SAIB

54th Annual Meeting Agentine Society for Biochemistry and Molecular Biology

LIV Reunion Anual Sociedad Argentina de Investigación en Bioquímica y Biología Molecular

CONICET

FONCYT
FONDO PARA LA INVESTIGACIÓN CIENTÍFICA Y TECNOLÓGICA

AGENCIA
NACIONAL DE PROMOCIÓN CIENTÍFICA Y TECNOLÓGICA

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Romina Uranga
Auditor
INIBIBB CONICET.
Universidad Nacional del Sur

DELEGATES OF SCIENTIFIC SESSIONS

CellBiology

Gustavo Chiabrando
CIBICI CONICET
Facultad de Ciencias Químicas
Universidad Nacional de Córdoba

Lipids

Hugo Gramajo
Facultad de CienciasBioquimicas y Farmaceuticas
Universidad Nacional Rosario
IBR-CONICET

Plants

Paula Casati
Facultad de Ciencias Bioquimicas y Farmaceuticas
Universidad Nacional Rosario
CEFOBI-CONICET

Microbiology

Monica Delgado
Instituto Superior de Investigaciones Biológicas - Instituto de Química Biológica “Dr. Bernabé Bloj”
Universidad Nacional de Tucumán

Signal Transduction

Mario Rossi
IBIOBA-CONICET

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FORMER PRESIDENTS SAIB

2016-2017	JOSE LUIS BOCCO
2014-2015	CARLOS ANDREO
2012-2013	LUIS MAYORGA
2010-2011	ALBERTO KORNBLIHTT
2008-2009	BEATRIZ CAPUTTO
2006-2007	NESTOR CARRILLO
2004-2005	ERNESTO PODESTA
2002-2003	NORMA STERIN DE SPEZIALE
2000-2001	RICARDO WOLOSIUK
1998-1999	DIEGO DE MENDOZA
1996-1997	RICARDO BOLAND
1994-1995	MIRTHA FLAWIA
1992-1993	ARMANDO J. PARODI
1990-1991	JUAN J. CAZZULO
1988-1989	HUGO MACCIONI
1986-1987	ISRAEL D. ALGRANATI
1984-1985	RICARDO FARIAS
1982-1983	JOSÉ SANTOME
1981-1981	HECTOR TORRES
1980-1980	JUAN DELLACHA
1979-1979	MARCELO DANKERT
1978-1978	FEDERICO CUMAR
1977-1977	ANTONIO BLANCO
1976-1976	HÉCTOR BARRA
1975-1975	RAÚL TRUCCO
1973-1974	ALEJANDRO PALADINI
1972-1972	HORACIO PONTIS
1971-1971	ANDRES STOPPANI
1970-1970	RODOLFO BRENNER
1969-1969	RANWEL CAPUTTO
1965-1968	LUIS F. LELOIR

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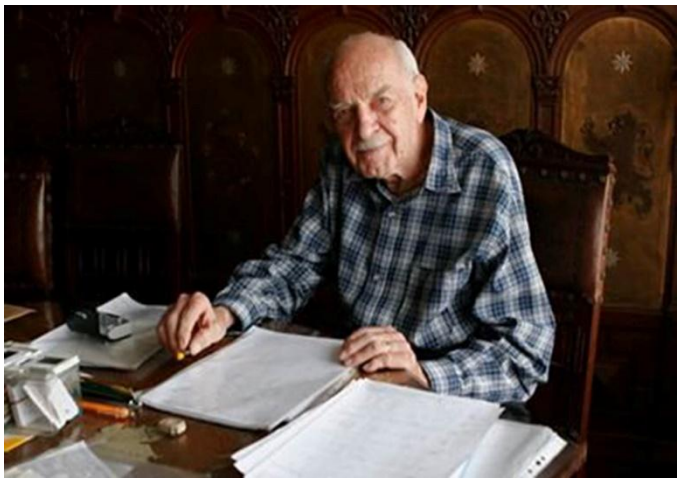
IN MEMORIAM OF RODOLFO R. BRENNER



Rodolfo R. Brenner, professor emeritus at the National University of La Plata and founding director of the Institute for Biochemical Research of La Plata, died on 3rd July 2018. He was an illustrious scientist and teacher of many generations of biochemists, with a distinguished career for his important discoveries in the field of lipid biochemistry.

He was born on 17th July 1922 in Banfield, Province of Buenos Aires, Argentina. As an outstanding student, he graduated at the Colegio Nacional de Buenos Aires in 1940 winning three gold medals due to his academic achievements. In 1946 he graduated as Doctor in Chemistry at the School of Exact, Physical and Natural Sciences (FCEFyN), of the University of Buenos Aires (UBA), obtaining another gold medal as best graduate. He had his first contact with lipids by means of his doctoral thesis 'Chemical composition of Argentinian olive oils', directed by Prof. Dr. Pedro Cattaneo.

During 1946 and 1954 he worked for the Department of Bromatology and Industrial Analysis of FCEFyN, first as a Graduate Assistant and then as an authorized Professor. At the same time, he was in charge of the Section of Industrial Toxicology at the Institute of Medical-Technological Investigations and at the Institute of Public Health. In this first period he studied the composition of lipids of several freshwater fish, a subject in which he directed five doctoral theses and published a dozen of original papers, mainly in the *Annals of the Argentine Chemical Association* and in *Industry & Chemist*.

In 1954 he obtained a postdoctoral fellowship of the British Council to work on 'Chemistry and Biochemistry of Lipids' with Professor John A. Lovern at the Torry Research Institute of Aberdeen in Scotland. Upon his return, he obtained by competitive examination the post of Head Professor of the Department of Biochemistry of the School of Medical Sciences until the year 1988. Almost from scratch, he created a research group in this Department which in the mid 1960s reached wide international renown, in special because of his works on biosynthesis of polyunsaturated fatty acids. In 1961, when the career of scientific investigator of the National Scientific and Technical Research Council (CONICET) was created, Dr. Brenner was accepted as Independent Investigator, and after subsequent promotions he became Superior Investigator in 1973. Being a prolific investigator, he directed 45 doctoral theses. He was the author of over 300 scientific works published in national and international journals, as well as many other communications presented at different conferences and scientific meetings. He lectured over 150 conferences in different countries of America, Europe and Asia.

In recognition of his work and career, he received more than 30 awards, among which we can highlight: Award of FundaciónCampomar in 1972; Herrero Ducloux Award of the National Academy of Exact, Physical and Natural Sciences in 1974, Konex Prize granted to the best 5 biochemists of Argentina in 1983; Gold Medal "G. Burns and Von Euler" granted in London in 1985; Awards "Alfredo Sordelli" in 1985 and "JJ Kyle" in 1990 of the Argentine Chemical Association; Supelco AOCS Research Award of the American Oil Chemists' Society in Baltimore in 1990; TWAS 2001 Award in Basic Medical Sciences of the Academy of Sciences of the Third World in New Delhi, India, 2002; 2009 Houssay Career Award in the area of Chemistry, Biochemistry and Molecular Biology in Buenos Aires, 2010; and the Distinguished Investigator of Argentine Nation, also in 2010. He was honorary member of the Society of Biology of Tucumán from 1987, of the Argentine Society of Biochemical Investigations (SAIB) from 1990, and of the Argentine Society of Biophysics (SAB) also in 1990.

He was Senior Investigator Emeritus of CONICET and Head Professor Emeritus of UNLP. He held the position of Established Academic of the National Academy of Exact, Physical and Natural Sciences, of the National Academy of Sciences of Buenos Aires, and of the National Academy of Pharmacy and Biochemistry, as well as the Medicine Academy of Córdoba, Argentina.

It is worth mentioning his productive role in the management and promotion of science and university teaching. In 1965, together with Drs. Luis F. Leloir, Andrés Stoppani and Federico Cumar created the Argentine Society of Biochemical Research (SAIB),

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being its President in the period 1971-72. He was Counselling Director of CONICET, Adviser and Substitute Dean of the School of Medical Sciences, UNLP, and member of several scientific and academic committees of CONICET, UNLP, UBA and the Committee of Scientific Research (CIC) of the Province of Buenos Aires, Argentina. He was the South American representative at the Steering Committee of the International Conferences on the Bioscience of Lipids (ICBL). Among his achievements and works, one of the most important ones was the creation (1982) and subsequent consolidation of the Institute of Biochemical Research of La Plata (INIBIOLP), of which he was the Director until 2003. Since 2015, this institution is called "Prof. Dr. Rodolfo R. Brenner" in recognition of his career.

He will remain for ever in the memory of all of us who had the privilege of knowing him and receiving his teaching.

Horacio A. Garda

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Schedule	Monday November 5	Tuesday November 6	Wednesday November 7	Thursday November 8
9:00- 11:00 hs		**Oral Communications *** **Room 1** ***Plants*** (PL-C01 to PL-C09) ***Biotechnology*** (BT-C01) **Room 2** ***Microbiology*** (MI-C01 to MI-C08) ***Enzymology*** (EN-C01 and EN-C02) **Room3** ***Lipids*** (LI-C01 to LI-C10)	**Oral Communications *** **Room 1** ***Cell Biology***(BC-C01 to BC--C10) **Room 3** ***Microbiology***(MI-C09 to MI-C16) ***Biotechnology***(BT-C02and BT-C03)	**Oral Communications *** **Room 1** ***Plants*** (PL-C10 to PL-C19) **Room 3** ***Signal Transduction*** (ST-C01 to ST-C10)
11:00-11:30 hs		**COFFEE-BREAK**		
11:30-12:30 hs		Conference ** **Andrés Aguilera** **Room A**	"HectorTorres" Conference **Sebastian Kadener** **Room A**	Conference **Miguel Ballicora** **Room A**
12.30- 14:30 hs		**LUNCH TIME**		
14:30-16:30 hs	**Congress Accreditation**	**Symposia** ***Microbiology**** **Room A** ***Protein Kinases**** **Room 1** ***Plant Biotechnology**** **Room 3**	**Symposia** ***Plant***** **Room A** ***Cell Biology***** **Room 3** ***Young investigators**** **Room 1**	**Symposia** ***Signal Transduction**** **Room 1** ***Applied Microbiology**** **Room A** ***Lipids***** **Room 3**
16:30-17:30 hs		**POSTERS** **COFFEE –BREAK** LI-P01 to LI-P07 SB-P01 to SB-P03 EN-P01 to EN-P13 ST-P01 to ST-P07 BT-P01 to BT-P16		
17:30-18:30 hs	**Opening Ceremony** *In memoriam* of **Rodolfo Brenner**	PL-P01 to PL-P15 BC-P01 to BC-P15 Mi-P01 to Mi-P20		PL-P16 to PL-P32 BC-P16 to BC-P30 NS-P01 to NS-P03 MI-P21 to MI-P39
18:30-19:30 hs	"Alberto Solís" Conference ** **Crisanto Gutierrez** **Room A**	"Ranwel Caputto" Conference **Claudio Fernández** **Room A**	Conference ** **SeongWook Yan** **Room A**	Closing Lecture Conference** **Pedro Aramendia** **Room A**
19:30 hs				**Closing Ceremony** **Room A**
	Cocktail 20 hs		**SAIB Assembly 19:45 hs**	**Pizza Party 21:30 hs**

*spanish ** english

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SAIB 2018

MONDAY, November 5, 2018

14:30-17:30 *REGISTRATION*

17:30-18:00 *OPENING CEREMONY*

Silvia Moreno

SAIB President

Room A

18:00-18:30 *IN MEMORIAM OF RODOLFO BRENNER*

Horacio Garda

INIBIOLP
Universidad Nacional de La Plata

Chairperson: Hugo Gramajo

Room A

18:30-19:30 *OPENING LECTURE* EMBO Conference

Crisanto Gutierrez

Factors linking genome replication, cell proliferation and chromatin dynamics

Centro de Biología Molecular Severo Ochoa (CSIC-UAM).
Madrid, España

Chairperson: Paula Casati

Room A

20:00 *WELCOMECOCKTAIL*

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TUESDAY, November 6, 2018

09:00-11:00 *ORAL COMMUNICATIONS*

Room 1: **Plants** (PL-C01 to PL-C09); **Biotechnology** (BT-C01)
Room 2: **Microbiology** (MI-C01 to MI-C08) **Enzymology** (EN-C01 and EN-C02)
Room 3: **Lipids** (LI-C01 to LI-C10)

ROOM1

Chairpersons: *Maria Elena Alvarez and Renata Reinheimer*

9:00-9:12
PL-C01
ROLE OF THE MIR394 PATHWAY IN THE REGULATION OF FLOWERING TIME IN *Arabidopsis* AND MAIZE
Bernardi Y; Ponso A; Medrano F; Vegetti A; Dotto M, Laboratorio de Biología Evolutiva y Molecular de Plantas, Facultad de Ciencias Agrarias, UNL, e-mail: mdotto@fca.unl.edu.ar

9:12-9:24
PL-C02
ROLES OF THE SUBUNIT 17 OF MEDIATOR COMPLEX IN THE UV-B INDUCED DNA DAMAGE RESPONSE
Giustozzi M; Jaskolowski A; Cerdán P; Casati P, Centro de Estudios Fotosintéticos y Bioquímicos (CONICET-UNR). Fundación Instituto Leloir, e-mail: gisutozzi@cefobi-conicet.gov.ar

9:24-9:36
PL-C03
FINE TUNING OF ARGONAUTE 1 STABILITY IS REGULATED BY CURLY LEAF
Re DA; Cambiagno DA; Arce AL; Tomassi AH; Manavella PA, Instituto de Agrobiotecnología del Litoral, UNL – CONICET. e-mail: delfina.re@santafe-conicet.gov.ar

9:36-9:48
PL-C04
DIVERGENT ROLES FOR AN ANCESTRAL HDZIP-I GENE OF *Marchantiapolymorpha*
Romani F[1]; Florent S[2]; Bowman JL[2]; Moreno JE[1], [1] Inst. Agrobiotecnología del Litoral (UNL-CONICET). Santa Fe-ARG. [2]Monash University. Australia .e-mail: javier.moreno@santafe-conicet.gov.ar.

9:48-10:00
PL-C05
PHENOTYPIC CHARACTERIZATION OF ATMSH7 DEFICIENT PLANTS
Chirinos Arias MC; Spampinato CP, Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI, CONICET-UNR), Suipacha 570, 2000 Rosario, e-mail: chirinos@cefobi-conicet.gov.ar

10:00-10:12
PL-C06
EPIDERMIS SPECIFIC EPIGENETIC MODIFICATIONS IN THE *Arabidopsis thaliana* ROOT UNDER SALT STRESS CONDITIONS
Beyrne CC; González RM; Iusem ND, IFIByNE – CONICET and FCEN – UBA, e-mail: cebeyrne@fbmc.fcen.uba.ar

10:12-10:24
PL-C07
SALICYLIC ACID HYDROXYLATION IN MAIZE
RighiniAramburu S; Falcone Ferreyra ML; Casati P .Centro de Estudios Fotosintéticos y Bioquímicos (CONICET-Universidad Nacional de Rosario, e-mail: righini@cefobi.gov.ar

10:24-10:36
PL-C08
OVEREXPRESSION OF AN ASPARTIC PROTEASE INCREASES DROUGHT TOLERANCE IN *Arabidopsis thaliana*
DIppolito S; Guevara MG; Frey ME; Tonon CV., Instituto de Investigaciones Biologicas, e-mail: dippolit@mdp.edu.ar

10:36-10:48
PL-C09
INSIGHTS INTO THE CHLOROPLASTIC UNFOLDED PROTEIN RESPONSE
Cantoia A; Ceccarelli EA; Rosano GL, Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET-UNR) – Rosario, Argentina, e-mail: cantoia@ibr-conicet.gov.ar

10:48-11:00
BT-C01
ENHANCED DROUGHT RESISTANCE OF *Nicotiana tabacum* BY COMPARTMENTALIZED MAIZE MALIC ENZYME EXPRESSION
Oitaven P; Müller G; Lara MV; Drincovich MF, Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI; CONICET-UNR). Rosario. Argentina, e-mail: oitaven@cefobi-conicet.gov.ar

ROOM2
Chairpersons: Claudia Studdert and Andrea Smania

9:00-9:12
MI-C01
IDENTIFICATION OF A *Streptomyces* NATURAL PRODUCT WITH POTENTIAL ANTI-VIRULENCE PROPERTIES
Bercovich BA; Bruna RE; Carabajal MA; Gramajo H; Rodríguez EJ; García Véscovi E, Instituto de Biología Molecular y Celular de Rosario (CONICET-UNR), e-mail: bercovich@ibr-conicet.gov.ar

9:12-9:24
MI-C02
***Salmonella*-SPECIFIC TRANSCRIPTION REGULATOR AFFECTING BIOFILM FORMATION AND VIRULENCE**
Vitor-Horen L; Echarren ML; Figueroa NR; Soncini FC, Instituto de Biología Molecular y Celular de Rosario, e-mail: luisinavitorh@gmail.com

9:24-9:36
MI-C03
ROLE OF RadA FACTOR IN THE GENETIC RECOMBINATION OF *Pseudomonas aeruginosa*
Moro C; Borgono VM; Monti MR; Argaraña CE, CIQUIBIC-CONICET, Dpto de Química Biológica, R Caputto. Fac de Cs Químicas, UNC. Córdoba, Argentina, e-mail: cmlmoro@gmail.com
9:36-9:48
MI-C04
BIOCHEMICAL CHARACTERIZATION OFCYCLOPHILINS IN*Brucella*
Muruaga EJ; Buffa GN; Briones G; Roset MS, Instituto de Investigaciones Biotecnológicas Dr. Rodolfo Ugalde; Universidad Nacional de San Martín, e-mail: emuruaga@iibintech.com.ar

9:48-10:00
MI-C05
***Bordetellabronchiseptica*BdcA REGULATES BIOFILM FORMATION IN A BrtA-DEPENDENT MANNER**
Ambrosis N; Fernandez J; Sisti F, IBBM, CCT La Plata CONICET, Dto. Cs. Biológicas. FCE. UNLP. La Plata, Buenos Aires, e-mail: nambrosis@hotmail.com

10:00-10:12
MI-C06
CHARACTERIZATION OF THE FIRST HOMOMERIC MULTIDOMAIN ACETYL-COA CARBOXYLASE FROM *Saccharopolyspora erythraea*
Livieri AL; Navone L; Gramajo H; Rodriguez E, IBR- Conicet. Facultad de Ciencias Bioquímicas y Farmacéuticas. UNR, e-mail: livieri@ibr-conicet.gov.ar

10:12-10:24
MI-C07
CATALASE ACTIVITY OF *Acinetobacter* sp. VER3 IS ESSENTIAL FOR PROTECTION AGAINST PEROXIDE AND UV
Sartorio MG; Steimbrüch B; Cortez N, IBR, Instituto de Biología Molecular y Celular de Rosario, FCByF, UNR & CONICET, e-mail: sartorio@ibr-conicet.gov.ar

10:24-10:36
MI-C08
A *Salmonella*-SPECIFIC TRANSCRIPTION FACTOR MODULATES BIOFILM FORMATION IN THE ENVIRONMENT
Tulin G; Soncini F, Instituto de Biología Molecular y Celular de Rosario, CONICET-UNR, Rosario, AR, e-mail: tulin@ibr-conicet.gov.ar

10:36-10:48
EN-P01
FUNCTIONAL CHARACTERIZATION OF ATYPICAL THIOREDOXINS FROM ENTAMOEBA HISTOLYTICA
Arias DG; Birocco F; Sasoni N; Guerrero SA; Iglesias AALaboratorio de Enzimología Molecular- IAL-UNL-CONICET, Santa Fe.E-mail: darias@fbcb.unl.edu.ar

10:48-11:00
EN-C02
BIOCHEMICAL AND GENETIC CHARACTERIZATION OF PYRUVATE DECARBOXYLASE FROM THE YEAST *CANDIDA ZEMPLININA*
Conti F; Raymond Eder ML; Rosa AL, IRNASUS–CONICET, Facultad de Ciencias Químicas, Universidad Católica de Córdoba. Argentina. E-mail: panchoconti@gmail.com

ROOM 3
Chairpersons: Gabriela Salvador and Maria Corvi

9:00-9:12
LI- C01
THE NUCLEAR-LIPID-DROPLET PROTEOME
Lagrutta LC[1]; Layerenza JP[1]; Trejo S[23]; Ves Losada A1[4], [1]INIBIOLP-CCT-La Plata-CONICET-UNLP; [2]UAB, Spain; [3]Y–TEC, Beriso; [4]Cs Biol. FCE, UNLP, Argentina, e-mail: lucialagrutta@hotmail.com

9:12-9:24
LI-C02
NITRO FATTY ACIDS: NOVEL CD36 LIGANDS WHICH MODULATE FATTY ACIDS METABOLISM
Vazquez MM; Gutierrez MV; Actis Dato V; Chiabrando GA; Bonacci, GCIBICI-CONICET. Dpto Bioquímica Clínica. Fac. Ciencias Químicas. UNC, e-mail: mvazquez@fcq.unc.edu.ar

9:24-9:36
LI-C03
PTEN ACTIVITY REGULATES TARGETING OF GP135 AND CELL DIFFERENTIATION IN MDCK CELLS
Pescio LG; Santacreu BJ; Romero DJ; Francisco MN; Favale NO; Sterin-Speziale NB, Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. IQUIFIB – CONICET, e-mail: lucilagpescio@ffyb.uba.ar

9:36-9:48
LI-C04
DUAL FUNCTION OF SPHINGOSINE-1-PHOSPHATE RECEPTOR 2 IN EPITHELIAL CELL MIGRATION
Romero DJ; Santecreu BJ; Pescio LG; Tarallo E; Chavez Flores JC; Favale NO, Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, IQUIFIB-CONICET, e-mail: danielaromero05@gmail.com

9:48-10:00
LI-C05
SPHINGOSINE KINASE 2 COORDINATES THE DISASSEMBLY OF CELL JUNCTIONS DURING CELL EXTRUSION
Santacreu BJ; Pescio LG; Romero DJ; Chavez Flores JC; Tarallo E; Sterin-Speziale NB; Favale NO, Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, IQUIFIB-CONICET, Argentina, e-mail: bsantacreu@ffyb.uba.ar

10:00-10:12
LI-C06
EFFECT OF CULTURE TEMPERATURE ON FATTY ACID COMPOSITION OF DIATOM *Cylindrotheca closterium,*
Almeyda D[3]; Scodelaro Bilbao P[13]; Constenla D[24]; Popovich C[13]; Leonardi P[13], [1]Dpto. BByF-UNS, [2]Dpto.IQ-UNS, [3]CERZOS, [4]PLAPIQUI - UNS-CONICET, e-mail: delfinaalmeyda@hotmail.com

10:12-10:24
LI-C07
CELLULAR LIPIDS CHANGES DURING ADIPOSE-DERIVED STEM CELLS OSTEOGENIC DIFFERENTIATION
Parra LG[1]; Casali CI[1]; Setton-Avruj PC[2]; Fernández Tomé MC[1], [1]BCM, FFyB, UBA, IQUIFIB-CONICET, CABA, Argentina [2]QBP, FFyB, UBA, IQUIFIB-CONICET, CABA, Argentina, e-mail: lparra@docente.ffyb.uba.ar

10:24-10:36
LI-C08
EXPRESSION OF GENES INVOLVED IN LIPID AND FATTY ACID METABOLISM IN *EX VIVO* CULTURED MOUSE TESTES
Oresti GM[1];Isoler-Alcaraz J[2]; Klampachas A[1]; Santiago Valtierra FX[1]; Aveldaño MI[1]; Del Mazo J[2], [1]INIBIBB, CONICET y Depto. BByF, UNS, Bahía Blanca, Argentina. [2]CIB, CSIC, Madrid, España., e-mail: gmoresti@criba.edu.ar

10:36-10:48
LI-C09
NOVEL ROLES OF MTP ON CANCER DEVELOPMENT, CELL SURVIVAL AND MIGRATION
Comanzo CG[1]; Lucci A[1]; Vera MC[1]; Lorenzetti F[1]; Ceballos MP[1]; Ferretti AC[2]; Alvarez ML[1]; Quiroga AD, [1]Instituto de Fisiología Experimental (IFISE-CONICET). [2]Área Morfología (FCByF-UNR), e-mail: comanzo@ifise-conicet.gov.ar

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10:48-11:00
LI-C10
PIP 2 PROMOTES MEMBRANE CURVATURE AND IS A SIGNALING HUB IN HUMAN SPERM ACROSOME EXOCYTOSIS
Altamirano KN; Suhaiman L; Lucchesi O; Ruete MC; Belmonte SA, Instituto de Histología y Embriología, IHEM-CONICET-FCMédicas-UNCuyo, e-mail: karina.altam@gmail.com

11:00-11:30 *COFFEE BREAK*

11:30-12:30 *PLENARY LECTURE ALBERTO SOLS*

Andrés Aguilera

Role of chromatin and DNA damage response functions in R loop-mediated genome instability

Centro Andaluz de Biología Molecular y Medicina Regenerativa-CABIMER,
Universidad de Sevilla, España

Chairperson: Silvia Moreno

Room A

12:30-14:30 *LUNCH*

14:30-16:30 *SYMPOSIA*

Room A
MICROBIOLOGYSYMPOSIUM
Chairpersons: Monica Delgado and Jorgelina Morán Barrio

Jorge Diego Marco
Recombinant antigens of* Leishmania *for the immunodiagnosis and immunoprophylaxis of American tegumentaryleishmaniasis
Instituto de Patología Experimental (UNSa-CONICET)

Augusto Bellomio
Study of the mechanism of action of lineal bacteriocins using suicide probes
Instituto Superior de Investigaciones Biológicas (UNT-CONICET)

Daniela Gardiol
Conserved mechanisms of viral pathogenesis: alterations of cell polarity and intercellular junctions
Instituto de Biología Molecular y Celular de Rosario (IBR)-CONICET, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Argentina

Hebe Dionisi
Brown algae polysaccharide assimilation potential in subantarctic sediments
Laboratorio de Microbiología Ambiental, Centro para el Estudio de Sistemas Marinos (CESIMAR-CONICET).

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Room 1

PROTEIN KINASES SYMPOSIUM

Chairpersons: Silvia Rossi and Daniela Albanesi

Ricardo Biondi

Small compounds modulating bi-directional allostery in protein kinases: a new grip on an old trick?

IBioBA-CONICET-Partner Institute of the Max Planck Society; Frankfurt University Hospital (Germany)

Paula Portela

Role of PKA in protein translation regulation during adverse environmental growth conditions

Departamento Química Biológica-FCEN-UBA. IQUIBICEN-CONICET, Buenos Aires. Argentina

Virginia Novaro

Study of PI3K/AKT/mTOR pathway in breast cancer progression

Instituto de Biología y Medicina Experimental, IBYME-CONICET, Buenos Aires.

Viviana Castilla

Involvement of RAF/MEK/ERK cell signaling pathway in Junín virus replication

Depto. de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires

Room 3

PLANT BIOTECHNOLOGY SYMPOSIUM

Chairpersons: Maria Valeria Lara and Elina Welchen

Raquel Chan

Successes and failures developing biotechnological tools in a model plant to improve crops. The long way from the growth chamber to the greenhouse and from the greenhouse to the field

Instituto de Agrobiotecnología del Litoral (UNL-CONICET) y FBCB (UNL).

