were characterized. Therefore, in this work stable biocatalysts was developed, allowing the design of an eco-compatible bioprocess, where a broad variety of pyrimidine nucleosides used in antiviral treatments and chemotherapeutics therapies were produced.

MI-P50
ANTIVIRAL COMPOUND BIOSYNTHESIS BY A STABILIZED MULTI-ENZYMATIC SYSTEM

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Ribavirin is a synthetic guanosine analogue with broad-spectrum of antiviral activity. It has been widely used for HCV treatment in combination with pegylated interferon-α and telaprevir. Cheaper and environmentally friendly processes had been studied for nucleoside analogues synthesis. For this purpose, a multi-enzymatic system stabilized by immobilization was developed. Phosphopentomutase (PPM) is a key enzyme involved in the salvage pathway of nucleoside synthesis which catalyzes the transfer of intramolecular phosphate on ribose. In combination with nucleoside phosphorylases (NPs), nucleoside analogues can be biosynthesized from simple substrates. In this work, PPM has been overexpressed in *Escherichia coli*, purified, stabilized at alkaline pH and immobilized on several supports. To conserve biocatalytic activity during immobilization process, different additives were assayed, being 10% (v/v) glycerol the best stabilizing agent, allowing PPM to conserve 86% of its initial activity at pH 10 after 18 h incubation; which favors covalent immobilization of this enzyme on glyoxyl-agarose with a high yield. This is the first time that PPM was immobilized on glyoxyl support. This derivative in combination with a PNP, was able to biosynthesize ribavirin from D-ribose-5-phosphate.

MI-P51
NEMATODE M01F1.3 AND C45G3.3 FUNCTIONALLY COMPLEMENT MICROBIAL MUTANTS IN LIPOYLATION PATHWAYS

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Lipoic acid (LA) is a covalently bound sulfur-containing cofactor present in multienzymes complexes involved in oxidative and one-carbon metabolism. The ways in which proteins become lipoylated are very well characterized in prokaryotes, while information concerning eukaryotes is scarce. Human patients with defective lipoylation pathways receive treatment just to alleviate symptoms. For this reason, we have started the study of LA metabolism in the model organism *Caenorhabditiselegans*. We have previously demonstrated that the worm is capable of synthesizing LA and that the enzyme M01F1.3 was involved in the process, as blocking its expression caused a reduction in protein lipoylation levels. In order to confirm its role, we expressed the worm protein in bacterial strains defective in different steps of lipoylation pathways. M01F1.3 was only able to rescue growth of *Bacillus subtilis* and *Escherichia coli*lipoate synthase (lipA) mutants. Another possible enzyme involved in nematode protein lipoylation is C45G3.3, which has considerable identity of primary structure with bacterial lipoate ligases. The worm protein was effective to complement a *Saccharomyces cerevisiae* lip3 mutant. We also found that when expressing C45G3.3 in a *B. subtilis* strain deficient in lipoate ligase (lplJ), it recovered its ability to grow in a minimal medium when supplemented with octanoate but not with LA.

NEUROSCIENCE

NS-P01
HIPPOCAMPAL BDNF IN RESERPINE-TREATED ADOLESCENT WISTAR RATS

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Reserpine is a drug that depletes central and peripheral monoamines, resulting in behavioral depression in laboratory animals. Memory processes -- which are known to be altered in depression -- are dependent on the integrity of the hippocampus, a brain structure expressing brain-derived neurotrophic factor (BDNF). The aim of this study was to investigate hippocampal BDNF in adolescent rats treated with reserpine. Twenty-eight adolescent Wistar rats (males and females) were given reserpine (1.0 mg/kg) or vehicle (0.0 mg/kg) every two days for 8 days. We then applied the elevated plus maze and the bar test to measure anxiety response and to dispell the possibility of reserpine-induced motor impairment, respectively. Hippocampal BDNF levels were obtained via western blot. The results indicated greater anxiety response in the absence of motor impairment. BDNF hippocampal levels tended to be lower in reserpine-treated animals, although this effect did not reach significance. This pattern of results was similar in males and females. Overall, the present results indicate reserpine-induced enhancement of anxiety (which is usually comorbid with depression), which was accompanied by a non-significant decrease in BDNF levels at hippocampus. These results validate the use of reserpine as a pharmacological model of depression.

NS-P02
ROLE OF C-FOS IN NEURONAL DIFFERENTIATION

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The oncoprotein c-Fos is a well-recognized member of the AP-1 family of transcription factors. In addition to this canonical activity we have previously shown that cytoplasmic c-Fos activates phospholipid synthesis by its association with particular lipid synthesizing enzymes at the endoplasmic reticulum. This particular function might be associated with the molecular mechanisms that allow the higher rate of membrane genesis required for the complex events that take place during neuronal differentiation. In our aim to study the role of c-Fos during neuronal differentiation, we found a strong co-localization with ER markers in specific structures at branching

sites of neuronal processes. Blocking either c-Fos expression or its activity provoked an impairment in differentiation with no development of axonal processes. As CCTβ2, an integral enzyme of the ER membranes that plays an important role in the formation of axon branches, has been shown to play a main role in axonal branching, we checked if it could be activated by c-Fos. We observed co-immunoprecipitation of c-Fos with the enzyme and a physical association between these two proteins as determined by FRET, indicating a possible mechanism for branching regulation. These results support our hypothesis that c-Fos mediated activation of phospholipid synthesis participates in neuronal differentiation.

NS-P03
NEUROINFLAMMATORY RESPONSES IN A MOUSE MODEL OF AUTISM SPECTRUM DISORDER (ASD)

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Rett Syndrome is an ASD caused by mutations in Methyl Cytosine Binding Protein 2 (MeCP2). Our main goal is to use a mouse monogenic model of ASD, which shows a highly reproducible phenotype, to evaluate the role of altered immunity in the pathogenesis of this disorder. We first evaluated the autoimmune/neuroinflammatory response in the context of the experimental autoimmune encephalomyelitis (EAE). Male MeCP2 WT and MeCP2 mutant (MT) mice were immunized with MOG35-55 peptide to induce EAE, scored daily for clinical signs and sacrificed at 12 dpi (acute stage) or at 56 dpi (chronic stage). MeCP2 MT mice showed an accelerated onset of the disease and more severe clinical scores than WT mice. IHC was performed in sections of spinal cord to detect activated microglia (Iba-1+) and to assess infiltration of immune cells into the CNS. At 12 dpi, cellular infiltrates in spinal cord were higher in MT-EAE than in WT-EAE mice. Also, isolated spleen lymphocytes were re-stimulated with MOG in vitro and cytokine production was assessed. We found high levels of pro-inflammatory cytokines (IL-6, IL-17, IFNγ) in both MT and WT EAE groups. Our results showed a more severe EAE clinical manifestations as well as neuro inflammatory response in the absence of MeCP2. Ongoing studies are addressing the specific role of Mecp2 in these immune processes.

NS-P04
DEFICITS OF HIPPOCAMPAL STRUCTURAL PLASTICITY IN A MOUSE MODEL OF MECP2 DEFICIENCY

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Methyl citosine binding protein-2 (MeCP2) is a chromosomal structural protein involved in the regulation of gene expression. Alterations in MeCP2 levels have been linked to neurodevelopmental and learning disorders. MeCP2 plays an important role in the maintenance of synaptic connections in the adult nervous system. In the adult hippocampus, the infrapyramidal tract (IPT) undergoes dynamic changes in response to neuronal activity and in addition to adult neurogenesis constitutes important events for pre-synaptic structural plasticity of the hippocampus. In the present work, we aim to define how the lack of MeCP2 interferes with these processes. Using mouse models of MeCP2 deficiency, we found that the volume of the IPT tract increases significantly 2 weeks after kainic-induced seizures in WT mice but not in MeCP2 MT (mutant) mouse. BDNF expression was up-regulated at both 6hrs and 2 weeks after kainic exposure in WT mice, but this increase was detected only after 6hrs in MeCP2 MT mice. Using BrDU injections and IHC we found no defects in kainic-induced adult neurogenesis in MeCP2 MT mice; however, the percentage of granule cells reaching maturity (NeuN+ Calbindin+) was significantly lower in this group. In addition, MT mice showed lower performance in a spatial learning paradigm (Barnes maze). These results shed light about the role of MeCP2 in neural circuit plasticity and learning

NS-P05
PKD1 REGULATION OF TRK RECEPTORS' TRAFFICKING: EFFECT ON NEURONAL DEVELOPMENT AND FUNCTIONALITY

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Previous works in polarized cells, such as neurons, have shown the main role of PKD1 in protein sorting, where blocking of this kinase activity was able to change normal dendritic markers' localization, directing them to the axon terminal, and showing at the same time a delay in dendritic growing. In this work, we analyzed how blocking of the PLC-PKC-PKD1 pathway would affect the transport of two neurotrophin receptors, TrkA/TrkB, and if it could also have an effect in their activity. Our work have shown that in neurons, blocking of this pathway led to retention of TrkA at the Golgi apparatus, similar as the effect described for membrane proteins in non-polarized HeLa cells. Moreover, when the pathway was inhibited, TrkB changed from a somatodendritic position to an apical dendrite localization. Additionally, sorting changes of these receptors have led to defects in their specific ligand response, and in the case of TrkB, it affected processes such as LTP and status epilepticus. Our results have revealed the complexity of trafficking regulation in polarized cells. At least in neurons, PKD1-dependent pathway would be participating not only in membrane fission regulation, but also in protein sorting to different compartments, such as axons, basal and apical dendrites. It is clear that PKD1 role in trafficking depends both on the type of transported protein and on its final destination.

NS-P06
IGF-1R AND PI3K-AKT SIGNALLING PATHWAY ARE ESSENTIAL FOR FORMATION OF BRAIN CORTEX

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The cerebral cortex is organized horizontally into six laminae oriented parallel to the brain surface and is patterned by the coordinated migration of inmature neurons. As they migrate, neuron morphology changes from multipolar to bipolar. Bipolar cells have a radially oriented leading process (a future dendrite) and a trailing axon, and move by locomotion along the radially oriented processes of glia or other neurons. In neurons in culture we showed that IGF-1 receptor activation is important for axonal formation. However, the possible roles of the IGF-1 receptor on neuronal differentiation and polarization in vivo in mammals have not yet been studied. Using "in utero" electroporation, we show here that the IGF-1 receptor is essential for neocortical development. Neurons transfected with a shIGF-1 receptor failed to migrate to the upper cortical layers and accumulated at the ventricular/subventricular zones. The change of the morphology from multipolar to bipolar cells was also affected and cells lacking the IGF-1 receptor remain arrested as multipolar forming a highly heterotopic tissue. The typical orientation of the migrating neurons with the Golgi complex pointing to the neocortex upper layers was also affected by transfection with shIGF-1 receptor. Finally, cells transfected with the shIGF-1 receptor were unable to form an axon and, therefore, neuron polarity was absent.

NS-P07
DIETARY SOY-BASED PROTEIN MODULATES THE OXIDATIVE AND INFLAMMATORY EFFECTS OF CADMIUM IN HIPPOCAMPUS

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Oxidative stress and inflammation can change hippocampus (Hp) biochemistry and physiology. Cadmium (Cd) is a neurotoxicant, but its effects on Hp are rarely informed and whether these effects can be modulated by the diet is unknown. Herein we hypothesize that low doses of Cd in the drinking water causes oxidative/inflammatory changes in the Hp and that soybean-protein-based diet may modulate these effects. To accomplish this goal, we fed 4 groups of female Wistar-rats for 60 days as follows: 1:casein-based diet (CBD) + tap water; 2: CBD + tap water with 15 ppm Cd (as Cl2Cd); 3: soybean-based diet (SBD) + tap water; and 4: SBD + tap water with 15 ppm Cd. In the Hp of CBD group, Cd exposure did not change superoxide dismutase (SOD) activity, TNF-α concentration and myeloperoxidase (MPO) activity. In the serum of rats fed a SBD, Cd increased SOD activity, whereas reduced the TNF-α concentration. Compared to CBD, the Hp of SBD showed enhanced MPO activity—a marker of leukocyte inflammation. On the other hand, in the Hp of CBD rats Cd induced endothelial nitric oxide synthase (NOS) and inducible NOS expression—markers of inflammation. This effect was not observed in SBD rats. Together our data suggest that SBD may trigger a compensatory antioxidant and inflammatory mechanism in the Hp of rats chronically exposed to low concentration of Cd.

NS-P08
STUDY ON VISUAL AND NON-VISUAL OPSINS IN A MODEL OF RETINAL DEGENERATION CAUSED BY LED LIGHTS

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The retina is a complex neural circuit that transduces light into a pattern of electrical Impulses to the brain. This tissue fulfils a visual role through rods and cones and the synchronization of circadian rhythms by a subpopulation of photosensitive ganglion cells (GC). The process of retinal degeneration (RD) by low intensity LED light exposure is not well characterized. Previously, we demonstrated rods and cones death and a reduced functionality of these cells after 4 days of light exposure.The goal of this work was to evaluate the constant low LED light stimuli effects over visual photoreceptors and GC, studying the oxidative stress mechanism, Rhodopsin (Rho), Opn4 and Opn5 expression and Rho phosphorylation. Male Albino Wistar rats were exposed to white LED light (200 lx) for 1 to 8 days. We found a significant increase in retinal reactive oxygen species production after 5 days of light. The number of GC was not altered, however, the expression of Opn4 increased and its location varied from neuritas to somas along the days. Opn5 was localized in GC and inner nuclear layer with higher expression levels after 8 days. We also found that Rho phosphorylation was reversible if animals were exposed to 48 hrs of darkness after light treatment. All this results suggest the occurrence of RD with a re- structuration in photoreceptor cells in low light exposure stress.

NS-P09
NOVEL PHOTORECEPTORS IN THE AVIAN INNER RETINA: HORIZONTAL CELLS EXPRESSING MELANOPSIN X

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In the vertebrate retina, three types of photoreceptors -visual photoreceptor cones and rods and the intrinsically photosensitive retinal ganglion cells (ipRGCs)-converged through evolution to detect light and regulate image and non-image forming activities. ipRGCs express the photopigment melanopsin (Opn4), encoded by two genes: the Xenopus (Opn4x) and mammalian (Opn4m) orthologs. In the chicken retina both Opn4 proteins are found in ipRGCs, and Opn4x is also present in inner retinal horizontal cells (HCs). Here we

investigate the intrinsic photosensitivity and functioning of these cells and show that Opn4x (+) HCs are novel photoreceptors requiring Opn4x expression and retinaldehyde as chromophore. HCs act through a rhabdomeric-like photocascade involving Gq protein and PLC activation, Ca^{2+} mobilization, and GABA release. Through these mechanisms HCs may potentially regulate non-visual tasks together with their sister cells, ipRGCs; and with visual photoreceptors, HCs modulate lateral interaction and inner retinal processing

NS-P10
ADULT HIPPOCAMPAL NOTCH ACTIVATION IMPAIRS Aβ CLEARANCE AND COGNITION IN A RAT MODEL OF ALZHEIMER

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Background: Along the entire lifetime, Notch is actively involved in dynamic changes in the cellular architecture and function of the nervous system. It controls neurogenesis, growth of axons and dendrites, synaptic plasticity, and neuronal death. The specific roles of Notch in adult brain plasticity and neurological disorders have begun to be unraveled in recent years, and experimental evidence suggest that Notch is operative in diverse brain pathologies including Alzheimer's disease (AD) however the mechanisms remain unknown. Objective: To study whether chronic Notch1 activation exacerbates Aβ associated pathology in a rat model of early AD. Methods: Two month-old transgenic McGill-R-Thy1-APP rats (Tg+/-) were injected with lentiviral particles (LVP) expressing the transcriptionally active proteolytic fragment of Notch1 (NICD) (n=9) or a fluorescent protein Tomato (TOM) (n=10). At 6 months, spatial cognition was assessed and hippocampal Aβ levels were quantified using a multiplex electrochemiluminescence ELISA test. Results: Tg-NICD rats displayed short-term spatial memory impairment, increments on hippocampal Aβ40/Aβ42 levels and decrements of neurotoxic Aβ species in cerebrospinal fluid (CSF) as compared to Tg-TOM. Conclusions: chronic expression of NICD in adult brain impacts on Aβ metabolism and worsen cognition suggesting a pathway linking Notch1 signaling and AD pathology

NS-P11
EXPERIMENTAL GUILLAIN-BARRE SYNDROME: ROLE OF THE CARRIER PROTEIN KLH

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Guillain-Barre syndrome (GBS) is considered a neuropathy associated with anti-gangliosides (Gg) antibodies (Ab). Little is known about the mechanisms involved in the origin of these Ab. Rabbits immunized with a single dose of an emulsion containing bovine brain Gg (BBG), KLH and complete Freund adjuvant develops the disease. In this study 4 groups of rabbits were immunized with the following mixtures. Group 1: KLH and BBG Group 2: BSAm and BBG Group 3: two emulsions, one with BSAm and BBG and another with KLH Group 4: KLH. All animals in Group 1 and 3 developed the neuropathy; however no animal became ill in Group 2. In animals on the Group 4 anti-GA1 Ab were detected, probably because the KLH possess a determinant also present in some Gg. These Ab are 100% cross-reactive with KLH, unlike Ab detected in Group 1, which are partially blocked and whose percent of cross reactivity changes over time. This would indicate a change of specificity, which was associated with the onset of neuropathy. Furthermore, a high percentage of anti-GA1 high affinity Ab in pre-immune serum was associated with an early onset of neuropathy. Two conclusions arise 1) In addition to Gg, KLH is a requirement for disease triggering and it would play a key role as specific and non-specific stimulator. 2) Anti-GA1 high affinity Ab on preimmune serum could be established as a possible "host susceptibility factor".

NS-P12
IMPAIRED AUTOPHAGY FLUX IN MÜLLER GLIAL CELLS EXPOSED TO HYPOXIA: IN VITRO AND IN VIVO MODELS

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Hypoxia is one of the main insults in Diabetic Retinopathy (DR) and Retinopathy of Prematurity (ROP), leading to neovascularization and neurodegeneration. In this pathological context, Müller Glial Cells (MGCs) has been reported to participate in retinal homeostasis maintenance by eliminating protein aggregates, activating stress proteins and detoxifying mechanisms. Here, we investigate whether autophagy is involved in MGCs response under hypoxia. MIO-M1, a human immortalized cell line of MGCs, were pre incubated with cloroquine 10 uM and exposed to CoCl2 250 uM for 24 h. In a similar experiment, MIO-M1 were exposed to 0,1% O2. Western blot and immunofluorescence analysis revealed increased levels of LC3B-II and p62/SQTSM1 but decreased colocalization of LC3 and lysotracker. In addition, autophagy flux was determined in the OIR mice model, which closely resemble ROP and DR. C57/BL6 mice exposed to 75% O2 from postnatal day (P)7 to 12, were brought to room air (RA) for additional five (P17) or nine days (P26). Age-matched mice maintained in RA were used as control. Western blot of neural retinas showed changes in autophagy flux, glutamine syntethase, total caspase 3 and GFAP levels at p12, p17 and p26. A distinct pattern of LC3 was observed at the end feet of MGCs by immunofluorescence staining demostrating that hypoxia modifies autophagy flux in MGCs during retinopathies.

PLANT BIOCHEMISTRY AND MOLECULAR BIOLOGY

PL-P01
MOLECULAR ANALYSIS AND SUBCELLULAR LOCALIZATION OF PEPTIDASE SILPEPSIN 2 FROM *Silybum marianum*

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Typical plant aspartic peptidases (APs) are comprised of both pre-and pro-regions as well as a C-terminal region containing a plantspecific insertion (PSI). APs from thistle flowers precursors have highly conserved signal peptides that direct the proteins to the secretory pathway. The aims of this work were to perform a molecular analysis of silpepsin 2, an AP from flowers of *Silybum marianum*,and to analyse its subcellular localization by confocal laser scanning microscopy. A structural model of the zymogen was built using MODELLER v9.14 based on the crystal structure of prophytepsin from barley as template(PDB: 1QDM), found with HHPred fold assignment method. The best model was assessed for quality using both energetic and structural criteria. Typical AP folding pattern was observed. The subcellular localization of silpepsin 2 was examined by transient expression of a red fluorescent protein (RFP) fusion protein in leaf epidermis of *Nicotiana benthamiana* accomplished by GATEWAY technology (vector pB7RWG2) and Agrobacteriummediated DNA transfer. 1, 2, and 3 days post-infiltration, cells were imaged by confocal laser scanning microscopy to assess the intracellular localization of the proteins. Results obtained indicate that silpesin 2 directs RFP to the vacuole in accordance with the expected localization in the secretory pathway.

PL-P02
MITOCHONDRIAL PPR-CONTAININGPROTEINS AREESSENTIAL TO SUSTAIN EMBRYOGENESIS IN*Arabidopsis thaliana*

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Plant embryogenesis involves a regular sequence of celldivision, differentiation and morphogenesis. Defects inmitochondrial proteins often show embryo lethal phenotypessuggesting that the mitochondria biogenesis is one of the keyfactors for normal embryo development. We are interested in therole of mitochondrial PPR proteins during embryogenesis. Twoallelic mutants for *At2g02150* (EMB2794) gene are beingstudied. Mutant plants show ~25% of shriveled seeds in maturesiliques. Homozygous mutants could not be obtained. Genesilenced plants were developed by using two independentartificial microRNAs. Due to PPR proteins are sequence-specificRNA-bindingproteins involved in many aspects of RNAprocessing primarily in organelles, the splicing profile of themitochondrial mRNAs was analyzed by quantitative RTPCRofmitochondrial transcripts in silenced plants. Results showsplicing defects for all exons of nad1 RNA (mitochondriallyencoded Complex I subunit NAD1), suggesting that EMB2794protein is a component of nad1 spliceosome. The annotation ofAt2g02150 locus has recently changed incorporating a secondsmall gene (At2g02148) covering the binding site of one of themicroRNAs. Due to the fact that both microRNAs are equallyeffective in silencing EMB2794 transcript, we propose that twodifferent overlapping mRNAs are being transcribed.