Juan Carlos Diaz Ricci

The elicitor AsES regulates ripening and enhances protection in avocado and strawberry fruit

Instituto Superior de Investigaciones Biológicas (INSIBI, CONICET-UNT), Facultad de Bioquímica, Química y Farmacia, UNT.

Maria Victoria Busi

Different targets for the design of biomass of plants

Centro de Estudios Fotosintéticos y Bioquímicos de Rosario (CEFOBI-CONICET-UNR)

Marina Clemente

Molecular farming to produce vaccines against human and veterinary coccidian parasites: improving the antigen expression in plants

Laboratorio de Molecular Farming y Vacunas, Instituto Investigaciones Biotecnológicas-Instituto Tecnológico Chascomús (IIB-INTECH, sede Chascomús), Provincia de Buenos Aires, Argentina.

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16:30-18-30 *COFFEE BREAK*

POSTER SESSION

MI-P01 to MI-P20

PL-P01 to PL-P15

BC-P01 to BC-P15

18:30-19:30 *RANWEL CAPUTTO CONFERENCE*

Claudio Fernandez

Structural and cell biology of synucleinopathies

Max Planck Laboratory of Structural Biology, Chemistry and Molecular Biophysics of Rosario (MPLbioR, UNR-MPIbpC), Rosario, Argentina; Instituto de Investigaciones para el Descubrimiento de Fármacos de Rosario (IIDEFAR, UNR-CONICET), Rosario, Argentina; Max Planck Institute for Biophysical Chemistry (MPIbpC-MPG), Göttingen, Germany

Chairperson: Gerardo Fidelio
Room A

WEDNESDAY, November 7, 2018

09:00-11:00 *ORAL COMMUNICATIONS*

Room 1: **Cell Biology** (BC-C01 toBC-C10)
Room 3: **Microbiology** (MI-C09 to MI-C16) **Biotechnology** (BT-C01and BT-C02

ROOM 1
Chairpersons: Javier Valdez Taubas and Cesar Casale

9:00-9:12
BC-C01
GLYCOGEN SYNTHASE KINASE 3 INHIBITION PREVENTS UV ELICITED TRANSCRIPTIONAL RESPONSE AND APOPTOSIS
Nieto Moreno N[1], Cuenca C[1], Villafáñez F[2], Soria G[2], Muñoz MJ[1], Kornblihtt AR[1], [1] IFIBYNE-UBA-CONICET, Argentina. [2] CIBICI-UNC-CONICET, Argentina, e-mail: nnietomoreno@fbmc.fcen.uba.ar

9:12-9:24
BC-C02
INTRAGENIC HISTONE ACETYLATION HELPS UPREGULATION OF *SMN2* EXON 7 INCLUSION BY SPINRAZA
Marasco LE[1], Krainer AR[2], Kornblihtt AR[1],1 IFIBYNE-UBA-CONICET, Buenos Aires, Argentina. 2 Cold Spring Harbor Laboratory, New York, USA, e-mail: lemarasco@agro.uba.ar

9:24-9:36
BC-C03
REGULATION OF THE SODIUM/IODIDE SYMPORTER (NIS) BY CREB3L1.
Di Giusto P, Martin M, Torres Demichelis VA, Sampieri L, Nicola JP, Alvarez C, Facultad de Ciencias uímicas, UNC. Departamento de Bioquímica Clínica / CIBICI-CONICET, e-mail: pablodigiusto91@gmail.com

9:36-9:48
BC-C04
TRANSLATIONAL CONTROL MEDIATED BY DIFFERENT DOMAINS OF ME31B IN *Drosophila*
Vilardo E, Rivera Pomar R, Layana C, Centro Regional de Estudios Genómicos, Fac. Cs Exactas, UNLP,e-mail: emilianovilardo@gmail.com

9:48-10:00
BC-C05
JOINT PROCESSING OF APOPTOTIC CELLS AND *Pseudomonas aeruginosa* BY MACROPHAGES
Jäger AV, Arias P, Pepe MV, Tribulatti V, Kierbel A, Instituto de Investigaciones Biotecnológicas "Dr. Rodolfo Ugalde" (IIIB-INTECH-UNSAM-CONICET), e-mail: avjager@gmail.com

10:00-10:12
BC-C06
LC3 OVEREXPRESSION MODULATES THE SECRETOME OF SENESCENCE TUMOR CELLS INDUCED BY RADIATION
Salvarredi LA[1]; Aguero H[1]; Marra F[2]; Millan E[2]; Lopez LA[2], [1]Fundación Escuela de Medicina Nuclear-CNEA. [2]IHEM CCT-CONICET, Mendoza, e-mail: leonardosalvarredi@yahoo.com.ar

10:12-10:24
BC-C07
CYTOSKELETAL DYNAMICS AT THE LEADING EDGE OF OVARIAN CANCER CELLS IS ENHANCED BY LA AND OL
Masner M[1]; Lujea N[1]; Bisbal M[2]; Acosta C[3]; Kunda P[1],[1] CIMETSA, IUCBC Córdoba [2] INIMEC-CONICET-UNC, Córdoba [3] IIHEM, FM, UNC Mendoza, e-mail: mmasner@gmail.com

10:24-10:36
BC-C08
MECHANISTIC ANALYSIS OF INFLUENZA A VIRUS GLYCOPROTEIN COMPENSATION
Drake A[1]; Garrido FM[1]; MorellattoRuggieri L[1]; Yewdell JW[2]; Magadan JG[1], [1]IHEM-CONICET, Fac. Cs. Médicas, UNCuyo. Mendoza, Argentina. [2]NIAID, NIH. Bethesda, MD, USA, e-mail: jmagadan@mendoza-conicet.gob.ar

10:36-10:48
BC-C09
EXPLORING THE DRIVING FORCES INFLUENCING S-ACYLATION OF PERIPHERAL PROTEINS AT THE GOLGI COMPLEX
Chumpen Ramirez S; Astrada MR; Daniotti JL, CIQUIBIC (UNC-CONICET), Fac. de Cs. Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina, e-mail: svchumpen@gmail.com

10:48-11:00
BC-C10
SUPINE FALLS IN *Ceratitis capitata*CORRELATE WITH DISTINCT GENE EXPRESSION PROFILES
Bochicchio PA; Pérez MM; Rabossi A; Cavaliere-Candedo V; Quesada-Allué LA, IIBBA-CONICET, FCEyN-University of Buenos Aires and Fundación Leloir, e-mail: pbocchicchio@leloir.org.ar.

BIOCELL 42 (suppl. 4), 2018
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ROOM 3

Chairpersons: Eduardo Rodríguez and Nestor Cortez

9:00-9:12

MI-C09

DISRUPTION OF CTL0175 HAMPERS *Chlamydia trachomatis* REPLICATION POST IFN GAMMA-INDUCED STRESS

Panzetta ME[1]; Lujan AL[2]; Bastidas RJ[3]; Damiani MT[2]; Valdivia RH[3]; Saka HA[1], [1]CIBICI-CONICET, UNC, Arg, [2]IMBECU-CONICET, UNCu, Arg., [3]Duke University School of Medicine, USA, e-mail: epanzetta@fcq.unc.edu.ar

9:12-9:24

MI-C10

INCREASE IN THE VIRULENCE FITNESS OF *Shigellaflexneri*: THE NOA POPULATION'S PREVALENT PATHOGEN

Torrez Lamberti MF; Ballesteros MF; Bonano M; Pescaretti MM; Delgado MA, INSIBIO, e-mail: mftorrezlamberti@gmail.com

9:24-9:36

MI-C11

EXPLORING INTERACTIONS OF THE S-LAYER PROTEIN OF *Lactobacillus acidophilus* ATCC4356

Fina Martin J[1]; Palomino MM[1]; Cutine A[2]; Allievi MC[1]; Zanini SH[1]; Mariño KV[2]; BarqueroA[1]; Ruzal SM[1], [1]UBA-FCEN-Qca Biológica CONICET-IQUIBICEN[2], IBYME-CONICET, Bs As, Argentina, e-mail: joaquinafinamartin@gmail.com

9:36-9:48

MI-C12

DIVERSITY AND EVOLUTION OF β-LACTAMASE ampC IN LONG-TERM *Pseudomonas aeruginosa* CHRONIC INFECTIONS

Colque CA[1]; Albarracin AG[1]; Hedemann G1; Hickman RA[2]; Sommer LM[3]; Molin S[3]; Johansen HK[2]; Smania AM[1], [1]Depto. de Qca. Biologica, FCQ, UNC, CIQUIBIC-CONICET. [2]Rigshospitalet. [3]CFB-DTU, e-mail: acolque@fcq.unc.edu.ar

9:48-10:00

MI-C13

IMMUNOPROPHYLACTIC EFFECT OF R-*Leishmaniabraziliensis* HSP70 IN EXPERIMENTAL CUTANEOUS LEISHMANIASIS

Moya Alvarez A; Bracamonte ME; Hoyos CL; Uncos DA; Acuña L; Basombrio MA; Barroso PA; Marco JD, Instituto de Patología Experimental, FCS, UNSa/CONICET, Salta, Arg, e-mail: elagus177@gmail.com

10:00-10:12

MI-C14

TAM SYSTEM IS INVOLVED IN CELL ENVELOPE HOMEOSTASIS IN α-PROTEOBACTERIA

Bialer MG[1]; Sycz G[1]; Ruiz-Ranwez V[1]; Estein S[2]; Zorreguieta A[1], [1]Fundación Instituto Leloir, IIBBA-CONICET. Bs As. [2]CIVETAN, CONICET-U.N.C.P.B.A. Tandil. e-mail: mbialer@leloir.org.ar

10:12-10:24

MI-C15

SYNERGISTIC MECHANISM BETWEEN INFLUENZA A VIRUS AND *Streptococcus pneumoniae* IN PNEUMOCYTES

Reinoso Vizcaino N[1]; Olivero N[1]; Cortes P[1]; Yandar N[1]; Hernandez Morfa M[1]; Perez DR[2]; Echenique J[1], [1]CIBICI-CONICET, Fac. Cs. Qcas, UNC. [2]College of Veterinary, University of Georgia, USA, e-mail: nreinoso@fcq.unc.edu.ar

10:24-10:36
MI-C16
DIFFERENTIAL YEAST POPULATIONS IN GRAPE MUSTS FROM DIFFERENT *Vitis* SPECIES IN A SHARED TERROIR
Raymond Eder ML; Conti F; Rosa AL, IRNASUS-CONICET, Facultad de Ciencias Químicas, Universidad Católica de Córdoba. Argentina, e-mail: marialraymond@hotmail.com

10:36-10:48
BT-C02
A GH 8 ENDOGLUCANASE FROM *Paenibacillus* sp. *A59* FOR APPLICATION IN BIOPROCESSES
Bradanini M; Ghio S; Ontañon O; Garrido M; Campos E, Instituto de Biotecnología, CICVyA. INTA- IABIMO CONICET, e-mail: mariabradanini@hotmail.com.ar

10:48-11:00
BT-C03
CHARACTERIZATION OF POLYHYDROXYALKANOATE PRODUCTION BY *KHS3*
Rodríguez AN[1]; D'Amico D[2]; Cyras V[2]; Studdert CA[1]; Herrera Seitz MK[3], [1]IAL-UNL-CONICET, Santa Fe, Argentina [2 3] INTEMA e IIB–UNMdP– CONICET, Mar del Plata, Argentina, e-mail: ailennatalirodriguez@gmail.com

11:00-11:30 *COFFEE BREAK*

11:30-12:30 *PLENARY LECTURE HECTOR TORRES*

Sebastian Kadener

Molecular and physiological functions of circRNAS

Brandeis University, Waltham, Massachusetts, USA

Chairperson: Luis Quesada Allué
Room A

12:30-14:30 *LUNCH*

14:30-16:30 *SYMPOSIA*

Room A

PLANT SYMPOSIUM
Chairparsons: Paula Casati and Caudia P. Spampinato

Erich Grotewold
Emerging patterns in plant gene regulation
Department of Biochemistry & Molecular Biology Michigan State University, East Lansing, Michigan USA

Åsa Strand
The role of retrograde signals during plant stress responses
Umeå Plant Science Centre, Dept. of Plant Physiology, Umeå University, Umeå, Sweden.

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Jorge Casal
Signalling dynamics and plant plasticity in complex environments
IFEVA, Universidad de Buenos Aires y Consejo Nacional de Investigaciones Científicas y Técnicas, Facultad de Agronomía, Argentina. Fundación Instituto Leloir, Instituto de Investigaciones Bioquímicas de Buenos Aires-CONICET, Argentina.

Javier Palatnik
The ups and downs of an* Arabidopsis *microRNA
IBR (Instituto de Biologia Molecular y Celular de Rosario), UNR-CONICET. Argentina.

Room 3

CELL BIOLOGY
Chairpersons:Gustavo Chiabrando and Claudio Fader

Claudio Fader
Erythropoiesis and autophagy: two closely related partners.
Laboratorio de Biología Celular y Molecular, Instituto de Histología y Embriología (IHEM), Universidad Nacional de Cuyo, CONICET, Mendoza, Argentina, Facultad de Odontología, Universidad Nacional de Cuyo, Mendoza, Argentina.

Galiano Mauricio
N-terminal post-translational arginylation regulates multiple roles of calreticulin
CIQUIBIC-Dpto. Qca Biológica RanwelCaputto, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.

Osorio-Fuentealba Cesar
Insulin-independent Glut4 trafficking in skeletal muscle: new mechanisms and advances to face metabolic diseases
Laboratorio de Biología Molecular, Celular y Metabolismo, Departament de Kinesiología, UMCE, Santiago, Chile, Centro de Investigación de Alcoholismo Adolescente (CIAA), Santiago Chile

Kashina Anna
Protein arginylation as a global regulator of intracellular protein trafficking and function
Department of Biomedical Sciences, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, USA

Room 1

YOUNG INVESTIGATORS
Chairpersons: Silvia Morenoand Eleonora Garcia Vescovi

Juan Pablo Fededa
Investigating the role of microRNAs during mammalian brain development
Instituto de Investigaciones Biotecnológicas, IIB-INTECH/CONICET-UNSAM, San Martín, Prov. de Bs. As., Argentina.

Nicolas Cecchini
The Azi1 subcellular targeting mechanism: how to anchor immune receptors to the plastid envelope
CIQUIBIC-CONICET, Departamento de Química Biológica-RanwelCaputto, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina.

Leticia Llarrull
Architecture of MecR1 of Staphylococcus aureus: clues to the signal transduction mechanism that unleashes resistance to β-lactams
Laboratorio de Sensores Bacterianos, IBR-CONICET-Universidad Nacional de Rosario, Argentina

Andrés Garelli
***Dilp8-Lgr3* pathway: a relaxin-like pathway controlling developmental transitions**
Instituto de Investigaciones Bioquímicas de Bahía Blanca (UNS-CONICET), Buenos Aires Argentina

Ignacio Cebrián
The endocytic pathway as a key modulator of antigen cross-presentation by dendritic cells
Facultad de Ciencias Médicas, Instituto de Histología y Embriología de Mendoza(IHEM)-CONICET, Universidad Nacional de Cuyo, Argentina

16:30-18-30 *COFFEE BREAK POSTER SESSION*

LI-P01 to LI-P07

SB-P01 to SB-P03

EN-P01 to EN-P13

ST-P01 to ST-P07

BT-P01 to BT-P16

18:30-19:30 *PLENARY LECTURE*

SeongWook Yan

Integration of light signaling into microRNA biogenesis

1Department of Systems Biology, College of Life Science and Biotechnology, Yonsei University, Seoul, 120-749, Korea

Chairperson: Pablo Manavella

Room A

19:45 *SAIB Assembly*

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THURSDAY, November 8, 2018

09:00-11:00 *ORAL COMMUNICATIONS*

Room 1: **Plants** PL-C10 to PL-C19)
Room 3: **Signal transduction** (ST-C01 to ST-C10)

ROOM 1

Chairpersons: Fabiana Drincovich and Maria Lorena Falcone Ferreyra

9:00-9:12
PL-C10
IS THE METABOLISM OF XENOBIOTIC COMPOUNDS REGULATED BY CIRCADIAN CLOCK?
Sosa Alderete LG[1]; Ronchi H[1]; Medina MI[1]; Guido ME[2]; Agostini E[1], [1]Dpto Biología Molecular-FCEFQyN UNRC, 5800- Rio Cuarto-Arg[2]CIQUIBIC-CONICET, FCQ UNC, 5000 Córdoba-Argentina,e-mail: lucasaureus@gmail.com

9:12-9:24
PL-C11
***Arabidopsis* KINESIN 13B INTERACTS WITH SEVERAL TRANSCRIPTION FACTORS INVOLVED IN GROWTH REGULATION**
Miguel VN; Ribichich KF; Chan RL, Laboratorio de Biología Vegetal, IAL, UNL, CONICET, CCT CONICET, Santa Fe, Argentina.e-mail: vmiguel@santafe-conicet.gov.ar

9:24-9:36
PL-C12
ROLE OF E2FC TRANSCRIPTION FACTOR DURING THE UV-B RESPONSES IN *Arabidopsis*
Gomez MS; FalconeFerreyra ML; Casati P, Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI), UNR – CONICET, Rosario, Santa Fe, e-mail: gomez@cefobi-conicet.gov.ar

9:36-9:48
PL-C13
THE LNK GENE FAMILY: AT THE CROSSROADS OF LIGHT SIGNALING AND THE CIRCADIAN CLOCK
Hernando CE; De Leone MJ; Romanowski A; Hourquet M; Casal J; Rugnone M; Mora Garcia S; Yanovsky MJ, Instituto de Investigaciones Bioquímicas de Buenos Aires - Fundación Instituto Leloir, e-mail: chernando@leloir.org.ar

9:48-10:00
PL-C14
ROLE OF MSH6 DURING DNA RECOMBINATION IN *Arabidopsis thaliana*
Gonzalez V; Spampinato CP, Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI, CONICET-UNR), Suipacha 570, 2000 Rosario, e-mail: gonzalez@cefobi-conicet.gov.ar

10:00-10:12
PL-C15
MECHANISMS INVOLVED IN THE CELLULAR ENERGY HOMEOSTASIS IN PLANTS
Blanco NE[1]; Liebsch D[2]; Jásik J[3]; Whelan J[4]; Strand Å[5], [1]CEFOBI/UNR-CONICET, [2]IBR/CONICET, [3]Institute of Botany, Slovakia, [4]Latrobe, Australia, [5]UPSC, Sweden, e-mail: blanco@cefobi-conicet.gov.ar

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10:12-10:24
PL-C16
LIGHT REGULATION OF ALTERNATIVELY SPLICED GENES DURING *Arabidopsis thaliana* SEED GERMINATION
Tognacca RS[1]; Servi L[2]; Botto JF[1]; Petrillo E[2], [1]IFEVA, CONICET-UBA.[2]IFIBYNE, CONICET-UBA., e-mail: rtognacca@agro.uba.ar

10:24-10:36
PL-C17
NON-THERMAL PLASMAS AFFECT SEED QUALITY, PLANT GROWTH AND DNA METHYLATION PATTERNS IN SOYBEAN
Pérez Pizá M[1]; Zilli C[1]; Ibáñez V[3]; Varela A[3]; Cejas E[2]; Prevosto L[2]; Marfil C[3]; Balestrasse K, [1]INBA (CONICET-FAUBA), [2]FRVT-UTN (CONICET), [3]IBAM (CONICET-UNCuyo), e-mail: macycecy@hotmail.com

10:36-10:48
PL-C18
SALICYLIC ACID SIGNALING PATHWAY AS KEY PLAYER IN THE EARLY ACTIVATION OF IMMUNE RESPONSES IN MAIZE
Agostini R[1]; Postigo A[1]; Rius S[1]; Campos Bermudez V[1]; Vargas W[2], [1]CEFOBI-CONICET. Rosario, Argentina. [2]YPF-Tecnologia-CONICET (Y-TEC). Berisso, Argentina, e-mail: agostini@cefobi-conicet.gov.ar

10:48-11:00
PL-C19
PAP-SAL1 CHLOROPLAST RETROGRADE PATHWAY MODULATES IRON DEFICIENCY RESPONSE IN ALKALINE SOILS
Balparda M; Gomez-Casati DF; Pagani MA, Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI – CONICET), Universidad Nacional de Rosario, e-mail: balparda@cefobi-conicet.gov.ar

ROOM 3
Chairpersons: Fabiana Cornejo and Ricardo Biondi

9:00-9:12
ST-C01
IT TAKES TWO TO TANGO: YvfTU AND DesKR TWO COMPONENT SYSTEMS REGULATE ABC TRANSPORTER TRANSCRIPTION
Fernández P; Albanesi D; De Mendoza D; Mansilla MC, Instituto de Biología Molecular y Celular de Rosario-CONICET. Facultad de Cs Bioq y Farm – UNR, e-mail: pfernandez@ibr-conicet.gov.ar

9:12-9:24
ST-C02
TcAMPK: IDENTIFICATION AND CHARACTERIZATION OF AN ENERGY REGULATORY HUB IN *Trypanosoma cruzi*
Sternlieb T1; Schoijet AC; Genta PD; Barrera NM; MassiminoStepñicka M; Alonso GD, Instituto de Investigaciones en Ingeniería Genética y Biología Molecular Dr. Héctor N. Torres, e-mail: tamara.sternlieb@gmail.com

9:24-9:36
ST-C03
CALCIUM SIGNALING: THE COMMUNICATION BETWEEN INTRACELLULAR CA2+ STORES IN HUMAN SPERM
Mata-Martínez E[1]; Arias RJ[1]; Treviño CL[2]; Mayorga LS[1]; Darszon A[2]; De Blas GA[1], 1IHEM-CONICET, UNCuyo, Argentina. 2IBT-UNAM, México, e-mail: ematamartinez@mendoza-conicet.gob.ar

9:36-9:48
ST-C04
THE ROLE OF MITOCHONDRIA IN CALCIUM SIGNALING AND HUMAN SPERM PHYSIOLOGY ACTIVATED BY PROGESTERONE.
Arias RJ[1]; Vargas S[2]; Mata-Martinez E[1]; Garcia A[2]; Härtel S[2]; Mayorga LS[1]; De Blas GA[1], 1IHEM-CONICET-UNCuyo, Mendoza, Argentina. 2CEDAI-Facultad de Medicina, Universidad de Chile, e-mail: rodojosearias@gmail.com

9:48-10:00
ST-C05
EXPRESSION REGULATION OF PROTEIN KINASE A SUBUNITS FROM *Saccharomyces cerevisiae*
Cañonero L[1]; Pautasso C[1]; Sigaut LI[2]; Ortolá MC[1]; Rossi S[1], [1]Química Biológica, FCEN, UBA, IQUIBICEN (CONICET-UBA) [2]Física, FCEN, UBA and IFIBA, CONICET, e-mail: lucianac@qb.fcen.uba.ar

10:00-10:12
ST-C06
ROLE OF AKR1B1 IN TUMOR AGGRESSIVENESS AND ITS INTERPLAY WITH THE P53 PATHWAY IN BREAST CANCER
Di Benedetto C1; BoriniEtichetti CM[1]; Bicciato S[2]; Menacho Márquez M3; Girardini JE[1], [1] IBR-CONICET, 2 Universidad de Módena, 3 IIDEFAR-CONICET, e-mail: girardini@ibr-conicet.gov.ar

10:12-10:24
ST-C07
ICMT COOPERATES WITH TUMOR AGGRESSIVENESS AND IT IS UNDER COMPLEX CONTROL BY p53 FAMILY MEMBERS
BoriniEtichetti CM[1]; Di Benedetto C[1]; Baglioni MV[2]; Bicciato S[3]; MenachoMarquez M[4]; Girardini JE[1], [1]IBR-CONICET. [2] IGE-Fac. Cs. Med. UNR. [3] Universidad de Módena. [4] IIDEFAR-CONICET, e-mail: girardini@ibr-conicet.gov.ar

10:24-10:36
ST-C08
DAL81 AND UGA3 TRANSCRIPTION FACTORS AND STRESS RESPONSE IN *Saccharomyces cerevisiae*
Muñoz SA; Mercau M; Gulías J; Valencia-Guillen J; Correa-García S; Bermúdez-Moretti M, Departamento de Química Biológica, FCEN, UBA - IQUIBICEN, CONICET. CABA, Argentina, e-mail: munozsebastiananibal@gmail.com

10:36-10:48
ST-C09 INTERACTIONS BETWEEN PROTEIN TYROSINE PHOSPHATASE 1B (PTP1B), EGFR AND FAK IN INTACT CELLS
Perez Collado ME; González Wusener AE; Arregui CO, IIB-INTECH, UNSAM-CONICET, Buenos Aires, Argentina, e-mail: mcollado@iibintech.com.ar

10:48-11:00
ST-C10
PKA AND HOG1 ROLE IN GENE EXPRESSION AND CELL SURVIVAL IN RESPONSE TO OSMOSTRESS IN *Saccharomyces cerevisiae*
Ojeda LE; Portela P, Departamento de Química Biológica, FCEN, UBA/IQUIBICEN–Conicet, e-mail: leojeda@qb.fcen.uba.ar

11:00-11:30 ***COFFE BREAK***

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18:30-19:30 *PLENARY LECTURE*

Miguel Ballicora

Biosynthesis of bacterial glycogen: Evolution of allosteric control

Department of Chemistry and Biochemistry, Loyola University Chicago, Chicago, IL, USA.

Chairperson: Alberto Iglesias

RoomA

12:30 -14:30 *LUNCH*

14:30-16:30 *SYMPOSIA*

Room 3

LIPIDS
Chairpersons: Hugo Gramajo and Nicolas Favale

Claudia Banchio
Specific phospholipids regulate the acquisition of neuronal and astroglial identities in post-mitotic cells
Instituto De Biología Molecular y Celular de Rosario IBR-CONICET Rosario, Argentina

Hector Alvarez
***Unraveling the molecular mechanisms involved in the regulation of lipid accumulation in oleaginous* Rhodococcus**
Instituto de Biociencias de la Patagonia (CONICET y Universidad Nacional de la Patagonia San Juan Bosco)

Richard Lehner
Regulation of lipid metabolism by endoplasmic reticulum-localized lipases
University of Alberta, Edmonton, Canada

SipioneSimoneta
Gangliosides in Huntington's disease and beyond
Alberta, Edmonton, Canada

Room A

APPLIED MICROBIOLOGY
Chairpersons: Sandra Ruzal and Christian Magni

Mariana Piuri
Fluorophages for rapid Tb-diagnosis in sputum samples and phenotypic drug susceptibility testing
Departamento De Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, IQUIBICEN-CONICET

Fadda Silvina
Study of lactic acid bacteria*—Escherichia coli *o157:h7 interaction and its contribution to bioprotection strategies in meat
Laboratorio de tecnología y desarrollo (CERELA-CONICET), Tucumán, Argentina

Serradell María de los Angeles
Probiotic lactobacilli as a source of proteins of biotechnological interest:* Lactobacillus kefiri *and its S-layer proteins
Cátedra De Microbiología, Departamento De Ciencias Biológicas, Facultad De Ciencias Exactas, Universidad Nacional De La Plata, instituto De Ciencias De La Salud, Universidad Nacional Arturo Jauretche

Eleonora Campos
Microbial enzymes and their application in cellulosic ethanol industry
Laboratorio de bioenergía y enzimas industriales, instituto de biotecnología-IABIMO, INTA-CONICET, Argentina.

Room 1

SIGNAL TRANSDUCTION
Chairpersons: Mario Rossi and Javier Girardini

Andrea Smania
Hipermutability and the evolution of small colony variants in* Pseudomonas aeruginosa *biofilms.
CIQUIBIC-CONICET, Dpto de Química Biológica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba

Vanesa Gottifredi
Specialized DNA polymerase iota coordinates DNA replication and checkpoint activation
Fundación Instituto Leloir. Instituto de Investigaciones Bioquímicas De Buenos Aires (Iibba), CONICET, Buenos Aires

Gaston Soria
A survival screen targeting the human kinome reveals synthetic lethal interactions with therapeutic potential for BRCA-deficient cancer cells
CIBICI-CONICET, Depto. de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina

Lucas Pontel
Novel targets of formaldehyde toxicity in cancer cells
IBIOBA-CONICET-Partner Institute of the Max Planck Society, Buenos Aires, Argentina

16:30-18-30 ***COFFEE BREAK***

POSTER SESSION

PL-P16 to LI-P32

BC-P16 to SB-P30

EN-P01 to EN-P03

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18:30-19:30 *CLOSING LECTURE*

Pedro Aramendia

Quantitative imaging in single molecule fluorescence localization

Centro de Investigaciones en Bionanociencias "Elizabeth Jares-Erijman" (CIBION-CONICET) y Departamento de Química Inorgánica. FCEN. Universidad de Buenos Aires

Chairperson: Maria Isabel Colombo

RoomA

19:30 *CLOSING CEREMONY AND AWARDS*

21:30 *CLOSING PARTY*

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ABSTRACTS:

All abstract will be published in:

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LECTURES AND SYMPOSIA ABSTRACTS

MONDAY, November 5, 2018

L-01
FACTORS LINKING GENOME REPLICATION, CELL PROLIFERATION AND CHROMATIN DYNAMICS

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The cell division cycle consists of a series of unidirectional and coordinated events that lead to the production of two daughter cells. Current evidence reveals that chromatin dynamics, including nucleosome remodeling, DNA and histone modifications and variants, are crucial for proper progression through cell cycle phases. Over the past years, we have mainly focused on two aspects of chromatin regulation during the cell cycle, namely, the initiation of DNA replication during S-phase and the impact of chromatin dynamics on cell proliferation potential during plant development.The large size of eukaryotic genomes poses serious challenges to maintain their stability every cell cycle. Faithful genome duplication relies on the activity of thousands of DNA replication origins (ORIs) that depends on the function of pre-replication complexes (pre-RC). Most of our knowledge derives from cultured cells, but identification of ORIs in an adult organism is of primary importance to learn about their biology and developmental plasticity. Our studies of the Arabidopsis "originome" revealed that ORIs associate with different chromatin landscapes, both active and repressed, although with different frequencies, and that they show a preferential usage depending on the developmental stage. Furthermore, the interaction of some pre-RC proteins with chromatin factors contributes to genome maintenance. These results are the basis for understanding ORI biology in response to environmental changes, developmental cues or mutant backgrounds. We have used live imaging and flow cytometry to visualize cell proliferation dynamics in a growing organ by exploiting the different dynamics of histone H3 proteins. We have found that the canonical histone H3.1, incorporated in S-phase, is maintained at high levels in actively proliferating cells in the root meristem but is massively evicted in cells undergoing their last cell cycle before differentiation. This allowed us to identify cell populations with different proliferation potential during organogenesis and patterning based on the ratio between H3,1 and the H3.3 variant. The H3.1 eviction takes place during the last G2, which is longer than in previous cell cycles. The last cell cycle before differentiation exhibits unique features not only in plant organogenesis but also during animal embryogenesis, revealing evolutionary conserved strategies to mark the acquisition of new transcriptional programs associated with the end of cell proliferation.

TUESDAY, November 6, 2018

LECTURES

L-02
ROLE OF CHROMATIN AND DNA DAMAGE RESPONSE FUNCTIONS IN R LOOP-MEDIATED GENOME INSTABILITY

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Coordination of DNA replication with DNA-damage sensing, repair and cell cycle progression ensures with high probability genome integrity during cell divisions. One important type of genome instability is that associated with transcription. R loops, structures formed by a DNA-RNA hybrid and the displaced single-stranded DNA (ssDNA) molecule, are transcriptional by-products that can be formed naturally as key intermediates in specific cellular processes. Nevertheless, they are also a major source of transcription-associated genome instability and compelling evidence supports that this is mainly caused by replication fork impairment. To explore further the role that replication functions and the DNA damage response (DDR) have on R loop-mediated genome instability we have determined the impact that R loops have on replication fork progression in normal cells and in cells accumulating high levels of R loops. Our results show that this transcription- and RNA-mediated genome instability is not necessarily caused or linked to a lower fork velocity, but to a higher level of obstacles that impair RF progression at sites scattered throughout the genome. Then, after a screen searching for new genes involved in R loop-mediated genome instability among a collection of genes involved in DNA metabolism, we selected 21 genes whose depletion in HeLa cells increased R loops apart of the previously reported role of the Fanconi Anemia/BRCA factors. Our in-depth analysis of R loop-mediated instability in human cells depleted of these factors as well as the THO complex involved in RNA biogenesis reveals a new role for chromatin modifications in R loop accumulation and DDR. The implications for the role of chromatin and DDR functions in both R loop formation and transcription-associated genome instability will be discussed.

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L-03
STRUCTURAL AND CELL BIOLOGY OF SYNUCLEINOPATHIES

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The aggregation of proteins into toxic conformations plays a critical role in the development of different neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), and Creutzfled−Jakob's disease (CJD). These disorders share a common pathological mechanism that involves the formation of aggregated protein species including toxic oligomers and amyloid fibrils. A hallmark of PD and a number of diseases collectively referred to as synucleinopathies is the aggregation of the protein α-synuclein (αS) into amyloid fibrillar formations. α-Synuclein is an intrinsically disordered protein (IDP) making up 1% of total brain-soluble proteins in humans and may play roles in uptake, storage, recycling of neurotransmitter vesicles and maintenance of dopamine. Currently, there is no preventive therapy for these diseases and the available therapeutic approaches are based on the treatment of the symptoms rather than the underlying causes of the disease. Accordingly, the aggregation pathway of these proteins represents a useful target for therapeutic intervention. Therefore, understanding the mechanism of amyloid formation, the biological factors promoting this process and/or its inhibition is of high clinical importance. The RanwellCaputto Conference will be centered on the structural, molecular and cell biology behind the amyloid aggregation of αS, with special focus on (a) the role of metal ions in amyloid assembly and neurodegeneration, and (b) the design of strategies leading to inhibition of amyloid aggregation.

SYMPOSIA

MI-01
CONSERVED MECHANISMS OF VIRAL PATHOGENESIS: ALTERATIONS OF CELL POLARITY AND INTERCELLULAR JUNCTIONS.

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Cellular polarity is maintained by the interconnected activity of polarity complexes whose components have defined cell localization, and are key regulators of the intercellular junctions. Proteins derived from different viruses can interact and interfere with the functions of polarity cellular proteins, resulting in significant biological alterations, important for virus entry, dissemination and pathogenesis. Human papillomavirus (HPV) infections are associated with the development of cervical cancer. We have focused on the oncogenic viral mechanisms from the point of view of the polarity disruption. Thus, we have characterized some of these cellular targets, such as the human Discs large oncosuppressor (DLG1) and the tight junction Partitioning defective 3 protein. Through the use of different methodological tools, such as traditional, histotypical and organotypical raft cultures, we were able to evaluate the changes in the expression of these cell proteins mediated by HPV. In particular, DLG1 not only regulates cell junctions and polarity, but also cell proliferation and migration. Remarkably, clear alterations in the levels and the distribution along the epithelium, as well as in the subcellular localization, were observed for this protein. The data obtained has encouraged us to analyzing the differential expression of DLG1 as a potential progression biomarker in HPV-associated cervical lesions. In addition, we extended our studies to other tumor viruses, such as the human T-cell lymphotropic virus type 1 (HTLV-1). Using fluorescence microscopy we evaluated the aberrant accumulation of polarity regulators in vesicle-like structures, in the presence of the HTLV-1 Tax oncoprotein, with potential significance in lymphocyte deregulation and in the development of lymphoid pathologies. Moreover, we could show for the first time the interaction between a viral and a polarity protein within the cell, using FRET methodology. On the other hand, disruption of cell polarity and alterations of the cell-to-cell contacts are not limited to tumor viruses. Thus, one of the group's current interests consists in the analysis of these processes during infections by regional arboviruses, mainly the Zika virus. The understanding of common mechanisms in viral pathogenesis could help not only for the comprehension of basic viral biology but also for the identification of special targets for novel diagnostic and therapeutic tools.

MI03
RECOMBINANT ANTIGENS OF *Leishmania* FOR THE IMMUNODIAGNOSIS AND IMMUNOPROPHYLAXIS OF AMERICAN TEGUMENTARY LEISHMANIASIS

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The leishmaniases are neglected parasitic diseases spread in 88 countries around the world, including Argentina, which control requires the development of new tools in diagnosis, vaccines and treatments. We applied the recently achieved *Leishmania* parasite genome/proteome knowledge and recombinant DNA techniques for the selection and synthesis of polypeptides for designing of high performance diagnostics or immunoprophylactics methods. Thus, through a seroproteomic analysis of the *L. (V.) braziliensis* amastigote extracts, three proteins were

selected as candidates for immunodiagnosis. The criterion of selection applied was based on their differential reactivity againstsera from patients with leishmaniasis and non-reactivity against sera from patients with Chagas disease, in two dimensional Western blots. In addition, five candidates for vaccines or immunotherapy were selected based on their differential induction of IgG2 or IgG3 antibodies detected by using sera from patients with tegumentaryleishmaniasis. These subtypes of human immunoglobulin are related with a Th1 or Th2 type responses, determining the disease cure o progression respectively. After having expressed in *Escherichia coli*, these candidates have been analyzed for immunodiagnostic purposes by imunobloting or ELISAs, or for vaccines development in murine models of tegumentaryleishmaniasis. This work describes the last results obtained, showing their applicability in the mentioned research fields.

MI04
BROWN ALGAE POLYSACCHARIDE ASSIMILATION POTENTIAL IN SUBANTARCTIC SEDIMENTS

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Polysaccharides are the most abundant and structurally diverse organic molecules in the oceans, and these resources are widely exploited by marine bacteria. Brown algae dominate high-latitude coastal environments, and part of their dead biomass rich in polysaccharides is buried in the sediments, where heterotrophic bacteria participate in its decomposition. These microorganisms represent an important component of the carbon cycle in cold coastal environments, as the degradation of the brown algae biomass by sediments bacteria prevents the long term carbon sequestration in the buried biomass. However, the mechanisms that marine bacteria use for the assimilation of these polysaccharides are poorly understood, in particular in yet-uncultured and difficult to cultivate taxa. At the Environmental Microbiology Laboratory of CESIMAR-CONICET, we are using metagenomic approaches to study the potential of sediment microbial communities to assimilate brown algae polysaccharides in Ushuaia Bay, a low-energy environment located within the Beagle Channel in Tierra del Fuego, Argentina. We analyzed the bacterial populations with the potential to degrade alginate, lineal polysaccharide that constitutes up to 40% of the brown algae biomass, using putative alginate lyase genes as biomarkers. These sequences were very abundant in a metagenomic dataset of Ushuaia Bay intertidal sediments (0.7 Gb), representing 1 every ~2,700 sequences. They belonged to 6 of the 7 polysaccharide lyase families (CAZy) described so far that include alginate lyase enzymes (in order of abundance, PL6 > PL7 > PL5 > PL14 > PL15 > PL17).The sequences were highly diverse within each family, and although 60% of them did not cluster with CAZymes, the overall tridimensional structure was similar to members of the family. The scaffolds containing putative alginate lyase sequences were assigned to 10 different phyla, with Bacteroidetes, Proteobacteria, Planctomycetes, Actinobacteria and Verrucomicrobia being the most abundant. Using the same approach, we analyzed the potential to degrade fucoidans in the sediments, which are branched polysaccharides containing mostly L-fucose and sulfate ester groups. Putative fucoidanase sequences were less abundant (1 every ~97,600 sequences), although they were probably underestimated due to the lack of sequence information for this enzyme (GH107, 8 sequences from 4 bacterial strains).These results provide a community-wide profile of the capability to degrade two abundant components of brown algae, and constitute the basis for the enzymatic characterization of novel CAZymes from uncultured bacteria, with various biotechnological applications such as the production of oligosaccharides with bioactive properties

MI05
STUDY OF THE MECHANISM OF ACTION OF LINEAL BACTERIOCINS USING SUICIDE PROBES

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Most of the antimicrobial peptides produced by bacteria, called bacteriocins, have a spectrum of action limited to bacteria phylogenetically related to the producing strain. They generally act at the cell membrane of target bacteriadissipating the transmembrane electrical potential by forming pores. In our laboratory, we study its mechanism of action and develop new bacteriocins with expanded spectrum of action. In recent years, it was found that the specificity of many bacteriocins is because they require a specific protein receptor in the cell membrane. To better understand which role the receptor plays in the mechanism of action of some linear bacteriocins, we built suicide probes. To construct the suicide probes, the *etpM* gene portion, which codes for the transmembrane helix of the EtpMbitopic protein, was fused with the structural gene of different lineal bacteriocins. These fusions were cloned under the control of the P_{BAD} promoter in *E. coli*. When the suicide probes are expressed by adding arabinose to the culture medium, the transmembrane potential dissipation occurs and the bacterium dies, even in the absence of the membrane receptor. The co-expression of the bacteriocin-specific immunity protein prevents the dissipation of the transmembrane potential and the cells remain viable. The results presented in this work agree with the hypothesis that one of the roles of the receptor protein would be to anchor the bacteriocins in the membrane so that they can penetrate the lipid bilayer and form the pore.

PK-01
SMALL COMPOUNDS MODULATING BI-DIRECTIONAL ALLOSTERY IN PROTEIN KINASES: A NEW GRIP ON AN OLD TRICK?

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Over the last 20 years we investigated the molecular mechanisms of regulation of a large group of kinases, termed AGC kinases (PDK1, PRKs, aPKCs, S6K, SGK, Akt/PKB, RSK, etc.). We identified a regulatory site in the small lobe of PDK1, termed “PIF-binding pocket” that

participates in the docking interaction of PDK1 with a subset of substrates, i.e. S6K, SGK, but not PKB/Akt. In addition, the PIF-pocket of PDK1 -and the equivalent PIF-pocket site in other AGC kinases- participates in the mechanism of activation and inhibition of these kinases, by phosphorylation or interaction with other domains. The binding of synthetic small compounds to the PIF-pocket can "close" the kinase domain and allosterically "activate" PDK1 in vitro, or allosterically affect the ATP-binding site and be allosteric inhibitors of other AGC kinases. I will describe the allosteric process, induced by compounds binding to the regulatory site and affecting the ATP-binding site and how this modulation can be "reversed" by small compounds binding to the ATP-binding site. Thus, different compounds binding with high affinity to the ATP-binding site can produce different "reverse" allosteric effects on the PIF-pocket of PDK1, i.e. displace or enhancing docking interactions, and ultimately can produce different effects in cell signaling. I will further present studies on the allosterc processes induced by small compounds in protein kinases outside of the AGC group of protein kinases, i.e. Aurora kinase, Polo-like Kinase 1 (PLK1) and others. The studies unviel the bi-directional use of the allosteric process for innovative drug discovery in protein kinases

PK-02
ROLE OF PKA IN PROTEIN TRANSLATION REGULATION DURING ADVERSE ENVIRONMENTAL GROWTH CONDITIONS

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In response to environmental stress conditions, the cellular protein content is readjusted through signalling pathways, such as cAMP-PKA, that alter different processes connected to transcriptional, translational and post-translational programs. In *S. cerevisiae*, PKA is a hetero-tetramer composed of two regulatory subunits encoded by the *BCY1* gene, and two catalytic subunits encoded by three genes, *TPK1*, *TPK2* and *TPK3*. We have reported that Tpk2 and Tpk3 differentially localize to mRNA processing bodies (PBs) and stress granules (SGs) in response to glucose starvation, strong osmotic stress, severe heat stress and stationary phase. Deletion of *TPK3* or *TPK2* genes differentially impacts on the capacity of cells to form PBs or SGs as wll as on the global translation and translational fitness of specific mRNAs. We also found that Tpk2 and Tpk3 showed different dynamics and mechanisms of interaction with SGs and PBs. Moreover, Tpk2 and Tpk3 kinase activity and the Tpk2 Q-rich domain are involved in the mechanism of assembly of PBs and SGs in a stress type-dependent manner. A global characterization of granular enriched fraction from mild and severe heat stress showed different protein composition under both conditions. The results suggest that Tpk2 and Tpk3 localized in PBs/SGs could interact with a complex network of distinct protein and potential substrates. Our findings contribute to the concept that different stress conditions induce specific cellular responses, and highlight a different potential role for each isoform of PKA on fundamental processes such as protein synthesis.

PK-03
STUDY OF PI3K/AKT/mTOR PATHWAY IN BREAST CANCER PROGRESSION.

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Deregulation in the PI3K/AKT/mTOR pathway is associated with breast cancer development. Using experimental models of breast carcinogenesis induced in mice, xenografts of tumor cell lines, and tumors from patients we found a differential role of AKT1 and AKT2 isoforms in breast cancer progression. That is, AKT1 regulates nuclear proteins related to cell proliferation, such as cyclin D1 and pS6, whereas AKT2 regulates proteins related to cell migration and invasion such as vimentin, integrin b1, F-actin and FAK. Furthermore, activation of AKT1 promoted the hormone-independent and endocrine resistant phenotype, whereas activation of AKT2 lead to a more aggressive phenotype and lung metastasis. We analyzed 98 luminal breast carcinomas and found that nuclear AKT1 associates with low grade tumors, while cytosolic AKT2 associates with high grade tumors. Furthermore, presence of cytosolic AKT2 was positively correlated with a shorter time to progression of the disease (earlier relapse). In addition, based on our results and data analysis from public databases of The Cancer Genome Atlas, we postulate that throughout the progression of the disease there would be a switch between AKT1 and AKT2 isoforms, which maintains AKT2 inhibited while AKT1 prevails in the early stages. In the more advanced stages, this inhibition is lost and AKT2 prevails. Specific miRNAs are good candidates involved in this regulation and are now been tested in our lab in different experimental and clinical conditions. We propose the use of AKT1 and AKT2 isoforms determined by immunohistochemistry as prognostic markers that could help to better stratify breast tumors and direct more specific therapies

PK-04
INVOLVEMENT OF RAF/MEK/ERK CELL SIGNALING PATHWAY IN JUNÍN VIRUS REPLICATION

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Viruses manipulate cell signaling machinery for their own benefit. Here we investigated the role of Raf/MEK/ERK signaling pathway in the multiplication of the arenavirus Junín (JUNV) in monkey and human cell cultures. We established that JUNV infection induces a biphasic activation of ERK and we proved that a specific inhibitor of the ERK pathway, U0126, impairs viral replication. U0126 also exerted inhibitory action against the arenaviruses Tacaribe (TCRV) and Pichindé. Moreover, treatment with known ERK activators, such as phorbol 12-myristate 13-acetate (PMA) and serum, increased viral yields. By contrast, ERK silencing by small interfering RNAs inhibited viral production. Initial steps of JUNV multiplication (adsorption, internalization and uncoating) were not affected by treatment with U0126. In contrast, the inhibitor caused a marked reduction in viral protein expression and RNA synthesis, whereas JUNV RNA synthesis was significantly augmented in the presence of PMA. Moreover, U0126 impaired the expression of a reporter gene in a TCRV-based replicon system, confirming the ability of the

compound to hinder arenavirus macromolecular synthesis. By using a translation cell-based assay, we determined that the inhibitor does not affect the translation of a synthetic TCRV-like mRNA. On the other hand, no changes in the phosphorylation pattern of the translation factor eIF2α were found in U0126-treated cells. Therefore, our study showed that Raf/MEK/ERK cascade is involved in arenavirus RNA synthesis and provide evidence to consider this cell signaling route as a potential host target for the development of novel antiviral strategies to deal with arenavirus infections.

PB-01
SUCCESSES AND FAILURES DEVELOPING BIOTECHNOLOGICAL TOOLS IN A MODEL PLANT TO IMPROVE CROPS. THE LONG WAY FROM THE GROWTH CHAMBER TO THE GREENHOUSE AND FROM THE GREENHOUSE TO THE FIELD.

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Worldwide research on Plant Molecular Biology has been carried out mostly working with model species. Among them, *Arabidopsis thaliana* is the most studied one due to several characteristics: first sequenced plant genome, short life cycle, small size, availability of genetic tools, etc. However, when the aim is to improve crops, using the knowledge acquired in such model, there is a long way full of obstacles and stop points to be traversed. An additional and independent complicated route appears towards the front when traits tested in crops in a culture chamber need to be evaluated in a greenhouse and field trials. HaHB4 is a sunflower transcription factor, shown as able to confer drought and salinity tolerance in Arabidopsis. It was introduced as transgene in soybean and in wheat. Culture chamber, greenhouse and field trials in different environments were conducted indicating that beneficial traits were conserved between the model and the crops. This technology has almost ended the long way and soybean HaHB4 became a rare successful case because it is expected to be released to the market in 2018/2019. Data from last summer in Argentina, where a severe drought occurred, indicated variations in biomass and seed yield for transgenic HaHB4, control and commercial genotypes in three environments. Under heat stress or drought, seed yield of HaHB4 plants was always the largest (26%-95% yield increase), outyielded by the commercial line only in one well-watered environment. On the other hand and in spite of robust experimental results, wheat HaHB4 could not achieve the final goal; its release was stopped in the Agroindustry Ministry. In our Institute, other sunflower transcription factors were introduced in crops aiming at testing their capability to promote benefits. In a few cases, the transgenes were not expressed as expected, probably due to inadequate genetic constructs used or silencing. HaHB11, which presents structural similarities with HaHB4, was shown as able to confer tolerance to flooding and increased yield in standard growth conditions. It was successfully introduced in maize, rice and soybean plants. Transgenic maize was assessed during two years in the greenhouse and in field trials showing increased biomass and yield at different extents. Transgenic HaHB11 rice was assessed only during one campaign showing promising results. Altogether, the assays performed with different transgenic technologies to improve crops performance indicated that once a technology is validated in a model, it is important to choose a suitable genetic construct and crop to transform and, most important, all the results must be classified as preliminary until repeated field trials are conducted in different environmental conditions

PB-02
DIFFERENT TARGETS FOR THE DESIGN OF BIOMASS OF PLANTS

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The consumption of biomass continues to increase worldwide due to the needs of food and energy provision. Therefore, it is considered as a need to generate more significant quantities of biomass or modified biomass that can meet this demand. For this purpose, biotechnological approaches emerge as advantageous since they are fast and well-known ways of improving. A potential biotechnological target for improving the production of biofuels is the modification of plant cell walls. This modification is achieved via several strategies, including, among others, altering biosynthetic pathways and modifying the associations and structures of various cell wall components. We modified the cell wall of *Arabidopsis thaliana* by targeting the starch-binding domains (SBD) of *A. thaliana* starch synthase III to this structure. The resulting transgenic plants (E8-SDB123) showed increased biomass, higher levels of both fermentable sugars and hydrolyzed cellulose and altered cell wall properties such as higher laxity and degradability, which are valuable characteristics for the second-generation biofuels and livestock feed industries. On the other hand, drought is one of the main causes of crop loss every year, and the current trend in global climate change is likely to exacerbate this situation in the years to come. A major challenge, therefore, is increasing non-food biomass without competing with food production, while also protecting dwindling water resources and enabling cultivation of crops on land not suitable for food production. The functions of SINA (*Seven in absentia*) homologues have been almost fully documented in animals, but the roles of these E3 ubiquitin-ligases are not well known in plants. Our work with SINAL7 (*Seven in absentia like 7*) from *A. thaliana*suggests its involvement in the regulation of glycolysis by modulating the localization and activity of GAPC1. Futhermore, we obtained evidence to show that the over-expression of SINAL7 causes changes in vegetative parameters such as increased biomass, a delay in senescence and the increased drought tolerance.

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PB-03
THE ELICITOR AsES REGULATES RIPENING AND ENHANCES PROTECTION IN AVOCADO AND STRAWBERRY FRUIT

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AsES (*Acremoniumstrictum*Elicitor Subtilisin) is a protein capable of inducing a defense response in strawberry (*Fragariaananassa*) and *Arabidopsis thaliana*, increasing the resistance against the hemibiotrophic fungal pathogen *Colletotrichumacutatum*, and the necrotrophic fungus (*Botrytis cinerea*). It was reported that the defense response observed was due to the activation the salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) signaling pathways. Since AsES activates the metabolism of ET which is involved in many physiological responses we were interested to investigate whether AsES can further regulate ripening of a climacteric fruit such as avocado (*Perseaamericana*), and a non-climacteric fruit such as strawberry. The study included the evaluation of ET production, respiratory rate (CO_2), weight loss, firmness, color, acidity, and soluble solids. Evaluation of ET formation on fruits treated with AsES showed a significant increase of ET production not only in climacteric fruits (avocado), but also in non-climacteric fruits (strawberry). However, the effect on avocado and strawberry were different. Whereas the ET and CO_2 production in avocado presented a peak 3 dpt, in strawberry the ET production was higher but steady along the time, and CO_2 exhibits no significant changes as compared with not treated fruits. Evaluations of weight loss showed that avocado fruits treated with AsES exhibited higher rate of weight loss, while strawberries presented lower rates. Firmness was only evaluated in avocado fruits, and results indicated that AsES causes a significant decrease of the firmness during the first 8 days evaluated. Color quality and acidity were only evaluated in strawberry. Results indicated that AsES causes lower color deterioration after the harvest during the first 3 days evaluated, and acidity showed no significant changes. Soluble solid determination revealed a significant increase in avocado and almost no change in strawberry fruit after the treatment. All these data clearly indicate that AsES affects key aspects of ripening and fruit quality either in avocado or strawberry fruit. It was also observed that AsES induced a protection effect against spontaneous natural post-harvest pathogens in avocado and strawberry fruits. Fruits treated with the ethylene competitive inhibitor 1-MPC (1-methylcyclopropene) previous to AsES suggested that the protection effect was due to the activation of the ET defense signaling pathway. These results uncover the potential use of AsES on the postharvest management of fruit ripening and quality, opening new research lines to study different immunization strategies to increase the crop protection to diseases.