PL-P03
TOWARDS UNDERSTANDING THE INTERPLAY BETWEEN PRODH AND ROS BURST IN PLANT HYPERSENSITIVE RESPONSE

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Proline dehydrogenase (ProDH) is the limiting enzyme in thetransformation of proline (Pro) into glutamic acid (Glu) occurringat mitochondria. This enzyme is required for normaldevelopment of the hypersensitive response (HR) against thebiotrophic pathogen *Pseudomonas syringae* pv*tomato AvrRpm1*, and the generation of ROS by the plasma membraneNADPH oxidase, RBOHD. The different subcellular localizationof ProDH and RBOHD excludes a direct interaction of theseenzymes. We here analyze different possibilities that couldexplain their coordination. ProDH generates Δ1-pirroline-5-carboxilate (P5C) and FADH2 loading electrons into themitochondrial electron transport chain (mETC), and its overactivation may affect mitochondrial ROS (mROS) and ATPlevels. Moreover, Pro catabolism could induce Pro synthesisthat takes place at the cytosol consuming NADPH, thusexchanging redox cofactors between both compartments. In this context ProDH could operate either with the second catabolic enzyme P5C dehydrogenase (P5CDH) cycling Pro and Glu, or with the second biosynthetic enzyme P5C reductase (P5CR) cycling Pro and P5C. To start unraveling the mechanisms connecting these mitochondrial and plasma membranes events we analyzed redox alterations derived from ProDH activation in the absence of P5CDH or P5CR.

PL-P04
USE OF NON-THERMALPLASMA FOR PATHOGEN CONTROL AND IMPROVEMENT ON THE BIOCHEMICAL QUALITY OF SEEDS

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Non-thermal plasma treatment is a fast, economic and pollution-free method. The objective of this study was to determine whether different non-thermal plasmas could be used for pathogen control and seed performance improvement or not. Soybean seeds were exposed to a multi filamentous DBD discharge. The dielectric barrier of the discharge consisted in a Pertinax 2.5 mm plaque and 2 0.1

mm layers of Mylar on top of which the seeds were deposited. Different gases (O_2 and N_2) were used to transport the active agents of the plasma. The seeds were irradiated for 1, 2 and 3 minutes using either O_2 or N_2. The fungal load and the growth of the seeds radicle with different treatments were compared with a pathogen containing control. To study the biochemical qualities of the seeds, different oxidative enzymes such as GPOX, SOD and CAT as well as lipid peroxidation (TBARS) and the ROS species generated were measured. When evaluating we observed that the 2 minutes treatment with O_2 had the least increase in the activities for most of the oxidative enzymes as well as the least amount of TBARS. In addition, we saw that the 2 minutes O_2 treatment was the one with the most growth of the seeds radicle. We conclude that the 2 min O_2 treatment is the most effective for the pathogen control. In addition, this treatment is the best for the improvement of the biochemical qualities of the soybean seeds.

PL-P05

POLYAMINES REDUCED GROWTH BY MODULATING REACTIVE OXYGEN SPECIES AND NITRIC OXIDE FORMATION IN WHEAT

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Polyamines (PAs), compounds involved in plant development, are able to generate reactive oxygen species (ROS) and nitricoxide (NO) during their normal catabolism. PAs increased levelsin plants have usually been related to abiotic stress tolerance. However, PAs are also well known to induce PCD or altergrowth or development upon depletion/overproduction withrespect to their physiological levels. The aim of this work was toevaluate If ROS and NO were involved in wheat growthinhibition after the exogenous addition of 1 mM putrescine (Put), spermidine (Spd) or spermine (Spm) or 100 µM of sodiumnitroprusside (SNP) as NO donor. All PAs and NO significantlyreduced seedling growth and increased NO formation. SNP andSpm produced the most drastic reduction in growth, and almostcompletely inhibited O_2- formation, increasing H_2O_2 levels.Catalase-dependent H_2O_2 removal reversed SNP-inducedgrowth repression and restored O_2.-generation,whereas theNO scavenger, cPTIO, recovered 50% of root length when wasadded with Spd, Spm or SNP. Aminoguanidine (DAO inhibitor) and 1,8-diaminooctane(PAO inhibitor) restored growth in thepresence of SNP and Put. Wheat growth reduction occurredafter PAs or NO exposure was mediated by an altered balanceamong the O_2.-,H_2O_2 and NO, produced by a modified NADPHoxidase activity and/or to an increased PAs catabolism mediated by DAO or PAO enzymes

PL-P06

EFFECT OF MAGNETITE NANOPARTICLES ON ALFALFA (*Medicago sativa* L.) PLANTS

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The use of nanomaterials has considerably increased duringthe last decade, however, their effects on plants and otherecosystem components are still controversial. Because theytypically exhibit high surface/volume ratio, metal nanoparticlesare expected to be more reactive than their corresponding bulkelements.The aim of this work was to evaluate if magnetite nanoparticles (Fe_3O_4NPs) are toxic to alfalfa plants. Alfalfa is a traditionalcomponent of argentine pastures dedicated to livestock. Two Fe_3O_4NPs doses (50 and 100 mg L^{-1}) were tested and wefocused on plant growth and oxidative damage. Total biomasssignificantly increased in alfalfa seedlings exposed to both Fe3O4NPs concentrations, likewise, root length and rootsurface area were significantly higher in NPs-exposedplants: root surface area almost doubled that of control plants.Chlorophyll content was also significantly enhanced in plantsexposed to NPs. No evidence of oxidative damage by exploringtraditional oxidative stress markers (H_2O_2, O_2-, MDA content) nor cell death (assayed by Evans blue staining) was detected inalfalfa tissues after exposure to Fe_3O_4NPs.We conclude thatat the doses tested, Fe_3O_4NPsnot only lack of phytoxicity onalfalfa plants, but they stimulate seedling growth and chlorophyllbiosynthesis, thus becoming good candidates for the design ofnew products targeted to agricultural use.

PL-P07

DROUGHT STRESS EFFECTS ON CARBON AND NITROGEN METABOLISM IN THE PEANUT-RHIZOBIA INTERACTION

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The peanut-rhizobiainteraction is valuable for the developmentof sustainable agriculture practices. However, such interactioncan be affected by intermittent episodes of drought stress. In thiswork, the impact of drought stress and rehydration on peanutplants and the role of carbon (C) and nitrogen (N) metabolism inthe response of drought-tolerantcultivars were analyzed. Thewater status, growth and biological nitrogen fixation (BNF); the gene expression by qPCR and the specific activity of C and Nmetabolism-enzymes; and the metabolite profile by GC-MSwere determined in drought-tolerantand drought-sensitivecultivars. The results revealed that nodulation and BNF weredecreased in the sensitive cultivar and unchanged in thetolerant cultivar, despite a similar water status. Then, it was suggested that the tolerant cultivar had the ability to trigger ametabolic response which underlies the tolerance to drought. Inthe tolerant cultivar, the content of compounds with a protectiverole against stress, such as gamma-aminobutyricacid, proline and trehalose were increased. Besides, the sucroseaccumulation was associated with a decreased expression andactivity of sucrose synthase although the N assimilation wasunchanged. Thus, the tolerant cultivar sustained the respiratory substrates for the highly-demanding BNF, a mechanism thatcontributed to the tolerance exhibited.

PL-P08
CONTRIBUTION OF TOMATO ELECTROPHILE COMPOUNDS TO THE THERMOTOLERANCE IN *Caenorhabditis elegans*

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Tomato has been recognized to provide health benefits basedmostly on their antioxidant property. In this work, we proposedthat electrophile compounds could also contribute to thebeneficial effects of tomato. Therefore, we have evaluated theelectrophilic activity in tomato fruit with different colors, and theability of electrophilic extracts to confer thermal stress resistance (thermotolerance) in a model of stress adaptation in*Caernohabditis elegans* (*C. elegans*). To quantify theelectrophilic activity in tomato fruit, we optimized aspectrophotometer method using p-Nitrobenzenethiolassubstrate. Electrophilic activity and pigment profile was detectedand measured in chloroformic but not in aqueous-methanolicextracts. Correlation analysis showed a strong associationbetween electrophilic activity and β carotene content. Moreover, we have used the electrophile compound 1-Chloro-2,4-dinitrobenzeneas an approach to demonstrate electrophile-inducethermotolerance in *C. elegans*. In addition, chloroformextracts from different tomato fruit manifested diversethermotolerance activities that were positively associated withtheir electrophilic activity. All these results provide evidence that β carotene-derivedelectrophiles could be contributing to thebeneficial effects of tomato fruit through stress adaptation.

PL-P09
CLONING AND SEQUENCING OF A NEW CYSTEINEPEPTIDASE FROM FRUITS OF *Bromelia hieronymi* MEZ

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Fruits of *Bromelia hieronymi* Mez (Bromeliaceae) possess highcontent of cysteine peptidases with potential uses in both foodand pharmaceutical industries. The aim of this work included cloning and sequencing of a new cysteine peptidase (CP) from this species. Unripe fruits were crushed in a mortar and pestleunder liquid nitrogen and 0.2 g were employed to extract totalRNA. cDNA was synthesized by retrotranscription and used asPCR template with specific primers designed from conserved N-terminalsequences of CPs. The amplified product of 989 bpwas cloned into pGEM-TEasy vector and transformed intochemically competent *E. coli* (Top10) cells. Several clones were sequenced. The deduced amino acid sequence (named BhCP2) encoded an enzyme of 229 residues with predictedmolecular mass of 24.6816 kDa, pI 5.71, and extinction molarcoefficient 58,705 M^{-1} cm^{-1}. BhCP2sequence displays thecharacteristic primary structure of plant CPs in addition toconserved catalytic residues as well as six Cys residues, involved in the formation of disulfide bonds. The peptidase shows 93% of identity with BhCP1, other peptidase from *B. hieronymi*, and ca. 80 % with *Ananas comosus* peptidases. This work is the first step to express this enzyme, which might be a promising biocatalyst for industrial processes.

PL-P10
ANALYSIS OF METABOLIC INTEGRATORS FROM *Nannochloropsis gaditana*

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Keeping up with demand for food, fuels and biological productsof interest require an urgent effort on increasing crop yields in asustainable way. Photosynthetic microorganisms such aseukaryotic microalgae possess several advantages over higherplants; higher growth rate, high photosynthetic conversionefficiencies, increased lipid and carbohydrate accumulation,and ease of manipulation are just a few. *Nannochloropsis gaditana* is a promising production microalga whose publishedgenome consists of 30 chromosomes and is under 30Mb intotal. It has high nutritional value with elevated protein andpolyunsaturated fatty acid (PUFA) content. Lipid accumulation accounts for more than half of its biomass. On the other hand, transcript profiling of carbon regulated-genesfrom *Arabidopsis thaliana* identified a myo-inositol-1-phosphatesynthase gene, whose expression correlates with increased biomass. In thiswork we assayed heterogeneous growth conditions of *N. gaditana* and analyzed the differential yields on protein content, hydrocarbons and lipids amounts as well as photosynthetic pigments in comparison to the expression of the algal myo-inositol-1-phosphatesynthase gene.

PL-P11
FUNCTIONAL CHARACTERIZATION OF PEACH FRUIT PPZAT12 AND *Arabidopsis* ATZAT12 TRANSCRIPTION FACTORS

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Refrigeration is used to prevent the rapid decay of peaches atambient temperature. However, low temperatures can generatechilling injury (CI), a group of physiological disorders whichseverely affects fruit quality. Many postharvest approaches weredeveloped to prevent CI, being heat treatment (HT) prior to coldstorage a proven efficient strategy. PpZAT12 mRNA was induced in HT-treated fruits and also after cold storage of CI-tolerant cultivars. It encodes a C2H2-typezinc finger transcription factor with an EAR repressor motif, as the reported systemic stress marker AtZAT12 from *A. thaliana*. Cold-andheat-driveninductions of AtZAT12 mRNA were confirmed indifferent organs. Comparative promoter analysis of phylogenetic-clusteredorthologues from different species allowed us to identify conserved cis elements which wouldaccount for the diversity of stimuli that generally alters ZAT12 expression. We obtained 35S::AtZAT12 and 35S::PpZAT12/KOAtZAT12 Arabidopsis transgenic lines and both had impaired growth, rendering smaller plants (compared to wild-type) withcurved leaves. Several targets of AtZAT12 were assessed, showing the different molecular

processes in which both genesmay be involved in. These results point that the transcriptionfactor PpZAT12 could have a protective role against CI in *P. persica*, achieved by similar molecular functionalities than AtZAT12 in *A. thaliana*.

PL-P12
ATTRAF: A NOVEL RNA CHAPERONE INVOLVED INTEMPERATURE STRESS RESPONSES IN *Arabidopsis*

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Once harvested, peaches are cold-storedin order to delayripening. However, refrigeration can produce chilling injury (CI) symptoms, affecting the quality of fruits. Among othersstrategies, heat treatment (HT) preceding cold storage resultedeffective in preventing CI symptoms. PpTRAF is a HT-andcold-inducedtranscript that encodes a TRAF-typeZn finger with aconfirmed RNA chaperone role in peach fruit. In this work, westudied AtTRAF: the *A. thaliana* uncharacterized PpTRAFprotein ortholog. Using an in vivo complementation test in *E. coli* RL211 strain, the RNA helicase activity of AtTRAF was confirmed. The promotor region of this gene was analyzed andseveral stress-relatedcis elements were found. To studyAtTRAF mRNA abundance, we measured its expression levelsby RT-qPCRin different organs and conditions. AtTRAF wasfound in several tissues, reaching the highest mRNA levels inflowers, and showed inductions after both low and hightemperatures. AtTRAF KO mutants and lines overexpressing thegene were obtained and analyzed regarding their ability togerminate and elongate the roots under cold and heat stresses, as well as in the presence of ABA, NaCl, H_2O_2 or mannitol.Several growth differences were addressed in comparison to wild-typelines. Overall, these results suggest that AtTRAF couldhave a molecular role as RNA chaperone under cold and heatstress in *A. thaliana*.

PL-P13
CHARACTERIZATION OF STONE HARDENING DURING THE PEACH FRUIT DEVELOPMENT

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Lignification of peach (*Prunus persica*) fruit endocarp involves astrictly coordinated process, which consists of ligninbiosynthesis, deposition in secondary cell wall andprogrammed cell death. Peach stones accumulate extremelyhigh lignin contents relative to other woody tissues. Therefore, understanding the mechanisms and regulations involved in lignin synthesis and the formation of stone could have importantimplications for forestry, forage and bioenergy crops.In the present work, total phenolic compounds, flavonoids andanthocyanins were quantified in different tissues (endocarp, mesocarp and exocarp) of the fruit during its development (from10 to 100 days after bloom) with the aim of evaluating the flow ofthese metabolites through the phenylpropanoid pathway. A sharp drop in the levels of phenolic compounds and flavonoids in endocarp in the earliest stages of development was observed. On the other hand, anthocyanins were not detected.The concentration of free amino acids in the different tissues ofthe fruit were also determined by high performance liquidchromatography in apolar phase, in order to correlate them tothe abrupt drop in total protein concentration recorded in thefruit during the early development. Interestingly, Asparagine ispresent in peaches at relatively high concentration in all stages and tissues analyzed.

PL-P14
THE TRANSCRIPTION FACTOR ATHB5 IS A NEGATIVE REGULATOR OF LIGNIN ACCUMULATION

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Homeodomain-leucinezipper transcription factors (TFs) are unique to plants and have been related to developmental processes associated to environmental conditions. It waspreviously reported that during Arabidopsis stems maturation, two members of this family (AtHB7 and AtHB12) are inducedwhereas AtHB5 is clearly repressed.

Aiming at unraveling the role of these TFs in stem developmentand lignification process, AtHB5 mutant and overexpressorplants were obtained. Transcript levels of AtHB6, AtHB7, AtHB12 and AtHB16 were upregulated in athb5 mutants and repressed in the overexpressors. AtHB5 mutants have adelayed life cycle and exhibit smaller rosette leaves and abended-branchphenotype compared with the WT. Moreover, mutant stems have more phloem vessels and less xylem thancontrols and a similar trait was observed in the leaves. In matureplants, mutant stems are wider and more lignified than the WTwhereas overexpressors show the opposite phenotype. Finally, plants obtained through crosspollinationbetween athb5mutants and plants transformed with the auxin reporterconstruction: PromDR5:GUS, indicates that auxins are involvedin the bended-branchphenotype. We conclude that AtHB5 is a negative regulator of AtHB7, AtHB12, AtHB6 and AtHB16 and of the lignification processduring stem maturation.

PL-P15
IMPROVEMENT OF STRESS TOLERANCE IN TOBACCO PLANTS BY EXPRESSING CYANOBACTERIAL FLAVODIIRON PROTEINS

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Flavodiiron proteins (Flvs) constitute a large family of enzymesdistributed among bacteria, archaea, protists and gymnospermsbut absent from angiosperms. In the cyanobacterium*Synechoystis* sp. PCC 6803, Flvs are encoded by four differentgenes (flv14) and form two functional heterodimers. The firstone, Flv1-Flv3,is a soluble NADPH-reductasewhich dissipatesthe excess of reducing power by

consuming NADPH andtransferring the hydrides to dioxygen to form water in a single step. The action of these enzymes helps to avoid oxidativestress under unfavorable growth conditions. Using Agrobacterium-mediated transformation we generated plants expressing the Flv1-Flv3dimer in chloroplasts. These plantswere used to evaluate stress tolerance and photosyntheticperformance under different growth conditions. The results of chlorophyll fluorescence assays show an improvement inphotosynthetic efficiency and faster dissipation of excessiveenergy in the transgenic lines compared with wild-typeplants, even under stress conditions. Moreover, transgenic plants display bigger size than controls as it was determined by comparing total leaf area and fresh/dry weight. Taken together, these observations suggest an increase in stress tolerance, faster adaptation to environmental fluctuations and enhancedbiomass production by expression of the cyanobacterial Flv1-Flv3 dimer in higher plants.

PL-P16
THE SUNFLOWER TRANSCRIPTION FACTOR HAHB11 INTERACTS WITH A KINESIN IN *Arabidopsis* TRANSGENIC PLANTS

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Helianthus annuus homeobox 11 (HaHB11) is a divergentmember of the sunflower HD-ZipI subfamily of transcriptionsfactors (TFs). When this TF was ectopically expressed in*Arabidopsis thaliana* plants, these plants were more tolerant toflooding, drought and salinity and, more important, they showeda significant increase in biomass and seed yield when theywere grown in standard conditions. Aiming at understanding themolecular mechanism by which these beneficial phenotypeswere generated, HaHB11' putative protein partners weresearched. A specialized service carried out yeast two-hybrid (Y2H) assays using HaHB11 as bait and protein domains of an *A. thaliana* expression library as preys. Through theseexperiments, several domain clones of a plant kinesin motorfamily protein with very high confident interaction were identifiedand then, confirmed by new Y2H assays. *Nicotiana benthamiana* leaves were transiently transformed with geneticconstructs bearing the kinesin and HaHB11 fused to differentfluorochromes and analyzed by confocal microscopy. Theseexperiments indicated co-localizationof both proteinssupporting the interaction between them. Kinesin mutant andoverexpressor plants were generated and their phenotype analysed. The role of the kinesin and its interaction withHaHB11 in the TF-generatedphenotypes are discussed.

PL-P17
THE HOMEODOMAIN-LEUCINE ZIPPER TRANSCRIPTION FACTOR ATHB23 PLAYS A ROLE IN ROOT DEVELOPMENT

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HD-Zip proteins are transcription factors (TFs) unique to plants.In *Arabidopsis thaliana*, HD-ZipI subfamily has 17 membersencoding proteins of 35 kDa, mainly associated primary withdevelopmental processes related to abiotic stress. AtHB23, belongs to this subfamily and it was found to be expressed in different parts of the plants like shoot meristem region, leaf junction, and flowers. It is know that AtHB23 could substitute itsparalogue AtHB13 in pollen when the latter is defective. Aiming at unraveling the natural role of AtHB23, differentexperimental strategies were applied, including histochemicalanalyses of transgenic plants containing a construct with aGUS::GFP reporter under the control of the promoter region ofAtHB23. GUS was detected in different regions in the main root, principally in the zone of the new lateral roots. In the same waywe observed expression of reporter GUS in the lateral roots. Onthe other hand, we generated AtHB23 silenced plants andectopic expression of AtHB23 in Col0 genetic background. The morphological changes detected in plants with altered levels of AtHB23 expression were related to roots development. AtHB23-silenced plants displayed shorter roots whereas 35S::AtHB23 plants presented longer roots compared with control plants.These results suggest that AtHB23 would have a functional role in root development.

PL-P18
PLEIOTROPIC EFFECTS INDUCED BY LIHSP83-SAG1 VACCINE ANTIGEN EXPRESSION IN TRANSPLASTOMIC PLANTS

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SAG1 is the main surface antigen of the intracellular parasite *Toxoplasma gondii* and is a promising candidate to produce ananti-*T. gondii* vaccine. Previously, we reported that the fusion of *Leishmania infantum* protein to SAG1 significantly increasedSAG1 expression levels in chloroplasts. However, LiHsp83-SAG1plants showed pleiotropic effects, like growth retardationand chlorotic phenotype. Previously, we showed that thechlorotic phenotype observed in the LiHsp83-SAG1plantscould be a consequence of damage in the photosystem II.Following with the characterization of the pleiotropic effects, wefound that LiHsp83-SAG140-60old-daysplants showed asignificant decrease of net photosynthesis and a low stomatalconductance compared to the wild type plants. According to this, we also found that the content of soluble sugars and starchwere highly reduced in LiHsp83-SAG1plants. Interestingly, thequantities of RuBisCo were diminished up to 50 %. Theseresults suggest that the pleiotropic effects in LiHsp83-SAG1plants produce not only injuries in the photosynthesisparameters but also in the metabolic processes. Despite of this, this line is able to produce the highest vaccine antigen yields.

PL-P19
ROLE OF 90KDAHEAT SHOCK PROTEIN IN PLANT IMMUNITY

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Several works demonstrated that plant Hsp90s have an activerole in the activation of the plant defense mechanisms againstpathogens, since the lack or deficit of these proteins impairsdefense against pathogenic bacteria and fungus. Hsp90s arefound in all plant cellular compartments examined to date, including cytosol, nucleus, endoplasmic reticulum, mitochondriaand chloroplasts. Recently, Hsp90 have also been found in theapoplast, even though their role in this location has not beendescribed. In this work, we added some light to this issue byassessing the effects on the defense mechanisms occurring in *Arabidopsis thaliana* plants infiltrated with a recombinantHsp81.2 protein from *A. thaliana* (rAtHsp81.2). After 24 h, cell death, ROS and callose were accumulated in infiltrated leaves, suggesting that the accumulation of the protein may participatein the induction of defense. In this sense, a 24h pre-treatmentofleaves with rAtHsp81.2 resulted in a reduction in theproliferation of *Pseudomonas syringae*pv. *tomato* DC3000 (PstDC3000), whereas the *in vitro* growth of the bacteria is notaffected in the presence of rAtHsp81.2. Our results suggest that infiltration with recombinant Hsp90 is important for mountingappropriate plant immune responses.