PB-04
MOLECULAR FARMING TO PRODUCE VACCINES AGAINST HUMAN AND VETERINARY COCCIDIAN PARASITES: IMPROVING THE ANTIGEN EXPRESSION IN PLANTS

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Molecular Farming refers to the production of recombinant proteins in plants (including pharmaceutical products, industrial proteins and other secondary metabolites). Over the years it was demonstrated that plants have the capability to express functionally active proteins from mammals and other eukaryotic organisms with therapeutic activity like human sera, growth factors, vaccines, hormones, cytokines, enzymes and antibodies. However, one of the most important challenges in the science community for using plants as a commercial productive platform is to improve the yields of recombinant proteins expressed within them. Therefore, the recombinant protein stability is considered the most important factor that limits the yields in molecular farming. Our lab. is specialized in Plant Biotechnology and Plant-based Vaccine. We aim to develop different strategies to optimize the heterologous protein expression in plants. Our study focuses on optimizing their use for the production of vaccine antigens and evaluating their potential for the delivery of these antigens. In particular,we are working with two relevant pathogens: *Toxoplasma gondii* and *Neosporacaninum.T. gondii* is considered a parasite of veterinary and medical importance, because it may cause abortion or congenital diseases in its intermediate hosts. Meanwhile, *N. caninum* is considered the most significant transmissible causes of reproductive failure in cattle; abortion and neonatal mortality result in significant economic losses within the cattle industry worldwide. Currently, we are developing strategies based on the fusion of the protein of interest to other peptide or protein that functions as a carrier. We selected a 90-kDa heat shock proteins (Hsp90s), which is related to the correct folding during the synthesis of certain proteins, as well as to the re-folding of denatured or partially denatured proteins. In addition, several studies showed that Hsp90s fused to antigenic peptides or proteins increase its humoral and cellular immune response demonstrating their activity as adjuvants. We demonstrated that Hsp90 from plants are an innovative alternative as a substitute of adjuvants based on toxins, not only for their immune-modulatory properties but also because they are excellent carriers of antigenic proteins and peptides expressed in plants. Therefore, we are developing a platform based on the use of tobacco and lettuce plants to produce vaccine antigens fused to Hsp90 from plants as a strategy to improve the expression of the protein of interest.

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LECTURES

L-04
MOLECULAR AND PHYSIOLOGICAL FUNCTIONS OF CIRCRNAS

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Circular RNAs (circRNAs) are highly abundant and evolutionary conserved RNAs of mostly unknown functions. Our lab has recently contributed to the understanding of their biogesis and function. Briefly, we showed that the majority of circRNAs are generated from protein coding exons, and produced co-transcriptionally. We also found that the introns flanking the circularizable exons carry the information for circRNA biogenesis. We also demonstrated that a subset of circRNAs in fly brain produce proteins. Further, to understand the physiology of circRNAs we generated *Drosophila* lines in which specific circRNAs were targeted for degradation using sh-RNAs. We showed that this tool is specific for the targeted circRNA with little or no off-target effects and generated 125 fly lines which allowed us to determine functions of circRNAs*in vivo*. Using these lines, we observed that some circRNAs are essential for fly development, whereas others involved in behavioral, neural, or muscular functions. Interestingly, downregulation of circMbl, the most abundant circRNA in flies, leads to partial male embryonic lethality, altered gene expression and a characteristic wing posture defect. Altogether, our results constitute a comprehensive study of biogenesis and functions of circRNAs in fly brain.

L-05
INTEGRATION OF LIGHT SIGNALING INTO MICRORNA BIOGENESIS

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Constitutive photomorphogenic 1 (COP1) is a RING-finger E3 ligase that plays a central role inphotomorphogenesis by destabilizing many light-regulated transcription factors and photoreceptors. Previously, we revealed a novel function for COP1 E3 ligase in controlling global miRNA biogenesis in Arabidopsis thaliana. In *cop1*mutants, the level of miRNAs is dramatically reduced because of the diminution of HYPONASTIC LEAVES 1 (HYL1), an RNA-bindingprotein required for precise miRNA processing. Under dark condition, HYL1 is rapidly destabilized by a protease, HYL1 CLEAVAGE PROTEASE 1 (HCP1) and 2 (HCP2), which specifically cleaves the N-terminal region from HYL1, thus neutralizing its function. Our results further show that the cytoplasmic partitioning of COP1 under light is essential to protect HYL1 against the proteases. Furthermore, we found that Dicer-like 1 (DCL1) is also up-regulated by dark-to-light transition. DCL1 is constitutively degraded by yet unknown proteolytic pathway in etiolated seedlings but dramatically stabilized in de-etiolated seedlings. However, the levels of many miRNAs are not correlated to the highly up-regulated microprocessor proteins. These results imply that miRNA biogenesis can be differentially regulated by dark-to-light transition. Based on detailed molecular and biochemical analyses, we discuss a new regulatory crosstalk between light signaling and miRNA biogenesis.

SYMPOSIA

PL-01
EMERGING PATTERNS IN PLANT GENE REGULATION

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We are interested in understanding how cells are 'wired', from the perspective of protein-DNA, protein-protein and protein-small molecule interactions. One of our long-term goals is therefore to understand the mechanisms by which plants control gene expression and to elucidate the structure and dynamics of the underlying gene regulatory networks (GRNs). We have used a number of cellular processes to explore the architecture of plant GRNs in model systems such as maize and *Arabidopsis*. The control of flavonoid biosynthesis is providing us with an unparalleled depiction of how combinatorial gene regulation is established, and the role of small molecules in modulating the assembly of transcription factor complexes. To understand GRN architecture, we are combining in maize transcription factor- (*e.g.*,ChIP-seq and DAP-seq) and gene-centered (*e.g.*, yeast one-hybrid) approaches to elucidate the players involved in the control of several branches of phenolic compound

biosynthesis (phenylpropanoids, flavonoids, etc). We have also started to investigate the emerging properties of GRNs with the goal to predict the architecture of GRNs from less-characterized organisms. Our lab hosts two highly-accessed public databases, AGRIS (http://agris-knowledgebase.org/) for *Arabidopsis* and GRASSIUS (www.grassius.org) for maize and other grasses. They contain information on transcription factors, promoters and their interactions, significantly facilitating the study and visualization of plant GRNs.

PL-02
THE ROLE OF RETROGRADE SIGNALS DURING PLANT STRESS RESPONSES

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Cells can sense changes in the environment by external cues that affect different receptors on the cell surface. Exposure to stress also inhibits metabolic activities and causes severe constraints on cellular energy homeostasis. Recovery of energy homeostasis by restoring respiration and photosynthesis is therefore essential for stress acclimation and plant productivity during stress. Organelles play crucial roles as stress sensors in the cell and communicate their status through so-called retrograde signals to regulate nuclear gene expression. Thus, the stress signalling response is not linear but rather a complex integration of signalling networks originating in different cellular compartments. The Mediator kinase module subunit CDKE1/CDK8 was identified through a screen for mutants that did not respond correctly to retrograde signals triggered by oxidative stress. The *cdke*/*cdk8* mutant demonstrated a genome-uncoupled phenotype in response to retrograde signals originating in both mitochondria and plastids. As a consequence the mutant showed severely impaired ability to recover energy metabolism following exposure to stress. CDKE1 is potentially a central nuclear component integrating mitochondrial and plastid retrograde signals playing a role in regulating energy metabolism during the response to stress

PL-03
SIGNALLING DYNAMICS AND PLANT PLASTICITY IN COMPLEX ENVIRONMENTS

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Plants are able to sense key features of their light microenvironment and adjust their body form and function to the prevailing conditions. For instance, the degree of shading by neighbour plants is perceived by phytochrome B (phyB). In turn, phyB modifies the activity of transcription factors such as PHYTOCHROME INTERACTING FACTORs both directly, by physical interaction, and indirectly by affecting the activity of other regulators such CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1), a RING type E3 ubiquitin ligase that acts either by itself or as substrate recognition subunit in a CULLIN 4 E3 ligase complexes. PIFs affect the hormone signalling status, connecting environmental and endogenous cues to control growth and development. We will present recent advances in our understanding of the signalling network that control plastic plant responses to the degree of shading caused by neighbouring vegetation. We will describe recently established connections within the network and key features of the network dynamics under scenarios that simulate the complex environment that plants have to face under natural conditions. The results show the occurrence of mechanisms of signalling integration, where different cues converge to define the output, as well as signalling homeostasis, where certain molecular components counteract the perturbation of the network caused by fluctuations in other factors.

PL-04
THE UPS AND DOWNS OF AN ARABIDOPSIS MICRORNA

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Plants and other multicellular organisms need a precise control of gene expression during development and response to stress. This regulatory capacity depends, in part, on small RNAs. We have been studying microRNA networks that regulate stem cell activity, cell proliferation and differentiation in plants. MicroRNA miR396 regulates transcription factors of the GRF class. In turn, GRFs form complexes with GRF-interacting proteins (GIFs), which are small transcriptional cofactors. Interestingly, GIFs directly associate to chromatin remodeling complexes, demonstrating an interplay of different regulatory layers in the control of gene expression. Our studies show that the miR396 network regulates the cellular programs in plants. Still, its precise roles depend on the specific cellular contexts. In leaves, the miR396 network is involved in the transition of proliferative to differentiating cells, while in roots miR396 regulates stem cells and their transition to active proliferative cells. A model of the participation of miR396 network in the control of gene expression during plant growth will be presented.

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BC-01
ERYTHROPOIESIS AND AUTOPHAGY: TWO CLOSELY RELATED PARTNERS.

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Erythroid maturation is a highly regulated process where immature cells from bone marrow go through a series of differentiation stages to become mature red blood cells. During this, essential intracellular modifications take place such as degradation of entire organelles that are non-necessary for erythrocyte functionality. Autophagy is a lysosomal degradative pathway where macromolecules and organelles are surrounded by double membrane vesicles called autophagosomes and then targeted to lysosomes for its degradation. Autophagy participates actively in erythropoiesis being responsible for engulfment and elimination of mitochondria (mitophagy) and ribosomes once all hemoglobin has been synthesized. Low density lipoprotein receptor-related protein 1 (LRP1) is a transmembrane receptor involved in a wide range of cellular processes such as proliferation, differentiation and metabolism. LRP1 participates as a scavenger receptor for hemin-hemopexin complex, leading to its endocytic internalization for metabolism. We have demonstrated that hemin, an erythropoiesis inductor, is able to generate an autophagic response (mitophagy) in erythroleukemia cells lines (K562 cells), inducing the expression of LRP1 and some autophagic genes such as LC3, Atg5 and Beclin1. Importantly, we have demonstrated that hemin target LRP1 to autophagosomes and this receptor is in part responsible for hemin autophagy activation. Moreover, hemin induces a traffic modification of LRP1, increasing its localization in later endosomal compartments including lysosomal vesicles. It has been proposed that autophagy manipulation is a feasible new therapeutic key in erythropoietic disorders as well as in fighting cancer. Taken together, our results suggest that hemin via LRP1 receptor, is favoring erythroid maturation by inducing an autophagic response in K562 cells, being a possible therapeutic candidate that helps in hematopoietic disorders as well as the chronic myelogenous leukemia(CML) treatment.

BC-02
N-TERMINAL POST-TRANSLATIONAL ARGINYLATION REGULATES MULTIPLE ROLES OF CALRETICULIN

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In cell, post-translational arginylation of proteins regulates many physiological pathways. The enzyme arginyltransferase (Ate1) catalyzes Arg transfer to proteins and polypeptides bearing an N-terminal Glu or Asp residues. Among different substrates, this modification facilitates ubiquitin-ligase recognition and degradation, protein-protein interaction or change of subcellular distribution of protein targets. In our research we identified calreticulin (CRT) as one Ate1 substrate. Different stressors induce retrotranslocation of this endoplasmic reticulum (ER)-chaperone to the cytosol, where CRT is modified by Ate1. Once modified, arginylated CRT (R-CRT) may associate with stress granules (SGs) or localize to the plasma membrane, participating of pro-apoptotic signals. Recently, we reported a correlation between R-CRT localization and the sensitivity to the proteasomal inhibitor bortezomib of glioma-derived cells. This drug induces enhanced mobilization of intracellular Ca^{2+} and ER stress, promoting increased arginylation of CRT and membrane exposure of R-CRT in bortezomib sensitive cells. Upregulation of cell death receptor DR5 is also associated with the R-CRT-mediated apoptosis mechanism induced by bortezomib. Our results suggest that increased R-CRT membrane exposure provides a novel mechanism to improve bortezomib effectiveness for glioma treatment and support Ate1 as a target for cancer therapy.

BC-03
INSULIN-INDEPENDENT GLUT4 TRAFFICKING IN SKELETAL MUSCLE: NEW MECHANISMS AND ADVANCES TO FACE METABOLIC DISEASES

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Muscle contraction stimulates muscle glucose uptake by facilitating translocation of the glucose transporter 4 (GLUT4) from an intracellular compartment to the plasma membrane of muscle cells, to achieve a net increase in surface The intracellular mechanisms regulating this process are not fully understood. It is well established that insulin causes cortical actin remodeling in muscle and fat cells, and that interfering with actin dynamics halts GLUT4 incorporation into the membrane. The small GTPase Rac1 governs actin remodeling, and Rac1 involvement in insulin-stimulated glucose uptake has been demonstrated. Recently, Sylow et al. further reported that Rac1 is activated during muscle contraction and exercise in mice and humans. Remarkably, Rac1 appears to be necessary for exercise/contraction-stimulated glucose uptake in skeletal muscle, since muscle-specific Rac1 knockout mice display reduced contraction- and exercise-stimulated glucose uptake into skeletal muscle. The underlying molecular mechanisms by which Rac1 regulates glucose uptake remain unclear, although there is evidence that Rac1 regulates a dynamic remodeling of the cortical actin cytoskeleton in response to insulin. Similarly, the causes of Rac1 activation during contraction remain elusive, although membrane stretch –which occurs during muscle contraction-, contributes to Rac1-dependent increase in glucose uptake. We previously reported that electrical stimulation leads to ATP release from rat primary myotubes, immortalized rat L6 myotubes and mouse adult muscle fibers (13). The released nucleotides in turn act autocrinely or paracrinely to activate purinergic P2Y receptors, which signal through phosphatidylinositol 3-kinase (PI3K) gamma (PI3Kγ). Like the other class I PI3K members (PI3K α and β), PI3Kγleads to Akt activation, and we showed that exogenously added ATP-stimulated PI3Kγleads to Akt activation and downstream phosphorylation, to promote GLUT4 mobilization and increased glucose uptake. We report that, in L6 rat skeletal myoblasts stably expressing *myc*-tagged GLUT4, exogenously added ATP increasedGLUT4 translocation and glucose uptake, and both responses required actin filament remodeling**.**ATP evokedcortical actin

polymerizationthat was mediated by Rac1 and also by Cdc42. Both GTPases were activated downstream of purinergic P2Y receptors acting via PI3K . Inhibiting the actin-branching Arp2/3 complex via CK869 or siRNA-mediated silencing of the Arp3 subunit reduced ATP-induced GLUT4 translocation. ATP also led to dephosphorylation (activation) of the actin-severing protein cofilin. Cofilin knockdown via siRNA partially inhibited GLUT4 translocation. We propose that ATP-dependent activation of Rac1 and Cdc42 engages both Arp2/3 and cofilin in a coordinated, dynamic cycle of actin branching and severing at the cell cortex, which is essential for ATP-mediated GLUT4 translocation.

BC-04
PROTEIN ARGINYLATION AS A GLOBAL REGULATOR OF INTRACELLULAR PROTEIN TRAFFICKING AND FUNCTION

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Protein arginylation mediated by arginyltransferase ATE1 is a key regulatory process essential for mammalian embryogenesis, cell migration, and protein regulation. Despite decades of studies, very little is known about the mechanisms of arginylation and the specificity of ATE1-mediated target site recognition *in vivo*. Using *in vitro* assays and computational analysis we dissected the tRNA recognition and target site specificity of mouse arginyltransferases and gain insight into the complexity of the *in vivo*arginylome. Our results suggest that ATE1 has a unique requirement for tRNA binding that is involved in regulating the balance between arginylation and protein synthesis. Moreover, we found that the four ATE1 isoforms have different, only partially overlapping target site specificity that includes more variability in the target residues than previously believed. Based on all the available data, we generated an algorithm for identifying potential arginylation consensus motif and used this algorithm for global prediction of proteins arginylated*in vivo* on the N-terminal D and E. Our analysis reveals multiple proteins with potential ATE1 target sites and expand our understanding of the biological complexity of the intracellular arginylome.

YI-01
INVESTIGATING THE ROLE OF MICRORNAS DURING MAMMALIAN BRAIN DEVELOPMENT

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The emergence of the cerebral cortex during evolution allowed the brain to enhance the sensing, interpretation and response to the surrounding world stimuli through a conscious state. Recently, it has been shown that microRNAs are essential for mammalian cortex development. However, the contribution of individual microRNAs in the regulation of brain differentiation mechanisms remains bastly unknown. To address this challenge, we developed *in vitro* screening methodologies coupled to *in vivo* studies in order to investigate the role of microRNAs in different processes related to corticogenesis, including regulation of cell division, migration and microRNA activity. Usinga high-content screen and mouse genetics, we identified miR-34/449 family as a key regulator of radial glial cell differentiation in the developing cerebral cortex. Analyzing miR-34/449 knockout (KO) mouse embryos, we found significant spindle misorientation phenotypes in cortical progenitors, resulting in an excess of radial glia cells at the expense of neurogenic intermediate progenitors, showing that miR-34/449 is required for the timely generation of cortical neurons. Using a similar strategy, we screened through all cortically expressed microRNAs and found that autistic syndrome associated miR-107 promotes cell migration *in vitro*. Finally, using a microRNA activity sensor, we screened the human kinome and found that several essential kinases for cortical lamination are necessary for microRNA activity. By coupling *in vitro* and *in vivo* assays, our data indicate that microRNAs and their regulators could be implicated in key cellular processes during cortical development.

YI-02
THE *AZI1* SUBCELLULAR TARGETING MECHANISM: HOW TO ANCHOR IMMUNE RECEPTORS TO THE PLASTID ENVELOPE

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Precise subcellular localization of defense factors is essential for plants immune system. The lipid transfer protein-like AZI1, is an important component for the systemic resistance and priming or immunological “memory” establishment. Recently, we have demonstrated that AZI1 is needed for the movement of the priming signal azelaic acid (AZA) and a pool of AZI1 exists at the site of AZA production, the plastid envelope. Furthermore, after systemic resistance-triggering infections, the proportion of plastid located AZI1 increases. However, AZI1 does not possess a chloroplastic transit peptide or any other recognizable targeting signal that can explain its localization. Here, we uncover a novel bipartite N-terminal signature that allows AZI1 to target plastids by using a signal anchor-like mechanism. We also show that the key defense-associated kinases MPK3/6 regulate the abundance of AZI1’s plastid pool in leaves and possibly in underground tissues. Interestingly, many Arabidopsis coded proteins display a similar N-terminal signature. Among them we found several NBS-LRR class of resistance (R) proteins, known to be essential plant immune receptors. Microscopy and subcellular fractionation data corroborate the plastid localization for some of them. These are the first R proteins proposed as functioning from plastids, which are crucial organelles for a successful defense response. Consistent with this,

preliminary results indicate that KO mutant plants for one of these R proteins are more susceptible to pathogen infections. Taken together, our results indicate the existence of an undescribed mode of plastid targeting probably related to defense responses against pathogens. Particularly with regards to the phenomena of priming, in which a well-placed ambush can be the difference between life and death.

YI-03
ARCHITECTURE OF MecR1 OF *Staphylococcus aureus:* CLUES TO THE SIGNAL TRANSDUCTION MECHANISM THAT UNLEASHES RESISTANCE TO β-LACTAMS

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Resistance to β-lactam antibiotics in the pathogen *Staphylococcus aureus* is due to the inducible expression of two enzymes: the serine-β-lactamase PC1 and the transpeptidase PBP2a, which is not inhibited by clinical concentrations of most of these antibiotics. The transmembrane sensor/signal transducer proteins MecR1 and BlaR1 detect the presence of the antibiotic and regulate the expression of the genes that result in manifestation of resistance. β-Lactam-acylation of the extracellular sensor domain of MecR1/BlaR1 is believed to be the initial event that unleashes activation of their metalloprotease domain through a yet inscrutable signal-transduction mechanism. The activated metalloproteases degrade the DNA-binding proteins MecI/BlaI. The latter are repressors of transcription of the genes of the operons. Hence, their degradation results in expression of PC1 and PBP2a. The mechanism of signal transduction used by MecR1 and BlaR1 is of great interest given that they are possible targets for the design of inhibitors that can restore the effectiveness of β-lactam antibiotics for the treatment of *S. aureus* infections. The complete proteins or, for that matter, the transmembrane/metalloprotease domains of MecR1 and BlaR1 remain not amenable for structural studies. In this study we combined homology modeling and co-evolution-based *de novo* structure prediction to generate a model of the transmembrane domain of MecR1. This model was tested through an experimental mapping of the topology of the loops of this domain in *E. coli*, using fusions to eGFP and Proteinase K/TEV-protease susceptibility assays. The model suggested that the gluzincin core of the metalloprotease domain would be anchored to the cytoplasmic side of the membrane by a reentrant helix, hypothesis that we confirmed by expression of the isolated gluzincin core in *E. coli* membranes. Finally, we docked the structure of the sensor domain of MecR1 into our model of its metalloprotease domain, guided by interactions reported by NMR between the sensor domain of the homologous protein BlaR1 and an extracellular loop of the transmembrane domain. Integration of all these results allow us to put forward a model for full-length MecR1 where the metalloprotease domain defines a hydrophilic transmembrane chamber in whose floor the zinc site sits, sealed on the periplasmic side by the sensor domain. A reentrant helix anchors the gluzincin core of the metalloprotease domain to the cytoplasmic side of the membrane and is poised to interact with the sensor domain. This model accounts for all the available biochemical and structural information on this class of sensors and gives the first insights into the way the presence of the antibiotic modulates the activity of the membrane-embedded domain.

YI-04
DILP8-LGR3 PATHWAY: A RELAXIN-LIKE PATHWAY CONTROLLING DEVELOPMENTAL TRANSITIONS

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The capacity to replace damaged tissues is a common characteristic in most metazoans. However, the regenerative competence of different tissues can vary significantly between animal groups and their life cycle stages. In arthropods, the ability to regenerate external structures is limited to the growth phases of their life cycles and is tightly coordinated with molting due to growth restrictions imposed by the hard exoskeleton. Accordingly, crabs, which can grow and molt after reaching maturity, can regenerate legs throughout their life, but insects lose this capacity as they reach adulthood and stop growing. The potential fitness impairment that results from the inability to regenerate injured structures is bypassed in insects by tissue-damaged triggered mechanisms that have evolved to extend the growth-competent period and provide extra time for regeneration. The molecular and cellular players mediating this coordination between tissue growth and developmental timing have been recently discovered in Drosophila. The insulin/relaxin-like peptide, Drosophila insulin-like peptide 8 (Dilp8), was identified as a factor communicating abnormal growth status of Drosophila larval imaginal discs to the neuroendocrine centers that control the timing of the onset of metamorphosis. We found that *dilp8* is highly and specifically expressed in imaginal discs in response to a variety of growth alterations and requires a neuronal relaxin receptor for this function, the Leucine rich repeat containing G protein coupled receptor, Lgr3. Imaginal disc-derived Dilp8 acts on four central nervous system Lgr3- positive neurons to activate cyclic-AMP signaling. This delays the induction of the transcription of enzymes in the ecdysone synthesis cascade and causes a reduction in ecdysone hormone production, which leads to an extension of the larval growth period and a simultaneous restriction of the growth rates of healthy imaginal tissues, promoting the generation of proportionate individuals. Similarities between the Dilp8-Lgr3 pathway controlling larval-to-pupal transitions and the hypothalamic-pituitary axis in vertebrates suggest that relaxins might be part of an ancient stress-responsive pathway coordinating animal growth and maturation timing. Analysis of *dilp8* expression pattern suggests that the Dilp8-Lgr3 pathway might have other functions at different life history stages. Dilp8 is expressed in the ovary and in multiple pulses close to the ecdysone surges that control major developmental transitions. The highest expression is reached at the larval to pupal transition, when the larva shortens and hardens its cuticle to acquire a typical barrel shape. Interestingly, both *dilp8* and *Lgr3* mutants show defective puparium contraction. Recent data show that *dilp8* is expressed in the epidermis and received by a population of Lgr3+ neurons different from those involved in growth coordination. Our results indicate that the Dilp8-Lgr3 pathway has multiple conserved roles in insect development which involve parallel neuroendocrine circuits.

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YI-05

THE ENDOCYTIC PATHWAY AS A KEY MODULATOR OF ANTIGEN CROSS-PRESENTATION BY DENDRITIC CELLS

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Cross-presentation by MHC class I molecules allows the detection of exogenous antigens by CD8+ T lymphocytes. This process is determinant to initiate cytotoxic immune responses against many pathogens (i.e. *Toxoplasma gondii*) and tumors. To achieve efficient cross-presentation, dendritic cells (DCs) have developed highly specialized adaptations of their endocytic network. Consequently, DCs are the most potent antigen presenting cell type to accomplish this immunological process. However, a complete view of the many molecular effectors involved in antigen cross-presentation is still missing. We have recently shown that the small GTPase Rab22a regulates the transport of MHC-I in DCs by stabilizing the intracellular pool of these molecules at the recycling center, allowing the normal delivery to phagosomes and guaranteeing an efficient recycling to the cell surface. Furthermore, we demonstrated that Rab22a modulates the acquisition of ER-derived proteins to endosomes but not to phagosomes in DCs. All these intracellular trafficking defects that take place in Rab22a deficient DCs drive to a significant impairment of antigen cross-presentation, including soluble, particulate and *T. gondii*-associated antigens. Recently, we have also started to study the role of sorting nexin (SNX) proteins during antigen cross-presentation. SNXs are characterized by the presence of a phox-homology domain that interacts with elements of the endocytic pathway enriched with phosphatidylinositol-3-monophosphate. In this way, SNXs control key features of endocytosis, as well as endosomal signaling, sorting and tubulation. In particular, SNX17 associates with compartments of the early endocytic network and participates in several processes of intracellular recycling. We have identified SNX17 as a main regulator of antigen internalization and cross-presentation by DCs. Our findings provide compelling evidence that Rab22a and SNX17 play central roles in the endocytic transport of DCs and are crucial molecules to guarantee an efficient antigen cross-presentation.