PL-P20
Colletotrichum acutatum PRODUCES A LOW MOLECULAR WEIGHT COMPOUND THAT SUPPRESSES-INDUCED DEFENSE

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AsES is a protein secreted by the isolate SS71 of *Acremonium strictum* that elicits a defense response in strawberry plants (*Fragaria annanasa*). Previously, we had observed that whenplants were treated simultaneously with AsES and a conidialsuspension of the pathogen *Colletotrichum acutatum* (isolateM11) a suppression of the AsES-inducedresponse occurred.The aim of this work was to evaluate the defense suppressionactivity in plants of strawberry of the cv. Pájaro using a purified *C. acutatum* M11 supernatant (SN-M11)extract by accumulationof ROS and phytopathological assays. Molecular weight estimation of the active compound was carried out by using a1KDa cut-off membrane. The probe DCFH-DA (2‘,7‘-dichlorodihydrofluoresceindiacetate) was used for intracellular ROS detection in strawberry leaf discs treated with: i) SN-M11,ii) filtered SN-M11(SN-M11f) and iii) culture medium (PDB, as control). The suppression of the defense response was evaluated by phytopathological assays, infecting strawberry plants with active conidia of the virulent isolate M11 previously treated with: i) AsES, ii) SN-M11+AsES, iii) SN-M11f+AsES and iv) H_2O_2. Results showed that the active defense response suppressing compound was lower than 1 kDa; SN-M11f suppressed intracellular ROS accumulation and plants treated with SN-M11f were unable to activate the defense response induced by the elicitor AsES.

PL-P21
SOFT MECHANICAL STIMULUS INDUCES RESISTANCE AGAINST *Botrytis cinerea* IN CULTIVATED STRAWBERRY

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SMS (Soft Mechanical Stimuli) is a type of abiotic stress inducedby mechanical touch. We have previously reported SMS-inducedprotection in strawberry plants (*Fragaria ananassa*) against *B. cinerea*. Thus, the aim of the present work was tofurther investigate characteristic biochemical defenseparameters induced by SMS during *F. ananassa-B. cinerea* interaction. For SMS treatment, leaflets were gently rubbed withfingers, whereas SMS-untreatedplants were used as controls.Then all plants were infected with *B. cinerea*($5x10^4$ conidia/ml)and disease symptoms were evaluated 2, 4, 6 and 8 days postinfection by quantification of lesion diameter (mm). During theplant-pathogeninteraction, fungal colonization, ROS burst (H_2O_2) and callose deposition, were evaluated. In line withSMS-inducedprotection, we observed that treated plants couldconsiderably restrict pathogen growth respect to controls, andaccumulated less H_2O_2. It was also observed that non-infectedtissue surrounding the lesion site presented high callosedepositions. It is likely that callose is acting as an induced-protectivebarrier that restricts *B. cinerea* infection whencompared to untreated control plants. Finally, based on theseresults, we can conclude that SMS-inducedprotection against *B. cinerea* is accompanied by some biochemical responses, which delay pathogen infection.

PL-P22
ASES INDUCES PHYSIOLOGICAL AND BIOCHEMICAL CHANGES OF AVOCADO FRUIT DURING RIPENING

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AsES is a plant elicitor protein that induces disease resistancein strawberry and *Arabidopsis thaliana* plants, triggering SA, JAand ET dependent signaling. The importance of ethylene inregulating fruit ripening has been clearly demonstrated fromstudies that suppressed ethylene biosynthesis or action. The objective of this study was to characterize the physiological andbiochemical responses of the climacteric fruit avocado (*Persea americana*) treated with AsES and to evaluate its utility inpostharvest for regulating the ripening of avocado fruit. Theresults showed that AsES treatment increases respiration rate, weight loss and soluble solids content, while decreasesfirmness of treated fruits, compared to the control not treatedfruits. In addition, ethylene production increases after AsES treatment at early stages of ripening (3 dpt), while in controlfruits the maximum production occurs later (6 dpt). This study is the first report of the influence of a defence elicitor on the physiological and biochemical changes regulated by ethylenein avocado fruit during

ripening, causing accelerated ripeningand softening during storage. These results uncover thepotential use of AsES in postharvest management of ripening, providing new insight for further research on the relationshipbetween fruit qualityand induction of disease resistance inAsES-treatedfruits.

PL-P23
HET ELLAGITANNIN ALTERS CELL REDOX STATUS AND ROS ACCUMULATION IN STRAWBERRY PLANTS

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It was reported that strawberry leaves exposed to various bioticand abiotic factors accumulate a phenolic compound identifiedas 1-0-galloyl-2,3;4,6-bis-hexahydroxydiphenoyl-β-D-glucopyranose(HeT) and that foliar applications of HeTincreased resistance toward *C. acutatum*, responsible ofanthracnose disease. In this paper we have investigated theearly cellular events that take place during the activation of theimmune response of strawberry plants treated with HeT.Antiradical activity of HeT was confirmed *in vitro* showing thatHeT acts as an antioxidant, scavenging superoxide radicals. Arapid decrease of the intracellular H_2O_2 in isolated mesophyllcells treated with HeT was observed 1 hour post treatment (hpt), suggesting that this effect may be due to the decrease of superoxide anion (substrate of the superoxide dismutase). With*in vitro* analysis it was also observed that HeT inhibits theactivity of the enzymes catalase and peroxidase. The latter may explain the accumulation of H_2O_2 observed 4 hpt in strawberry plants treated with HeT. The addition of HeT to mesophyll cellsalso caused a rapid hyperpolarization of the plasmaticmembrane, possibly due to the movement of ions through themembrane provoked by the alteration of the cell redox status.Finally, a quick accumulation of nitric oxide in the chloroplasts ofcells treated with HeT was observed.

PL-P24
EFFECT OF BRASSINOSTEROIDS TREATMENT IN STRAWBERRY DEFENSE MARKERS

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Brassinosteroids (BRs) are steroidal compounds that areessential for plant growth and development. It was shown thatBRs can also provide plants tolerance/resistance to a widerange of biotic and abiotic stresses in a dose dependentmanner. The aim of this work was to evaluate some defencebiochemical markers such as H_2O_2, O_2^-andcallose instrawberry plants of the cv. Pájaro treated with the BRs 24-epibrasinolide (EP24) and the commercial formulationBIOBRAS 16 (BB16). Defence related oxidative burst wasevaluated by two histochemical staining, and H_2DCF-DAfluorescent probe. Callose deposition was studied by histochemical staining with aniline blue. The influence of BRs on stomatal aperture was evaluated in epidermal strips of *Vicia faba*. Results show that strawberry plants treated with EP24 and BB16 showed increased production of H_2O_2, and O_2^-, and higher callose deposition as compared to the control plants. However, stomatal closure was only observed in leaf stripstreated with BB16. These results reveal that BRs can also beused for the activation of innate immunity in strawberry plants, hence could be used as a new strategy for the control ofstrawberry plants diseases, environmentally friendly and alternative to agrochemicals.

PL-P25
STRUCTURAL DETERMINANTS INVOLVED IN THE REDOX REGULATION OF THE *Arabidopsis* FUMARASES ENZYMES

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Fumarase (FUM) is a homotetrameric enzyme that catalyzes thereversible hydration of fumarate to L-malate,a reaction of thetricarboxylic acid (TCA) cycle occurring in aerobic organisms. Inplants, the regulation of carbon flux through the TCA cycle isespecially important since mitochondrial activities have to becoordinated with photosynthesis. *Arabidopsisthaliana* has twoFUM genes, *At*FUM1 encoding the mitochondrial isoform and*At*FUM2 encoding for a cytosolic one. In previous studies, wehave carried out the biochemical characterization ofrecombinant forms of these enzymes. The emerged regulatoryproperties linked AtFUM1 and -2with the nitrogen metabolismand the cytosolic pH regulation. In this work, we have exploredthe redox modulation of AtFUMs, as several enzymes of TCAcycle have been reported as thiorredoxin targets *in vitro*. Inorder to identify the residues involved in the redox modulation,MALDI-TOFanalysis of oxidized and reduced AtFUMs as wellas site-directedmutagenesis approaches were performed. Theresults obtained for AtFUM1 indicated the participation ofCys219 and Cys306 in the formation of interchain disulfidebridges and suggested that Cys332, Cys389 and Cys435 arealso involved in the redox regulation. Our results provide newinsights into the regulatory mechanisms of FUM of plants thatcould link them with photosynthesis and other subcellular process.

PL-P26
DIFFERENTIAL METABOLIC REARRANGEMENTS AFTER COLD STORAGE OF DIFFERENT PEACHFRUIT VARIETIES

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Peaches are highly perishable and deteriorate quickly atambient temperature. Therefore, refrigeration is commonly usedto prevent fruit decay. The molecular changes that take placedue to cold storage impacts on the way fruits ripen during thefollowing shelf-life.Few studies have been devoted to theanalysis of the overall metabolite reconfiguration duringripening after cold storage of fruits prior to consumption.Metabolite profiling of six peach fruit varieties was performed.Metabolic content after normal ripening and after ripeningfollowing cold treatment was compared. Both common anddistinct metabolic responses among the six varieties werefound;

common changes include isomaltose, raffinose andxylose increase; Ser and Phe increase; the polyamineputrescine and spermidine increase; and fumaric aciddecrease. Among the differential responses it is interesting tonote that the variety Elegant Lady presents a marked increasein sucrose, glucose and fructose sugars; and malic and citricacids, all of which are closely related to the flavor of ripe fruits.Overall, results indicate that peach fruit differential metabolicrearrangements due to cold treatment are variety dependent.The identified metabolic changes that are associated with coldstorage may aid in the improvement of peach fruits, with thegoal of engineering fruits with higher quality for consumers.

PL-P27
FIRST ANALYSIS OF THE WHOLE PUTATIVE THIOREDOXIN FAMILY MEMBERS IN *Zea mays*

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Thioredoxins (Trxs) are ubiquitous small proteins containing anextremely reactive site with a highly conserved sequence, participating in several reactions that require reduction ofdisulphide bonds on selected target proteins. Trxs form amultigenic family encoding numerous isoforms which areusually classified in relation to their function, structure andsubcellular localization. Plants possess the greatest Trx familyfound in all organisms, however, little is known about this familyin plant kingdom. In this work, we present the characterization ofthe whole maize putative thioredoxin family members. Byperforming sequence homology search, gene structure andphylogenic analysis in comparison with the alreadycharacterized Arabidopsis Trxs family we found twentysixmaize putative Trxs, the largest family known until present.These Trxs were classified in seven groups corresponding totheir possible evolutionary origin. RT-qPCR of the wholeputative maize chloroplast Trxs analyzed in different tissuesshowed a predominant leaf expression. Besides, *in silico*analysis of two chloroplast Trxs promoters showed that ZmTRXCMwould be involved in response to ethylene while ZmTRXCVVwould be regulated by light, participating in light-dependentpathways like photosynthesis. These results are thestart point to unravel the diverse routes in which maize Trxsisoforms are involved.

PL-P28
FUNCTIONAL CHARACTERIZATION OF A DC1-DOMAIN PROTEIN ESSENTIAL FOR EARLY GAMETOPHYTIC DEVELOPMENT

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In this work we identified At2g17740 as a DC1-domainproteinpresent in the endomembrane system that is essential for bothfemale and male gametophyte early development in*Arabidopsis thaliana*. At2g17740 was originally annotated as agene encoding a DC1-domainprotein with unknown function.As no homozygous mutant plants could be identified, we showin hemizygous insertional mutant lines that nearly half of themale and female gametophytes arrest after meiosis. At arrestedstages both mutant gametophytes contain only one nucleus(FG1 for female and microspore for male), as seen with DAPIstaining techniques. In order to functionally characterize thisgene we generated expression reporter lines pAt2g17740-GUSfor promoter activity and pAt2g17740-At2g17740-GFPfor co-localizationassays. GUS activity was detected in cotyledons, leaf primordia and roots of young seedlings, while it was alsoobserved in inflorescence stem and filaments of mature plants.Co-localizationwith RFP-Rha1revealed that At2g17740-GFPislocalized in prevacuolar compartments in transientlytransformed *Nicotiana benthamiana*. In addition, At2g17740was proven to interact with AtPVA12, a member of the SNAREvesicle associated protein family, and with LTL1, a GDSL-motiflipase by means of two-hybridand bimolecular fluorescencecomplementation assays. Supported by ANPCyT (PICT 1524),CICPBA,Conicet and UNMdP.

PL-P29
OPTIMIZATION OF RECOMBINANT MAIZE CDKA PRODUCTION IN *Escherichia coli*: A STATISTICAL APPROACH

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The complex formed by the cyclin dependent kinase A (CDKA)and cyclin D is responsible for the G1-Stransition of cell cycle.Oxidative post-translational modifications of these proteins blockcell cycle progression, and thus contributing in plant growthinhibition during abiotic stress. As part of a project to determinepossible sites of CDKA protein oxidation, CDKA from *Zea mays*L (maize) was cloned and expressed in *E. coli*. The presentstudy describes the optimization of the production of the proteinusing as statistical approach: Response Surface Methodology.A 24 full factorial central composite rotary design for fourindependent variables, each at five levels was employed to fit asecond order polynomial model which indicated that 30experiments were required for this procedure. Level 0 ofvariables used was 26°C, 0,6 OD 600nm before induction, 0,75mM isopropyl β-D-1-thiogalactopyranoside(IPTG) concentrationand 5h post-inductiontime. An optimization in CDKA productionin the soluble fraction was achieved. Results obtained are notvalid for all recombinant proteins.

PL-P30
CITRATE METABOLISM IN OIL SEEDS

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Different plant species use different pathways to generateprecursors for the biosynthesis of seed reserves. In turn, in eachspecies would coexist more than one route to the input ofcarbon, energy and the required reducing power In this work,we studied the contribution of citrate metabolism to the fillingprocess in the model species *Arabidopsis thaliana* and insoybean, a legume of great economic importance. In thegeneral framework, the aim of this project is to findbiotechnological tools for improving oil yields from oilseedcrops. Based on previous studies, organic acids would beactively metabolized during seed maturation. In this context, citrate synthase, enzyme responsible for mitochondrialsynthesis of citrate, and ATP-citratelyase, which catalyzes itscleavage to generate acetyl-CoA,the direct precursor of fattyacids, are good candidates for improvement in soybean. Thus, profiles of enzyme activity and gene expression of the differenttranscripts were comparatively evaluated at different stages ofseed maturation in both species.

PL-P31
PHOSPHATE DEFICIENCY IN PLANTS: THE ROLE OF EXTRACELLULAR AND INTRACELLULAR RIBONUCLEASES

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Phosphorus deficiency is a widespread problem in agriculturalareas. Most soils contain significant amounts of P; however, less than 1% of it is available for plant root uptake because inorganicphosphate (Pi), the only form in which plants can assimilate P, israpidly immobilized in soils. Plants growing under Pi limitationtrigger a complex set of responses, among which is theinduction of ribonucleases (RNases) to scavenge Pi from ARN.In *Nicotiana alata*, intracellular NnSR1 (Nicotiana non S-RNase1) and extracellular RNase NE are, thus far, the onlyRNases known to be induced under Pi starvation. To discern thePi threshold triggering such induction, plants were cultivatedunder different Pi concentrations. The induction of RNase NE at10 µM Pi, assayed by RT-PCRand western blot, suggests thatthe extracellular RNA may be the first source of P used by thecell under Pi stress. To understand the functional role of NnSR1and RNase NE, a post transcriptional silencing strategy was developed. The hypervariable sequence of each RNase wasinserted in opposite directions into an RNAi binary vector.These constructs were introduced into Nicotiana leaf disks viaAgrobacterium infection. The level of RNase NE, which isconstitutively expressed in floral organs, was assayed on regenerated plants. The analysis of silenced plants growingunder conditions of Pi deficiency is discussed.

PL-P32
THE CYSTEINE DESULFURASE ATNFS1 IS INVOLVED IN FE-SCLUSTER ASSEMBLY AND IRON METABOLISM

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The *Arabidopsis thaliana* cysteine desulfurase AtNfs1 has anessential role in cellular Fe-Scluster assembly. It has beenreported a relationship between this protein and ironmetabolism. In order to further understand this, we have studiedthe phenotype of two mutant lines (KD501 and KD681) and twooverexpressing lines (AtNFS-1and -2)of AtNfs1 in *Arabidopsis thaliana*. The mutant lines show a decrease in biomasscorresponding to a smaller foliar area and main root lengthwhile overexpressing lines show an increase in theseparameters compared to wild type. No change has beenmeasured on chlorophyll content in these lines. An increase inthe amount of total iron has been observed in theoverexpressing lines compared to wild type and mutant linesbut no significant change in total iron has been detectedbetween mutant and wild type lines. Thus, we analyzed theexpression levels of iron metabolism related genes. Resultsshowed an increase of the expression of several genes relatedto iron homeostasis. Taken together, our results suggest that AtNfs1 plays a key role not only in cellular Fe-Sclusterassembly but also in iron uptake and assimilation.

PL-P33
TWO MITOCHONDRIAL SCO PROTEINS DIFFERENTIALLY AFFECT SALT STRESS RESPONSES IN *Arabidopsis thaliana*

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Cytochrome c oxidase (CcO) is the last complex of themitochondrial electron transport chain. Arabidopsis HCC1 is ahomolog of Sco proteins involved in the delivery and insertion ofcopper into CcO. Another Sco protein, HCC2, is only present inplants and does not have a copper binding domain. To furtherstudy the role of HCC1 and HCC2, we analyzed globalexpression profiles of knockout mutants in HCC2 (hcc2) andantisense plants for HCC1 (asHCC1). Both plant genotypespresent opposite transcriptional profiles in numerous GeneOntology terms. For instance, hcc2 plants exhibit higher levelsof transcripts related to stress responses, while asHCC1 plantsshow decreased levels. In agreement with this, they showdifferential responses to salt stress: hcc2 plants are moretolerant than wild-typeand asHCC1 plants are more sensitive.Likewise, transcripts of stress-responsivegenes show earlier orincreased induction in asHCC1 plants after long term treatmentsbut the short term response, which is known to be essential forplant survival, is diminished or abolished. An opposite behavioris observed in hcc2 plants. This points to a role of mitochondriain the modulation of the response of plants to stress.Particularly, Sco proteins seem to haveeveloped roles beyondCcO assembly during plant evolution.

PL-P34
TCP14 AND TCP15 PARTICIPATE IN TEMPERATURE-DEPENDENT DEVELOPMENTAL RESPONSES IN *Arabidopsis thaliana*

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TCP transcription factors are exclusive to plants and are mainlyinvolved in the regulation of developmental processes andhormone responses. They can be grouped into two majorclasses, I and II. In this work, we studied the role of two closelyrelated class I TCP proteins, TCP14 and TCP15, in theresponse to temperature in *Arabidopsis thaliana.* Loss-of-functionmutants in TCP14 and TCP15 showed a reducedresponse to an increase in temperature from 22°C to 29°C, reflected in reduced hypocotyl and petiole elongation. Theinduction of the auxin biosynthesis gene YUC8 was not affectedin these plants, but the expression of members of the auxin-responsive SAUR family was reduced. In addition, theexpression of TCP15 is reduced in the PIF4 mutant pif4-2that isunable to respond to a raise in temperature. Mutants in TCP14and TCP15 also showed a reduced response of hypocotyls elongation to gibberellin or auxin treatments. Our results suggest that TCP14 and TCP15 participate in hormonalresponses during temperature-inducedgrowth in Arabidopsis.

PL-P35
SURF-1MODULATES HYPOCOTYL GROWTH BY INFLUENCING AUXIN AND GIBBERELLIN ACTION IN *Arabidopsis*

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Cytochrome c oxidase (COX) biogenesis is a complex processthat requires the action of multiple factors including SURF1proteins. SURF1 deficiency is a mitochondrial disorder and isthe most frequent cause of Leigh syndrome associated withCOX deficiency in humans. The role of SURF1 in plants still remains unknown. SURF1 is encoded by two genes, SURF1-1and SURF1-2, in Arabidopsis. Heterozygote surf1-1 mutant plants produce 25% abnormal seeds, showing that SURF1-1 isessential for plant development. surf1-2 mutants exhibit shorterhypocotyls under low light intensity compared with those of wild-typeplants. In contrast, SURF1-2overexpressing plants haveelongated hypocotyls under identical conditions. Histochemical analysis using SURF1-2promoter GUS fusions showed thatexpression levels increase in elongating hypocotyls. We found that genes encoding auxin and gibberellin (GA) biosynthesisenzymes are significantly repressed in surf1-2 when grown atlow light. Activity of the auxin reporter DR5-GUS was lower in the surf1-2 background, indicating reduced auxin response insurf1-2hypocotyls. Interestingly, normal elongation of surf1-2 hypocotyls was observed in the presence of exogenous GA and auxin. Our results suggest that SURF1-2is capable of regulating the elongation of Arabidopsis hypocotyls acting onGA and auxin homeostasis.

PL-P36
OVEREXPRESSION OF STAP PSI-DOMAIN IN *Arabidopsis thaliana* INCREASES RESISTANCE TO *Botrytis cinerea*

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Saposin-likeproteins (SAPLIPs) are members of a large proteinsuperfamily widely distributed among of living organisms. Theseproteins may exist for itself independently as a functional unit oras a part of a multi domain protein. Plant specific insert (PSI) is adomain present in the precursors and monomeric mature typicalplant aspartic proteases (APs). This domain has structuralhomology with SAPLIPS with antimicrobial activity and it hascytotoxic activity towards plant pathogens. In the current study, we evaluate the resistance degree to *Botrytis cinerea* in six*Arabidopsis thaliana* transgenic lines overexpressing *St*AP-PSIdomain. Results obtained show that the level expression of*St*AP-PSI has a positive correlation with the decrease in the foliar area affected by *B. cinerea*. Also, we determinate that*St*AP-PSI has a direct cytotoxic effect on *B. cinerea* spores and hyphae. In addition, qPCR analysis demonstrated that theexpression profiles of various disease resistance genes (PR1, PR2, PR5 and WRKY33) in the transgenic *A. thaliana* lines were increased respect to the wild type plants. These findings suggest that *St*AP-PSI expression enhances resistance to *B. cinerea* in *A. thaliana*, in a direct and indirect manner.

PL-P37
STUDYING THE CYTOSOLIC GA3PDHASE IN AUTOTROPHIC AND HETEROTROPHIC *Chlorella* CELLS

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The unicellular green algae *Chlorella* accumulates lipids and starch in varying proportions due to metabolic changesoccurring in different trophic culture conditions. This fact is notwell understood but is potentially useful for biotechnologicalapplications. Glycolysis, a central metabolic pathway providingenergy and building blocks in the cell, occurs in the cytosol andplastids of photosynthetic organisms. In this scenario, glyceraldehyde-3-phosphate (Ga3P) is a key glycolyticprecursor being partitioned inside the cell. In this work, westudied the cytosolic (NAD^+ dependent) Ga3P dehydrogenase (Ga3PDHase; EC 1.2.1.12) that catalyzes the reversibleoxidation of Ga3P to 1,3-bisphosphoglycerate.We cloned thetwo genes coding for Ga3PDHase in *Chlorellavariabilis* and expressed the recombinant proteins in *Escherichia coli*. Bothpurified enzymes were characterized in their respective kinetic and regulatory (posttranslational) properties. Also, we determined that Ga3PDHase exhibited particular protein profiles in protein extracts from *Chlorella* cells grown under different trophic conditions, with 6-fold higher activity in heterotrophic regarding autotrophic

conditions. Heterotrophic cells contained 3-fold more lipids and less starch than autotrophic cells, pointing out a correlation between the lipid accumulation and the Ga3PDHase activity.

PL-P38
ALTERNATIVE SPLICING REGULATION AND RNAPOLYMERASE II ELONGATION MEDIATED BY LIGHT

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We have previously shown that light/dark conditions affectseveral alternative splicing events in *A. thaliana*, including thatof the splicing factor RS31. Treatment of seedlings withtrichostatin A, a drug that inhibits histone deacetylases, mimics the effect of light on RS31 alternative splicing (AS). This led usto investigate whether chromatin modifications play a role in theregulation of AS by light. However, chromatinimmunoprecipitation assays showed no light-dependentchanges in H3K9Ac or H3K9me2 along the RS31 gene.Knowing that the elongation rate of RNA polymerase II (RNAPII) modulates AS in mammalian cells (de la Mata *et al*, 2003) weasked whether a similar mechanism is involved in the lightregulation of RS31 AS. First, we observed that treatment of seedlings with camptothecin, a drug that inhibits RNAPII elongation, mimics the effect of darkness on RS31 AS. Second,we found that mutant plants defective in the elongation factorTFIIS do not respond to light/dark conditions as wild type plants,mimicking the effect of darkness on AS. Finally, preliminaryresults aimed at detecting changes in RNAPII elongationthrough quantifications of pre-mRNAat proximal and distalregions of the RS31 gene support a scenario in which lightcontrols RS31 AS by increasing RNAPII elongation.

PL-P39
STUDY OF CALMODULIN BINDING PROTEIN IQ67-DOMAIN CLASS IV (IQD) IN *Arabidopsis thaliana*

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Calmodulins (CaM) proteins are principal sensors ofintracellular calcium fluctuation, which are generated by plants in responses to abiotic and biotic stimuli. A novel class ofputative plant-specific CaM targets is the IQ67-Domain (IQD) family protein. Surprisingly, little is known about the biological roles of these proteins. We have cloned, expressed and purified three members of *Arabidopsis thaliana* IQD family (IQD28, -30and -32).The recombinant proteins were used to test their ability to bind CaM *in vitro* and found that IQD28, -30and -32 interact with CaM in a Ca^{2+}-independent and –dependent manners. Also, we have studied their transcriptional response to different conditions. Their transcripts levels were repressed in leaves after salicylic acid treatment. In contrast, there were no differences of transcripts levels between control and treated roots with abscisic acid (ABA). On the other hand, transcripts levels of these genes were induced in roots after osmotic treatment. Roots of knock out lines were phenotypicallycharacterized in response to similar stresses. Our data reveal a difference of primary root length between wild type (WT) and knock out lines (IQD32) under NaCl and ABA conditions. The length of the primary root was lower in knock out lines (IQD30) than in WT lines under control conditions. IQDs may affect root growth by binding to cytoskeletal components.

PL-P40
LIPID PROFILING OF PEACH LEAVES FROM GENOTYPES WITH CONTRASTING SUSCEPTIBILITY TO *Taphrina deformans*

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Peach Leaf Curl disease, caused by *T. deformans*, affects yieldand plant longevity. Only a few cultivars are considered tolerant.We conducted a lipidomic analysis of healthy leaves from aresistant (DR) and a susceptible (DS) genotype, and ofasymptomatic DS leaves in which the pathogen was detected (DS^+). By using ultra-performance liquid chromatography coupled to Fourier-transform mass spectrometry, we detected 132 lipids. Principal component analysis revealed the lipidome of DR clearly differs from that of DS, and that of DS significant changes upon *T. deformans* infection. DR shows higher levels of MGDG 34:5 (37%), MGDG 34:6 (33%), TAG 60:3 (16%), PI,PC PE, PG and SQDG and lower levels of DAG 36:3 (8%), DAG36:6 (7%) and MGDG 36:2 (8%) than in DS. Comparing infected and healthy leaves of DS, while the behavior is variable within each member of the different groups of lipids (TAGs, DAGs, SQDGs and phospholipids), the levels of MGDG is not affected and those of DGDG decrease in infected leaves. Galactolipidsare important constituents of chloroplastic membranes and have also associated with the medicinal and nutritional properties ofvegetable plants. Here, results obtained suggest that galactosyldiacylglycerol lipids (MGDG and DGDG) may have a critical role in the response of *Prunus persica* against *T. deformans*.

PL-P41
REGULATION OF PLANT DEVELOPMENT BY A NON-CODING RNA TRANSCRIBED BY THE BIDIRECTIONAL HAWRKY6 PROMOTER

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MicroRNAs (miRNAs) are small 21-nucleotide RNAs that post-transcriptionally regulate gene expression, controlling development and adaptation of multicellular organisms to the environment. The miRNA-mediated regulation of transcription factors is critical for the correct response to stress conditions. For instance, the miR396-mediated regulation of HaWRKY6 controls the sunflower response to temperatures. We observed that *Arabidopsis thaliana* plants expressing a reporter gene under the control of the HaWRKY6 promoter present an unusual phenotype accompanied with a reduction in the miRNAs levels.We discovered that this promoter works bidirectionally, it transcribes HaWRKY6 in sense and a non-codingRNA (ncRNA) in antisense.This ncRNA, the cause of the phenotype, adopt a fold back structure characteristic of miRNA precursors.Small RNA Sequencing (sRNA-Seq), allowed us to identify

a 21nt small RNA derived from this ncRNA. Transcriptomic analysis of plants expressing such ncRNA is allowing us to identify the potential targets of this ncRNA that could explain the phenotype. Interestingly, we have observed that the phenotype triggered by this ncRNA disappear with the successive plants generations, unless transformed into siRNA deficient mutants, suggesting that this region of the HaWRKY6 undergoes epigenetic silencing, fact confirmed by bisulfate treatment in sunflower.

PL-P42

MECHANISMS OF LOADING, SELECTION AND RETENTION OF THE MICRO RNAS STRANDS IN PLANTS

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MicroRNAs (miRNAs) are small 21-nucleotides RNAs that post-transcriptionallysilence messengers RNAs. During the miRNAbiogenesis process a double-strandedduplex is excised. It has been shown that the RISC complex can retains both strands of the duplex and triggers silencing. This is the case of the *Arabidopsis thaliana* miR171 duplex, in which each strand canbe alternatively loaded into AGO1 and silence different targetsin a tissue specific manner. How the RISC complex selects andretains one of the two strands remain largely unclear. To identify the mechanisms and factors involved in this process, wedesigned a double trigger reporter system that allows us todiscern which strand of an artificial miRNA is loaded into AGO1.In this system, the guide strand of the artificial miRNA wasdesigned to target the gene encoding the BASTA Resistance while the miRNA* target an engineered version of the FireflyLuciferase. Arabidopsis transgenic plants carrying this systemwere generated and the reporter system extensively tested.Homozygous selected lines are mutagenized by EMS looking for altered strand-selectionmutants to identify genes involved insuch process. Furthermore, we are using a miR171 strand-dependent reporter system, previously designed in the lab, to explore environmental conditions affecting the miRNAs strand selection.

PL-P43

FINDING NEW COMPONENTS OF THE MIRNA BIOGENESIS IN *Arabidopsis*

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In Arabidopsis, the miRNA biogenesis machinery is composed by a few core proteins, such as DICER-LIKE1, HYPONASTIC LEAVES-1 and SERRATE, plus a combination of regulatory factors recently identified. Even when the miRNA pathway is well known, its regulation remains largely unknown. In order to identify new components of the miRNA biogenesis that help us to explain how it is regulated, a forward genetic screening was designed. Plants carrying a LUCIFERASE-based miRNAreporter system were mutagenized by EMS and used to isolate putative miRNA deficient mutants. Using this approach, anumber of new cofactors in the pathway, subtly affecting thepath, were studied and recently described in our lab. Now, weturned our attention to a set of mutants, obtained in the same screening, that present severe developmental defects. As expected, our analysis revealed that either the mature miRNAspopulation or their action is severely impaired in these mutants.Whole genome sequencing follow by SHORE mapping allowedus to identify the region involved in such miRNA deficiency. Adeeper analysis revealed that a few genes, including thoseencoding an RNA helicase, a WD40 protein and an unknownprotein, are mutated in this region. By studying them, we are inthe process to identify new components of miRNA biogenesisthat severely affect the pathway.

PL-P44

RELATIONSHIP BETWEEN PII PROTEIN AND ENVIRONMENTAL STRESSES IN *Arabidopsis*

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PII proteins are central regulators of nitrogen (N) metabolism in eubacteria and cyanobacteria. They also coordinate the relationship between N and carbon metabolism and sense cell energy status. In eukaryotes, PII homologs have been only reported in red algae and plants. Plant PII is targeted to plastids and is encoded by a nuclear gene (GLB1). The Arabidopsis PII mutant is viable and has not severe phenotype. Since its identification in photosynthetic eukaryotes, many efforts have been made to unravel its biological function. To gain insights in the PII protein role in plants, we submitted an Arabidopsis mutant lacking PII to different abiotic (salt and oxidative) and biotic (infection with the fungal pathogen *Fusarium graminearum*) stresses. Different phenotypic and biochemical analysis were performed. Our results showed that even though PII is not essential, the lack of that protein caused modifications in the plant response to abiotic and biotic stresses. One of the most relevant effects was the increased tolerance to Fusarium infection exhibited by the PII mutant. We also analyzed the PII transcript levels under control and stress conditions in Arabidopsis wild-type by RT-PCR experiments. In conclusion, our data indicate that PII protein could have a role in the response to environmental stresses in Arabidopsis. Supported by CONICET, ANPCyT, UNMdP and FIBA.

PL-P45

TEMPERATURE STRESS TOLERANCE IN *Arabidopsis thaliana*: A ROLE FOR ALTERNATIVE SPLICING

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In the last year earth global temperature has reached anhistorical record, exceeding in 1°C the value estimated for the19th century. As a consequence, a profound effect on plants distribution and survival is expected, so crop improvement orientated to high temperature tolerance seems an interestingalternative in order to mitigate the deleterious effects of heatstress in plants. It is well known that plant´s physiological and metabolic plasticity is given by deep molecular changes in gene expression. Although the importance of these processes instress responses is widely accepted, little is known about howpost-transcriptionalregulatory mechanisms contribute to

stresstolerance. With the aim of identifying components of the pre-mRNAprocessing machinery involved in the establishment ofhigh temperature tolerance, we performed an in-silicoscreeningin public data bases. As a result of this approach, we were ableto find an *Arabidopsis thaliana* splicing factor whose expressionis altered under both high and low temperatures. By performing a physiological and molecular characterization of Arabidopsis plants bearing a mutation in this splicing factor, we uncovered the importance of alternative splicing in stress tolerance. Our results highlight the role of both alternative splicing and its regulation in establishing high temperature tolerance in *Arabidopsis thaliana*.

PL-P46
CONTROL OF BRASSINOSTEROID SIGNALING BY SHADE AND TEMPERATURE CUES

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Plants respond to the shade imposed by neighbors by modifying their growth patterns to produce a more competitivebody form. Shade reduces the activity of photosensoryreceptors phytochromes and cryptochromes and henceincreases the activity of PHYTOCHROME-INTERACTING FACTORS (PIFs), which are bHLH transcription factors that regulate the expression of growth-related genes. Warm temperature also increases PIF activity and yield growthpatterns that resemble shade responses.Brassinosteroids are potent promoters of growth, and mutants affecting this pathway barely respond to shade signals.BRASSINAZOLE-RESISTANT1 (BZR1) and BRI1-EMS-SUPPRESSOR1 (BES1) are positive regulators of the BRsignaling pathway that physically interact with PIFs and synergistically regulate the expression of growth-promoting genes. We investigated whether shade and/or temperature cues affect brassinosteroid signaling. We will report the dynamics of BES1protein levels and phosphorylation status and of BES1 nuclearabundance in hypocotyls and cotyledons of plants expressing pBES1:BES1:GFP. Current results support a model where shade and temperature signals modify the activity of BES1.

PL-P47
NEW GENES FROM *Xanthomonas citri* SUBSP. *citri* INVOLVED IN BACTERIAL EPIPHYTIC SURVIVAL

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Xanthomonas citri subsp. *citri* (Xcc) is the causal agent of citruscanker. In this work we analyzed genes from Xcc with unknownfunction present in several plant pathogens, such as XAC0100and XAC4007. Expression of these genes in bacteria grown inXVM2, a minimal medium that simulates conditions of theintercellular apoplast of leaves, was analyzed by RT-qPCRandthe expression of both genes showed an induction compared tobacteria grown in rich medium. Moreover, RNA was obtainedfrom Xcc recovered from citrus leaves at 0, 3 and 6 days postinfection (dpi) and after RT-qPCR, results revealed that bothgenes were induced at 6 dpi. These results suggest that thesegenes have a role in plant-pathogeninteraction. To characterizethe function of XAC0100 and XAC4007 during diseasedevelopment, we constructed non-polarXcc mutants in thesegenes generated by double cross-overevents. The resultingΔXAC0100 and ΔXAC4007 mutant strains were tested for theirability to trigger disease in citrus leaves and hypersensitiveresponse in non-hostpepper leaves. No differences wereobserved in host and non-hostinteractions. However, ΔXAC0100 and ΔXAC4007 mutants displayed impairedepiphytic survival on citrus leaves, indicating that these geneswere required to maintain bacteria on the host plant tissue.

PL-P48
FRATAXIN OLIGOMERIZATION AND METAL BINDING PROPERTIES IN PLANTS

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Frataxin is a highly conserved protein involved in FeSclusterbiosynthesis, iron homeostasis, heme metabolism andprotection against oxidative damage. In humans, defects infrataxin levels lead to Friedreich's ataxia, a progressiveneurodegenerative disease. Previous studies in *E. coli*, *S. cerevisiae* and human frataxin indicate that these proteinspresent different oligomerization behavior and different metalsbinding properties, which are specific to each species. Also, ithas been reported that the N-terminaltransit sequence thatdirects its transport to the mitochondria participates in theseprocesses. In plants, frataxin is found in both chloroplast andmitochondria. In the present work, we characterized *A. thaliana* (AtFH) and*Zea mays* (ZmFH1 and ZmFH2) frataxin proteins. Recombinantproteins in full length, in an intermediate form and in matureform without transit peptide were synthesized, and then theywere studied by ESI-MSspectroscopy. Results indicate thatfrataxins from both plants present common oligomerization andmetals binding properties. In addition, we determined that the N-terminalregion is important in these processes, and our findings suggest the existence of self-cleavage to reach the mature form.

PL-P49
GATA TRANSCRIPTION FACTORS DURING HEAT STRESS IN *Arabidopsis thaliana*

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High temperature is a major environmental stress that causesplant growth arrest and that greatly reduces the yield and theproduct quality of crops worldwide. In our lab, we found thatheat stress (HS) triggers a cell death pathway that resemblesferroptosis, an iron-dependenttype of cell death described firstin tumor cells. Ferroptosis can be specifically prevented bytreating cells with the iron-chelatingagent ciclopiroxolamine (CPX) or with the lipophilic antioxidant ferrostatin-1(Fer-1).RNAseq experiments and differential expression studies showedthat *GATA*, *TCP* and *AP2*/*EREBP* transcription factor genes areup-regulated in plants treated with ferroptosis inhibitors. Here, we examine whether the overexpression of differenttranscription factor (TF) genes is able to prevent

ferroptosis-likecell death in Arabidopsis after HS stress. Transgenic linesoverexpressing four different *GATA* genes are studied anddifferent aspects of development and stress response areshown.

PL-P50

CONTROL OF PLANT DEVELOPMENT BY THE TRANSCRIPTIONAL COACTIVATOR AN3

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In *Arabidopsis thaliana*, GRF-INTERACTING FACTORS (GIFs) gene family consists of AtGIF1, also known as ANGUSTIFOLIA3 (AN3), AtGIF2 and AtGIF3. They have homology to human SYNOVIAL TRANSLOCATOR transcriptional coactivator and have been identified as transcriptional coactivators. GIFsproteins interact with the GROWTH REGULATING FACTORS transcription factors and BRAHMA, a central ATPase component of the chromatin remodeling complex. The *AN3*gene has a central role in the control of cell proliferation and therefore in plant size. *AN3* mutants have lanceolated leavesand a modified meristematic activity. AN3 is a small protein that can move from cell to cell through plasmodesmata. Here, we studied the importance of AN3 movement in different biological processes. To do this, we prepared translational fusions of AN3 genomic sequence (*gAN3*) to single (*gAN3GFP*) or triple molecules of GFP (*gAN33XGFP*).The later chimeric protein cannot move from cell to cell due to its larger size. We expressed these proteins from their own regulatory sequences as well as tissue specific promoters. We analyzed the ability of AN3 to move across different cells and to complement *AN3* mutants. The results provide new insights about the control of AN3 expression and the role of AN3 movement during development.

PL-P51

INTERACTIONS BETWEEN GROWTH REGULATING SYSTEMS IN *Arabidopsis thaliana*

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Leaf growth and development involves the concerted action ofvarious small RNA and transcription factor networks. InArabidopsis, the GROWTH-REGULATINGFACTOR (GRF) family is composed of nine members and seven of them are regulated by microRNA miR396. The GRF transcription factors act as growth promoters and plants with higher GRF activity have larger leaves, which senescence later. Conversely, *ARF2*, which belongs to the AUXIN RESPONSE FAMILY of transcription factors, is a repressor of growth. *ARF2* is in turn regulated by transacting (ta-siRNA) small RNAs. The *arf2* knockout mutants have an increase in the size of the leaves and seeds, and a delay in senescence. Here, we studied the participation of the miR396-GRF and ta-siRNA in the control of leaf development. First, we tested the genetic interaction between the two systems by crossing mutants and transgenic plants with modified levels of miR396and GRF, with plants modified in the ta-siRNA/*ARF2* system. We made a phenotypic analysis of the crosses in relation to the parental lines, by analyzing organ size and cell number. In addition, we evaluated the expression levels of the different genes under study by RT-qPCR assays and reporter genes. A model showing the integration of these networks in leaf development will be presented.

PL-P52

BIOCHEMICAL AND MOLECULARCHARACTERIZATION OF TWO *Citrus reticulata*VARIETIES: MURCOTT AND ELLENDALE

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Argentina exports about 100,000 tons of tangerines (*Citrus reticulata*) annually. More than a third of this is constituted byMurcott and Ellendale varieties. Commercial success of thisactivity depends in the preservation of internal and externalquality.The goal of this research was to analyze thebiochemistry of these two *C. reticulata* varieties in order to shedlight on the molecular basis that determine internal fruit qualityduring postharvest storage. Assays were performed on theflavedo. Primary metabolites, phospholipids and secondary metabolites relative contents between both varieties were analyzed by gas or liquid chromatography followed by massspectrometry. Free amino acids were also analyzed. Principalcomponent analysis showed a clear separation of both varietiesby one principal component. Fumaric acid, octadecanoic acidand hesperidine (on the positive side), and melibiose (on thenegative side) were the main contributors for the division. Anassessment of the antioxidant capacity showed a higher level ofsuperoxide dismutase in Ellendale. In conclusion, it is possibleto define a precise metabolic profile that discriminates betweenvarieties. This information can assist future research dedicatedto the creation of new varieties with specific quality traits andexplain different postharvest behaviors.

PL-P53

UV-B LIGHT ENHANCES ANTIMICROBIAL ACTIVITY OF POSTHARVEST LEMON PEEL AGAINST *Penicillium digitatum*

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UV-B radiation treatment previously standardized in our laboratory contributes to prevent green mold on postharvestlemons, caused by *Penicillium digitatum*. The aim of this workwas to assess *in vitro* the effect of flavedo extracts from UV-Bexposed lemons (UV-BFEx) and from control lemons (CFEx) on*P. digitatum* conidia. Several parameters of conidia viabilitywere evaluated after treatments. Moreover, HPLC and HPLCMS/MS analysis of phenolic compounds were carried out on both flavedo extracts. Conidia viability was markedly affected byUV-BFEx in respect to CFEx, in assays using the same concentrations. Conidia treated with UV-BFEx showed an inhibition of germination and oxygen consumption, an increased membrane permeability and ROS and TBARS production. On the other hand, two fractions of the flavedo extracts were detected and isolated by HPLC, due to a stable

chromatographic behavior of two peaks in response to the UV-B exposure. The different phenolic composition in those fractions could explain the differential antifungal activities of the extracts. In conclusion, UV-B exposure increases lemon peel natural defenses, by activating the differential synthesis of phenolic compounds and thereby raising the resistance against the phytopathogen.