THURSDAY, November 9, 2016

LECTURES

L-06

BIOSYNTHESIS OF BACTERIAL GLYCOGEN: EVOLUTION OF ALLOSTERIC CONTROL

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The bacterial glycogen biosynthetic pathway is a case study for the structure and evolution of regulatory (allosteric) function. Its similarity with the synthesis of starch in plants and unicellular algae makes it attractive for evolutionary and biotechnological purposes. They enzyme ADP-glucose pyrophosphorylase (ADP-GlcPPase) controls these synthetic pathways. ADP-GlcPPase has evolved to satisfy the regulatory requirements for the particular metabolism of the organism in different species. Different effectors regulate this enzyme depending on the major metabolic pathway of the species. The allosteric control of this enzyme family is distinct because its richness in evolutionary divergence. For instance in photosynthetic eukaryotes (unicellular and multicellular), we have observed that different subunits have evolved to acquire different catalytic and regulatory roles, and we were able by mutagenesis to resurrect some of those ancient roles. With computational, structural, site-directed mutagenesis, and kinetic studies we studied the transmission of the allosteric signal and the interaction of the allosteric effectors in the enzyme family. Our results indicate the presence of two separate allosteric sites with different levels of synergistic effects. These distinct sites have allowed the enzyme to evolve with greater flexibility without changing the underlying mechanism of activation. Despite the divergence in regulation among plant and bacterial subunits, there seem to be a common structural element that works as a switch, but also an alternative allosteric signal guided by a secondary site. Our current knowledge of this enzyme family allows us to interpret the allosteric mechanism by which previously obtained random mutants of the *Escherichia coli* enzyme were highly active. In the past, these random approaches were instrumental to obtain transgenic plants with improved production of starch. For all these reasons, our research is seeking to contribute fundamental knowledge to understand the evolution of the ADP-GlcPPase and its rational manipulation to obtain photosynthetic organisms for both nutritional value and for the production of biofuels. This work was supported by a grant from the National Science Foundation, USA (NSF MCB 1616851).

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L-07
QUANTITATIVE IMAGING IN SINGLE MOLECULE FLUORESCENCE LOCALIZATION

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Fluorescence microscopy is a widely used technique to study cellular structure and dynamics. The introduction of methods breaking the diffraction limit of optical resolution, named super resolution microscopy or optical nanoscopy, allowed tens of nanomenter lateral resolution to provide structural details unattained before. These achievements posed new challenges for the design and photochemical studies of fluorescent probes with specificity for a certain substrate, fluorophores whose fluorescence could be turned on and off, photochemical studies under high irradiation conditions. Soon it was also evident the need to introduce methods to analyze images to precisely locate single molecules and to evaluate molecular association. In this talk I will deal with two problems we are working at, using optical nanoscopy, in CIBION in collaboration with IBioBA (Instituto de Biomedicina de Buenos Aires-MPSP). On one side we studied the GPCR membrane receptor corticotropin release hormone receptor 1 (CRHR1). For that, we designed and synthesized a fluorescent probe that works as an antagonist and developed a method to evaluate the molecular binding constant in the cellular environment. On the other side, we undertook the study of the interaction between HIF (Hypoxia inducible factor), VHL (von Hippel-Lindau complex), RSUME (RWD-domain-containing sumoylation enhancer) in the hypoxia adaptation syndrome of cancer cells. In the beginning of this project we characterize the distribution of the proteins in the cell

SYMPOSIA

ST-01
HIPERMUTABILITY AND THE EVOLUTION OF SMALL COLONY VARIANTS IN *Pseudomonas aeruginosa BIOFILMS*.

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During chronic lung infections in cystic fibrosis (CF) patients *Pseudomonas aeruginosa* (PA) grows as biofilm communities, where it undergoes extensive evolutionary diversification. Among the biofilm-adapted specialists, small colony variants (SCVs) are frequently observed, which may associate with worse patient prognosis. SCVs show a great instability when grown outside biofilms, thus revealing the ability to switch between phenotypes. We've previously described that hypermutator Mismatch Repair System (MRS)-deficient strains of PA show an increased phenotypic diversification, particularly in biofilms. In this work, we explored the adaptive potential of PA and the role of hypermutability in SCV phenotypic switching by carrying out experimental evolution assays and comparative genomics. Compensatory mutations clustered in pathways related to the synthesis/degradation of the second messenger c-di-GMP in both, wt and *mutS*hypermutator lines and this parallelism suggests that they evolved through convergent pathways. However, we found that the adaptive potential of hypermutators was notably higher, enabling them to bypass genetic constraints imposed on the successive phenotypic switching. We propose that the limits imposed by the continual SCV bimodal switching are relieved by the existence of multiple loci contributing to control the levels of c-di-GMP, with an enhanced access to these loci enabled by the increased mutation rate. Our results have implications for hypermutator management in clinical settings and may help to better understand the high prevalence of PA isolates exhibiting a hypermutable phenotype in CF chronic pulmonary infections

ST-02
SPECIALIZED DNA POLYMERASE IOTA COORDINATES DNA REPLICATION AND CHECKPOINT ACTIVATION

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The DNA damage response (DDR) is a multifaceted network of signals which is activated by structural and chemical alterations of the DNA. Between others, central pathways for DDR are the DNA damage tolerance by specialized DNA polymerases and the checkpoint. It is unclear if all specialized DNA polymerases have complete overlapped functions. Here we show that the alternative polymerase iota, but no other alternative pols, has a role in checkpoint activation which is unrelated to a role in DNA damage tolerance. In pol iota depleted samples, nascent DNA elongation increases being such accelerated elongation symmetric in speed. In addition, pol iota depleted samples show lower levels of single-stranded DNA (ssDNA) accumulation implying that elongation of DNA is continuous. While nascent DNA elongation seems untroubled by pol iota depletion, the low levels of ssDNA correlate with a global reduction on DDR markers including chromatin-bound RPA, gamma H2AX, 53BP1,phosphor-Kap1 and even Chk1 activation. Defective DDR in the absence of pol iota triggers excessive origin firing which in turn causes increased genomic instability of pol iota-depleted cells. Our results reveal a crucial role of pol iota in the coupling of nascent DNA elongation and the generation of ssDNA–mediated DDR signals. Such a novel function of pol iota is relevant for the genomic stability and the survival of cells.

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ST-03
A SURVIVAL SCREEN TARGETING THE HUMAN KINOME REVEALS SYNTHETIC LETHAL INTERACTIONS WITH THERAPEUTIC POTENTIAL FOR BRCA-DEFICIENT CANCER CELLS

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Fundamental metabolism provides energy and molecules required for cell growth and development. Some biological reactions such as histone and nucleic acid demethylations or the one carbon cycle can also generate genotoxic metabolites such as endogenous formaldehyde (EFA). This simple aldehyde avidly reacts with electron-rich groups adducting proteins, nucleic acids and thiols. To prevent EFA toxicity, mammals harbour the enzyme alcohol dehydrogenase 5 (ADH5) that converts EFA into the less reactive molecule formate. The Fanconi Anemia DNA repair pathway protects the genome from EFA-induced DNA damage. The inactivation of these two systems in mice leads to hematopoietic stem cell loss, karyomegaly, liver and kidney dysfunction, and cancer, revealing the cytotoxic and carcinogenic potential of EFA. DNA damage is likely one of the main drivers of EFA toxicity, however the strong reactivity of EFA may affect other cellular targets and biomolecules. To further understand how EFA damages cells, we have established a colorectal carcinoma cancer model deficient in ADH5. These cells are exquisitely sensitive to EFA, which also alters the formation of in vitro tumour spheroids. The characterization of ADH5-deficient cells uncovers novel targets of EFA toxicity and a conserved protection mechanism to counteract the most simple and one of the most reactive endogenous aldehydes.

ST-04
NOVEL TARGETS OF FORMALDEHYDE TOXICITY IN CANCER CELLS

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Fundamental metabolism provides energy and molecules required for cell growth and development. Some biological reactions such as histone and nucleic acid demethylations or the one carbon cycle can also generate genotoxic metabolites such as endogenous formaldehyde (EFA). This simple aldehyde avidly reacts with electron-rich groups adducting proteins, nucleic acids and thiols. To prevent EFA toxicity, mammals harbour the enzyme alcohol dehydrogenase 5 (ADH5) that converts EFA into the less reactive molecule formate. The Fanconi Anemia DNA repair pathway protects the genome from EFA-induced DNA damage. The inactivation of these two systems in mice leads to hematopoietic stem cell loss, karyomegaly, liver and kidney dysfunction, and cancer, revealing the cytotoxic and carcinogenic potential of EFA. DNA damage is likely one of the main drivers of EFA toxicity, however the strong reactivity of EFA may affect other cellular targets and biomolecules. To further understand how EFA damages cells, we have established a colorectal carcinoma cancer model deficient in ADH5. These cells are exquisitely sensitive to EFA, which also alters the formation of in vitro tumour spheroids. The characterization of ADH5-deficient cells uncovers novel targets of EFA toxicity and a conserved protection mechanism to counteract the most simple and one of the most reactive endogenous aldehydes.

AM-01
FLUOROPHAGES FOR RAPID TB-DIAGNOSIS IN SPUTUM SAMPLES AND PHENOTYPIC DRUG SUSCEPTIBILITY TESTING

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Tuberculosis (TB) is a major cause of human mortality with 9 million new cases and nearly two million deaths annually; approximately two billion people are infected with the causative agent, *Mycobacterium tuberculosis (M.tb).* The emergence of resistant strains has become a serious public health problem worldwide complicating treatment and control of the disease. The World Health Organization (WHO) estimates that 40% of tuberculosis cases go undiagnosed and consequently not treated. In 2016, WHO recommended the use of rapid molecular tests to speed up tuberculosis drug susceptibility testing (DST) although due to the cost of equipment and supplies, ZiehlNeelsen staining of *Mycobacterium spp.* in sputum, with subsequent culture to determine viable bacilli and DST using the proportion method is often the method of choice. Culture methodology is laborious and takes 3-6 weeks to report the presence of viable mycobacteria in the sample and a few additional weeks for DST. Fluoromycobacteriophages (or Fluorophages) are reporter mycobacteriophages containing a fluorescent gene. These phages are a simple and rapid mean of revealing the metabolic state of *M.tb* cells, and therefore their response to antibiotics. We have constructed a new Fluorophage, $mCherry_{bomb}\phi$, with higher sensitivity and less time to detection of signal in *M. tb*. Using this improved Fluorophage we have developed a simple microscopy-based methodology for detection of viable *Mycobacterium spp*. and phenotypic determination of rifampicin resistance within just 3-5 days from sputum sample collection. Fluorophage methodology is compatible with regularly used protocols in clinical laboratories for TB diagnosis and paraformaldehyde fixation after infection reduces biohazard risks with sample analysis by fluorescence microscopy. Using clinical isolates of pre-XDR and XDR-TB strains, we tested $mCherry_{bomb}\phi$ for extended DST and we compared the antibiotic resistance profile with those predicted by whole genome sequencing. Our results emphasize the utility of a phenotypic test for *M. tuberculosis* extended DST. We had also set up the conditions for infection of pure cultures in a 96-multiwell format in the presence of increasing concentrations of drugs monitoring the appearance of fluorescence as a function of time using a fluorimeter. Using this fluorimeter, a complete DST of *M. tb* could be done from pure culture in 6 or 30 hs (when pre-incubation with the drug was required). We found a good correlation between the MIC values obtained with this technique and the proportion method used as gold standard for TB. Overall, we have developed a simple and inexpensive assay for rapid detection and determination of rifampicin resistance of *M.tb* in sputum samples and we had optimized the conditions for an automated phenotypic assay to test in a short time susceptibility of pure cultures to different drugs used for TB treatment and as a high sensitive method for HTS of new anti TB drugs.

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AM-02
STUDY OF LACTIC ACID BACTERIA—*Escherichia coli*O157:H7 INTERACTION AND ITS CONTRIBUTION TO BIOPROTECTION STRATEGIES IN MEAT

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Human infection by Enterohemorrhagic *Escherichia coli* (EHEC) occurs through the ingestion of contaminated foods such as milk, vegetable products, water-based drinks and particularly minced meats. In addition, 5-10% of patients infected with EHEC develop Hemolytic Uremic Syndrome (HUS), the main cause of renal failure and transplantation in children under 5 years of age. Argentina is, worldwide, the country with the highest incidence of this pathology. Therefore, EHEC constitutes a serious threat to public health and a major concern for the sustainability of the meat industry as well as for its entire production chain. Presently, consumers assumed a crucial role requiring safer and healthier foods with minimal chemical additives. This context highlights the need to provide the meat industry with sustainable and eco-friendly solutions to limit and prevent future risks surrounding this problematic. The aim of this research is to provide with a technological solution to control EHEC contamination in meat by a biological approach based on the use of lactic acid bacteria (LAB). In order to proceed towards an efficient bioprotective culture as strategy of EHEC inactivation, it is necessary to have a highly competitive strain to fight the pathogen. In this work the assayed LAB strains were examined for antagonistic activity towards *E. coli* O157:H7 NCTC12900 by using a simple and rapid method and by analyzing the growth kinetics of co-cultures (LAB-EHEC) in a meat-based medium. *Enterococcusmundtii* CRL35 was selected as the candidate due to its optimal ability to fight the pathogen, triggering its death after 8 h and reducing more than 2 log units EHEC viability after 96 h of co-cultivation. This effect proved to be independent of the action of bacteriocin, acid or other soluble agent produced by the LAB, suggesting cell-cell interaction as one of the mechanisms involved in the inhibitory action. From the proteomic results it is highlighted that, during the first hours (6 h) of co-culture with the pathogen, *Ent. mundtii*, evidenced the over-expression of proteins related to the metabolism of sugars and nitrogen which allowed it to compete optimally with the pathogen even triggering its death phase. When adhesion to proteins related to the extracellular matrix (ECM) of meat was assessed, results evidenced differential adhesion of both microorganisms suggesting a competitive advantage of *Ent. mundtii* over EHEC during the adhesion / colonization of meat. Finally, with the aim of evaluating the bioprotective action of *Ent. mundtii* in real scenario, a ground meat-based system was designed. Results showed that *Ent. mundtii* CRL35 exerted a bacteriostatic effect over EHEC in ground beef after 48 h. Even when additional technological studies have to be performed in fresh meat to optimize the bioprotective action of this strain, this research lays the foundations of the molecular basis of the interaction between *Ent. mundtii* CRL35 and *E. coli* NTCC12900, as well as of the strategies of competition applied by both microorganisms. Finally, this work opens new perspectives for the application of this bioprotective LAB to control *E. coli* O157:H7 in meat products.

AM-03
PROBIOTIC LACTOBACILLI AS A SOURCE OF PROTEINS OF BIOTECHNOLOGICAL INTEREST: *Lactobacillus kefiri*AND ITS S-LAYER PROTEINS

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Lactobacillus kefiri is one of the most important species of lactobacilli retrieved from “kefir”, a dairy product obtained by fermentation of milk with kefir grains. The health-promoting properties of several *L. kefiri* strains were demonstrated by *in vitro* and *in vivo* studies. Besides its application as a probiotic, *L. kefiri* can be considered as a source of proteins of biotechnological interest. Some years ago, the presence of S-layer, a nanostructured (glyco)-proteinaceous envelope constituted by subunits that self-assemble to form a two-dimensional lattice that covers the surface of different species of *Bacteria* and *Archaea*, was described in *L. kefiri* strains by our workgroup. The S-layer proteins (SLPs) have been shown to possess exceptional physicochemical properties which make them singular organizational structures with high potential application in different areas of life and non-life sciences. The SLPs from *L. kefiri* are glycosylated and show high heterogeneity among strains at the primary structure level. Considering the amino acid sequence of the SLPs from 16 different *L. kefiri* strains available in our laboratory, the total length of the mature proteins varies from 492 to 576 amino acids, and all SLPs have a calculated pI between 9.37 and 9.60. The 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. The O-glycosylation site SASSAS was found in all *L. kefiri* SLPs. The analysis of monosaccharides of the O-glycosidic residues of from 4 different strains, revealed the presence of glucose as the main component. Moreover, the presence of N-glycans in a lactobacilli SLP was described by first time in the strain *L. kefiri*CIDCA 83111. Regarding the biotechnological applications of these glycoproteins, we have recently demonstrated that SLP from *L. kefiri* CIDCA 8348 (SLP-8348) enhanced the LPS-induced response on murine macrophages. There was a significant increase in the expression of surface cell markers such as CD40, CD86 and MHC-II, and secretion of IL-6 and IL-10 in comparison with LPS-stimulated cells. This synergism is abrogated by EGTA, a Ca^{+2} chelating agent, indicating the involvement of glycan moieties in that effect. Indeed, the carbohydrates present in SLP-8348 are crucial for recognition by cellular receptors and its subsequent internalization by macrophages, since that process is inhibited by the presence of glucose, mannose or EGTA. Recent *in vivo* studies carried out on BALB/c mice using ovalbumin as a model antigen, showed that glycoconjugates are in part responsible for both the immunogenicity and the adjuvant capacity of SLP-8348. These interesting findings, along with its known ability of self-assembly, do make SLP-8348 a unique structure with high potential in vaccinal applications.

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AM-04
MICROBIAL ENZYMES AND THEIR APPLICATION IN CELLULOSIC ETHANOL INDUSTRY

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Lignocellulosic biomass recalcitrance is based on the complex polymeric network of cellulose, hemicellulose and lignin that provides plants with strength and resistance. For this reason, enzyme-mediated hydrolysis of lignocellulose, to release soluble and fermentable sugars, is a key step for lignocellulosic based biofuels. Lignocellulosic biomass is deconstructed by the concerted action of multiple enzymes, mainly endo and exo-acting glucanases (EC 3.2.1.91; EC 3.2.1.74), xylanses (EC 3.2.1.8), β-glucosidases (EC 3.2.1.21) and β-xylosidases (EC 3.2.1.37) as well as enzymes with auxiliary or debranching activity (such as LPMOs and arabinofuranosidases). These enzymes are collectively referred to as CAZymes (carbohydrate active enzymes). The general objective of our group is the study of the enzymatic mechanisms of hydrolysis of polysaccharides in order to develop novel and improved enzymes for industrial applications in the degradation of residual biomass, especially for the production of bioethanol. We have thoroughly characterized the CAZome (all CAZymes encoded in the genome) and secretome of two bacterial cellulolytic isolates of the genera *Cellulomonas*sp and *Paenibacillus* sp. Both bacteria secrete a repertoire of enzymes necessary for the degradation of cellulose and hemicellulose, when grown on lignocellulosic biomass, although there are differences in their strategies for polysaccharides utilization. We have also purified xylanases from both bacterial groups as well as from the fungus *Pycnoporussanguienus*. GH10 and GH11 xylanases present distinct structures and have different mode of action. When assayed on lignocellulosic biomass, addition of a β-xylosidase greatly imporved the conversion to xylose. Moreover, when xylanases were added to a cellulolytic commercial cocktail, cellulose conversion to glucose was improved, indicating synergy between the enzymes involved. By improving our knowledge on enzymatic deconstruction of biomass we are able to optimize the enzymatic cocktails for different applications.

LI-01
SPECIFIC PHOSPHOLIPIDS REGULATE THE ACQUISITION OF NEURONAL AND ASTROGLIAL IDENTITIES IN POST-MITOTIC CELLS

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Up to now, the known mechanisms underpinning cell-fate specification act on neural progenitors, affecting their commitment to generate neuron or glial cells. Here, we show that particular phospholipids supplemented in the culture media modify the commitment of post-mitotic neural cells *in vitro*. Phosphatidylcholine (PtdCho)-enriched media enhances neuronal differentiation at the expense of astroglial and unspecified cells. Conversely, phosphatidylethanolamine (PtdEtn) enhances astroglial differentiation and accelerates astrocyte maturation. The ability of phospholipids to modify the fate of post-mitotic cells depends on its presence during a narrow time-window during cell differentiation and it is mediated by the selective activation of particular signaling pathways. While PtdCho-mediated effect on neuronal differentiation depends on cAMP-dependent kinase (PKA)/calcium responsive element binding protein (CREB), PtdEtn stimulates astrogliogenesis through the activation of the Raf/MEK/ERK signaling pathway. Considering the poor capacity of neural stem cells to differentiate into neurons in the injured microenvironment, we evaluated the effect of PtdCho in the presence of reactive oxygen species and inflammatory mediators. Collectively, our results provide an additional degree of plasticity in neural stem cell specification and further support the notion that cell differentiation is a reversible phenomenon. They also contribute to our understanding of neuronal and glial lineage specification in the central nervous system, opening up new avenues to retrieve neurogenic capacity in the brain.

LI-02
UNRAVELING THE MOLECULAR MECHANISMS INVOLVED IN THE REGULATION OF LIPID ACCUMULATION IN OLEAGINOUS *Rhodococcus*

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Rhodococcus bacteria are able to synthesize significant amounts of triacylglycerols (TAG), which are accumulated as lipid droplets (LD). Global "omics" studies demonstrated that the massive synthesis and accumulation of TAG by oleaginous rhodococci demands a complex metabolic network involving several reactions at different metabolic levels. The TAG biosynthetic machinery of oleaginous rhododocci may include enzymes involved in different reactions of metabolism, transporters and structural proteins associated to LD. Moreover, cells must possess efficient mechanisms for controlling the availability of key intermediates in the metabolism, while ensuring adequate supply of the necessary energy, reducing equivalents and precursors. These processes are probably tightly regulated in cells by a regulatory circuit integrated by global as well as specific regulatory proteins, which finely coordinate the shift from the vegetative cellular state to storage status. The mechanisms that control the regulation of TAG metabolism in oleaginous rhodococci are largely unknown. Recent studies suggest that TAG accumulation is regulated directly and indirectly at different molecular levels. Glycogen metabolism, which is modulated allosterically through the control of the ADP-glucose pyrophosphorylase enzyme in *R. jostii* RHA1, provides a pool of carbon able to be re-routed to produce TAG under nitrogen limiting conditions. The availability of glycolytic intermediates regulates glycogen synthesis, which in turns control phosphorylated sugar fluxes through glycolytic pathways for ensuring the supply of energy, reducing equivalents and precursors for lipogenesis. On the other hand, the regulatory protein called NlpR increases the rate of carbon flux into lipid metabolism in *R. jostii* RHA1. NlpR is a pleiotropic regulator that contributes to the allocation of carbon into the different lipid fractions, including TAG, DAG, fatty acids and phospholipids, in response to nitrogen levels. It activates large modules of lipid metabolism, regulating the flux within the module when carbon is available. Its regulon in RHA1 include genes involved in fatty acid synthesis (FASI and FASII), and the Kennedy pathway for TAG and

phospholipid synthesis, among others. Results indicated that NlpR is not essential for TAG accumulation; however it provides a stronger redirection of carbon flux toward lipid metabolism under nitrogen limiting conditions. Recently, other regulatory protein related to TAG accumulation has been identified in *R. jostii* RHA1. The protein called MLDSR regulates the expression of MLDS, which is a protein associated to the LDs with a structural function. The expression of the cluster containing genes coding for MLDSR and MLDS seems to be controlled by the availability of fatty acyl-CoA intermediates in cells, indicating that fatty acid synthesis and LD assembly are tightly coup.

LI-03
REGULATION OF LIPID METABOLISM BY ENDOPLASMIC RETICULUM - LOCALIZED LIPASES

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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 (NAFLD) and cardiovascular disease. NAFLD is characterized by excessive TG deposition, which is the leading cause of abnormal liver functions. Hepatic TG levels are regulated by synthesis, hydrolysis (lipolysis) and secretion. We have shown that endoplasmic reticulum-localized lipid hydrolases play a significant role in regulating lipid trafficking to various metabolic fates. Carboxylesterase 1d (Ces1d, also known as Ces3/TGH) participates in the provision of substrates for very-low density lipoprotein (VLDL) assembly. Mice lacking Ces1d have decreased blood TG levels, improved glucose metabolism and are protected from high fat diet-induced fatty liver, atherosclerosis and inflammation. On the other hand, carboxylesterase 1g (Ces1g, also known as Es-x), which shares 76% amino acid sequence identity with Ces1d, exhibits different function to Ces1d. Mice lacking Ces1g show hallmarks of metabolic syndrome including insulin resistance, hyperinsulinemia, increased lipogenesis, hepatic and adipose lipid accumulation and hyperlipidemia. The endoplasmic reticulum in the liver and small intestine also harbours a type II membrane protein arylacetamide deacetylase (AADAC), which shares amino acid sequence homology with hormone-sensitive lipase. Mice lacking AADAC accumulate excessive neutral lipid stores in the liver and intestine during Western type diet supplementation. Therefore, endoplasmic reticulum-localized lipid hydrolases regulate lipid storage and trafficking. Ces1d plays a pro-atherogenic, pro-steatotic and pro-inflammatory role, while Ces1g is protective against the development of hyperlipidemia, hyperinsulinemia and insulin resistance, and AADAC protects against diet-induced steatosis.

LI-04
GANGLIOSIDES IN HUNTINGTON'S DISEASE AND BEYOND

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Huntington's disease (HD) is a neurodegenerative disorder caused by the expansion of a CAG trinucleotide repeat in the first exon of the *HTT* gene. The resulting mutant huntingtin (mHTT) protein acquires toxic conformations and aggregates within the cells, leading to neuronal dysfunction and death. There is no cure for HD and current treatments are palliative. We have shown that levels of ganglioside GM1, a glycosphingolipid highly enriched in the brain, are decreased in HD models. Administration of exogenous GM1 reduces levels of soluble and aggregated mutant huntingtin in HD mouse brains slows down neurodegeneration and corrects motor as well as cognitive and psychiatric-like dysfunctions in HD mice. Preliminary data suggest that, at least in part, the beneficial effects of GM1 might be mediated by increased secretion and clearance of mHTT through extracellular vesicles. Overall, our data suggest that GM1 has profound disease-modifying properties in HD mouse models and could be a novel treatment for HD.