PL-P54
CONTRIBUTION OF *Gluconacetobacter diazotrophicus* PAL5 TO PHOSPHORUS NUTRITION IN STRAWBERRY PLANTS

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Intensive strawberry crop production requires the use ofphosphate fertilizers to cover deficiencies of solublephosphorus (P) in soil. *Gluconacetobacter diazotrophicus*, aplant growth promoting bacteria, is able to solubilizephosphates through a non-phosphorylatingextracellularoxidative pathway, which oxidizes glucose to gluconic and 2-ketogluconicacids. It has been demonstrated in our laboratorythat *G. diazotrophicus* PAL5 can solubilize phosphate fromdifferent sources. The aim of this work was to analyze if PAL5contributes to P nutrition in strawberry plants. Plants were grownin sterile substrate and watered with nutrient solution in theabsence of P and with the addition of soluble or insoluble P.After 20 days, plants grown with insoluble P were inoculatedwith PAL5. Plants incubated in the absence of P presenteddwarfism, low dry weight, growth index and P content; dark green leaves and poor root development. A similar deficientphenotype was observed with insoluble P. Conversely, plantsincubated with soluble P or insoluble P and PAL5 had high dryweight, growth index and P content; big, vigorous and light-greenleaves and abundant roots. Beneficial effects were achieved even if bacteria were inoculated after the appearanceof deficiency symptoms. In conclusion, PAL5 contributes to Pnutrition in strawberry plants by improving growth in lowavailable P medium.

PL-P55
DIFFERENTIAL SENSITIVITY OF *Medicago sativa*, *Zea mays* AND *Raphanus sativus* TO OLIVE CAKE AND SOIL

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Olive cake is a toxic and complex effluent derived from the oliveoil industry. Its effect on plants growth has been studied byapplying it in mixtures with different substrates. Here, the aimwas to analyze the sensitivity of different plant species to freshand 40 days-compostedmixtures of olive cake and soil (FM andCM, respectively). *Medicago sativa* (lucerne), *Zea mays* (corn) and *Raphanus sativus* (radish) were grown in 5, 10 and 20% ofFM and CM during 20 days. Photosynthetic pigments, leaf area, biomass, and anatomical structure of roots were evaluated. *M. sativa* was the most affected, especially in the presence of theFM, showing a strong diminution of photosynthetic pigments,biomass and leaf area and being killed in 20% FM. *R. sativus*showed a less marked decrease of those parameters, withsimilar values for FM and CM. *Z. mays* was the less affected,showing a slight growth inhibition. In root cross-sections,the most notable effects of the FM and CM were the development ofaerenchyma in corn, and the cell wall thickening of the layer ofcortex cells adjacent to endodermis in radish. Thus, *Z. mays* and *R. sativus* developed mechanisms of resistance against olivecake. Corn aerenchyma could be induced by anoxia in compactsoil mixtures; while the wall thickening would be associated withthe restriction of entrance of toxic compounds from the mixturesto radish plants.

PL-P56
IDENTIFICATION OF CELL CYCLE REGULATORS IN PLANTS

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Plant organ growth occurs through an initial stage of cellproliferation followed by another of cell expansion anddifferentiation. Therefore, the control of the cell cycle (CC) is critical to determine the magnitude of plant growth. CCregulators often have oscillating activities regulated bytranscriptional and posttranscriptional mechanisms. Forexample, E2F or MYB3R transcription factors control theexpression peaks of several genes during the G1/S or G2/Mphases, respectively. Genomic studies indicated that only asubset of the genes expressed in the G1/S or G2/M phases areregulated by these already described pathways, suggesting thatother transcriptional regulators remain to be identified. Theobjective of this work is to identify genes that control the CCusing *Arabidopsis thaliana* roots as model system. To this end, we analyzed the transcriptome of cells in the G2/M transition.These cells were obtained by cell sorting of protoplastsprepared from roots expressing a GFP reporter of a mitoticcyclin. Analysis of these data yielded ca. 400 genes withexpression enriched in G2/M cells, of which only 60 havepreviously been described as CC regulators. Analysis of theremaining genes enabled us to identify new potential regulatorsof the CC. The initial characterization of some of these candidates, with special emphasis on the transcription factorsidentified, will be discussed.

PL-P57
PROTEOMICS OF THE CHLOROPLAST STROMA OF *Lotus japonicus* UNDER COLD STRESS

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Low temperature is one of the most important factors affecting plant growth, directly altering the photosynthetic process andleading to photo-inhibition.With the aim to address thephotosynthetic acclimation response of *L. japonicus* under coldstress, two ecotypes (MG-1and MG-20) were studied. Previousdata showed that photo-inhibitionoccurs in stress anddifferentially between ecotypes, being MG-1more affected thanMG-20.In this study, we used a proteomic approach to evaluatechanges in the chloroplast stroma of both ecotypes after 24 h oflow temperature. A total of 450 proteins were identified, with 325uncharacterized in the *L. japonicus* protein database.

Proteinswere classified and 168 and 86 were differentially accumulatedin MG-1and MG-20, respectively, in the stress treatmentcompared to controls. Of these, only 3 and 4 proteins were moreabundant, while 165 and 82 showed a reduction in itsaccumulation at low temperature. A functional annotation andclassification was made by Gene Onthology, KEGG MetabolicPathways and blastp. The results obtained showed that down-regulated proteins were mainly related to amino acid, carbohydrate and energy metabolisms. However, particular metabolic pathways were different regulated in each case and for each ecotype. Data obtained may be useful in identifying proteins with a key role in low temperature acclimation in legumes.

PL-P58
MULTIPLE MYB TRANSCRIPTIONAL FACTORS ARE INVOLVED INTO CONDENSED TANNIN BIOSYNTHESIS REGULATION

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Condensed tannins (CTs) biosynthesis in plants has beenshown to be transcriptionally regulated through the MBWcomplex. This complex is formed by the physical interaction ofthree transcription factors (TFs) belonging to the R2R3-MYB, bHLH, and WDR protein families. It appears to be redundancyfor the bHLH cofactors, while the MYB protein generallyprovides specificity. In *L. japonicus*, three MYBs involved in toCT regulation (TT2a, TT2b and TT2c) have been described.However, our transcriptional studies performed on crop specieswith polymorphism for this trait (*L. tenuis* (Lt) and *L. corniculatus*(Lc)) suggest that almost another six MYB proteinscould be form part of different MWB complex related to CTbiosynthesis regulation.Whole transcriptome sequencing analysis (Illumina Hiseq1500) was performed from Lt and Lc shoots samples andtranscripts were obtained with *de novo* assembly methodology.Orthologue sequences from several MYB TFs involved in TCbiosynthesis regulation from different species were obtained.We selected nine MYB TFs related to *TT2*, *MYB14*, *MYBPA* and*MYB5* genes. The relative expression of MYBs candidates (RT-qPCR) was measured in Lt, Lc and Lt x Lc genotypes usingspecific primers.The gene expression and its correlation with CTlevels suggest that four of them (*TT2b*, *TT2d*, *MYBPA2* and*MYB5*) are the best candidates as regulators of Pasbiosynthesis in *Lotus spp.*

PL-P59
REGULATION OF PHOTOSYNTHETIC MALIC ENZYMES IN C_4 MILLETS

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Setaria italica (foxtail millet) and *Panicum virgatum* (switchgrass) belong to the group of millets that carry outphotosynthesis C_4. In these species the initial CO_2 fixationyields C_4 acids within the mesophyll cells which are thentransported to the bundle sheath cells where they aredecarboxylated. The resulting CO_2 is incorporated into theCalvin cycle. In *S. italica* the decarboxylating enzyme thatprovides most of the CO_2 to the assimilative process is achloroplastic NADP-dependentmalic enzyme (NADP-ME) while in switch-grass a mitochondrial NAD-dependentmalic enzyme (ME-NAD) is the main decarboxylating enzyme. In this study weanalyzed the biochemical and molecular processes thatregulate the expression and activity of each decarboxylase. The aim of this work was to identify the key processes linked with theefficient decarboxylation of C_4 compounds in vascular sheathcells. The characterization of these processes is a central pointin the main objective of introducing mechanisms concentrationof carbon dioxide in C_3 species to increase the efficiency ofcarbon assimilation.

PL-P60
ANALYSIS OF MITOCHONDRIAL ALKALINE/NEUTRAL INVERTASE MUTANTS

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Alkaline/Neutral Invertases (A/N-Invs) are sucrose hydrolyzing enzymes with an optimal pH range from 6.8 to 8.0. They are the least studied enzymes in sucrose metabolism. In the last decade it was shown that A/N-Invs localized not only in the cytosol but also in mitochondria and/or plastids. The functional role of organellar isoforms is still not fully elucidated. Previous studies in Arabidopsis suggested that mitochondrial A/N-Invs have significant roles in root growth and development, and in abiotic stress adaptation. We investigated the role of three mitochondrial A/N-Invs from Arabidopsis by performing i) the functional characterization of a non-described A/N-Inv isoform from Arabidopsis, ii) the comparison of phenotypes of single and multiple mutants generated in this study, and iii) expression analysis by qPCR. Interestingly, the three single mutants exhibited differential phenotypes regarding growth and development (root length, germination and flowering time). To date, a fully segregated triple mutant could not be obtained and we cannot discard that the presence of these mitochondrial enzymes are essential for survival. Supported by CONICET, ANPCyT (PICT 1288), UNMdP(EXA645) and FIBA.

PL-P61
EFFECTS OF PHENOL TREATMENT ON CLOCKGENES EXPRESSION IN TOBACCO HAIRY ROOTS

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As a consequence of changing environmental conditions, most species contain an endogenous circadian clock which allows them to daily adapt and optimize physiology and metabolism.Our main objective was to evaluate the effects of phenol treatment (PT) (100 mg/l) on expression of clock genes (CG) and putative genes involved in metabolism of xenobiotic compounds (MXC) such as peroxidase (NtPXC8), cytochrome monooxygenase (Cyp71D21), glucosyl transferase (GT) and glutathione S-transferase (GST). For

this, hairy root cultures (HRC) of *Nicotiana tabacum* of 3 weeks synchronized by a12:12 light dark photoperiod were used. HRC subject to PT for 24 h showed a significant decrease in patterns of CG expression (LHY, TOC1, FKF1, GI and PRR9) as compared with water treated controls. When expression profiles of putative genes for MXC were assessed, we found that PT up-regulated the expression of all genes analyzed. The highest expression of NTPXC8 and GST was observed at dark phase (ZT 19) while GT and Cyp71D21 exhibited an increased expression at ZT 19 as well as at light phase (ZT3 and ZT 11). These findings show for the first time that PT down-regulated CG expression but significantly up-regulated expression of putative MXC genes across time. These results also suggest a putative circadian control on MXC gene expression, improving our understanding of phenol metabolism in plants.

PL-P62
EFFECT OF ZINC STRESS ON PLANT MISMATCH REPAIR

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Zn is an essential micronutrient that is involved in the functional activities of various proteins. However, metal excess may resultin toxicity due to the formation of reactive oxygen species (ROS) by an indirect mechanism. Accumulation of ROS can induceperoxidation of lipids and oxidation of proteins and DNA bases, with 8-oxoguanine(8-oxoG)being the most frequent baselesion. Unrepaired 8-oxoGmay be mutagenic since theoxidized base can direct incorporation of either C or A. Evenmore, the dGTP pool can also be oxidized. To minimize theburden of 8-oxoGseveral DNA repair pathways exist. One ofthem is the mismatch repair (MMR) system. The first step of thepathway involves recognition of the DNA lesion by MutSproteins (MSH2, MSH6 and MSH7). To investigate the role ofplant MMR system on the processing of oxidative DNA damageinduced by Zn, we first evaluated the transcripts levels of MMRproteins by qRT-PCR.Our results demonstrate that MSH6 isinduced by Zn in *A. thaliana* leaves, while MSH7 is repressed.Then, we identified *msh6* T-DNAinsertion mutants. Plantsdeficient in MSH6 show altered chlorophyll content and rootelongation relative to wild-typeplants. Finally, we generated transgenic plants that express β-glucuronidase (GUS) under thecontrol of the MSH6 promoter. Taken together, our results suggest that *MSH6* may be involved in Zn-induced DNA damage recognition.

PL-P63
METABOLOMIC RESPONSE OF SOYBEAN LEAVES TO *Fusarium tucumaniae*

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Sudden-deathsyndrome (SDS) of soybean can be caused atleast by 4 different Fusarium species: *F. brasiliense*, *F. crassistipitatum*, *F. tucumaniae* and *F. virguliforme*, with *F. tucumaniae* being the dominant specie in Argentina. Infection and disease development are highly dependent on environmental conditions. Argentinean soybean germplasm show various levels of resistance to *F. tucumaniae*. Here, two soybean cultivars, one susceptible (S) and one partially resistant (R) to SDS were inoculated (I) with an isolate of *F. tucumaniae* grown in sorghum grain, using the layer method. Uninoculated controls (C) were included for both genotypes. Plants were grown for 7 days. To detect early metabolic markers that could potentially discriminate between S and R genotypes, leaf metabolite profiling by gas chromatography mass spectrometry was performed. Compounds were putatively identified by comparison of their retention index and mass spectrum with those present in the commercial mass spectra library NIST. These analyses identified amino acids, organic acids, soluble sugars, alcohols, fatty acids and miscellaneous compounds. Metabolite levels were normalized to the ribitol internal standard and compared between samples. Levels of two soluble sugars (psicose and rhamnose), glycerol and isothiocyanate were increased after infection and this increment was dependent on the genotype.

PL-P64
ENDOGENOUS NO MODULATES SPATIAL DISTRIBUTION OF PIN2 DURING GRAVITROPISM IN *Arabidopsis*

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Nitric oxide (NO) is an endogenous signaling moleculeimplicated in several key auxin-mediateddevelopmentalprocesses including gravitropism. The asymmetry in the auxindistribution during the gravitropic response is established andmaintained by the spatio-temporalregulation of the PIN2 auxintransporter facilitator abundance. Our results showed anasymmetric NO accumulation in epidermic cells of gravi-stimulatedArabidopsis roots. Dynamic changes of NOdistribution co-localizedwith the asymmetric auxin abundancein the upper and lower sides of the root tip. The regulation ofPIN2 distribution by NO during the gravitropic response wasinvestigated in PIN2-GFPtransgenic plants subjected to NOdonor (NO-Cys)and the scavenger (cPTIO) treatments.Endogenous NO was critical in the asymmetric abundance ofPIN2 during gravitropism. Preliminary results indicated thatcompared with wild type, the double mutant *nia1*/*nia2* showed areduced root bending after 135° root reorientation. Only 44% of mutant plants showed the expected orientation towards gravity.We are carrying out genomic and physiological researches to investigate the role played by the coordinated action of auxin and NO during gravitropism. Supported by CONICET, ANPCyT,UNMdP.

PL-P65
TIR1 S-NITROSYLATION IS A REGULATORY COMPONENT IN TEMPERATURE-DEPENDENT *Arabidopsis* SEEDLING GROWTH

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Plasticity allows plants to modulate their development inresponse to widely fluctuating environments. A mild increase inenvironmental temperature stimulates the growth of Arabidopsisseedlings by promoting biosynthesis of the plant hormoneauxin. A recent study has revealed that the accumulation of auxin is only a part of the response. Temperature also promotes stabilization of the auxin co-receptorTIR1 through its interactionwith the molecular chaperone HSP90. The aim of this work was to determine whether nitric oxide (NO) throughout S-nitrosylationof TIR1 is involved in the temperature-inducedhypocotyl elongation in Arabidopsis. Plants overexpressing tir1proteins mutated in cysteine residues targets of S-nitrosylation, tir1 C140A and tir1 C480A, showed an auxin-resistant response quantified as hypocotyl elongation. Temperature-induced phenotype was impaired in two different overexpressing transgenic lines of tir1 C140A. Although the introduction of tir1C480A in the *tir1-1* background partially restores auxin sensitivity, tir1 C140A did not recover TIR1 functionality. These data provided evidence about the relevance of post-translationalmodification in TIR1, and added a novel regulatorycomponent in the temperature-dependenthypocotyl elongation.Supported by ANPCyT, CONICET and UNMdP.

PL-P66
BDIRCN4 REGULATES MERISTEM FATE IN *Brachypodium distachyon*

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In higher plants, a phase transition from vegetative toreproductive development is tightly coordinated throughnetworks that integrate internal and external stimuli. TERMINALFLOWER1 (TFL1) controls flowering time and inflorescencearchitecture in *Arabidopsis thaliana*, acting as a negativeregulator of the phase changes of the shoot apical meristem (SAM) from vegetative to inflorescence meristem (IM), and fromIM to floral meristem. Here we show functional analyses ofBdiRCN4, homologue to TFL1 in *Brachypodium distachyon*.Arabidopsis plants expressing the 35SCaMV::BdiRCN4construct show a strong delay in SAM to IM transition, as well asa longer maintenance of the IM, leading to a longer life cyclecompared to wild type. These plants also exhibit a highlybranched inflorescence shoot and bracts instead of flowers, which correlates with lower expression levels of the ABCDE genes LFY, AP1and PI. Some of these inflorescences eventually develop fertile flowers. In Brachypodium, insertional mutantscarrying a 4xCaMV35S enhancer sequence display a similarphenotype. Higher expression levels of BdiRCN4 promoteextended vegetative phase and few or absent production ofspikelets. These results suggest that BdiRCN4 acts as floral repressor. Furthermore, function of TFL1 homologues in the regulation of meristem transition and identity seems to be conserved between monocots and dicots.

PL-P67
IDENTIFICATION OF DOWNSTREAM TARGETS OF ATERF019 TRANSCRIPTION FACTOR IN DROUGHT STRESS

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AtERF019 is a member of the APETALA2 (AP2)/ETHYLENE RESPONSE FACTOR (ERF) gene family, which was induced under oxidative stress originated in chloroplast. Adult plants overexpressing *AtERF019* (ERF019 lines) showed enhanced drought tolerance compared to Col-0 plants. This tolerance could be explained in part by a lower transpiration rate in ERF019 lines owed to smaller stomatal aperture and lower cell wall permeability. Using a proteomic approach, the branched-chain-amino-acid amino transferase 3 (BCAT3), a protein known to be produced in response to stress, was identified in ERF019 lines under control condition. This enzyme is plastid localized and is involved in both BCA and glucosinolate synthesis.Additionally, a *bcat3* mutant was more sensitive to drought stress than Col-0.Several genes encoding proteins present in ERF019 control or drought conditions but not in Col-0 plants were predicted to be targets of AtERF019. Among them are the transcription factor *OXS2* and genes related to cell wall metabolism. These data indicate that a number of responses involving the cell wall appear to be important in adapting cells to water-deficit stress.

PL-P68
PLD/PA MODULATES PROLINE AND H_2O_2 LEVELS IN BARLEY EXPOSED TO SHORT-AND-LONG-TERM CHILLING STRESS

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PLD hydrolyses phospholipids to produce PA and a head groupand it is involved in the response to various environmental stresses. In this work, we investigated the roles of PLD/PA inresponse to short and long-termchilling stress through theregulation of proline and ROS levels in barley. Seedling grown at 25ºC for 4d were maintained at 4ºC for 0.536h. Short-termchilling stress increased rapidly and transiently the PLD activityand modulated proline and ROS levels in young leaves. Pre-treatmentwith 1-butanolaugmented the proline synthesis, andthe treatment with PA exogenous amplified H_2O_2 formation. In contrast, long-term chilling stress reduced enzymatic activity ofPLD; while the proline synthesis and the ROS signal were stimulated in roots. H_2O_2 application stimulated the proline levelwhile the exogenous proline decreased the ROS level.Bioinformatic search of the barley database revealed thepresence of highly homologous sequences of P5CS and ProDHArabidopsis protein. Ours results indicate that PLD contributesto the signalling pathway during 0.53hat 4°C through theregulation of balance between P5CS activity and H_2O_2 level.On the contrary, reduced enzymatic activity of PLD during 2436hat 4°C could not contribute to the proline level. Thissuggests that PLD/PA signal is involved in the relationshipbetween ROS signalling and proline metabolism.

PL-P69
THE TCP DOMAIN MEDIATES THE ANTAGONISTIC ACTION OF TCP8 AND TCP23 ON FLOWERING IN *Arabidopsis*

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TCP transcription factors are plant-specificproteins that sharethe TCP domain, responsible for DNA binding and dimerization.TCPs are key factors in plant growth and development and theyare classified in two classes: I and II. We found that twomembers of class I from *Arabidopsis thaliana*, TCP8 andTCP23, regulate flowering time in an opposite manner. A loss-of-functionmutant in TCP8 has a late flowering phenotype whileplants that express TCP8 from the 35SCaMV promoter flowerearlier that wild type. On the contrary, overexpression lines ofTCP23 show a delayed transition to flowering. Analysis of theexpression patterns conferred by the TCP8 and TCP23promoter regions revealed that both genes are expressed in theapical meristem. To analyze the molecular basis governing theopposite action of these proteins, we overexpressed fourchimeric proteins between TCP8 and TCP23 in plants andfound that the TCP domain would be the responsible for the observed effects on flowering. While both TCP domains arehighly similar, TCP23 presents a substitution in a highly conserved residue that may alter the secondary structure of the HLH motif. We are currently investigating how this substitution changes the functional properties of the TCP domain.

PL-P70
STUDY OF MITOCHONDRIAL PROTEINS ENGAGED IN GROWTH AND DEFENSE IN *Arabidospsis thaliana*

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The aim of our group is to elucidate the mechanisms throughwhich mitochondria perform non canonical functions associatedwith hormonal growth control and pathogen defense in plants.We identified a family of new proteins involved in defensemechanisms against oxidative stress in Arabidopsis. Plants overexpressing (OE) one of our candidates exhibit interesting phenotypic characteristics like larger size, higher biomass and increased resistance to pathogen infection compared to wildtype (WT) plants. As was previously observed in yeast andhumans, by confocal microscopy we demonstrated that ourprotein is also specifically localized in plant mitochondria undercontrol growth conditions. Interestingly, plants with modifiedexpression levels of this protein exhibit altered content ofhormones like Citokinins (CK) and Salicylic acid (SA).Furthermore, by using a combination of plants with different backgrounds (ahp1,2,3,4; arr3,4,5,6,7,8,9; ipt3,5,7; oeCKX2,oeCKX1, pCKX1:GUS) we demonstrated that the sensitivity andsignaling pathways of these hormones are also modified in ourplants. We conclude that our protein of interest constituteanother example regarding to the interplay betweenmitochondrial activities, hormonal control of growth anddefenses in plants.