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ORAL COMMUNICATIONS

BIOTECHNOLOGY

BT-C01
ENHANCED DROUGHT RESISTANCE OF *Nicotiana tabacum* BY COMPARTMENTALIZED MAIZE MALIC ENZYME EXPRESSION

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Water availability decrease has negative effects in agriculture production. When soil water content drops beyond certain level, plants reduce the stomata aperture, with a concomitant dismiss in growth and yield. A maize NADP-malic enzyme (NADP-ME) was expressed in guard cells and vascular tissues of *Nicotiana tabacum*, driven by the *Arabidopsis thaliana* Potassium channel 1 promoter. The biochemical modifications in those cells and tissues, produced by maize NADP-ME, are traduced in important phenotypic modifications in the whole plant. Compared to Wild-Type plants (WT), the transgenic tobacco lines have higher levels of sucrose, glucose, and fructose in phloem exudates and veins, produce significantly more biomass per water used and flower earlier than WT. Besides, CO_2 fixation rates at CO_2 levels above 400 ppm and light intensity above 200 μmol m^{-2} s^{-1} are significantly higher in transgenic tobacco lines. Here, we aimed to study stomatal aperture, stomatal conductance and stress resistance during a shortage of water. Transgenic lines show significantly smaller stomata pores than WT in normal irrigation conditions and are not modified during water stress. Stomatal conductance study shows significant differences within WT and transgenic lines before the stress and after the rehydration. Drought injury was significantly higher in WT than in transgenic lines. These findings show that the NADP-ME cell-specific expression causes important variations in transpiration rates and water use efficiency, emerging as an effective strategy to enhance drought resistance in the C_3 plant *Nicotiana tabacum.*

BT-C02
A GH 8 ENDOGLUCANASE FROM *Paenibacillus* sp. *A59* FOR APPLICATION IN BIOPROCESSES

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In order to deal with the energy problems affecting the modern world, sustainable alternatives to fossil fuels are being under study. Second generation bioethanol production still requires to overcome cost issues and technical limitations because the process requires highly effective cellulases and hemicellulases and efficient fermentative microorganisms. One of the most studied approaches is the prospecting of bacteria capable of degrading lignocellulolytic biomass to obtain enzymes to use during the saccharification process. In this work, an endoglucanase from the hemicellulolytic isolate *Paenibacillus sp. A59* was expressed as recombinant protein, purified in a soluble form and biochemically characterized. The protein, which we named Cel8Pa, has 409 amino acids including a conserved domain from glycosyl hydrolases family 8. By homology with previously characterized proteins, we identified the aminoacids Glu95 and Asp156 as the catalytic residues. The tertiary structure corresponded to a regular (alpha/alpha) 6 barrel formed by six inner and six outer alpha helices, determined by molecular modeling. The mature protein fused to a 6 His N-terminal tag was expressed in E. coli, and purified in a native form by IMAC, with an apparent molecular weight of 46 kDa. To determine the substrate specificity of Cel8Pa we evaluated its activity on several substrates. It had activity on barley beta-glucan (BG) (45 UI/mg), phosphoric acid swollen cellulose (PASC) (14 UI/mg), chitosan (5 UI/mg) and carboxymethyl cellulose (CMC) (4 UI/mg), while it did not have beta-glucosidase activity. The resulting main products obtained from BG hydrolysis were cello-oligosaccharides (COS) with a degree of polymerization (DP) ≥ 2, while PASC, CMC, C4, C5 and C6 were completely hydrolyzed to cellotriose and cellobiose, identified by TLC assays. Cel8Pa did not hydrolyze insoluble cellulosic substrates such as bacterial cellulose, Avicel or filter paper, in accordance to its endoglucananase activity profile. Noteworthy, long-term assays (17 h) on xylan resulted in xylose, xylobiose and xylo-oligosaccharides (XOS) of DP≥3, although no beta-xylosidase activity was observed at any time, indicating Cel8Pa has also low levels of endoxylanase activity (0.4 UI/mg). The optimal reaction conditions on BG were 40°C and pH 4.5, maintaining 50% of activity after 30 hours under these conditions. Kinetic studies indicated a Vmax of 197 ± 70 IU/μM, a KM of 0.99 ± 0.47 μM and the catalytic constant Kcat of 215.18 sec-1. We also assayed Cel8a activity on barley straw (pre-treated by extrusion), which resulted in COS/XOS of different DP after 17 h of hydrolysis. In conclusion, Cel8Pa is an endoglucanase/endoxylanase enzyme, active at moderate temperatures and acid pH, which may be used as part of an enzymatic cocktail in simultaneous saccharification and co-fermentation processes (SSCF) for lignocellulosic biomass deconstruction

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BT-C03
CHARACTERIZATION OF POLYHYDROXYALKANOATE PRODUCTION BY *KHS3*

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Hydrocarbon-degrading strain *Halomonastitanicae KHS3* was isolated from contaminated seawater in Mar del Plata harbour*Halomonas*species have been described as microorganisms that are able to degrade a variety of xenobiotic compounds and accumulate polyhydroxyalcanoates (PHAs). We have obtained the genomic sequence of *H.titanicaeKHS3* and found homologues of the phaA and *phaC* (PHA synthase), *phaP* (phasin), acetyl-CoA reductase, *phaR* (regulatory protein of PHA synthesis) and phaZ (depolymerase) genes. The aim of this work was to evaluate and characterize the ability of *H.titanicae KHS3* to produce and accumulate PHA when grown in minimal medium with excess of a single carbon source (glucose 1%). In this condition, we observed accumulation of PHA in different points along the growth curve. In order to identify the type of PHA produced by *H. titanicae KHS3*, analysis of the FTiR, DSC and RMN were conducted and the results indicated that in this growth condition *H.titanicae*accumulatespolyhydroxybutyrate (PHB). The polymer was separated from cells using sodium hypochlorite and its amount was estimated by gravimetric determinations (dry cell and PHA weights) and by spectrophotometric measurements of crotonic acid after acid hydrolysis. Our results indicated that, when grown on glucose,*H. titanicae KHS3* is able to accumulate PHB since early stages of growth, reaching up to 50 - 60 % of dry cell weight. In the stationary phase of growth, an approximate yield of 1g PHB/culture liter was obtained. The determination of mechanical properties of the produced PHBHt and its molecular weight are under way.

CELL BIOLOGY

BC-C01
GLYCOGEN SYNTHASE KINASE 3 INHIBITION PREVENTS UV ELICITED TRANSCRIPTIONAL RESPONSE AND APOPTOSIS

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UV irradiation induced DNA lesions trigger a transcriptional response that includes RNAPII hyperphosphorylation, a decrease in RNAPII elongation rate and changes in gene expression, including changes in alternative splicing patterns that lead to apoptosis. We recently described that UV induced cyclobutane pyrimidine dimers (CPDs) trigger a signal transduction cascade, mediated by ATR that ends in the above mentioned transcriptional response. To unveil other kinases involved in this cascade, we developed an alternative splicing fluorescent reporter system that allowed us to perform a screening with the Public Kinase Inhibitors Library (PKIS2) from GlaxoSmithKline. From the almost 700 inhibitors screened, 12 resulted to affect the UV induced transcriptional response and glycogen synthase kinase 3 (GSK-3) came out as the most prominent common target. Further validation of the role of GSK-3 was obtained with the highly-specific commercial GSK-3 inhibitors AR-A014418 and CHIR 99021. We found that GSK-3 inhibition prevents RNAPII hyperphosphorylation as well as the decrease in RNAPII elongation rate, the changes in alternative splicing patterns and apoptosis. Altogether, our results indicate an essential role for GSK-3 in the UV induced transcriptional response.

BC-C02
INTRAGENIC HISTONE ACETYLATION HELPS UPREGULATION OF *SMN2* EXON 7 INCLUSION BY SPINRAZA

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Spinal muscular atrophy (SMA) is caused by mutations on the *SMN1* gene causing the loss of function of the protein it encodes. Humans have a paralog gene named *SMN2*, that cannot compensate for the deficiency in the SMN protein because exon 7 (E7) is poorly included in its mature mRNA. A successful approved therapy for SMA restores normal levels of SMN expression by the use of antisense oligonucleotides (ASOs, Spinraza) designed to increase E7 inclusion in the SMN2 transcript. Our studies aim at understanding how modulation of chromatin structure as well as transcriptional elongation affect *SMN2* E7 inclusion and to design therapies complementary to Spinraza based on drugs affecting chromatin-regulated alternative splicing.We found that *SMN2* E7 inclusion is sensitive to changes in transcriptional elongation. Our results reveal that slow elongation promotes *SMN2* E7 skipping. An immediate prediction of this result is that treatments that increase RNAPII elongation should promote *SMN2* E7 inclusion, which was confirmed by the treatment of cells with the histone deacetylation inhibitor trichostatin A (TSA), which caused an increase in E7 inclusion. Furthermore, we found that the combined treatment of cells in culture with TSA and an anti-sense oligonucleotide (ASO) with similar sequence as Spinraza, promotes a bigger inclusion of E7 inclusion than treatment with each reagent separately. Our results indicate that the ASO is acting at two levels with apparently opposite effects. At the post-transcriptional splicing regulation level, it promotes *SMN2* E7 inclusion by displacing the negative splicing factors hnRNPA1 and A2. In parallel, at the co-transcriptional level, by creating a more compact chromatin structure characteristic of higher H3K9me2 levels, the ASO may inhibit RNAPII elongation, which in turn would cause lower *SMN2* E7 inclusion, counteracting the positive post-transcriptional effect. These findings would explain why by opening the *SMN2* chromatin structure, TSA is able to potentiate the upregultation of E7 inclusion by the ASO.

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BC-C03
REGULATION OF THE SODIUM/IODIDE SYMPORTER (NIS) BY CREB3L1.

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NIS is required for thyroid iodide uptake. Its expression is fundamental for the use of radioiodine in the treatment of thyroid carcinomas. The BRAF(V600E) mutation is the most common genetic alteration in papillary thyroid carcinomas. This oncoprotein confers cancer cells with a more aggressive phenotype and decreased thyrotropin (TSH)-responsiveness. In agreement, BRAF(V600E) mutation correlates with low expression levels of thyroid-specific genes, including *NIS*. We have described that in thyroid cells, the transcription factor CREB3L1 regulates the expression of transport factors in response to TSH. In addition, in bone cells, CREB3L1 regulates the expression of transport factors as well as the expression of bone-specific genes. However, the function of CREB3L1 in the regulation of thyroid-specific proteins is still unknown. Here, we analyzed the effect of CREB3L1 overexpression on NIS levels and activity (125I uptake assay) in thyroid cells. Our results show that CREB3L1 overexpression increases NIS levels and iodide uptake. Furthermore, we examined CREB3L1 in a BRAF(V600E) inducible system, where NIS levels decrease after the induction of the oncogene. Interestingly, CREB3L1 levels increase after 5 days of BRAF(V600E) induction but then decrease after 7 days. Taken together, our results indicate a dual behavior of CREB3L1 in a normal context and an oncogenic context. But CREB3L1 regulation and its impact on thyroid function remains to be characterized

BC-C04
TRANSLATIONAL CONTROL MEDIATED BY DIFFERENT DOMAINS OF ME31B IN *Drosophila*

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Development of an organism implies regulation of gene expression at several levels. The eukaryotic initiation factor 4E (eIF4E) is a key factor involved in mRNA metabolism. D. melanogaster genome encodes 8 eIF4E isoforms. The canonical isoform, eIF4E-1, plays a role in translation in the formation of P-bodies, and is a translational repressor in oogenesis when it interacts with Me31B. While eIF4E-3 is expressed in testis and control translation initiation during male germ line development. d4E-HP (4E-8) is a translational repressor in the embryo and interacts with Bicoid to inhibit the translation of caudal mRNA. Previous results of our laboratory showed that Me31B interacts with eIF4E-1 and eIF4E-3, both in the yeast two-hybrid system and in S2 cells by FRET. We demonstrate that Me31B is also expressed during spermatogenesis and co-localize with eIF4E-3 in the male germ cells.Here we have further investigated the molecular interaction of Me31B and eIF4Es using two-hybrid assay. We generated Me31B truncated proteins and point mutation by site directed mutagenesis. Our results indicate that the binding sites of Me31B and eIF4E-1 or eIF4E-3 are located in a different domain inside the Me31B sequences. Specifically eI4E1 binds to D1 domain ($Me31B^{1-96}$) while eIF4E3 binds to D2 domain ($Me31B^{366-459}$). We also showed that the 4E mutants that delocalize the PBs ($eIF4E\text{-}1^{W117A}$ y $eIF4E\text{-}3^{F103A}$) are not able to interact with Me31B.These results suggest that the development of the germ line requires the mRNA silencing mediated by the interaction of specific factors depending on whether it is the female or the male, through differential domains of interaction

BC-C05
JOINT PROCESSING OF APOPTOTIC CELLS AND *Pseudomonas aeruginosa* BY MACROPHAGES

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Pseudomonas aeruginosa (PA) is an opportunistic pathogen that infects vulnerable patients such as those with cystic fibrosis (CF). CF is characterized by an increased number of apoptotic cells in the airways as well as impaired efferocytosis: a process in which apoptotic cells are engulfed and dispose of by other cells. We have shown that PA binds to apoptotic cells and is internalized by epithelial cells through efferocytosis. Bacteria are then eliminated intracellularly. Here we demonstrate that bacteria-laden apoptotic cells are targets for J774 macrophage-like cells and Bone Marrow-Derived Macrophages (BMM). Macrophages internalize apoptotic cells and PA individually or together, constituting three differentiated cell populations: macrophages with apoptotic cells, with PA, and with both. Classically or alternatively activated macrophages display enhanced phagocytic or efferocytic programs, respectively. Our aim is to evaluate the response elicited by macrophages facing both stimuli (i.e. bacteria and apoptotic cells) simultaneously. To study bacterial clearance we measured intracellular survival over time. Both types of macrophage cells eliminate PA, which was found inside LAMP1-positive vesicles suggesting that its final fate is elimination inside lysosomes. Also apoptotic material was found inside these vesicles. We also analyzed the phagocytic and efferocytic efficiency. The presence of PA diminishes apoptotic cell uptake. However, the presence of apoptotic cells does not affect the uptake/elimination of bacteria, suggesting that macrophages displayed rather a phagocytic phenotype non-withstanding the presence of apoptotic cells.

BC-C06
LC3 OVEREXPRESSION MODULATES THE SECRETOME OF SENESCENCE TUMOR CELLS INDUCED BY RADIATION

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Senescence is a tumor suppression mechanism actioned by stimulus that affects cell genome integrity. However, senescence cells (SCs) promoted tumor cell growth and migration by pro-inflammatory factors secretion known as SASP (Senescence Associated Secretome Phenotype). Ionizing radiation exposition during radiotherapy is a stress stimulus that induces senescence, autophagy and other cell responses. In

this way, autophagy induction by radiation may inhibits senescence or alter SASP composition. Microtubule-associated protein 1A/1B-light chain 3 (LC3), is a canonical factor involved in autophagy induction. Objective: To study the effect of B16F0 tumor cell LC3 overexpression on radiation induced senescence and SASP activity. Methodology and Results: Cell cultures of murine melanoma cell line B16F0 were seeded and 24h later transiently transfected or not with pRFP-LC3 plasmid (B16F0-LC3). B16F0 and B16F0-LC3 cells were exposed or not to 10Gy gamma radiation (iB16F0, iB16F0-LC3 and B16FO respectively). After 72h irradiation, senescence was evaluated by β-galactosidase assay. Radiation increased the percentage (SCs/total cells) (82,4 ± 9, iB16F0, 79,5 ± 4, iB16F0-LC3 vs 7 ± 3, B16F0; $p \leq 0,01$). To evaluate the tumorigenic potential of SASP, conditioned media (CM) from B16F0 (control CM), iB16F0 (iCM) and iB16F0 LC3 cultures (iLC3 CM) were obtained. A wound healing assay was made incubating arrested B16F0 cell with these CMs. The assay showed that iCM increased cell migration (iCM vs control CM; $p \leq 0,05$) while LC3 overexpression partially reverted this effect (iLC3CM vs iCM $p \leq 0,05$; iLC3CM vs control CM; $p \geq 0,05$). Conclusions: B16F0 tumor cells in gamma radiation induced senescence produce a secretome with a tumorigenic activity that can be modulated by LC3 overexpression. Further studies should be done to understand the mechanisms involved and its potential therapeutic phenomenon.

BC-C07
CYTOSKELETAL DYNAMICS AT THE LEADING EDGE OF OVARIAN CANCER CELLS IS ENHANCED BY LA AND OL

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Tumoral cell migration is one of the main mechanisms of metastatic pathogenesis in ovarian cancer. We studied the effect of unsaturated Fatty Acids (FA) in vitro on migrating ovarian cancer cells and the role of the cytoskeleton on the regulation of this process. We used the human ovary cancer SKOV-3 cell line, in a scratch wound setting to assess different variables regarding cell migration. Low dose (16 μM) of Linoleic (LA) and Oleic fatty acids (OL) promoted migration, while Linolenic acid did not. Single cell tracking revealed that LA and OL treated cells migrate faster, more straight, polarized and orientated toward the wound closure than control. Microtubule and centromere analysis of migrating cells also showed a time dependent increase in polarization that was enhanced in the presence of LA and OL. Confocal video microscopy of EB3-Cherry transfected SKOV-3 cells exhibited an increase in microtubule +end speeds at the cell front. LifeAct-GFP SKOV-3 cells showed that FAs administration induce an increase in number, length and protrusion speeds of fillopodia at the cell front. FA treated cells featured higher lamellipodial actin dynamics with increased retrograde actin waves speed and frequency. FRAP analysis displayed an increment of actin turnover when compared with control. All these data together indicate that LA and OL modify cytoskeletal actin and tubulin protrusion dynamics at the leading edge of migrating ovarian cancer cells enhancing cell migration rate. We propose that FA composition of the peritoneal fluid could alter metastasicbehaviour in ovarian cancers due to alterations in cell migration.

BC-C08
MECHANISTIC ANALYSIS OF INFLUENZA A VIRUS GLYCOPROTEIN COMPENSATION

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Influenza viruses spread around the world causing seasonal epidemics, resulting in about 3 to 5 million infections and 250,000 to 500,000 deaths annually, and also sporadic pandemics, which have historically occurred every 10-40 years. The continued threat of influenza viruses in the human population is associated with its ability to escape protective immunity, to be transmitted through respiratory droplets, and the frequent emergence of antigenically novel viral strains from avian and non-avian animal reservoirs. Given the large public health and economical burden due to circulating influenza viruses, their improved control through effective anti-viral strategies and efficient immunization protocols is immediately needed. However, the performance of new-released therapeutic and preventive approaches is usually compromised due to the rapid acquisition of amino acid substitutions in two viral surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). This phenomenon, also known as “antigenic drift”, allows influenza viruses to evade adverse conditions that may affect their proper replication and propagation, like the host immune humoral response and anti-viral therapies. Despite of their beneficial outcome, mutations that affect HA or NA functions can also drastically impair viral fitness. Due to HA and NA opposite functions during the viral life cycle, influenza viruses frequently incorporate secondary or “compensatory” changes in one or another glycoprotein in order to maintain in equilibrium the delicate HA/NA balance and thus maximize viral replication. Our results indicate that under certain conditions of selective pressure (exposure to anti-HA neutralizing antibodies), influenza A virus (IAV) compensates harmful mutations in HA by incorporate new amino acid substitutions in NA, which not only leads to anti-viral drug resistance but also impairs NA incorporation into nascent virus particles, and by inference, NA activity per virion. Consistent with this observation, we also found that such mutations in NA have a profound impact at the host level, mainly affecting NA conformation stability and its intracellular trafficking from the endoplasmic reticulum (ER) to the plasma membrane, the primary place where the new viral progeny is assembled. Our observations are original and important in several ways: 1) they suggest a previously uncovered, multilayered mechanism, by which drifted IAVs not only maximize viral fitness compensating glycoprotein activity but also acquire drug resistance; 2) in addition, our comprehensively characterization of the cellular pathways and critical factors involved in the maturation and trafficking of NA undoubtedly offers an excellent opportunity for discovering potential therapeutic targets

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BC-C09
EXPLORING THE DRIVING FORCES INFLUENCING S-ACYLATION OF PERIPHERAL PROTEINS AT THE GOLGI COMPLEX

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S-acylation of proteins is a post-translational modification that consists in the addition of long chain fatty acids to cysteines, through a reversible thioester bond. For soluble proteins, this modification confers stable attachment to membranes, influencing their trafficking and subcellular distribution. S-acylation is catalyzed by a family of transmembrane Acyl Protein Transferases (PATs) distributed along the secretory pathway, mainly at the Golgi complex. Myristoylation or farnesylation of many soluble proteins constitute the first transient membrane adsorption before being S-acylated. On the other hand, some S-acylated soluble proteins do not possess hydrophobic modifications involved in the initial membrane interaction, as observed in GAP-43, a neuronal protein involved in axonal growth and regeneration. We reported that the signals for GAP-43 S-acylation are contained in the first 13 amino acids (N13GAP-43), including two acylatable cysteines (C3, C4) embedded in a hydrophobic region followed by a basic cluster. By biochemical, fluorescence microscopy and time-lapse imaging experiments, we found that mutation of critical basic amino acids drastically reduced membrane interaction and hence S-acylation of N13GAP-43 both in neuron-like and epithelial cells, without affecting substrate recognition by PATs as revealed in a forced Golgi complex interaction of the mutated protein via binding to PI4P. Moreover, pharmacological depletion of PI4P markedly reduced GAP-43 membrane binding, strongly suggesting that few basic residues around C3 and C4 mediate electrostatic interactions with anionic lipids at the Golgi complex, where GAP-43 is S-acylated.

BC-C10
SUPINE FALLS IN *Ceratitis capitata*CORRELATE WITH DISTINCT GENE EXPRESSION PROFILES

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The peculiar supine behavior of young males of the pest-fly *Ceratitiscapitata*, measured in experimental laboratory conditions, seems to be a presumptive indicator of longevity. The different profiles of supine falls correlate with a number of behavioral and molecular parameters. When during six days-long experiments flies showing frequent supine falls were compared to those showing low frequency of falls (or not falls at all), using an RNA extraction and sequencing approach, significant differences were found in gene expression. Certain stress genes change more than twice their expression; as well as genes related to ataxias in other animals, including vertebrates. Surprisingly, certain glycosyl transferases, lectins and genes related to the innate immune system might change several-fold their expression. Main data were confirmed by RT-PCR and/or protein biochemistry. Data on supine behavior were also correlate with interaction behavior of the Medflies in three-flies experimental laboratory minimal lek models using a specially designed arena and recording device that allows the analysis of two-hour bouts, dayly during six days. In many experiments, the age of death was recorded to be correlated with other individual parameters within the lek or compared to similar flies in other leks.

ENZYMOLOGY

EN-C01
FUNCTIONAL CHARACTERIZATION OF ATYPICAL THIOREDOXINS FROM *Entamoeba histolytica*

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Entamoeba histolytica, a unicellular parasite, usually lives and multiplies within the human gut, under reduced oxygen pressure. During tissue invasion, it is exposed to increased amounts of reactive oxygen species, which are highly toxic for the parasite. The metabolic pathways used by this organism to cope with such environmental changes and redox homeostasis are a matter of our work. Recently, we characterized in *E. histolytica* its functional thioredoxins system, composed by thioredoxins (TRXs) and thioredoxin reductase (TRXR). In this work, we present the functional characterization of three atypical TRX from *E. histolytica* (*Eh*TRX111, *Eh*TRX212 and *Eh*TRX289). *Eh*TRX111 (a tail-anchor protein) and *Eh*TRX289, but not *Eh*TRX212, were able to *in vitro* reduction (via *Eh*TRXR) of cystine, CySNO, insulin and *Eh*2CysPrx. However, *Eh*TRX212 was able to coordinate iron-sulfur cluster (ISC) by an *in vitro*reconstitution assay. By gel filtration chromatography and UV-Vis spectroscopy experiments were detected *Eh*TRX212-ISC complexes. In other hands, we observed the nitrosationCySNO-dependent of *Eh*TRXs. This redox modification generated a partially reversible inactivation of the disulfide reductase activity of these proteins. This suggests that this modification could act as a possible regulation of their activity. This work strongly supports the occurrence in *E. histolytica* of new TRXs, which were not previously described in the parasite. Our results extend the knowledge regarding to *Eh*TRX function and suggest that these proteins have important functions in redox and iron metabolism of this pathogen parasite. Granted by ANPCyT (PICT2014-2103 and PICT2016-1778).

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EN-C02
BIOCHEMICAL AND GENETIC CHARACTERIZATION OF PYRUVATE DECARBOXYLASE FROM THE YEAST *Candida zemplinina*

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Pyruvate decarboxylase (PDC) is the enzyme responsible for the non-oxidative decarboxylation of pyruvate to acetaldehyde and carbon dioxide. PDC plays an essential role in the yeast fermentation pathway leading to ethanol production. Saccharomyces cerevisiae has three active PDC isoenzymes (PDC1, PDC5 and PDC6) which, similar to PDC from other yeasts species, exhibit substrate activation. Here we present the first biochemical and genetic characterization of PDC from the yeast *C. zemplinina* (syn., *Starmerellabacillaris*). CzPDC activity was readily detected in total cell lysates from *C. zemplinina* type strain 9494 as well as from several indigenous *C. zemplinina* isolates from spontaneously fermenting grape musts. Characterization of kinetic parameters of PDC1 and CzPDC, through steady state measurement, showed that CzPDC does not present substrate activation but a standard Michaelis-Menten kinetic behavior. In silico analyses of the recently available complete genome sequence of several *S. bacillaris* isolates, including *C. zemplinina* type strain 9494, suggest the existence of a single gene encoding CzPDC. Further analyses of the conceptually translated amino acid sequence of CzPDC revealed that critical amino acids at the PDC catalytic site are highly conserved among CzPDC and S. cerevisiae PDC1, PDC5 and PDC6. However, critical residues C221, H92 and H225, involved in PDC substrate activation and present in PDCs from several yeast species, are absent in the *C. zemplinina*enzyme. This observation supports the results obtained from the kinetic analyses of CzPDC. This report represents the first description of a yeast PDC lacking substrate activation.

LIPIDS

LI- C01
THE NUCLEAR-LIPID-DROPLET PROTEOME

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Nuclear-lipid droplets (nLD) are a dynamic organelle that store neutral lipids in a hydrophobic triacylglyceride-cholesterol-ester core enriched in oleic acid (OA) surrounded by a monolayer of polar lipids, cholesterol, and proteins. nLD are probably involved in lipid homeostasis as a buffer that provide or incorporate lipids and proteins in signaling pathways, as transcription factors and enzymes of lipid metabolism and nuclear processes. In nLD proteome analysis, we hypothesized that nLD-monolayer proteins could be involved in similar functions as cytosolic LD (cLD). We analyzed rat-liver-nLD proteome under physiological and nonpathological conditions by GeLC-MS2. Since isolated nLD are highly diluted, a protein-concentrating isolation protocol was designed. 35 proteins were identified within the functional categories: cytoskeleton and structural (31%), transcription and translation (23%), histones (20%), protein-folding and posttranslational modification (8.5%), cellular proliferation and/or cancer (8.5%), lipid metabolism (6%), and transport (3%). nLD contained an enzyme from lipid metabolism, carboxylesterase 1d (Ces1d/Ces3), whose intranuclear localization was confirmed by fluorescence microscopy in HepG2 cell line. Ces1d/Ces3 was observed to be involved in nLD- and cLD-population dynamics upon stimulation by external-OA. These results—the first describing nLD proteome—demonstrate that a tandem-GeLC-MS2-analysis protocol facilitates similar studies on rat-liver nuclei. A diversity of cellular-protein functions was identified indicating the direct or indirect nLD participation and involving Ces1d/Ces3 in LD-population dynamics.

LI-C02
NITRO FATTY ACIDS: NOVEL CD36 LIGANDS WHICH MODULATE FATTY ACIDS METABOLISM

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CD36 is a high affinity receptor that facilitates the binding and uptake of long-chain fatty acids and modified-LDL into the cell. These CD36 ligands have also been described to trigger cell signaling with different effects on cell metabolism. Since nitro-fatty acids are electrophilic lipid mediators which exhibit anti-inflammatory and cytoprotective actions in experimental models of atherosclerosis and other inflammatory diseases, we have hypothesized that nitrolipids may bind and signal through CD36 receptor. Hereby, we demonstrated that Nitro-Oleic Acid (NO2-OA) displays CD36-mediated intracellular signaling via Src/ERK and AMPK pathways. Pharmacological strategies to inhibit both ligands binding to CD36 (SSO) and CD36 downstream signaling pathway for ERK (PD98059) and Src (PP1), allowed us to elucidate the specificity of the pathway involved. Therefore, to study their interaction, an in vitro assay was developed using recombinant CD36 (rCD36) and biotinylated-NO2-OA. This reaction, revealed by Western blot, exhibited that NO2-OA interacts with CD36. Competition assays with increasing molar concentration of OA, GSH and unbiotinylated NO2-OA exposed the reversibility of this interaction. Similar experimental settings showed that NO2OA impairs modified-LDL binding to rCD36, which was supplemented with experiments in RAW264.7 macrophages cell line, where pre-incubation with NO2-OA showed a decrease in cholesterol accumulation after modified-LDL treatment. Altogether this data suggests that NO2-OA acts as a CD36 ligand and triggers its downstream signaling to modulate modified-LDL uptake and fatty acid metabolism in macrophages.

LI-C03

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PTEN ACTIVITY REGULATES TARGETING OF GP135 AND CELL DIFFERENTIATION IN MDCK CELLS

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MDCK cell differentiation is characterized by the development of a mature apical membrane enriched in gp135 and primary cilium formation. The correct apical targeting of gp135 is necessary for MDCK cells to organize a single lumen at the apical surface. We have previously demonstrated that the correct localization of PTEN, a key enzyme in the metabolism of the polyphosphoinositides, depends on glycosphingolipid metabolism, and this feature is essential for MDCK cell differentiation. In this study we analyzed the development of primary cilium and the distribution of gp135 in cells cultured under hypertonicity (inductor of cell differentiation) in the presence of SF1670, a selective PTEN inhibitor. MDCK cells stably transfected with GFP- tagged gp135 (gp135 - GFP) cultured under hypertonicity 48 h post - confluence developed a differentiated phenotype with apical accumulation of gp135 and primary cilium, like wild type cells. Interestingly, gp135 - GFP transfected cells cultured under hypertonicity treated with SF1670 revealed aberrant lumens at the lateral domain and mistargeting of gp135 - GFP to this structure, and the same effect was found for endogenous gp135. Furthermore, the treatment with SF1670 also altered primary cilium formation. These effects were observed when SF1670 was added together with hypertonicity, but not when it was added once the cells were already differentiated. In conclusion, the disruption of epithelial cell differentiation induced by PTEN inhibition suggests an interplay between phosphoinositides and glycosphingolipids that govern the correct sorting of polarity proteins.

LI-C04
DUAL FUNCTION OF SPHINGOSINE-1-PHOSPHATE RECEPTOR 2 IN EPITHELIAL CELL MIGRATION

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Epithelial cell differentiation is a process that involves the mesenchymal to epithelium transition (MET) and includes cell cycle arrest, cell-cell junction maturation in addition to changes in cell migration capacity. Previous results from our laboratory showed that Madin-Darby Canine Kidney (MDCK) cultured in hypertonic medium activate a cell differentiation program that depends on changes in sphingolipid metabolism. One of the most important sphingolipids is sphingosine-1-phosphate (S1P) that can act both intracellularly as second messenger and extracellularly as ligand for cell surface receptors (S1PRs). In the present work we evaluated the importance of S1P in the modulation of cell migration and its association with cell differentiation. We performed wound healing, colony dispersal and immunofluorescence studies using proliferative, polarized and differentiated MDCK cells. S1P receptor 2 (S1PR2) inhibition mediated by the specific antagonist JTE-013 increased the migration capacity of polarized and proliferative cells, while the opposite effect was found in differentiated cells. In colony dispersal assays, proliferative cells overexpressing S1PR2 failed to migrate and formed tightly clustered colonies. These results suggest that S1PR2 plays a dual role, acting both as a migration inhibitor and promoter depending on the differentiation stage of epithelial cells.

LI-C05
SPHINGOSINE KINASE 2 COORDINATES THE DISASSEMBLY OF CELL JUNCTIONS DURING CELL EXTRUSION

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The maintenance of the epithelial integrity is essential to prevent inflammation, inappropriate growth factor signaling, and invasion by pathogens. Epithelial integrity requires a high cell turnover given by coordination between cell division and cell extrusion. Cell extrusion acts as key regulator of cell density homeostasis by removing the cells and preserving tissue integrity. This process is triggered by the release of sphingosine-1-phosphate (S1P) which activate S1P receptor 2 and produces the contraction of an actomyosin ring in the neighboring cell. The contraction squeezes the cell out apically while drawing together neighboring cells and preventing any gaps to the epithelial barrier. Previously, we demonstrated that cell extrusion is triggered by sphingosine kinase 2 (SK2) in differentiated MDCK cells. The goal of this work was to study the implication of SK2/S1P pathway in the disassembly of cell junctions that occurs as a mandatory process before cell extrusion. For this end, differentiated MDCK cells were subjected to SK inhibitors or knocked down for SK2. After incubation, focal adhesion (talin and vimculin) and adherens junction (E-cadherin and β-catenin) proteins were visualized by fluorescence microscopy in extruding cells. We found that SK2 participates in the disassembly of cell-cell and cell-matrix junction. Moreover, we found that the inhibition of SK2 impairs the degradation of E-cadherin. These results show that SK2 / S1P pathway besides activating the process in neighboring cells also has an effect on the extruded cell itself, regulating in a global and coordinated manner the entire cell extrusion process.

LI-C06

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EFFECT OF CULTURE TEMPERATURE ON FATTY ACID COMPOSITION OF DIATOM *Cylindrothecaclosterium*

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Fish oil is widely used as a source of essential long chain poly-unsaturated omega-3 fatty acids, such eicosapentaenoic (EPA) and docosahexaenoic (DHA), for aquaculture. However, there is increasing interest in reducing the aquaculture industry's dependence on this resource due to its unsustainability and variable cost and supply. Marine microalgae naturally produce EPA and DHA fatty acids and their content can be modified by manipulation of the growth conditions. The aim of this study was to assess the effect of culture temperature on fatty acid composition of the marine diatom *Cylindrothecaclosterium*. To this end, *C. closterium* was grown in a photobioreactor: a) at 20 °C (control) and b) lowering the temperature from 20°C to 11°C in the stationary growth phase. Total lipid content and lipid fractions were determined spectrophotometrically and gravimetrically. Gas chromatography was performed to analyze fatty acid composition. *C. closterium* growth was not affected by temperature variation, as showed by cell density and dry weight determinations. When the temperature was lowered, triacylglyceride content significantly increased compared to the control condition. In addition, among omega-3 fatty acids, DHA and EPA showed a marked increase. Thus, these results evidence the potential of this strain as an alternative and sustainable source for aquaculture purposes.

LI-C07
CELLULAR LIPIDS CHANGES DURING ADIPOSE-DERIVED STEM CELLS OSTEOGENIC DIFFERENTIATION

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Adipose-derived Stem Cells (ASC) constitute a promising tool for many applications in regenerative medicine. In the last years, a large number of works describing the mechanism underlying ASC differentiation was published but the role of lipid metabolism in this process is still unclear. The aim of this work was to evaluate whether osteogenic differentiation of ASC involves changes on lipids content and biosynthesis. For this, adipose tissue was removed from adult Wistar rats and ASC were isolated and culture in low glucose DMEM through 3 passages. After that, ASC were characterized by measuring their capacity to differentiate into the osteogenic and adipogenic lineages and the expression of CD90. Third passage ASC was cultured and in vitro differentiated or not to an osteoblast phenotype with low glucose DMEM containing 100 nM dexamethasone, 10 mM β-glycerophosphate and 5 µg/ml ascorbic acid 2-phosphate. After 21 days, cellular lipids and phospholipid (PL) species were quantified. To evaluate lipid synthesis, cells were incubated in the presence of 0,1 µCi [14C(U)]-glycerol for 3h, and then lipids biosynthesis was determined. After treatment, differentiated cells showed a decrease on lipid synthesis and phospholipid content compared to control cells. Total PL content decreased by 54%, while PL species content varied in a different manner, leading to a different PL profile with enrichment in sphingomyelin in differentiated cells membranes. PL, Diacylglycerol (DAG) and Triacylglycerol (TAG) synthesis decreased by 90, 87 and 86%, respectively. Results show that ASC osteogenic differentiation is accompanied by changes on lipid synthesis and content

LI-C08
EXPRESSION OF GENES INVOLVED IN LIPID AND FATTY ACID METABOLISM IN *EX VIVO* CULTURED MOUSE TESTES

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Spermatogenesis has been achieved in vitro using neonatal mouse testes maintained in a gas-liquid interphase culture system. In this setting, it is possible to manipulate lipid metabolism to know its role during the spermatogenic process, thereby gathering information potentially useful for ex vivo spermatogenesis technology. Here, the progression of spermatogenesis was followed in mouse explants at cytological and histological levels and the gene expression of some proteins involved in lipid metabolism were compared in vitro vs. in vivo. Two PUFA elongases (Elovl2 and Elovl4), fatty acid 2-hydroxylase (Fa2h), two fatty acid binding proteins (Fabp3 and Fabp9) and a diacylglycerol acyltransferase (Dgat2) were examined by RT-qPCR. Testis explants from 6 days-old CD1 mice were cultured for 22 days. Primary spermatocytes (PS) appeared at around days 10-12, and the first round spermatids (RS) emerged after day 18 to become abundant at day 22. The whole process showed a delay compared with that in vivo. Interestingly, akin to findings in vivo, mRNA levels of Elovl4 were high in the pre-meiotic phase to decrease thereafter, while those of Elovl2 steadily increased from days 1 to 22. Fabp3 mRNA also increased with time in the explants, linked to interstitial cells differentiation. Maximal expression of Fa2h, Fabp9, and Dgat2 occurred at day 22 in culture, associated with the increase in RS numbers. Our results suggest that finding influences that promote lipid metabolism (growth factors, hormones) will be a promissory way to optimize spermatogenesis in explants. Supported by the MCIyU, Spain [BFU2013–42164-R, BFU2017–87095-R to JdM], PGI-UNS [24/B272 to GMO].

LI-C09

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NOVEL ROLES OF MTP ON CANCER DEVELOPMENT, CELL SURVIVAL AND MIGRATION

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Microsomal triacylglycerol transfer protein (MTP) locates in the lumen of the endoplasmic reticulum and participates in the secretion of lipids from the liver as very low density lipoproteins. There is evidence that MTP might be involved in other cellular processes, including the pathogenesis of different diseases; however, no studies were performed yet to evaluate if MTP plays a role in cancer. Therefore, the objective of this work was to study the participation of MTP on liver cancer. First, we assesed MTP mRNA and protein expressions in livers from rats subjected to a 2-phase model (initiation-promotion – IP) of chemical hepatocarcinogenesis. We observed an increase in MTP expression in IP group vs. control (C) group. Then, we analyze MTP protein expression in C57Bl/6 mice euthanized 6 (early stage) or 40 weeks (late stage) after hepatocarcinogenic treatment. Liver MTP protein levels were significantly higher on early stages, compared to C animals, whereas differences in protein expression were less evident between both groups on the late stages. Additionally, we studied the effects of an MTP inhibitor (Lomitapide) on hepatocellular carcinoma cell lines Huh7 and HepG2. MTT assay showed that Lomitapide significantly reduced cellular viability in a dose-dependent manner compared to untreated cells in both cell lines. This result was confirmed by clonogenic survival assay. Wound healing assay showed that also the cellular migration was significantly reduced in a dose-dependent manner upon Lomitapide treatment. Conclusion: although preliminary our results demonstrate a novel role for MTP on cancer development, cell survival and migration

LI-C10
PIP 2 PROMOTES MEMBRANE CURVATURE AND IS A SIGNALING HUB IN HUMAN SPERM ACROSOME EXOCYTOSIS

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The human sperm has a secretory vesicle that undergoes exocytosis when challenged with different stimuliknown as acrosome reaction (AR),which requires the fusion of the outer acrosome membrane and plasma membrane. We reported that DAG stimulates AR by feeding into a PKC and PLD1-dependent positive loop that supplies PIP2. We hypothesize that PIP2 synthesis is required to produce DAG and IP3, and to induce a change in the acrosomal membrane curvature. Our Molecular Dynamics simulations and TEM experiments demonstrated that PIP2 increase induces the formation of deep acrosomal membrane invaginations; although it was not able to induce acrosome swelling, AR or membrane disruption. However, for the AR to proceed, PIP2 needs to be hydrolyzed. Previous work led us to propose the following signaling pathway: cAMP–>Epac–>Rap1–>PLCε. The hydrolysis of PIP2 generates IP3, which binds IP3-sensitive channels and releases Ca2+ from the acrosome. We demonstrate the presence of a nucleotide exchange factor activated by DAG (RasGRP1), described to activate Rap1 in secretory cells, by WB and IFI. Also, we proved RasGRP1 ability to trigger AR in a dose-dependent manner. Furthermore, as shown by Far-IFI, DAG was able to activate Rap1 indicating that it is involved in the pathway described previously. Our findings highlights the dual role of PIP2 in exocytosis and report a direct evidence of the presence and function of RasGRP1 in the signaling module cAMP–>Epac–>Rap1–>PLCε in AR.

MICROBIOLOGY

MI-C01
IDENTIFICATION OF A *Streptomyces* NATURAL PRODUCT WITH POTENTIAL ANTI-VIRULENCE PROPERTIES

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Salmonella is an enteropathogen responsible for a wide range of diseases in humans and animals. In *S.* Typhimurium, the PhoP/PhoQ two-component system (TCS) controls both adaptation to low Mg2+ environments and key virulent phenotypes. Hence, and considering that TCS-signalling is not present in humans, PhoP/PhoQ constitutes an optimal target for the identification of new compounds with pharmacological potential against *Salmonella* infections. The aim of this work was to carry out a screening of culture supernatants from a library of *Streptomyces* species -well known for their ability to produce secondary metabolites with clinical value- to identify molecules that could modulate PhoP/PhoQ TCS, and therefore inhibit the virulence of *S.* Typhimurium. 118 *Streptomyces* strains were grown and their corresponding supernatants were tested against *Salmonella* strains carrying a *lacZ* transcriptional reporter fusion to PhoP-activated genes. Samples that significantly repress reporter activity and did not affect *Salmonella* growth were further analyzed. Highest repression effect was founded for the *Streptomyceseurocidicus* culture supernatant. Differential solvent organic extractions allowed toanalyze different fractions of this extract by TLC, which were subsequently revealed by bioassays based on the inhibitory activity of PhoP-activated genes. These assays together with a purification of this extract, suggested that a nitroimidazole scaffold is responsible for the repression activity. In sum, we identified a naturally secreted compound from *S. eurocidicus* with potential anti-virulence properties over *S.* Typhimurium.

MI-C02

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Salmonella-SPECIFIC TRANSCRIPTIONAL REGULATOR AFFECTING BIOFILM FORMATION AND VIRULENCE

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Salmonellosis is among the most common foodborne diseases, with millions of human infections occurring worldwide every year. A key aspect of*Salmonella*'s life cycle that contributes to its high prevalence is its ability to form biofilms, bacterial communities embedded in a self-produced extracellular matrix that allows them to adhere to each other and to diverse surfaces. This multicellular behavior facilitates persistence and transmission between hosts, and survival in the environment. In*Salmonella*, the extracellular matrix is composed mainly by cellulose and curli fimbriae whose synthesis are controlled at transcriptional level through the expression of its master regulator, CsgD. Expression of this transcriptional activator is in turn finely regulated by several transcription factors that integrate different environmental signals. We identified a previously uncharacterized*Salmonella*-specific transcription factor, MlrB, that affects biofilm-formation by controlling the induction of the Csg regulon, and hence the switching between planktonic and sessile lifestyles. We purified MlrB and determined that it expression is maximal under conditions relevant to the infection, which agrees with the regulation observed for members of the SPI-2 locus. We also established that MlrB is essential for intracellular survival in macrophages, allowing us to postulate it as a link between the biofilm formation and*Salmonella*pathogenesis

MI-C03
ROLE OF RadA FACTOR IN THE GENETIC RECOMBINATION OF *Pseudomonas aeruginosa*

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Genetic recombination in bacteria plays an important role in the repair of chromosomal DNA breaks and is one of the causes of resistance to the clastogenic effect produced by some antibiotics. We have analyzed DNA recombination mechanisms in the human opportunistic pathogen*Pseudomonas aeruginosa*, and found the coexistence of both RecA dependent and independent pathways in this species. In order to determine the factors that mediate these mechanisms, we selected a number of genes encoding proteins with known/suspected involvement in recombination, including the*radA*gene whoseArchaeaand*E. coli*homologues encode a recombinase and a helicase, respectively. Using a genetic system previously developed in our laboratory, we determined *in vivo* recombination rates for several mutant strains. In particular, a*radA*mutant showed recombination rates as low as that observed in*recA*mutants (200-fold decrease compared to the WT strain). Also, analysis of the β-galactosidase reporter present in our genetic system indicated that the loss of enzyme activity (mutagenesis) previously detected in recombinant clones of WT and other strains is not observed in RadA deficient cells. Lastly, we found that the *radA* strain showed higher levels of cisplatin sensitivity than the WT strain, consistent with the recombination rates measured for this strain. In conclusion, we report here results indicate that RadA plays a key role in the recombination mechanisms of *P. aeruginosa*, not previously described. We are currently studying the *in vitro* activity of RadA; as well as its interplay with the main recombination player, the RecA recombinase.

MI-C04
BIOCHEMICAL CHARACTERIZATION OFCYCLOPHILINS in *Brucella*

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*Brucella*is an intracellular bacterial pathogen that causes the worldwide zoonotic disease brucellosis.*Brucella*virulence relies on its ability to transition to an intracellular lifestyle within host cells. Comparative proteomic studies identified two overexpressed proteins during*B. abortus*intracellular life characterized as potential cyclophilins by sequence analysis, called CypA and CypB. Cyclophilins are enzymes that catalyze *cis*/*trans* isomerization of peptide bonds that involve prolines (PPIase).*Brucella*'s cyclophilins play an important role in stress adaptation and virulence. While CypA shares homologies with cyclophilins of Gram-negative bacteria, CypB has primary protein structure characteristics of eukaryotic cyclophilins, thus, inhibible by Cyclosporine A (CsA). The relation with pathogenesis coupled with the sequence similarity to eukaryotic cyclophilins is strongly suggestive of CypB being deployed by*B. abortus*as an effector that mimics the host Cyclophilins. We analyzed PPIase activity and inhibition by CsA of recombinant proteins and evaluated*in vitro*and*in vivo*chaperone role performing *Nde*I residual activity assay and a bacterial stress survival comparison, respectively. Additionally, we examined formation of cyclophilin oligomers using SDS-PAGE, gel filtration and bacterial two-hybrid system. Our results showed that recombinant CypA and CypB present PPIase and chaperone activities and that PPIase activity of CypB is more inhibited by CsA than PPIase activity of CypA, supporting in silico information of activity and homology. We observed that CypB dimerized*in vitro*and*in vivo*, while CypA did not, suggesting distinct functions.

MI-C05
*Bordetella bronchiseptica*BdcA REGULATES BIOFILM FORMATION IN A BrtA-DEPENDENT MANNER

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The genus*Bordetella*is composed of multiple species involved in airways tract infections.*B. bronchiseptica*is able to persist into their hosts during long periods of time and its biofilm formation ability is proposed to be involved in this process. In order to advance in the understanding of this host-pathogen interaction we analyzed the contribution of the c-di-GMP regulated adhesin BrtA in physiologically relevant culture conditions.Hewllet and co-workers have recently shown that physiological concentrations of albumin and calcium (BSACa) elicit a massive

increase in the RTX-family protein Adenylate Cyclase (AC) secretion. Like AC, the BrtA adhesin is a RTX-family protein so we hypothesized that BSACa could also have an effect on BrtA secretion. Our western blot results showed that BSACa also increased BrtA secretion. We also studied BrtA mediated biofilm formation in the presence of BSACa using multiple approaches. We used microtiter dishes, glasses and plastic tubes and analyzed the results using CV staining and Scanning Electron Microscopy. We observed a significantly reduction in biofilm formation in the Δ*brtA* strain, while Δ*lapG* strain showed a significantly enhanced biofilm formation compared with wild type strain. To get a deeper understanding on the role of c-di-GMP in this process we performed biofilm formation assays with deletion mutants in two putative diguanylate cyclases involved in BrtA localization. Using biofilms and dot blots techniques we conclude that BdcA but not BdcG is involved in BrtA-dependent biofilm formation on these physiological conditions.

MI-C06
CHARACTERIZATION OF THE FIRST HOMOMERIC MULTIDOMAIN ACETYL-COA CARBOXYLASE FROM *Saccharopolyspora erythraea*

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Acetyl-CoA carboxylases (ACCs) catalyze the biotin dependent carboxylation of acetyl-CoA to give malonyl-CoA, a key extender unit for the committed step of the de novo fatty acids biosynthesis and polyketide natural products in actinobacteria. ACCs are composed by three main catalytic domains and few non-catalytic domains. In Prokaryotes and plastids of most plants, these domains are encoded by separate subunits, establishing heteromerics complexes. However, cytosolic ACCs of Eukaryotes and the ones from plastids of graminaceous monocots are encoded by a single polypeptide, establishing homomericmultisubunit complexes. To date there are no description of any homomeric multidomain ACC from bacteria. Recently, bioinformatics analysis allowed us to identify*sace_4237*gene of*Saccharopolysporaerythraea*as a putative homomeric multidomain ACC. We were able to purify the protein by recombinant expression, obtaining an active homomeric enzyme as described for most eukaryotic ACCs. Kinetic studies have demonstrated its *in vitro* activity as ACC as well as propionil-CoA carboxylase. In addition, the construction of a conditional mutant allowed us to study the physiological role of SACE_4237. Growth of the conditional mutant was dependent of SACE_4237 expression or oleic acid supplementation to the media. All together, these results demonstrate that SACE_4237 is the first homomeric multidomain acyl-CoA carboxylase described from bacteria which main role is provide malonyl-CoA for the novo fatty acid biosynthesis in*S. erythraea.*

MI-C07
CATALASE ACTIVITY OF*Acinetobacter* sp.VER3 IS ESSENTIAL FOR PROTECTION AGAINST PEROXIDE AND UV

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High Altitude Andean Wetlands (HAAW) are a group of shallow lakes and salterns located above 4400 masl, exposed to high UV radiation, high salinity and elevated heavy metals content. These ecosystems emerged as an amazing source of biological strategies developed by microorganisms to survive under extreme conditions. The UV-resistant*Acinetobacter sp.*Ver3 isolated from HAAW displays catalase activity levels up to 15 times higher than control strains (Di Capua*et. al.*, 2011). After genome pyrosequencing and gene annotation, two related ORFs encoding catalase activity were identified:*katA*and*katB*. Our results show that the*katA*product accounts for most of the catalase activity observed, being localized in cytosol. On the other side,*katB*encodes a protein carrying a signal targeting peptide responsible not only for periplasmic emplacement but also for correct folding and enzyme activity. Experiments in*E. Coli*secretion deficient strains, including heterologous expression and subsequent periplasm isolation, revealed that KatB is delivered by the TAT system.We constructed a*kat*-insertional mutant of*Acinetobacter baylyi*ADP1 highly sensitive to peroxide and UV. When transformed with a plasmid carrying the full*katA*gene the wild-type phenotype was restored, suggesting this protein plays a main role in the tolerance to such pro-oxidant agents.When Ver3 cells were challenged with peroxide an increase of KatB was observed by inmunostaining and, in lesser extent, also of KatA. When cultures were exposed to UV, only the rise of KatB was distinguishable.Our results indicate that polyextremophile*Acinetobacter sp.*Ver3 takes advantage of both a cytosolic, highly active catalase, and an inducible periplasmic enzyme as efficient antioxidant barrier.

MI-C08
A*Salmonella*-SPECIFIC TRANSCRIPTION FACTOR MODULATES BIOFILM FORMATION IN THE ENVIRONMENT

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*Salmonella*is a food-borne pathogen associated with animal and human infections ranging from gastroenteritis to enteric/typhoid fever. The infections caused by*Salmonella*constitute an important problem for public health. It is postulated that the ability of*Salmonella*to survive in the environment and its persistence and transmission between hosts relies to its capacity to form biofilms. This lifestyle's change, from motile to sessile cells attached to diverse solid surfaces, implies a drastic metabolic rearrangement, and depends on the master transcriptional regulator CsgD. This regulator activates the two major components of extracellular matrix in*Salmonell*a: the curli fimbriae and the exopolysaccharide cellulose. We have previously identified a*Salmonella*-specific transcription factor, that we named BioR, important for both biofilm production and *csgD* transcription. To gain insight into the optimal expression conditions of this factor, we generated *lacZ* fusions allowing us to define that low temperatures and minimal medium are the optimal expression conditions for this regulator. In these conditions, BioR is essential for cell-

adhesion and biofilms persistence *in vitro*. We also determined that this regulator is necessary for the correct cellulose and curli production. Furthermore, we identified target genes that are responsible of this production. A mutant with a deletion of this regulator also affects swimming and swarming motility. Altogether, these results define conditions that trigger the activation of this regulator, that resemble the harsh conditions present in the environment, where*Salmonella*survive between hosts transmission

MI-C09
DISRUPTION OF CTL0175 HAMPERS *Chlamydia trachomatis* REPLICATION POST IFN GAMMA-INDUCED STRESS

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Chlamydia trachomatis (CT) is the most common sexually transmitted bacterial pathogen globally. CT causes asymptomatic, persistent infections leading to serious complications, particularly in young women. CT is an obligate intracellular bacterium alternating between two developmental forms: the infectious elementary body (EB) and the non-infectious, replicative reticulate body (RB). In presence of stressors such as interferon-gamma (IFN), CT enters a viable but non-cultivable or "persistent" state. Once the stressors are removed, CT resumes replication and continues to propagate. In a high throughput screen to identify chlamydial genes involved in persistence, we previously found that null mutations in ctl0175/ptr (encoding an uncharacterized hypothetical protease), lead to a significant decrease in the generation of infectious progeny after IFN-induced stress. In Chlamydia, this may be due to reduced genome replication or to blocked RB/EB differentiation. We generated a ptr::GII (ptr knockout) strain and quantified the rate of genome accumulation during IFN-treatment and recovery, compared to the wild type L2 strain. We found that ptr::GII exhibits reduced genome replication during recovery post IFN-induced stress, indicating that ptr is required for engaging rapid exit of persistence and replication upon IFN removal. We used a female genital tract model of infection in mice and surprisingly found increased chlamydial burden at 14 days post-infection for the ptr::GII strain. Overall, these results point that a reduced replication rate upon IFN removal enhances the ability of this bacteria to establish a long-lasting infection in the uterus.

MI-C10
INCREASE IN THE VIRULENCE FITNESS OF *Shigella flexneri*: THE NOA POPULATION'S PREVALENT PATHOGEN

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The Ministry of Health of the Nation, informed that in Argentina almost of 1,200,000 children suffer diarrhea disease per year. These cases are directly related to the incidence of enteropathogens as *Shigella*, the main etiological agent of foodborne diseases (FBD).It was reported that 30% of these cases are register in the northwest of the country (NOA). In previous studies, we have determined *Shigella flexneri* 2 as the prevalent pathogen responsible of infantile diarrhea in the NOA region. Here, we perform in vitro studies of two clinical isolates (CI) from our bank of strains, CI133 and CI172, isolated in 2013 and 2017 respectively. On the bases that *Shigella* causes piroptosis in macrophages to reach the basolateral cells of the intestinal epithelium, we determined the cytotoxicity generated by these strains in a Caco-2 and RAW 264.7 cell lines infection model. For this purpose, we determined the levels of lactate dehydrogenase (LDH) after different infection times with the clinical isolates CI133 and CI172, using *Shigella flexneri* isolated during 2000, as reference. On the other hand, we performed in vitro infection assay using the same cell lines to determine the virulence capacity of these isolates. We observed that the CI133 and CI172 strains showed increased cytotoxicity levels related to those observed in the reference strain. In concordance, both isolates displayed higher replicative power within Caco-2 cells than the control, while their elimination from phagocytic cells occurred faster. Taken together, these results allowed us to suggest that the virulence fitness of the FBD's prevalent pathogens is improved over time, in the NOA population.