PL-P71
PLANT GAMMA-CARBONIC ANHYDRASES AND THEIR ROLE IN GROWTH AND EMBRYOGENESIS: THE IMPORTANCE OF CA3 PR

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In *Arabidopsis thaliana*, attached to the respiratory Complex I, there is a spherical domain named Carbonic Anhydrase (CA) domain, which is composed by five gamma-typeCarbonicAnhydrases (CA1, CA2, CA3, CAL1 and CAL2). This domainwas demonstrated that is involved in Complex I assembly, photorespiratory pathway and embryo development. In ourlaboratory, we are working with multiple mutants in these genesin order to elucidate new roles of CA domain, its composition inCA proteins and the attachment site within Complex I.Apparently, CA3 protein is outside of CA domain, which isintriguing because double mutants, *ca1ca3* and *ca2ca3* showlethal and slow growth phenotypes, respectively. The delay inembryo development is most likely due to low mitochondrial activity (as mitochondrial membrane potential). This delay provokes abnormal seed shapes and impairs seed germination.However, *ca1ca3* embryos can be rescued by sowing inartificial medium complemented with sucrose. On the other hand, *ca2ca3* mutant exhibits slow growth and late flowering being partially recovered by sucrose or high CO_2 atmospheres.Preliminary results show that Complex I activity is lower than WT in *ca2ca3*, and totally absent in *ca1ca3*. Our results and observations focus on CA3 role in CA domain composition, and its importance in Complex I functions.

STRUCTURAL BIOLOGY

SB-P01
3D-STRUCTURE PREDICTION AND STUDY ON THE INTERACTION BETWEEN RAT CALTRIN AND MODEL MEMBRANES

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Rat caltrin (calcium transport inhibitor), the small and basic protein of the seminal plasma, binds to sperm cells during ejaculation and inhibits the extracellular Ca^{2+} uptake. Thus, it prevents the sperm spontaneous acrosomal exocytosis along the female reproductive tract. Although the sequence and some biological features of rat caltrin were studied, its 3D structure and physicochemical properties are still unknown. We predicted rat caltrin 3D structure by molecular homology modeling and threading. The molecular structure was

further characterized by circular dichroism. Surface electrostatic potentials and electric fields were calculated using the Poisson-Boltzmann equation. Several different bioinformatic tools and available web servers were used to deeply analyze physicochemical characteristics such as Kyte and Doolittle Hydropathy score and helical wheel projections. Equilibrium spreading pressure was obtained by Gibbs adsorption isotherms. Interactions between rat caltrin and phospholipids model membranes were defined by penetration (cut off) studies. We observed that rat caltrin is able to penetrate into the membranes, mainly in negatively charged surfaces and expanded lateral phase states. Results presented have significant relevance to further understanding the molecular mechanisms of caltrin to modulate physiological processes associated with fertilization.

SB-P02
A TACHYLECTIN AND A PORE-FORMING MACPF PROTEINS COMBINED INTO A SNAIL EGG NEUROTOXIN

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Pomacea is a group of snails with aerial oviposition where adult females lay conspicuously colored egg masses that are ignored by most predators, which is associated to the presence of multifunctional proteins with defensive properties. Among them is the neurotoxin PcPV2 present in the eggs of *P. canaliculata*. A structurally similar protein (PmPV2) also with neurotoxic properties was recently found in the eggs of the sympatric species *P. maculata*. The aim of the present work was to determine the molecular and functional features of PmPV2. PmPV2 is composed by two subunits of ≈30 and ≈68 kDa. Full cDNA sequencing and analysis of the 30-kDa subunit show it is related to the tachylectin family of lectins. Its structure predicted by *in silico* modelling consists of a six-bladed beta propeller, a common arrangement in this family. Besides, PmPV2 showed agglutinating activity on rabbit erythrocytes. The 68-kDa subunit belongs to the MACPF family, particularly related to C8 and perforins from mammalian immune system. The predicted structure of this subunit presents the characteristic MACPF domains, including the transmembrane helixes (TMH) related to the insertion of the protein into the membrane. PmPV2 exhibit the “AB toxin” structure found in bacterial attack and plant defensive toxins and restricted to PcPV2 in animals, composed of a delivery lectin covalently linked to a toxin (MACPF).

SB-P03
LECTINS AS DEFENSES. CHARACTERIZATION OF THE MAJOR EGG PROTEIN OF THE SNAIL P*omacea diffusa*

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Pomacea freshwater snails lay eggs above the water line, exposing them to high temperatures, desiccation and predators. Recent studies showed that *Pomacea* egg carotenoproteins provide not only embryo nutrition, but also unexpected defenses against predation. In this work we isolated and characterized PdPV1, the most abundant egg protein of *P. diffusa*. The carotenoprotein was isolated from egg homogenates by ultracentrifugation and size exclusion chromatography (SEC). PdPV1 molecular weight and global shape were estimated by PAGE, SEC and small angle X-ray scattering (SAXS). Thermal stability was evaluated by fluorescence and absorption spectroscopy and SAXS. Carbohydrate content determined by colorimetry. PdPV1 resistance to gastrointestinal digestion was evaluated *in vitro* and its lectin activity by hemagglutination assays. PdPV1 is an anisometric oligomer of 422 KDa, composed by 5 subunits of 23, 25, 29, 30 and 33 KDa. The protein is highly glycosylated (8.9% w/w), thermostable (up to 65ºC) and resists 2h of simulated gastrointestinal digestion. PdPV1 was able to agglutinate rabbit erythrocytes. The presence of an egg carotenoprotein with lectin activity resistant to gastrointestinal digestion suggests a protective role against predation similar to that well established for defensive lectins of plants. In animals, lectins alike were only reported in a related snail.

SIGNAL TRANSDUCTION

ST-P01
ACSL4 PROMOTER CHARACTERIZATION AND REGULATION BY SHP2 IN BREAST CANCER CELLS

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In breast cancer Acyl-CoA synthetase 4 (ACSL4) and the tyrosine phosphatase SHP2 play a role on tumor aggressiveness. ACSL4 is up-regulated in triple negative breast cancer cell lines and tumors. In order to study the mechanism involved in the regulation of expression of the enzyme, we previously showed differences in ACSL4 promoter regulation in MDA-MB-231 and MCF-7, being 3´ end a possible responsible region of its over expression in MDA-MB-231. In this work we demonstrated by successive deletions of the promoter leaving fixed one end, that the last 43 bp of the 3´ end is a positive regulatory region only in MDA-MB-231. By Genomatix tool, we identified transcription factors consensus sites on the promoter. Results of RORα mutant site suggest it is involved in the inhibition in the 5' end in both cell lines. Results of E2F transcription factor 2 mutant sites showed it could enhance the promoter activity in the 3' end only in MDA-MB-231. Moreover, we treated MCF-7 with estradiol benzoate and a decrease in promoter activity was observed, effect reversed by ICI 182780. We expressed ERα in MDA-MB-231 and had a signal decrease, suggesting this receptor is responsible of the estrogenic effect. We demonstrated that the MEK signaling pathway could be involved in the regulation of ACSL4. We also demonstrated a role of SHP2 on ACSL4 expression and promoter activity in breast cancer cells.

ST-P02
SEXUAL PHEROMONE MODULATES THE FREQUENCY OF CYTOSOLIC CA 2+ BURSTS IN *Saccharomyces cerevisiae*

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Transient and highly regulated elevations of cytosolic Ca 2+ control a variety of cellular processes. In budding yeast, Ca 2+ has been implicated in the response to several environmental challenges. Bulk measurements using radioactive Ca 2+ and the luminescent sensor aequorin have shown that in response to pheromone, yeast cells experience a rise of cytosolic Ca 2+ that is mediated by two import systems composed by the Mid1-Cch1- Ecm7 protein complex, and the Fig1 protein. Although this response has been largely studied, there is no report on Ca 2+ dynamics at the single cell level. Here, we adapted protein calcium indicators to yeast and show that both vegetative and pheromone-treated cells exhibit discrete and asynchronous Ca 2+ bursts. Most bursts reach maximal amplitude in 1-10 secs, span between 7 and 30 secs and decay fitting a single exponential model. In vegetative cells bursts are scarce but preferentially occur when cells are transitioning G1 and S phase. Upon pheromone presence Ca 2+ burst occurrence increases dramatically, persisting during cell growth polarization. Pheromone concentration modulates burst frequency in a mechanism that depends on Mid1, Fig1 and a third, still unidentified, import system. Moreover, we show that the calcineurin-responsive transcription factor Crz1 experiences nuclear localization bursts during the pheromone response.

ST-P03
A NOVEL PHOSPHATASE REGULATES STRESS GRANULES DYNAMICS

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Stress granules (SGs) are liquid organelles that form transiently upon cellular stress. SGs contain repressed mRNAs and RNA-binding proteins involved in reprogramming mRNA translation and decay. SGs are highly dynamic and their formation and dissolution is regulated by specific RNA-binding proteins, translational regulators, chaperones and molecular motors. We performed a high-throughput RNAi screen in Drosophila cells to identify novel signaling pathways that regulate these factors thereby affecting SG dynamics. Here we focus on the role of a novel phosphatase (NP) that showed a high score in the primary screen. We found that the KD of NP induced an increase in SGs upon oxidative stress in both Drosophila and mammalian cells. Conversely, NP over-expression impaired SGs assembly. Furthermore, NP localized to discrete microdomains in the nucleus, where NP and SF2 -a splicing speckle-marker- partially colocalized. Upon oxidative stress, the distribution inside the nucleus became homogeneous, and the NP microdomains vanished. In addition, NP was mobilized to the cytosol and was not significantly recruited to SGs. We hypothesize that NP affects the biogenesis, processing and/or quality control of unknown transcripts, either dephosphorylating 5 'triphosphate RNAs or phospho-proteins involved in nuclear metabolism of RNPs. We thank ANPCyT, CONICET and UBA, Argentina for funding.

ST-P04
IGF 1 MODULATES ZEB1 STABILITY DURING EPITHELIAL MESENCHYMAL TRANSITION (EMT)

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ZEB1 is a transcription factor (TF) involved in EMT and oncogenesis. Previously we showed that an N-term fragment of ZEB1(NZEB1) was able to induce EMT by itself in mouse epithelial cells NMuMG. We intend to determine the mechanisms involved in regulating NZEB1 during EMT. NMuMG-NZEB1 cells were treated with specific inhibitors and activators of signaling pathways for 48h. Immunoblots against NZEB1, E-cadherin and α-tubulin (loading control) and the activity of pathways were assayed. None of the reagents modified the effect of NZEB1 on E-Cadherin expression. However, incubation with 5μM NVP (IGF1R inhibitor) for 48 h reduced NZEB1 expression ($P<0.05$). Confocal microscopy and IF of tested cells also revealed a changed pattern of F-actin filaments to a more epithelial-like distribution than controls. Coincidently, invasive and migration assays showed a strong reduction in their values after NVP treatment compared to controls ($P<0.001$). The protein stability of NZEB1 was also assessed in NMuMG-NZEB1 cells treated with NVP + 5μM MG132 for 48 h. Immunoblots showed that MG132 reverted NVP effect and increased NZEB1 expression suggesting that NVP reduces ZEB1 stability by proteasome degradation. Coincidently, IGF1 treatment for 48h, increased ZEB1 stability. The results strongly suggest that IGF1R pathway could regulate ZEB1 expression and therefore EMT by regulating the TF stability.

ST-P05
EVOLUTION OF CELL CYCLE MEDIATED REGULATION OF YEAST SEX

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Timing is an important aspect of yeast sex. In Saccharomyces cerevisiae timely mating is guaranteed by strict regulation via the cell cycle of Ste5, a key protein of the mating pathway. Recruitment of Ste5 to the plasma membrane (PM) is necessary and sufficient for activation of the mating response, but is blocked during late G1 and S phases by the G1/S-specific cyclin Cln2. Cln2 docks to a specific motif in Ste5 and promotes a series of phosphorylations around its PM-binding domain, which prevents Ste5 from attaching to the PM. Here we investigate the evolution of this cell cycle mediated regulation of Ste5. We use computational sequence analysis tools to compare Ste5 homologs from a wide range of yeasts, and note that both the PM domain and its flanking phosphorylation sites are relatively well conserved features among yeasts, whereas a clear Cln2 docking site is only found within the Saccharomyces genus. It

has previously been reported that many yeasts only mate under strictly specific growth conditions, such as nutrient starvation. This is in clear contrast to S. cerevisiae which shows a broad responsiveness to mating pheromone. It could be that cell cycle regulated mating may have evolved relatively recently, allowing S. cerevisiae to mate efficiently irrespective of nutrient availability. Interspecies Cln2-Ste5 interaction studies could help support this idea.

ST-P06
AN IMAGE-PROCESSING PROTOCOL TO QUANTIFY AKT LOCALIZATION IN DIFFERENT SUBCELLULAR COMPARTMENTS

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Akt is a kinase involved in a great variety of processes such as cell proliferation, survival and malignant transformation. Several posttranslational modifications (PTMs) as well as numerous subcellular localizations have been reported for Akt recently. However, it is unclear how and why the localization of Akt is regulated, and how this information is encoded in the repertoire of Akt PTMs ("Akt molecular code"). In order to shed light on these questions we studied how Akt variants (WT and mutants) are recruited to different organelles. Qualitatively, we observed differences between Akt mutants that simulate or abolish certain PTMs. In particular, we found that Akt is recruited to Golgi, a localization that has not been previously reported. We found new substrates and PTMs associated to this localization. However, cell-to-cell variability in Akt localization patterns leads to the necessity of improving the methods to quantify robustly the presence of Akt in different subcellular compartments. To this end, we plan to develop a protocol for quantitative measurement of Akt localization and its substrates in the nucleus, cytosol, mitochondria and other organelles, by performing image-processing that combines cellular segmentation in Cell Profiler and co-localization analysis in Matlab. Here we show preliminary measurements and the strategies taken to improve the protocol.

ST-P07
THE GPCR STE2 MEASURES FRACTION OF OCCUPIED RECEPTORS BY BOTH ACTIVATING AND INHIBITING G PROTEIN

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In theory a cellular response to a ligand depends on the amount of agonist-receptor complex. This model predicts that changes in the abundance of receptors should also change the response curve. However, the response to pheromone in Saccharomyces cerevisiae is robust (unchanged) to increases or reductions in the number of the GPCR Ste2, indicating that this pathway responds not to the absolute abundance but according to the fraction of occupied receptor. We determined experimentally that this signaling mode originates in the G protein activation cycle. In yeast, Ste2 binds to Sst2, the inhibitory RGS (Regulator of G-protein Signaling), forming a complex with opposing activities (a so-called Push-pull motif). According to our mathematical model, this GPCR-RGS complex "computes" the ratio between bound and unbound Ste2 and thus it is the key for fraction measurement.Here, we tested this prediction using a set of mutant strains with different abundance of Ste2, heterologous RGS proteins, which we engineered to interact with Ste2, and Gα mutants. We show extensive evidence supporting the notion that fractional receptor occupancy signaling depends on GPCR-RGS interaction, thus validating our model. In eukaryotes, many RGSs bind to specific GPCRs, suggesting that complexes with opposing activities, that detect fraction occupancy using Push-pull motifs might be ubiquitous.

ST-P08
ANTIGENIC VARIATION IN *Giardia lamblia*: ROLE OF VSPS TRANSMEMBRANE DOMAIN IN SENSING AND SIGNALING

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Giardia lambliatrophozoites undergo antigenic variation, where one member of the Variant-specific Surface Protein (VSP) family is expressed on the surface of proliferating trophozoites and periodically replaced by another. VSPs comprise an N-terminal variable domain and a highly conserved C-terminal region that includes a hydrophobic transmembrane domain (TMD) and a short cytoplasmic CRGKA tail. Despite the high degree of conservation, the VSP TMD has received relatively little attention, possibly due to the fact that TMDs have often been viewed as passive anchor sequences that span the lipid bilayer. Accumulating evidence has implicated TMDs in helix-helix interactions leading to dimerization and signal transduction. We have carried out TOXCAT assays to assess the ability of VSP TMDs to self-interact and found that mutations of highly conserved aminoacids within this sequence can alter VSP TMD interactions. Our results suggest that dimerization of VSPs via their TMDs play a role in the mechanism of signal transduction in antibody-triggered antigenic variation.

ST-P09
ANTINEOPLASTIC EFFECT OF ERK 1/2 AND AKT INHIBITION BY VDR AGONISTS IN VGPCR ENDOTHELIAL CELLS

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The Kaposi's Sarcoma-associated Herpes virus G Protein-Coupled Receptor (vGPCR) is a key molecule in the pathogenesis of Kaposi Sarcoma. In the present work, we studied the role of ERK1/2 and Akt regulation by VDR agonists, 1α,25(OH)2D3 and TX 527, of

antiproliferative actions in vGPCR cells.The results showed that incubation with the MEK inhibitor PD98059 (10 μM) or Akt inhibitor, Inhibitor IV (2.5 μM) decreased cell number after 72 h treatment. Western blot analysis of time (24-48 h) and dose response studies (0.1- 100 nM) showed that 1α,25(OH)2D3 or TX527 (10 nM) decreased ERK1/2 and Akt protein phosphorylation. MKP-3, a specific phosphatase that attenuate ERK1/2 signaling, was found increased in western blot and qRT-PCR time response studies after 1α,25(OH)2D3 or TX527 treatment. This was correlated with p-ERK1/2 inhibition. ERK and Akt antineoplastic participation was evaluated using pharmacological inhibition. qRT-PCR studies (24h) revealed that 1α,25(OH)2D3, TX 527 (both 10nM) and PD (10 μM) treatment decreased IL-6, A20 and NF-κB gene expression while the opposite effect was observed for Bim. However, all genes studied exhibited an mRNA increased in cells incubated with Inhibitor IV (2.5 μM). All together, these results suggest that 1α,25(OH)2D3 and TX 527 inhibit proliferation involving ERK and Akt inhibition as part of an anti-angiogenic and anti-inflammatory mechanism

ST-P10
QUERCETIN DECREASES THE PROLIFERATION OF ENDOTHELIAL CELLS TRANSFORMED BY KAPOSI SARCOMA HERPESVIRUS

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The Kaposi's Sarcoma-associated Herpes virus G Protein-Coupled Receptor (vGPCR) is a key molecule in the pathogenesis of Kaposi-Sarcoma. Persistent expression and activity of vGPCR is required for NF-κB pathway activation and tumor maintenance in endothelial cells. The flavonoid quercetin possesses potent anti-inflammatory effects due in part to its anti-NF-κB activity, which we supposed to be part of the antiproliferative mechanism. In the present work, we studied the effect of quercetin in the proliferation of vGPCR cells and the regulation of ERK1/2, Akt and NF-κB pathway. Quercetin dose response studies (1-20 μM) in vGPCR cells showed a reduction on cells number after 24 h treatment. Under contrast phase microscopy, cells exhibited apoptotic features as quercetin concentration was increased. Under the same conditions, Western blot studies showed that incubation of vGPCR cells with quercetin increased IκBα and decreased NF-κB expression. These changes were accompanied by a reduction of p-Akt and p-Erk at low concentration (1-5 μM) of quercetin while the opposite effect was observed with higher (10-20 μM) quercetin concentration. All together, these results suggest that quercetin has antiproliferative and anti-inflammatory effects on endothelial murine cells transformed by vGPCR

ST-P11
COMPARATIVE ACTIONS OF 1α,25(OH)2D3-GLYCOSIDES AND SYNTHETIC 1α,25(OH)2D3 DURING MYOGENESIS

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The presence of glycoside derivatives of 1α,25(OH)2D3 endows plants to gradual release of the free bioactive form of 1α,25(OH)2D3 from its glycoconjugates by endogenous animal glycosidases. This results in increased hormone half-life in blood when purified plant fractions are administered for therapeutic purposes. In this work, we compared the effects of 1α,25(OH)2D3-glycosides enriched natural product (Solbone A) with synthetic 1α,25(OH)2D3 in skeletal muscle cells C2C12 undergoing differentiation and the role of vitamin D receptor (VDR) on this process. Results showed that Solbone A and 1α,25(OH)2D3 (10 nM) increased VDR expression. To explore VDR dependence on C2C12 differentiation, cells were transfected with a shRNA against VDR and then treated with Solbone A or 1α,25(OH)2D3 (10 nM, 48 h). qRT-PCR analysis indicated that VDR expression was greatly decreased and microphotographs showed that VDR knockdown cells failed to differentiate. Furthermore, likewise the synthetic hormone, Solbone A increases myogenin, a differentiation marker that was absent in VDR knockdown cells. In conclusion, our results demonstrated that VDR expression is crucial for C2C12 differentiation. Moreover, Solbone A exhibits equal or greater effects on early myoblast differentiation as the synthetic hormone, suggesting that plant glycosides could be an effective substitute to promote muscle growth.

ST-P12
A FLUORIMETRIC POPULATION ASSAY SHOWS THAT MEMBRANE POTENTIAL OF HUMAN SPERM DEPEND ON PKA A

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Physiological changes that endow mammalian sperm with fertilizing capacity are known as sperm capacitation, which involves, among other events, changes in ion membrane-permeability. This ion permeability changes impact on the plasma membrane potential (Em) of any animal cell, which is typically negatively charged inside. Em hyperpolarization associated to capacitation is well described for mouse sperm, where it depends on potassium fluxes and it is both necessary and sufficient for sperm to undergo the acrosome reaction. However, little is known in human sperm, where mostly qualitative flow cytometry analyses have been conducted. Our results show for the first time a set up for measuring human sperm membrane potential, by means of the cationic carbocyanineDiSC(3)5 in a fluorimetric population assay. The equilibrium potential for potassium was for each sperm sample calculated using the Nernst equation and considering an intracellular human sperm potassium concentration of 120 mM. Our results show a high variation of absolute values among different samples donors. However, hyperpolarization of Em was consistently observed, regardless of the initial non-capacitated Em value. This hyperpolarization is, as in the case of mouse sperm, dependent on PKA activity. Our experimental conditions have the potential to add a strong diagnostic tool to reproductive clinic.

ST-P13
IN VIVO OLIGOMERIC STRUCTURE AND PARTNERS OF YEAST PKA-R SUBUNIT THROUGH PULL-DOWN PROTEOMICS

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PKA is a tetramer formed by a dimer of regulatory subunit (R2), which binds cAMP, and two catalytic subunits. In mammals, the N-terminus of R2 (DD) is responsible for dimerization and binding to AKAPs. We have shown that Bcy1, the yeast PKA R subunit, binds specific proteins through its N-terminus and that a purified Tag-Bcy1(1-50) version of the DD forms tetramers both in crystals and solution. To investigate the oligomerization state of Bcy1in vivo, we overexpressed the Tag-DD in yeast, with the rationale that it would interact with endogenous Bcy1 if a tetramer existed within the cell. A pull-down protocol was established comprising biological replicates of a WT strain with or without overexpression of the Tag-DD. Crude extracts were loaded onto Ni-agarose columns, the Tag-DD and its binding partners were eluted with 200 mMimidazol, and analyzed by nanoHPLC-ESI-Orbitrap. Bcy1 and the catalytic subunits (Tpk1,Tpk2,Tpk3) were detected in the replicates, indicating that in vivo, the overexpressed DD could interact with the Bcy1 subunit and with the PKA holoenzyme. We predict that this interaction is via the formation of a tetramer through the DD domain. The semi-quantitative comparison of the results yielded a set of specific proteins differentially observed in cells with the overexpressed DD, possible binders of Tag-DD, BCY1 or a new surface in the tetramer DD2/BCY12

ST-P14,
SEQUENTIAL ERK PHOSPHORYLATION IN TYROSINE AND THREONINE DETERMINES ITS CELLULAR DISTRIBUTION

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The canonical ERK1/2 cascade is stimulated upon the binding of growth factors to their receptors; then MEK phosphorylates ERK at threonine (pThr) and tyrosine (pTyr) residues. pThr183 and pTyr185 fully activate ERK2 (2pERK) which translocates to the nucleus. Reactive oxygen species,such as H2O2, control ERK oxidation state and function. Low H2O2 (1μM) promotes cell proliferation while high H2O2 (50μM) arrests the cell cycle, a consequence of differential ERK translocation to the mitochondria and the nucleus, in LP07 lung tumor cells. The aim of this work is to study mechanisms involved in ERK intracellular traffic under different redox conditions in LP07 cells. Low oxidant status prompts a fast cytosolic pTyr185 and a later appearance in mitochondria, seen by immunoblot against pTyr. 2pERK transiently augments in mitochondria with a decrease at 60 min of H2O2 treatment to shuttle to the nucleus, promoting cell proliferation. In contrast, at high oxidative conditions, 2pERK accumulates in mitochondria with little passage to the nucleus. We showed by confocal microscopy that the ERK2 mutant Y185A (Tyr replaced by Ala) does not overlap with mitochondria but T183A (Thr by Ala) accumulates in the organelle, meaning that pThr183 is needed for ERK to leave mitochondria. These results suggest that under proliferative redox conditions, pTyr185 allows ERK to interact with mitochondria achieving pThr183 to drive ERK to the nucleus.

ST-P15
ERK-SPECIFIC PHOSPHATASE MKP-3 SPLICE VARIANTS DIFFER IN REGULATION AND SUBCELLULAR LOCALIZATION

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MAP kinase phosphatase 3 (MKP-3) plays an essential role in cell proliferation. It belongs to a subfamily of three cytoplasmic dual-specificity MKPs and, unlike other subfamily members, is an ERK-specific enzyme. In human tissues and cell lines, two splice variants of MKP-3 are expressed in different proportions: the full transcript or isoform L, and isoform S, where exon 2 is skipped. The lack of important regulatory sites in S could thus affect the L/S ratio and, consequently, impact cell biology. This work analyzes and compares different aspects of both isoforms mRNA and protein. In breast cancer cell line MDA-MB-231, mRNA decay evaluated by RT-PCR showed higher stability for the L isoform. Western blot analysis revealed both L and S protein expression, L levels being upregulated by a MEK inhibitor. MKP-3 S and L were cloned for the expression of flag-tagged proteins under a constitutive promoter and the recombinant vectors transfected in HEK293 cells. Both recombinant isoforms displayed activity against P-ERK. Kinetics profile analysis in serum-stimulated cells showed that flag-S protein was accumulated over time, while flag-L remained constant. Immunofluorescence microscopy showed both proteins in the cytosol and flag-S protein also in the nucleus. Therefore, S and L variants exhibit different properties which could affect L/S ratio and its consequences in cell behavior.

ST-P16
CENTRAL SIGNALING ROLE FOR THE CONSERVED GLYCINE HINGE OF BACTERIAL CHEMORECEPTORS

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Chemotaxis requires the transmission of information from the environment to the flagellar motors. Chemoreceptors are dimeric transmembrane proteins with a periplasmic domain for ligand binding and a cytoplasmic domain consisting of a long hairpin that forms a four-helix coiled coil bundle. The activity of the CheA kinase, attached to the tip of the cytoplasmic domain, is modulated in response to external signals. The mode of signal propagation along the long chemoreceptor rod is still under study. In this work we

focus on the role of three conserved glycine residues, two in the N-terminal (G340-G341) and one in the C-terminal (G439) helix of the hairpin, that conforms a hinge in the serine receptor Tsr. We carried out random-codon mutagenesis and selected the non-functional variants. We obtained 14 different replacements, 13 of which retained native receptor interactions and subcellular localization, but were defective in kinase control, as assessed by flagellar rotation assays. All the mutants in G439 were unable to activate the kinase and showed a hyper-methylated pattern, indicative of a locked-OFF receptor conformation. Second-site revertants showed alterations on the methylation region or near the mutated glycine and recovered the ability to activate the kinase. These results indicate that the glycine hinge is implicated in the receptor ON-OFF transition during signaling.

ST-P17

PKA ROLE IN TRANSLATIONAL RESPONSE TO HEAT STRESS IN *Saccharomyces cerevisiae*

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In Saccharomyces cerevisiae the cAMP dependent Protein Kinase (PKA) is involved in multiple steps of translation regulation in response to nutrient availability. PKA is a hetero-tetramer composed of two regulatory subunits encoded by BCY1 gene, and two catalytic subunits encoded by three genes, TPK1, TPK2 and TPK3. Previously, we demonstrated that mild heat stress induces the aggregation of Tpk3 and of closed mRNA loop complexes into SGs. Severe heat stress leads to the formation of PBs and SGs that contain Tpk2 and Tpk3 and directs the accumulation and aggregation of the initiation translation complex 48S. Tpk1 showed mostly cytoplasmic localization. Here, we analysemRNP dynamics, translational arrest and translational fitness of CYC1, HSP42, HSP30 and ENO2 genes; whose tranlational fitness, regulated by heat stress, showed that TPK3 or TPK2 deletion affects the translational response to severe heat stress. TPK2 deletion leads to strong translational arrest, an increment in mRNPagreggation and translational hypersensitivity to heat stress. TPK3 deletion inhibits mRNP formation as well as translational arrest and affects the translational response of analyzed genes. Therefore, our results provide evidence that Tpk2 and Tpk3 would have opposite role on heat stress translational adaptation.

ST-P18

TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL REGULATION OF PKA SUBUNITS IN *Saccharomyces cerevisiae*

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Protein kinase A (PKA) is a broad specificity protein kinase that controls a physiological response following the increment of cAMP as a consequence of a particular stimulus. The specificity of cAMP-signal transduction is maintained by several levels of control acting simultaneously. One of these control levels is the regulation of the expression of each PKA subunit which is regulated at the activity of their promoters, mRNA levels and posttranscriptional processing as degradation. Stabilities of individual transcripts can be regulated in response to environmental conditions such as rapid shifts to different carbon sources and cellular stress. S.cerevisiae PKA holoenzyme contains two catalytic subunits encoded by TPK1, TPK2 and TPK3 genes, and two regulatory subunits encoded by BCY1 gene. We demonstrate that the promoter of each isoform of TPK and of BCY1 is differentially activated during heat shock stress. TPK1 promoter activity is the only one that is positively regulated during this stress. We also measure the half-lives of yeast PKA TPK1 and BCY1 mRNAs upon heat stress. Both mRNAs have different half-lives, being BCY1 mRNA more stable than TPK1 mRNA. During heat shock, TPK1 and BCY1 mRNAs half-lives increase in comparison with the normal situation. We suggest that the exonuclease Xrn1 is involved in the degradation of both BCY1 and TPK1 mRNA degradation.

ST-P19

CHROMATIN REMODELING REGULATION OF PROTEIN KINASE A TPK1 SUBUNIT IN *Saccharomyces cerevisiae* BY STRESS.

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Chromatin remodeling is required for efficient transcription of eukaryotic genes.This process is performed by a large class of ATP-dependent chromatin remodeling complexes as SWI/SNF, ISWI, CHD and INO80 protein families. Yeast PKA is composed of two catalytic subunits encoded by TPK1, TPK2 and TPK3 genes, and two regulatory subunits encoded by BCY1 gene.The transcription of TPK1 is regulated in response to osmostress and heat-shock. Using MNase protection assay we demonstrate that three positioned nucleosomes reside only over the TPK1 promoter. Upon heat-shock and osmostress this nucleosomes are no longer detectable, and TPK1 is induced. Using β-galactosidase reporter assay and ChIP we demonstrate that remodeling of TPK1 chromatin structure requires SWI/SNF, INO80, and RSC during heat-shock and SWR1 during osmostress. It had been described that inositol polyphosphates can modulate the activities of several chromatin remodeling complexes. To identify additional factors important for remodeling chromatin in TPK1 promoter we analyze TPK1 promotor activity and mRNA synthesis in null mutant strains for the synthesis of soluble inositol polyphosphates pathway. TPK1 promoter activity and mRNA levels decreased in the plc1Δ, ipk2Δ, plcΔ and ksc1Δ strains but not in the ipk1Δ strain. These results indicate that it is likely the lack of PP-IP4 production that affects the TPK1 transcription.

ST-P20
INVOLVEMENT OF HISTONE METHYLTRANSFERASE-1 IN *Giardia lamblia* DIFFERENTIATION

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In eukaryotes, histone lysine methylation is associated either with active or repressed chromatin states, depending on the status of methylation. Even though the amino-terminus of Giardia lamblia histones diverges from other organisms, these regions contain lysine residues that are potential targets for methylation. When we examined the role of the histone methyltransferase 1 (HMT1) in the regulation of the encystation process by HMT1 overexpression or downregulation in transfected trophozoites, we observed an increase or decrease of cyst production, respectively, compared with wild-type trophozoites. Time-lapse analysis of encystation showed that overexpression of HMT1-HA induced an earlier and faster process than in wild-type cells together with an up-regulation of the mRNA expression of cyst wall proteins. Localization studies indicated that HMT1-HA was mainly associated with the nuclear and perinuclear region during growth and encystation of the parasite, in agreement with bioinformatic analysis showing that HMT-1 of Giardia possesses nuclear localization signals in addition to the classical SET- and post-SET domain. Altogether, these findings suggest that the function of HMT1 is critical for the success and timing of the encystation process and reinforce the idea that epigenetic marks are critical for cyst formation in G. lamblia.

ST-P21
TOLL CONTROL: IMPORTINS AS SENTINELS OF NUCLEAR/CYTOPLASMIC SHUTTLE IN *Giardia lamblia*

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In eukaryotic cells, proteins larger than 50 kDa with nuclear localization signals (NLS) are transported across the nuclear envelope by a family of transport proteins called karyopherins or importins. This process first involves the recognition of NLSs by the adaptor importin α and binding by its N-terminal importin-β-binding (IBB) domain to importin β. In this mechanism also participates RanGTP, nuclear transport factor 2 and RCC1. In the early divergent protozoan Giardia lamblia, we have found the existence of all of these components. Particularly, we found two genes GL50803_16202 and GL50803_15106, which match by in-silico analysis with importin α and β, respectively. Immunofluorescence studies of growing transfected trophozoites over-expressing these proteins showed the expected perinuclear localization. Interestingly, during the differentiation process (encystation), cells over-expressing importin α showed similar cyst production to wild-type cells while cells over-expressing importin β shown a 2-fold increase in the cyst production. The use of importazol (importin β inhibitor) showed a dramatically reduced cyst production. These results suggest that the classical nuclear transport is functional in Giardia, being this mechanism essential during the differentiation process.

ST-P22
INDOMETHACIN MODULATES BMMC MIGRATION THROUGH PROSTAGLANDIN AND/OR PPARϒ AFTER SCIATIC NERVE INJURY

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Our group has demonstrated that indomethacin inhibited the migration of systemically transplanted bone marrow mononuclear cells (BMMC) to the lesion area, and promoted a decrease in prostaglandin E2 (PGE2) biosynthesis and a significant increase in PGD2 and PGJ2 levels in control and injured nerves. Considering that PGJ2 is an endogenous modulator of PPARϒ, the aim of the present work was to evaluate whether indomethacin prevents the inflammation associated with injury through cyclooxygenase 1 or 2 (COX -1 or -2) activity or PPARϒ activation.To this end, adult Wistar rats subjected to sciatic nerve crush were treated with indomethacin and the expression of Cox-1 and -2 and PPARϒ were evaluated, as well as the synthesis of PGs at different times previous and post crush, through qPCR, immunohistochemistry and PG radioconversion, respectively. Naïve animals treated with indomethacin were used as controls.The increase in IOD levels associated to COX-1 induced by the injury was blocked by indomethacin. COX-2 expression was detected only at the mRNA level from day 4 post injury. PPARϒ immunofluorescence distribution was modified by indomethacin.Our results suggest that indomethacin prevents BMMC migration not only through PG biosynthesis inhibition but also affecting the expression/localization of PPARϒ. Further experiments are necessary to confirm this dual effect of indomethacin.

ST-P23
A SIGNAL TRANSDUCTION PATHWAY RELATED TO VIRULENCE REGULATION IN *Leptospira*

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Signal transduction allows microorganisms to sense the environment and adapt central cellular processes. Reversible protein phosphorylation is a ubiquitous mechanism of protein regulation, in bacteria mediated by His kinases in two component systems (TCSs) and by S/T protein kinases and phosphatases. Our research focuses on *Leptospira*,the agent of leptospirosis, the most extended zoonotic disease. The project is set to study a signaling pathway that regulates *Leptospira*virulence. Genetic approaches identified gene *lic20111* encoding one of the proteins of this pathway, present only in pathogenic *Leptospira*. Based on similarity to the stress-regulator RsbU in *B. subtilis*, we hypothesize that LIC20111 is a transmembrane sensory phosphatase that, together with other proteins encoded within the same operon, regulates the expression of virulence factors *via* modulation of an alternative sigma-factor. Cloning

of several constructs allowed most of the target proteins to be expressed and purified to homogeneity. Crystallographic studies are underway and protein:protein interactions will be analyzed to uncover the scheme linking these partners. Features from TCSs as well as from sigma-factor-related regulatory pathways are intertwined in a fascinating way to control virulence. We anticipate these studies to provide valuable information to tackle leptospirosis in novel and effective ways.

ST-P24
ADAPTATION MECHANISMS REGULATING THE SECRETORY PATHWAY IN RESPONSE TO A SECRETORY STIMULUS.

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Our work aims to analyze molecular and cellular mechanisms that are involved in the adaptation of the secretory pathway to a higher secretory demand. Rat thyroid cells (FRTL5) were used as a secretory model. FRTL5 are stimulated with thyroid-stimulating hormone (TSH) to induce expression of thyroid-specific cargo proteins [ex: sodium iodide symporter (NIS)] that require the secretory pathway to reach their final destination. Moreover, TSH increases the expression of sterol regulatory element-binding proteins (SREBP1c and SREBP-2), master transcriptional regulators of cholesterol and fatty acid synthesis. We have showed (SAIB 2014) that TSH stimulation enhances the level of proteins required for membrane transport as well as the Golgi volume. We now show that inhibition of SREBPs activity, induced by 25-HC cholesterol, modifies the increase in level of proteins required for membrane transport induced by TSH. Also, overexpression of CREB3L1 regulates expression of SREBP1c. Our data suggest that TSH-activated pathways coordinately synchronize the expression of lipids and transport factors necessary to amplify the structure and function of the secretory pathway.

ST-P25
INVOLVEMENT OF BOX2 IN THE REGULATION OF CHKA EXPRESSION IN NEURO-2A CELLS AND IN HUMAN TUMOR CELLS.

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Choline kinase α (CKα) is the first enzyme of the phosphatidylcholine-biosynthetic pathway (Kennedy pathway). We demonstrated that CKα levels increase during retinoic acid (RA)-induced neuronal differentiation and overexpression of this enzyme was also observed in certain types of human tumors. There are two motives in the Chka gene promoter, named Box1 and Box2, which are involved in its transcriptional regulation. Box2 is essential for maintaining low level of expression under proliferating condition, and mutations or its deletion causes induction of Chka expression, thus this region negatively regulates transcription. In this study, we showed that histone demethylase KDM2B interacts with the Box2 both in vitro and in vivo by EMSA and ChIP assays. To get insight in the mechanism of CKα overexpression in human tumors, we searched for mutations in the sequence of the Box2 that could impair KDM2B binding and/or regulation. This analysis was performed by DNA sequencing on samples obtained from different biopsies and tumor cell lines. Likewise, we examined CKα and KDM2B levels by Western Blot and qRT-PCR. To conclude, this study contributes to elucidate the mechanism by which CKα is regulated in different scenarios like RA-induced neuronal differentiation and in tumor cells regulating its aggressiveness.

ST-P26
STUDYING THE ROLE OF CPSF6 AS A MUTANT P53 EFFECTOR IN TUMOR AGGRESSIVENESS

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Most human cancers inactivate the tumor suppressor p53 through missense mutations, leading to the expression of full-length point mutant proteins. Besides losing the tumor suppressor function of the wt protein, p53 mutants may acquire novel activities that promote aggressiveness and metastasis. Understanding the molecular mechanisms underlying mutant p53 oncogenic function may help to identify molecular ways and targets for cancer treatment. We have previously found that Pin1 cooperates with mutant p53 to alter gene expression in breast cancer cells. In particular, both proteins cooperate to induce the expression of 10 genes (Pin1/mutant p53 signature) whose expression is associated with the development of aggressive breast tumors. Among them we found CPSF6, which codes for a 68 kDa protein, belonging to the CFIm processing factor that regulates mRNA polyadenilation. Although several aspects of CFIm function in mRNA processing were described, little is known regarding the role of CPSF6 in human cancer. In order to identify biological effects of CPSF6 that may cooperate with tumor progression, we used zebrafish embryos as biosensors to study the consequences of Cpsf6 overexpression in vivo. As a mean to investigate the mechanisms regulating the expression of Daniorerio Cpsf6, we analyzed the expression of Cpsf6 deletions lacking the N-terminal, C-terminal and proline-rich domains.

ST-P27
BMP5 INDUCES IN VITRO MIGRATION OF BOVINE OVIDUCTAL EPITHELIAL CELLS

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Bone morphogenetic proteins (BMPs) have an essential role in organogenesis and tissue repair. We have previously reported the expression of a number of BMPs and their receptors in the bovine oviduct epithelial cells (BOEC). The knowledge about their functions in the oviduct is scarce. In the present work, by using the in vitro wound healing assay, the potential role of BMP5, as an

autocrine factor, was evaluated in primary BOEC cultures. In vitro cultured BOEC monolayers from isthmus were cultured for 24 h in serum-free medium in the presence of human rBMP5 (100 ng/ml) after a scratch made in the monolayers; the scratched area was measured at 0, 3, 6, 12 and 24 h (n=3) and compared with non-treated control groups. BMP5 increased significantly the cells ability to close the scratched area at 24 h. No significant differences between treated and control groups were observed until 12 h. RT-qPCR assays were performed to evaluate the ID2 and SMAD6 mRNA levels after 24 h of incubation. Although no significant differences were detected for these direct targets of BMP signaling, results suggest that BMP5 and other BMP should be autocrine factors regulating the repair and renovation of the bovine oviductal epithelium in physiological and pathological states

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Helguera PR CB-C06
Henkel T L-06
Henning MF CB-P26
Heras H CB-P29/SB-P02/SB-P03
CB-P28
Heredia V LI-P04
Hernandez LE MI-P07
Hernando CE PL-P45
Herrera L BT-P04
Herrera Marrero LM BT-C02
Herrera Seitz MK ST-P16
Hilal M PL-P53/PL-P55
Hildebrandt P SB-C03
Hoehne M PL-C03
Hosseini S PL-C02
Hovanyecz P ST-P25
Huarte Bonnet C LI-P032

I

Iannone MF PL-P06
Ibarra SM CB-P63
Idrissi A El NS-01
Iglesias AA EN-C03/EN-P01/EN-P03
EN-P11/EN-P12/EN-P13
MI-P16/PL-P37
Iglesias Gonzalez PA LI-P30

Iglesias MJ PL-C06
Imelio J ST-C06
Insani M EN-P04/EN-P05
Irazoqui P ST-P11
Isaia A MI-P07
Ishihara H PL-C03
Isla MI PL-P53
Ispizua JI CB-P32/ CB-P34
Ituarte S SB-P02

J

Jovellano J LI-P29
Juárez MP LI-C08/LI-P25
Jurkin J L-06

K

Kahmann R PL-01
Katz M CB-P56
Katz MJ ST-C01
Kelly H PL-P04
Kilmurray C MI-P06
Kleiman FK NS-01
Klinke S SB-C03
Koltan M BT-P13
Kornblihtt AR CB-P30/PL-P38
Kourdova L CB-P42
Krainer AR CB-P30
Krapf D ST-C03/ST-P12
Krapf DK ST-C04
Krapp AR PL-C20
Krause U PL-C03
Kubaczka MG PL-P38
Kumar S LI-C08
Kunda P CB-P61
Kunitomi M L-05
Kurzchalia T LI-P08

L

Labadie G LI-P08
Lachenicht JA MI-P26
Lacunza E LI-P17
Lagrutta LC LI-P19
Lalle M CB-P50
Lamattina L MI-P03
Lami MJ BT-P17
Lanfredi-Rangel A MI-P37
Lanteri ML PL-C15
Lanver D PL-01
Lanza L BT-P03
Lapasset Laumann A BT-P18
Lara MV PL-P11/PL-P12/PL-P13
PL-P26/PL-P40/PL-P39
Lareo C BT-P06
Larrieux N ST-C05
Lascano CI CB-P67
Lavandera JV LI-P28/LI-P29
Lavarías S LI-P19
Lavatelli A MI-P51
Leal MC NS-P10
Lechner L PL-P60
Ledda A LI-P16
Ledesma MD NS-04
Legris M PL-C12
Lehner R LI-02
Lemes Santos F BT-P15
Leonardi P LI-P31
Leonhard V LI-P04
Lepek VC MI-P08/MI-P09
Levi V CB-C07/CB-P43
Levy GV MI-P29
Lezcano V ST-P10
Lian J LI-02
Liang L PL-01
Libisch G CB-01
Liggieri CS PL-P01/PL-P09
Lima MA CB-04
Liporace F | BT-P21
Lisa MN ST-P23
Livieri AL MI-P17
Lizarraga E CB-P31
Llames ME MI-P13
Llorens MC ST-P04
Lo Presti L PL-01
Lobertti C PL-P59
Lodeiro AR MI-C03
Lodeyro A PL-P15
Lopez Diaz FJ CB-02
Lopez FE MI-P42
Lopez LA CB-P31
Lopez PF ST-P01
Lopez PV MI-P21
Lorch MS CB-P32/CB-P33
Lorenzetti F LI-P27
Lovaisa N PL-P54
Loyola A CB-03
Lozano M MI-P49
Lozano ME CB-P32/CB-P33/CB-P34
Lucca A CB-P10
Lucero LE PL-C10
Ludwig N PL-01
Lujan H CB-P35
Lujan HD BT-P12/CB-P36/CB-P37
CB-P38/CB-P39/ST-P08
Lujea N CB-P61
Lunn J PL-03
Lunn JE PL-C03
Luque AG PL-P63
Luque GM ST-C03
Luquez JM LI-C02/JM LI-C05/JM LI-P14

M

Machado LS CB-04
Machado R PL-P66
Madzzudulli G LI-P02
Magni C MI-P41/MI-P44
Maiale SJ PL-P18/PL-P57
Malamud M MI-P48
Malan AK BT-P05/BT-P06
Malcolm M CB-P20
Malizia F LI-P03
Maloberti PM ST-P01/ ST-P15
Malvicini R LI-C01/LI-P12
Mamaní de Marchese AI PL-P23
Mammi P CB-P48
Manavella PA PL-C11/PL-C16/PL-P41
PL-P42/PL-P43/PL-02
Mancovsky S LI-P12
Mandile MG BT-P14
Mangano S PL-04
Mangialavori IC EN-P14/EN-P15

Mansilla MC MI-P51
Mansilla N BT-P16/PL-P33
Marasco LE CB-P30
Marchetti F PL-P02/PL-P71
Marco F PL-P58
Marder M EN-P15
Margara LM MI-P14
Margarit E PL-P52
Marini MS CB-P43
Marizcurrena JJ BT-C01
Marotte C CB-P53
Márquez JÁ SB-C02
Marrero Diaz R EN-P05
Marshall M NS-02
Martín G NS-P07
Martín JM CB-P18
Martín L LI-P31
Martín M EN-C02/EN-P02/PL-C21
Martín MG L-07
Martin MJ CB-P35/ST-P08
Martin MV PL-P60
Martín PR CB-P28
Martina MA CB-P36/CB-P37
Martinez J L-06
Martinez SJ CB-P47
Martinez W BT-C01
Martínez Zamora MG PL-P21/PL-P22/PL-P24
Martínez-Noël G PL-P44/PL-P60
Martino Adami PV NS-C02/NS-P10
Martino G1 MI-P41
Martino RA BT-P12/CB-P37
Martos GG PL-P20/PL-P23
Marzol E PL-04
Mas P PL-P61
Masin M CB-P63
Masner M CB-P61
Massazza DA ST-P16
Massot F BT-C03
Mateos J PL-C14
Mateos MV LI-C06/LI-P20/LI-P21
Mateyca C MI-P32
Matveev E CB-P04
Maugeri D EN-P08
Max F ST-02
Mayol G ST-P20/ST-P21
Mayorga LS CB-P40
Meakin S NS-P05
Mechaly A ST-C05
Medina MI PL-P61
Meini MR MI-P31
Meirelles FV CB-04
Melani M CB-P56
Melli L MI-P24
Mencia R PL-P70
Mendez AA PL-P29
Menendez AB MI-P13
Mengucci F MI-C03
Meringer MV MI-P10
Merini L BT-P21
Merini LJ BT-C03
Merkis CI MI-P22
Michaut M CB-P41
Michavila G BT-P15
Miguel VN PL-P16
Millan ME CB-P31
Millán-Pacheco C MI-02
Minahk CJ BT-P03/MI-C01/MI-P25
Minen RI EN-P11/EN-P12
Minjárez-Sáez M MI-02
Miranda A CB-P42
Miranda MR MI-P30
Miretti M CB-P45
Moi D CB-P04
Molinari PE PL-C20
Moliva M MI-P23
Moliva MV MI-P22
Monchietti P PL-P62
Monesterolo NE CB-P13
Mongiardini EJ MI-C03
Monjes NM LI-P18
Montanaro MA CB-P26/LI-P17
Montaner A CB-C01/LI-P03
Montecchiarini ML PL-P52
Montero-Silva, F MI-03
Montes CL CB-P62
Montes G LI-P23
Montes MR EN-P16
Monteverdi L CB-P10
Monti LL PL-P26
Monti MR MI-P14/MI-P19
Mora García S PL-P45/PL-P46
Morales D BT-C01
Morales LL EN-P02
Morbidoni HR LI-P22
Morduchowicz NN CB-P14
Morel Gomez E LI-P13
Morelli S ST-P10
Morelli L NS-C02/NS-P10
Moreno AS PL-P52
Moreno G BT-P14
Moreno JE PL-C07
Moreno JE PL-C17/PL-P16
Moreno S PL-P25/PL-P57/ST-P13
Morera LP NS-P09/NS-C01
Mori Sequeiros Garcia MM ST-P15
Moriconi DE LI-C08/LI-P25
Moro B PL-C14
Moro C MI-P19
Morozova V NS-01
Mortera P MI-P41
Moscoso V LI-P34
Mosquillo MF CB-P59
Moyano AJ CB-P12
Moyano S CB-P50
Mroginski M SB-C03
MRSA-Group CB MI-P33
Mu H SB-P02
Muchut RJ EN-P01
Muchut S PL-P66
Muhlberger T CB-P13
Müller GL PL-P13
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N

Nardella GN CB-P47
Navarro SA MI-P28
Navone L MI-P17
Negretti D CB-P42
Nejamkin A MI-P03
Neuman MI LI-P01
Nicola JP MI-P34
Niderhaus C EN-P04
Nieto Guil AF NS-P06

Nieto M NS-P06
Nieva AS MI-P13
Nievas RY CB-P01
Nikel PI MI-04
Nittolo AG MI-P29
Nocelli N MI-P12
Nores GA NS-P11
Nudel CB LI-P23
Nudler SI ST-P15
Nusblat AD LI-P23

O

Ochoa AL EN-P06/EN-P07
Ogara MF CB-P43
Ohya Y ST-03
Oitaven PA PL-P27
Olivetti CE BT-P09/BT-P10
Oms S BT-P12
Oms SR CB-P39
Ontañón O EN-P05
Orest GM LI-C02
Oresti GM LI-C06/LI-C05/LI-P14
Orlando UD ST-P01
Orlando UO CB-P14
Orozco A CB-P25
Ostersetzer O PL-P02
Otegui M PL-P64
Otegui ME BT-P07
Otero LH MI-P12/SB-C03
Ottado J PL-C19/PL-P47/PL-C18

P

Pagani MA PL-C13/PL-P32/PL-P48
Paggi RA MI-P01
Pagnussat G PL-C21/PL-P02/PL-P71
Pagnussat GC PL-P28/PL-P49
Palatnik J PL-C14
Palatnik JF PL-P50/PL-P51/PL-P56
Palomer E L-07
Panizza S L-06
Pansa MF CB-P07/CB-P08/CB-P09 CB-P11
Panzetta de Dutari GM CB-P42
Panzetta-Dutari GM CB-C02/CB-P21/CB-P22 CB-P23/CB-P12
Paolocci F PL-P58
Paris R PL-P64
Parodi A CB-01
Parody JP LI-P27
Parra L ST-P22
Parra LG LI-C01
Pasqualini ME LI-P02
Pasquare S LI-C04
Pasquaré SJ LI-P24
Paternoste M CB-P51
Patop I ST-P07
Paulucci NS MI-P06
Pautassi R CB-P60/NS-P01
Pautasso C ST-P18
Pavlovic T PL-P30
Paz C ST-P09/ST-P14/ST-P15
Paz García EC BT-P13/MI-C04
Paz MC NS-P12
Pecci A CB-C07/CB-P43
Pedetta A ST-P16
Pedraza RO MI-P05/PL-P54
Pedrini N LI-C08/LI-P25
Pellon-Maison M CB-P26/LI-C03
Pena LB PL-P29
Peñalva DA LI-C05/LI-C07
Penkov S LI-P08
Pepe A BT-P11/PL-P36
Peppino Margutti MY PL-P68
Peralta DA PL-C05
Peralta DO CB-P37
Peralta DR MI-C04
Peralta I BT-P10
Peralta J PL-P07
Peralta JM MI-P07
Perato SM PL-P22
Percaretti MM MI-P11
Perea Garcìa A PL-P58
Pereira CA MI-P30
Pereyra D PL-P10
Perez Chaia A MI-P43
Perez Ipiña E ST-P02
Pérez M1 CB-C03
Pérez Pizá C PL-P04
Pérez-Cañamás A NS-04
Pérez-Díaz L CB-P59
Perez-Pepe M ST-C01/ST-P03
Perez-Perri J CB-P56
Perna O BT-P09/BT-P10
Perotti MF PL-P17
Perotti V PL-P52
Perrone AP PL-P50
Perusia P MI-P12
Pescaretti MM MI-P20
Pescio LG LI-P26
Peterson G LI-P07/LI-P19
Petrillo E ST-02
Phillips G NS-01
Pianzzolla MJ MI-P22
Piattoni CV PL-P37
Piazza A PL-C19
Piccinni F EN-P05
Piegari E ST-P06
Pierella Karlusich JJ PL-C02
Piñero GM ST-P22
Pinto NP ST-C04
Pinto P L-06
Pires NS CB-P67
Pisani G LI-P27
Pitossi FJ NS-P10
Plateo Pignatari MG NS-P07
Podbilewicz B CB-P04
Poderoso C ST-P01/ST-P14
Poderoso JJ ST-P14
Podestá EJ CB-P14/ST-P01
Podestá FE EN-P13/PL-P52
Poetsch A MI-P01/MI-P02
Polo M LI-P07
Pomares M BT-P15
Pomares MF BT-P13/MI-C04
Ponce Dawson S ST-P02
Ponce JC LI-P032
Popovich C LI-P31
Popow J L-06
Porporatto C MI-P23
Porrini L EN-P09
Porta E LI-P08
Portela P ST-P17/ST-P18

Pozzi B CB-P48
Prado Acosta M MI-P47
Presman DM CB-C07
Previtali G EN-P06/EN-P07
Prevosto L PL-P04
Prez GM LI-P08/LI-P09
Principe A MI-P27/MI-P40
Prucca CG CB-P44/CB-P45/CB-P46
Pryciak PM ST-04/ST-C02
Puga Molina L ST-P12
Puga Molina LC CB-P55
Puga Molina LP ST-C04
Pujol-Lereis L CB-C03

Q

Qiu JW SB-P02
Quelas JI MI-C03
Quesada-Allué L CB-C03
Quevedo C BT-P21
Quinteros-Quintana ML NS-P08
Quiroga A LI-02
Quiroga AD LI-P27
Quiroga IY LI-C03
Quiroga M MI-P43
Quiroga S NS-P06

R

Rabossi A CB-C03/NS-C02
Racagni GE MI-P10/PL-P68
Racca AC CB-P12/CB-P44/CB-P46
Racca S PL-C09
Radi R NS-C02
Raineri J BT-P07
Ramallo Guevara C MI-P01
Ramello MC CB-P62
Ramirez DC CB-C04/NS-P07
Ramírez Tapias YA BT-P18
Ramírez-Mata A MI-02/MI-P04
Rapisarda VA MI-P05/MI-P45/PL-P53 PL-P54/PL-P55
Raviolo JM MI-P23
Ré DA PL-C16
Reca S ST-P19
Recalde L PL-P05
Reggiani PC LI-P06
Rego N CB-01
Reguera YB MI-P06
Reigada C MI-P30
Reinoso E MI-P23
Reinoso EB MI-P22
Reinoso N MI-C02/MI-P15
Reissmann S PL-01
Repetto MV ST-C02
Repossi G LI-P02
Requena Serra FJ PL-P20/FJ PL-P21
Reutemann A PL-P66
Revuelta V ST-P21
Rey Serantes D MI-P24
Reyna L CB-C02/CB-P22/CB-P23
Reyna M PL-P68
Ribichich KF PL-P16
Ribone PA PL-C08/PL-P17
Ridano ME CB-C02/CB-P22/NS-P12
Riesco AS EN-P14
Rinaldi D EN-P14/EN-P15
Rinaldi JJ SB-C03
Ríos Colombo NS MI-P25
Rios DN BT-P12/CB-P38/ST-P08
Ríos MN CB-P20
Risso G CB-P48
Ritagliati C ST-C03/ST-P12
Rivelli JF EN-P06/EN-P07
Rivera C CB-03
Rivero CW BT-P19/MI-P50
Rizzi YS PL-C01/PL-P03
Rizzo A CB-P58
Robello C CB-01
Rodenak Kladniew B LI-P06
Rodrigues Capítulo A LI-P19
Rodriguez AV MI-P43/MI-P46
Rodríguez Berdini L NS-P02
Rodriguez CE PL-P13
Rodriguez EJ MI-P17
Rodríguez Gastón JA BT-P08
Rodriguez RE PL-P50/PL-P56
Rodríguez Sawicki L LI-P34
Rodriguez-Walker M CB-P16
Rojas A PL-C14
Rojas BE EN-P13
Rojas ML CB-P21
Roldán J PL-C21
Roldán JA PL-P49
Roldán Olarte EM ST-P27
Romani FA PL-C07
Romano PS CB-P47
Romero DJ LI-10/LI-P11/LI-P26
Romero H CB-P04
Ronco MT LI-P27
Rópolo A ST-P20/ST-P21
Rópolo AS MI-P39
Rosati RG PL-P63
Roser LG MI-P29
Rossi F CB-C03
Rossi FR PL-P19
Rossi JI PL-P06/EN-P14/EN-P15
Rossi RC EN-P14/EN-P16
Rossi S ST-P13/ST-P18/ST-P19
Rota RP CB-P33
Roth GA NS-P03/NS-P04
Ruggiero FM CB-P03
Ruiz OA MI-P13/PL-P57/PL-P58
Ruiz P CB-P60
Ruiz P NS-P01
Ruiz V PL-P53
Ruiz-Ranwez V MI-C05
Rumbo M BT-P14
Rupil LL CB-P36/CB-P37/CB-P39
Russo de Boland A ST-P11
Russo DM MI-C05
Ruzal SM MI-P47

S

Saad LF CB-P20
Saavedra F CB-03
Saban T LI-P12
Sabatino ME CB-P08/CB-P10
Saigo M PL-P30/PL-P59
Saín J LI-P28/LI-P29
Saint Martin M EN-P16
Salamanca JÁ LI-P25
Salamanca JE LI-C08

Salazar PB MI-P25
Salazar SM PL-P24
Salerno G PL-P44
Salerno GL PL-P60
Salomón RA MI-P26
Salusso A ST-P20
Salvador G LI-C04/LI-P31
Salvador GA LI-P20/LI-P21/LI-P30
Salvatierra Fréchou DM CB-P54
Salzman V CB-P51
Sampieri L CB-P05/ST-P24
Sánchez DO CB-P49/MI-P29
Sanchez Granel ML LI-P23
Sánchez I ST-P05
Sanchez López EF PL-P19
Sanchez M BT-P13/PL-P48
Sánchez MC CB-P15/CB-P18/NS-P12
Santacreu BJ LI-P10/LI-P 11
Santana Medrini N LI-P032
Santander VS EN-P07
Santi E CB-P58
Santiago Valtierra FX LI-C02/LI-C05/LI-C06/LI-P14
Santillan LD CB-C04
Santos L EN-P17
Saparrat MCN LI-P032
Saran A BT-C03
Sarquiz A BT-P19
Sasoni N EN-P03
Saura A CB-P39
Saveanu L CB-P28
Sayago P MI-P18
Saye M MI-P30
Scaglia N LI-P33/LI-P34
Scanarotti IG LI-P28
Scandiani MM PL-P63
Scarpeci TE PL-P67
Schapire A PL-C14
Scherma ME LI-P02
Schipper K PL-01
Schor I CB-C05
Schurig-Briccio L MI-C01
Schwarzbaum PJ CB-P54
Scodelaro Bilbao P LI-P31
Seeger M MI-03
Sequeira M PL-P07
Serradel MC BT-P12/CB-P36/CB-P37 CB-P39
Serradell MA MI-P48
Setton-Avruj CP ST-P22
Shahinnia F PL-C02
Silva RA LI-P02
Silvarrey C BT-P20
Silvestre D BT-P14
Silveyra MX PL-C15
Siri MI MI-P22
Smania AM CB-P12/MI-P18/MI-P31
Smith LC CB-04
Sola C CB-P12/MI-P33/MI-P34
Solari CA ST-P17
Soler SB LI-P17
Sonnewald S PL-C02
Sonnewald U PL-C02
Soria G CB-P07/CB-P09/CB-P11
Soria GR CB-P08/CB-P12
Sorianello E CB-P56
Sorroche F MI-P12
Sosa Alderete LG PL-P61
Sosa-Alderete L LI-C04
Soto D PL-P71
Soto PA ST-P22
Sottile AE SB-P01
Spampinato CP PL-P62/PL-P63
Sparwasser T CB-P36
Spinsanti LI CB-P33
Srebrow A CB-P48
Stadler M CB-P57
Stegle O CB-C05
Stella CA MI-P38
Stellberger T MI-C06
Sterin-Speziale NB LI-P26
Stitt M PL-C03
Stival C ST-P12
Stival VC ST-C03
Stockwell B PL-C21
Stortz M CB-P43
Stortz MD CB-C07
Stringa P CB-P26
Strologo L PL-P11/PL-P12
Studdert CA ST-P16
Suares A ST-P09
Subirada Caldarone PV NS-P12
Svetaz L PL-P40
Sycz G MI-C05

T

Taboga O BT-P08
Talia P EN-P05
Tapia C ST-P09/ST-P10
Tarkowski N ST-P02
Taruselli MA CB-P64/CB-P65
Tassetto M L-05
Tekiel V CB-P49
Tellado M CB-C08
Tello M MI-03
Tempesti T CB-P45
Tempesti TC NS-C01
Temprana CF BT-P14
Tenaglia A ST-P26
Tenaglia AH CB-P38/ST-P08
Tenconi PE LI-P20/LI-P21
Terrile MC PL-C06/PL-P64/PL-P65
Therrien J CB-04
Thomas Graw RH PL-P23
Tito FR BT-P11/PL-P36
Tobares RA MI-P18
Todaro LB CB-P64/CB-P65
Tofolón E ST-P13
Toledo J LI-P16
Tomas Grau RH PL-P20/PL-P21
Tomassi AH PL-P41/PL-P42
Tornero P PL-P01
Torre M. CB-P58
Torres Demichelis V LI-P32
Torres Demichelis VA CB-P05/ST-P24
Torrez Lamberti MF MI-P42
Torri A CB-P38
Torti P BT-P16
ToselloBoari J CB-P62
Touz MC CB-P50/MI-P37
Trajtenberg F ST-C05
Trelles JA BT-P19/BT-P18/MI-P50/ MI-P49
Triassi A PL-P39

Trípodi KEJ PL-P52
Troetschel C MI-P01
Tronconi MA PL-P25
Trotschel C MI-P02
Tulino MS LI-P26
Turdera L CB-C03
Turowski VR PL-P32

U

Uberti Manassero N PL-P66
Uchikawa E SB-C02
Ugalde JE MI-P21/MI-P24
Uranga RM LI-P30
Urtreger AJ CB-P64/CB-P65
Usach V ST-P22

V

Vaglienti MV ST-P04
Vairo Cavalli SE PL-P01/PL-P09
Valacco MP ST-P13/PL-P57
Valacco P PL-P25
Valansi C CB-P04
Valdecantos PA ST-P27
Valdez Taubas J CB-P02/CB-P03/MI-P39
Valentini G PL-P26
Valentini H PL-P40
Valera-Vera EA MI-P30
Valko A CB-P56
Valle EM PL-P67
Valles AS LI-P15
Valsecchi WM EN-P08
Valz Gianinet JN ST-P27
Vargas-Straube MJ MI-03
Vasen G ST-P07
Vasquez Gomez ME CB-P25
Vázque S BT-P21
Vázquez A PL-P05
Vazquez CT CB-P32/CB-P34
Vázquez DE PL-P52
Vázquez MM CB-P15/LI-P05/PL-P64
Vazquez Ramos JM PL-P29
Vegetti A PL-P66
Velázquez F SB-C03
Velazquez FN CB-P44/CB-P45
Vena R PL-P56
Venturino A CB-P67
Vera C L-08
Verstraeten SV CB-P52/CB-P53/CB-P54 CB-P55/LI-P13
Ves-Losada A LI-P19/LI-P33/LI-P34
Vierstra R PL-C12
Vila AJ MI-P31
Vilas JM PL-P18
Vilas JM PL-P57
Vilcaes AA LI-P32
Villafañez F CB-P08/CB-P11
Villafañez MF CB-P07/CB-P09
Villalba N ST-P14
Villasuso AL MI-P10/PL-P68
Villaverde MS CB-C08/CB-P66
Villegas JM MI-P45
Vincent P BT-P15
Vincent PA BT-P13/BT-P17/MI-C04
Viola IL PL-P34/PL-P69
Viruega-Góngora VI MI-P04
Visconti PE ST-C03
Vitanescu M CB-P57
Vizoso Pinto MG MI-C06
Vranych C PL-C19

W

Wagner PM LI-C04
Wallinger ML NS-C02
Wappner P CB-P56/ST-C01
Weber K ST-P22/LI-C01/LI-P12
Weiss L NS-P06
Weitzer S L-06
Welchen E PL-C09/PL-P33/PL-P70
Welte MA LI-01
Whitfield Z L-05
Wilke N LI-04
Winters MJ ST-04/ST-C02
Wirth S EN-P04

X

Xiqui-Vazquez MLMI-P04

Y

Yandar NY MI-C02/MI-P15
Yanovsky MJ PL-P45

Z

Zabaleta E PL-C21/PL-P02/PL-P71
Zabaleta EJ PL-P49
Zalguizuri A MI-P08
Zalosnik MI NS-P03/NS-P04
Zampini IC PL-P53
Zamponi E CB-C06
Zamponi N ST-P20/ST-P21
Zanor MI PL-P67
Zanotto SC CB-P13
Zapata CA PL-P14
Zappa Villar MF LI-P06
Zawoznik MS PL-P06
Zechmann B PL-01
Zenoff AM BT-P17
Zilli C PL-P04
Zinni MA MI-P50
Zlocowski N ST-P20
Zorreguieta A MI-C05
Zubimendi JP PL-P25
Zuljan FA MI-P44
Zurbriggen M PL-C02/M PL-C20