MI-C11
EXPLORING INTERACTIONS OF THE S-LAYER PROTEIN OF *Lactobacillus acidophilus* ATCC4356

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Surface layer (S-layer) proteins are the outermost cell envelope of some species of lactobacilli, and they have been involved in modulating the host immune system and inhibit pathogenic virus and bacteria adhesion. This attribute has made them interesting from a prophylactic point of view, to prevent infection. Here, we investigate interactions of purified S-layer proteins from Lactobacillus acidophilus ATCC 4356 cells, and chimerical GFP–S-layer fusion proteins containing different portions of the SlpA protein both with prokaryotic (peptidoglycan and lipoteichoic acids) and eukaryotic (mucin, fibronectin, collagen) macromolecules, as well as with viruses and bacterial, yeast and blood cells. The lectin capacity of S-layers was analysed by means of Dot blot analysis, hemagglutination, flow cytometry and solid phase assays. Our results show that the C-terminal portion of the S-layer protein recognizes carbohydrates, interacting with different glycoconjugates, being the LTA (lipoteichoic acids) and mannose-derived structures the preferred ligands. The interaction of the SlpA protein with viral particles was studied by means of a virulent activity test of herpes simplex virus type 1 (HSV-1), vesicular stomatitis virus (VSV), human adenovirus type 5 (AdV5) and bacteriophage J1. No virucidal activity was observed. However, interaction with viral particles enables to co-precipitate them with the protein after centrifugation, decreasing the viral titer without affecting bacteriophage titer, suggesting that S-layer proteins interact with glycosylated proteins of the virus. All together, these results may explain the pathogen exclusion effect reported for SlpA.

MI-C12

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DIVERSITY AND EVOLUTION OF β-LACTAMASE ampC IN LONG-TERM *Pseudomonas aeruginosa* CHRONIC INFECTIONS

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Chronic *Pseudomonas aeruginosa* (PA) infections in the airways of patients with cystic fibrosis (CF) offer extraordinary opportunities to study bacterial evolution in natural environments. Continuous antibiotic treatment is a major factor in PA evolution, producing antibiotic-resistant bacterial lineages that expand under high selective pressure. We previously studied the evolutionary trajectories of two PA mutator lineages for 20 years of CF infection. Comparative genomic characterization showed that PA populations underwent extensive within-patient genomic diversification. However, PA isolates from one patient (CFD), who was intensively treated with β-lactam antibiotics, showed mutations that convergently target the β-lactamase encoding ampC gene, which may optimize pathogenic fitness. Here, we used a combination of phenotypic and molecular analysis, to inquire into the genetic diversity of ampC within long-term PA evolved populations. Sequential collections of isolates obtained from single sputum samples from the CFD patient were used, extending our study to 26 years of evolution. We obtained a time-resolved map that shows that evolution is still occurring and driven by antibiotic treatment and that the ampC sequence is highly diverse across populations. Furthermore, some ampC allelic variants, which dominate the populations, were associated to high resistance towards cephalosporins and monobactams. Remarkably, by ultra-deep sequencing of ampC from sputum samples, we show that some positions in the ampC sequence are frequently hit by mutations across different CF patients suggesting a key role of these mutations in β-lactam resistance.

MI-C13

IMMUNOPROPHYLACTIC EFFECT OF R-*Leishmania braziliensis* HSP70 IN EXPERIMENTAL CUTANEOUS LEISHMANIASIS

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Tegumentary leishmaniasis (TL) is a disease caused by parasites of the genus *Leishmania.* There are no effective human vaccines and the use of recombinant proteins seems to be a safe strategy. Seroproteomic approaches were applied for the selection of *Leishmania (Viannia) braziliensis* antigen as immunoprophylactic candidates. A recombinant HSP70 antigen (rAg) was evaluated on a model of TL using Al(OH)3 as adjuvant (Adj). BALB/c mice were distributed in four groups: 1) rAg (49.5µg) + Adj (1mg), 2) rAg alone, 3) Adj alone and 4) control with PBS (Buf). They were challenged in the footpad with *L. (L.) amazonensis* promastigotes and then DTH was measured at 24, 48 and 72h. The footpad swelling (FPS) was measured weekly until euthanasia in the 16thweek post-infection (PI). The footpad lesions were taken to measure parasite load. IgG1 and IgG2a were quantified before challenge and at 16th week PI. DTH in group rAg+Adj at 24h (0.2±0.04 mm) was higher ($p>0.043$) than Adj (0.14±0.05mm). At week 8 PI, the FPS in group rAg+Adj was 0.78±0.29mm, lower ($p>0.006$) than Adj (1.67±0.26mm) and rAg (2.07±0.9mm). However, these differences did not last until week 16 PI and parasite load did not show any differences. Before challenge, the levels of IgG1 (DO492=0.9±0.04) and IgG2a (DO492=0.17±0.11) of the two immunized groups were higher ($p>0.0009$) as compared to Buf. These differences persisted until euthanasia. The immunization with the rAg was not protective, inducing high levels of IgG1, associated with a non- healing Th2 response, as it was observed in humans. But, combined with Adj, the exacerbated FPS was controlled over time; despite it did not affect antibody levels.

MI-C14

TAM SYSTEM IS INVOLVED IN CELL ENVELOPE HOMEOSTASIS IN α-PROTEOBACTERIA

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The ability of Gram-negative pathogens to survive and invade host cells depends on the correct assembly of the cell envelope, especially the outer membrane (OM). The aim of this work is to understand the genetic and molecular bases that determine the correct assembly of the cell envelope in diderm bacteria from *Alphaproteobacteria* phylum, particularly *Brucella suis* and *Ochrobactrumanthropi*. We have identified a locus -*map*AB- encoding the TAM machinery, which is proposed to participate in the translocation of autotransporters (ATs) through the OM in ɣ-proteobacteria. B. suis ΔmapB strain showed enhanced sensitivity to lysozyme, Triton X-100 and polymyxin B, indicating that the cell envelope integrity is compromised. Western blot and proteomic approaches showed that the absence of MapB lead to a deficient assembly of an AT adhesin in the OM and to a reduction in the relative amounts of a protein subset, including proteins from the Omp25/31 family. Electron microscopy revealed that Δ*mapB* cells exhibit multiple anomalies in cell morphology, indicating that the absence of MapB in *B. suis* severely affects cell division. Δ*mapB* cells were also impaired in macrophage infection and showed an attenuated virulence phenotype in the mouse model. Some of these phenotypes have also been observed in Δ*mapB* of *O. antrophi*. Collectively, our results indicate that the role of MapB is not restricted to the translocation of ATs across the OM but that it is essential for OM stability, protein composition and that it is involved in cell envelope biogenesis, a process that is inherently coordinated with cell division.

MI-C15

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SYNERGISTIC MECHANISM BETWEEN INFLUENZA A VIRUS AND *Streptococcus pneumoniae* IN PNEUMOCYTES

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Influenza A Virus (IAV) and *Streptococcus pneumoniae* (Spn) are considered as two of the most important human pathogens. Co-infections with both microorganisms usually lead to severe respiratory disease, and occasionally, death. Although it has been described a clear synergism between these two pathogens, the mechanism of how they interact during infection of eukaryotic cells is poorly understood. We set up a co-infection model using A549 pneumocyte cells, and we observed that when cells were previously infected with IAV, the intracellular survival of Spn duplicated in comparison with non-IAV-infected cells. It has been reported that Spn can be eliminated by the autophagic pathway in pneumocytes. Our hypothesis was that the increased Spn survival in IAV-infected cells is due to a blockage of the autophagosome/lysosome fusion caused by the viral M2 protein. This was confirmed by over-expression of M2 in A549 cells, where we observed an increased Spn survival. In addition to this host factor, we also proposed that Spn should sense IAV-induced changes at intracellular level in pneumocytes to increase its survival, and these changes should be sensed by a two-component system (TCS) to induce a bacterial response to these stress conditions. We screened TCS mutants and we found that the ΔvisRH did not increase its survival as wt cells. An RNAseq analysis revealed that VisRH regulates the expression of many genes that are involved in the acidic/oxidative stress response. Taken together, these results contribute to elucidate the Spn survival mechanism in IAV-infected pneumocytes.

MI-C16
DIFFERENTIAL YEAST POPULATIONS IN GRAPE MUSTS FROM DIFFERENT *Vitis* SPECIES IN A SHARED TERROIR

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The study of indigenous microbial communities in *V. vinifera L.* ecosystems constitutes a major research area in oenology. Few studies, however, consider the yeast communities present in non-vinifera Vitis ecosystems. Moreover, there are no comparative studies concerning yeast communities in V. vinifera L. and non-vinifera Vitis ecosystems in a shared terroir. In this work, we report the identification and characterization of the main indigenous yeast species present during spontaneous fermentation of Malbec (*V. vinifera L.*) and Isabella (*V. labrusca L.*) grapes harvested from neighboring vineyards. Our studies showed that *Hanseniasporauvarum* was the predominant non-*Saccharomyces* species in both Malbec and Isabella ecosystems. *Hanseniasporavineae*, *Metschnikowiapulcherrima* and *Torulasporadelbrueckii*, yeast species commonly found in *V. viniferaL*. grape musts, were isolated only from the Malbec ecosystem. Candida californica, on the other hand, was only isolated from the Isabella ecosystem. Phenotypic analyses of four randomly selected *H. uvarum*, *Starmerellabacillaris* and *Saccharomyces cerevisiae* isolates, as well as microsatellite genotyping of *S. cerevisiae* isolates from each Malbec and Isabella grape musts, suggest that *V. vinifera L.* and *V. labrusca L.* ecosystems could potentially harbor yeast strain populations that are specific to each *Vitis*species. Non-conventional *Vitis*ecosystems could constitute a relevant research system for the ecological and evolutionary study of wine yeast species.

PLANT

PL-C01
ROLE OF THE MIR394 PATHWAY IN THE REGULATION OF FLOWERING TIME IN *Arabidopsis* AND MAIZE

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The microRNA miR394 regulates the accumulation of a transcript coding for LCR, a member of the F-BOX family. These proteins are part of SCF complexes responsible for the addition of ubiquitin residues to a target protein, marking it for degradation by the proteasome. The miR394 pathway participates in the regulation of leaf morphology, in the development of the shoot apical meristem and in the response to abiotic stress produced by drought and salinity in *Arabidopsis thaliana*. We generated Arabidopsis mutant plants in the two endogenous *MIR394* genes (*mir394a/mir394b*) and maize mutant plants in the two *ZmLCR*genes regulated by miR394 (*zmlcr1/zmlcr2*) and determined that this pathway is also involved in the regulation of flowering time in both species. In this work, we present the characterization of *mir394a/mir394b* mutant plants, which show an early flowering phenotype which correlates with a higher expression of the floral integrators *FT* and *SOC1* during plant development, compared to wild-type Col-0 plants. Conversely, a late flowering phenotype was observed for *zmlcr1/zmlcr2* mutants. Moreover, we used a phylogenetic approach to identify the maize orthologue of the MLP proteins shown to be marked for degradation by the mR394 pathway in Arabidopsis and characterized their expression in maize mutants in the components of this pathway

PL-C02

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ROLES OF THE SUBUNIT 17 OF MEDIATOR COMPLEX IN THE UV-B INDUCED DNA DAMAGE RESPONSE

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Mediator complex is multi-subunit protein, conserved throughout eukaryotes; which functions as a molecular bridge between gene specific transcription factors bound at enhancers and RNA polymerase II. Several reports demonstrate that the Mediator complex in yeast acts as regulatory protein linking transcription with DNA repair. In particular, in yeast and humans, mutants in the subunit 17 of the Mediator complex have defects in the nucleotide excision repair (NER) system. In *Arabidopsis thaliana*, mediator is composed by 34 subunits and acts as a signal integrator. In recent years, ozone depletion in the stratosphere has resulted in an increase in ultraviolet-B radiation levels at the earth's surface, causing DNA lesions, induction of stress responses and inhibition of photosynthesis. Repair of DNA damage is essential to maintain the genomic integrity. Thus, the aim of this work is to explore the participation of Mediator in DNA damage responses induced by UV-B radiation in *A. thaliana* plants by using *med17* mutants and transgenic plants overexpressing AtMED17. Our results show that *med17* plants accumulate more damaged DNA after UV-B exposure in the absence of white light than Wild Type Col-0 plants. When DNA damage level is not repaired in cells, programmed death cell (PDC) is triggered, consequently, our interest is to analyze if MED17 is involved in PDC after UV-B. Therefore, *med17* mutants show a lower number of meristematic death cells in root tips after UV-B exposure, while the opposite was observed in overexpressing MED17 roots. Together, our results suggest that AtMED17 has an important role in the DNA damage response in *Arabidopsis*.

PL-C03
FINE TUNING OF ARGONAUTE1 STABILITY IS REGULATED BY CURLY LEAF

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CURLY LEAF (CLF) is a methlytransferase that reversibly regulates gene expression by tri-methylating H3K27 of target genes. This member of the Policomb Repressor Complex 2 was recently found in a screening designed to identify miRNA-pathway co-factors. We studied the connection of CLF to the miRNA pathway and we observed that CLF regulates ARGONAUTE1 (AGO1) stability. This protein loads mature miRNAs and slices the mRNA target or stops its translation, being the main effector of miRNA-mediated silencing. Thus, we observed that *clf* mutant plants, with reduced levels of AGO1, have a global defect in miRNA-mediated silencing, with deregulated miRNA-targets transcript levels. Another interesting observation was that AGO1 degradation in *clf* mutants is even stronger when AGO1 is loaded but hampered with not cleavable targets. We found a novel connection between epigenetics and the miRNA pathway and evidence of a potential mechanism to release not cleavable targets

PL-C04
DIVERGENT ROLES FOR AN ANCESTRAL HDZIP-I GENE OF *Marchantiapolymorpha*

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Land plants evolved multiple independent mechanisms to tolerate abiotic stress. The number of transcription factors (TFs) increased with plant radiation, but TFs families remained largely stable. Here, we explored the case of the Class I *HOMEODOMAIN LEUCINE-ZIPPER* (C1HDZ) genes. These TFs play a role in plant tolerance to abiotic stress in angiosperms, but it is not clear if this function is conserved in early divergent plants like *Marchantiapolymorpha*. Here, we present a preliminary characterization of a *Marchantia*mutant (*mpc1hdz*) lacking a functional C1HDZ. The abiotic stress response of *mpc1hdz* was similar to the wild type. The abiotic treatments, including drought, NaCl and osmotic stress, induced similar growth inhibition on both genotypes. However, the mutant was impaired in non-conserved phenotypes. We found that *mpc1hdz* developed gemmae cups and reproductive structures with abnormal morphology, and no detectable oil bodies in gemmae cups. Then, we performed an RNA-seq analysis of *mpc1hdz* mutant plants. I will discuss these results in the context of the eco-physiological consequences for Marchantia and their putative conservation in angiosperm plants

PL-C05
PHENOTYPIC CHARACTERIZATION OF ATMSH7 DEFICIENT PLANTS

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The mismatch repair (MMR) system maintains genome integrity by correcting replication-associated errors and inhibiting recombination between divergent DNA sequences. In plants, MMR is initiated by the binding of heterodimeric MutS homologue (MSH) complexes, MSH2-MSH6, MSH2-MSH7 and MSH2-MSH3 to mismatches or unpaired nucleotides. MSH2-MSH7 is also involved in meiotic recombination. In order to study the effects of the absence of MSH7 in *Arabidopsis thaliana* (Col-0), a phenotypic characterization of the first generation of knockout *msh7* mutant plants has been carried out in early stages of the development and in reproductive organs. We observed that the length of the siliques and the number of seeds per silique of mutant plants are lower than in Wild Type Col-0 (WT). Additionally, a low percentage of mutant seeds (0.008-0.010%) show deformations and color changes. Seed morphology and color change were not observed in WT. STRING-DB analysis showed that MSH7 shows a predicted interaction with the DNA polymerase epsilon catalytic subunit (ESD7/POL2A/TIL1/ABO4), an enzyme that is essential for viability of the embryo and is involved in abscisic acid (ABA) signaling. One of the central regulators in ABA

signaling is ABI3, a transcription factor that interacts genetically with other proteins and controls accumulation of chlorophyll and anthocyanins. Accordingly, we found that some of the mutant seeds show chlorophyll fluorescence. We conclude that *msh7* mutants show decreased seed yield due to less siliques containing less seeds and altered seed morphology and color, suggesting that MSH7 is involved in establishment and seed production in *Arabidopsis*.

PL-C06
EPIDERMIS SPECIFIC EPIGENETIC MODIFICATIONS IN THE *Arabidopsis thaliana* ROOT UNDER SALT STRESS CONDITIONS

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Epigenetic modifications are changes in DNA or in the associated chromatin that do not imply changes in their sequence. These modifications are reversible and sometimes inheritable.The plant root epidermis develops root hairs, which are responsible for the uptake of water and nutrients from the soil. The *Glabra2* gene is involved in epidermal cell differentiation: trichoblasts, which will originate root hairs and the atrichoblasts, which will not.In the present work, specific primers for the amplification of a portion of the *Gl2* gene after bisulfite DNA treatment were designed. These primers were able to specifically amplify DNA molecules from epidermal cells. Under 20 mMNaCl saline stress conditions, there is a marked decrease in CG methylation, only when the DNA of epidermal cells is analyzed. This epidermal specific effect is lost when the stress conditions are more severe (75 mMNaCl). In this case, the decrease in CG methylation were noticed even when primers that amplify DNA of all root cell types are used.Another finding was that plants daughters of plants that had undergone salt stress (but had not suffered it themselves) had similar level of CG methylation as their parents when considering the epidermis exclusively, which shows that these modifications are heritable.These results show that environmental changes can produce epigenetic variations in specific cell types, in this case the epidermis, which is the outermost cell layer of the root in direct contact with the soil. This influences the production of root hairs, according to the saline concentration of the medium.

PL-C07
SALICYLIC ACID HYDROXYLATION IN MAIZE

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Salicylic acid (SA) plays roles both in plant physiological responses and defense. Although much research has been carried out on SA biosynthesis, some aspects of its catabolism remain unknown. Recently, a salicylic-3-hydroxylase, a 2- oxoglutarate dependent dioxygenase (2-ODD), was characterized in *Arabidopsis thaliana* (AtS3H). In vivo, this enzyme converts SA to 2,3dihydroxybenzoic acid. In order to study this enzyme in maize, we searched for putative 2-ODD enzymes in the maize genome based on sequence homology. We identified one putative gene encoding a S3H, and its protein sequence was used in phylogenetic reconstructions with several other plant 2-ODD proteins, primarily involved in phenolic metabolism. The tree showed different clusters; and because the putative ZmS3H grouped with the characterized AtS3H, we aimed to demonstrate that the identified gene encoded a S3H enzyme. First, to determine its activity *in planta*, we generated *A. thaliana* transgenic plants expressing ZmS3H in the *s3h* background (mutants in the S3H gene). Then, we investigated the role of the putitative ZmS3H in pathogen infection responses by infection of three independent transgenic lines with *P. syringaepv tomato* DC3000. Transgenic plants were more susceptible to the pathogen infection than Wild Type Col-0 plants, suggesting that these plants would have decreased SA levels due to higher hydroxylation of it, supporting ZmS3H activity in planta. Moreover, to characterize ZmS3H activity, we cloned the ZmS3H coding region and expressed it in *E. coli* for further *in vivo* activity assays. We are testing its activity by feeding SA as substrate followed by the identification of products by HPLC

PL-C08
OVEREXPRESSION OF AN ASPARTIC PROTEASE INCREASES DROUGHT TOLERANCE IN *Arabidopsis thaliana*

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In last years, it has been reported that plant aspartic proteases might have a role in the adaptation of plants to an environment with less water availability. In our laboratory we determined that an *Arabidopsis thaliana* gene that encodes an aspartic protease (At1g11910) is expressed in guard cell and that is induced under deficit water conditions and that *At1g11910*mutant plants were more susceptible under water stress conditions. The aim of this work was to evaluate the drought tolerance in*At1g11910* overexpressing *A. thaliana* plants. We compared the response of Col-0 and *At1g11910* overexpressing plants in a mild water deficit condition (MWD). Seedlings of 14 days were putted in pots with an equal substrate quantity and were watered until saturation during 10 days. Then, treated plants were watered until reached a 26% of the maximum substrate capacity during the next 20 days. We evaluated the phenotype of each plant under stress conditions and quantified different hydric parameters. Our results indicated that overexpressing plants were more tolerant than Col-0 to a MWD. These plants showed an increment in the total area leaf and had a lower water loss (60%) and consumption (25%). In addition, we observed that *At1g11910* overexpressing plants showed a reduction in the stomatal aperture even before to be treated with the stomata closure-inducing hormone ABA.These results allow us to suggest that *At1g11910* would participate in the tolerance to drought. Currently we are completing the characterization of this gene to understand the molecular mechanisms that could be involved in this process

PL-C09

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INSIGHTS INTO THE CHLOROPLASTIC UNFOLDED PROTEIN RESPONSE

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Proteolysis plays a key role in maintaining cellular proteostasis. It is very important in organelles where protein turnover and replacement are highly elevated. Chaperones and proteases recognize and remove unnecessary, unfolded or aggregated proteins. In certain situations the quality control system is overloaded and cells trigger a response to deal with the accumulation of unwanted proteins. This process is called "unfolded protein response" (UPR), which is a mechanism that activates signaling pathways leading to increased expression of specific chaperones and proteases, with the aim of restoring proteostasis. The UPR in endoplasmic reticulum and mitochondria has been described, yet there are no reports of a chloroplastic UPR. We propose that the accumulation of misfolded proteins in chloroplasts can trigger a chloroplastic UPR in plants. To test this, we constructed a mutant of ferredoxin NADP+ reductase (FNR) bearing a deletion in Asp289, Trp290 and Ile291 (Taq3-FNR). This resulted in a marked decrease in solubility. Taq3-FNR was cloned into a binary vector with a chloroplastic transit peptide. Taq3-FNR was transiently expressed in *Nicotianabenthamiana* after *Agrobacterium*-mediated infiltration. The expression of the chloroplastic chaperone ClpB was assessed by Western blot. A two-fold increase in ClpB expression was found in leaves infiltrated with Taq3-FNR in comparison with leaves that express FNR wild type and mock-infiltrated samples. Our results suggest that Taq3-FNR generates a specific response in chloroplasts. As ClpB is a nuclear encoded protein, the response involves chloroplasts-to-nucleus communication, a common feature in other UPRs.

PL-C10
IS THE METABOLISM OF XENOBIOTIC COMPOUNDS REGULATED BY CIRCADIAN CLOCK?

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Plants as sessile and light-sensitive organisms have developed endogenous mechanisms such as the circadian clock (CC) that allow them anticipate daily environmental changes adjusting their physiology at the appropriate time of the day. However, the role of CC on the metabolism of xenobiotic compounds (MXC), like phenol is still few known. Therefore, the main aim of this work consists to analyze the possible circadian regulation of the expression of genes related to MXC. For this, hairy root cultures from *Nicotianatabacum* of 3 week-old, synchronized by light/darkness cycles, were used. Our results showed that phenol treatment (PT) differentially affected the temporal expression profiles of most of the selected genes related to MXC. PT was able to induce, at certain times (zt), the expression of genes related to MCX in the light phase, for instance the *NtCYPG8H* (zt 7), *NtPOD72* and *NtUGTBeta* genes (zt 3), as well as in the dark phase, for example *NtGSTZ* gene (zt 19). Moreover, PT decreased the expression of the *NtCYP710* (zt 23) and *NtUGT89* (Zt 7-19) genes. These results would suggest that although the genes associated to MXC conserve temporal expression profiles regulated by the CC, PT affected such regulation modifying the expression profiles of these genes either by induction or repression. Thus, phenol is able to trigger the loss of temporality in the expression of several genes. The fact that these genes related to MXC were induced or repressed by phenol, give us a key knowledge about the appropriate time of the day that the enzymes encoded by them (CytP450s, PODs, GSTs and GTs) would be more active for the removal of such environmental pollutant

PL-C11
Arabidopsis KINESIN13B INTERACTS WITH SEVERAL TRANSCRIPTION FACTORS INVOLVED IN GROWTH REGULATION

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Kinesins constitute a superfamily of microtubule-based motor proteins and they have diverse cellular functions such as the transport of membranous organelles, protein complexes and mRNAs. In Arabidopsis thaliana this family is composed by 61 genes; however, only a few have been characterized so far. Searching by Y2H for proteins able to interact with homeodomain-leucine zipper transcription factors, we detected kinesin13B as one of such interacting proteins and corroborated the interaction by BiFC analyses. The expression pattern of kinesin13B was determined using Arabidopsis plants transformed with its promoter fused to the GUS reporter gene indicating expression in cotyledons and leaves of 10-day-old seedlings. A Threading bioinformatic analysis revealed the existence of alpha helices and a loop between them conforming the putative interaction region. To further investigate the role of kinesin13B in plants, we obtained and characterized mutant and overexpressor lines (OE). Three independent mutant lines did not show significant differential phenotypes, probably due to an induction of kinesin 13A. Notably, OE lines showed shorter and wider stems compared with controls as well as more branches in the main stem. Moreover, such plants have more rosette leaves at bolting and increased biomass, a similar phenotype to that exhibited by OE of several kinesin13B-interacting transcription factors. Crosses between kinesin13B mutants and interacting transcription factors OE were performed and analyzed.Altogether, the results indicate that kinesin13B has a role regulating the action of specific transcription factors which modulate plant growth and development.

PL-C12

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ROLE OF E2FC TRANSCRIPTION FACTOR DURING THE UV-B RESPONSES IN *Arabidopsis*

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Plants are normally exposed to a constantly changing environment, which influences the developmental programs executed throughout their lifetime. Solar radiation is one of the most important environmental factors affecting plant growth, but its spectrum includes UV-B radiation, a high-energy component to which plants need to adapt. High UV-B intensities induce damage responses in plants, affecting cell cycle and development. Inhibition of leaf growth is one of the most consistent responses. The Retinoblastoma-E2F pathway (RBR/E2F/DP) plays an important role in the regulation of the cell cycle and in several differentiation processes. In this work, we particularly analyzed the role of E2Fc, one of the six E2F transcription factors in *Arabidopsis*, in the response after high UV-B radiation. This transcription factor acts as a repressor of cell proliferation and is a positive regulator of the endoreduplication onset. For this purpose, we analyzed the effect of UV-B light in the development of *Arabidopsis thaliana* transgenic lines with altered expression of E2Fc. By phenotypic studies and microscopic analysis in leaves and roots, together with molecular biology experiments, we show that E2Fc regulates the inhibition of cell proliferation at UV-B intensities that cause DNA damage. In addition, our results demonstrate that E2Fc regulates the expression of genes involved in the activation of programmed cell death without having a direct participation in DNA repair.

PL-C13
THE LNK GENE FAMILY: AT THE CROSSROADS OF LIGHT SIGNALING AND THE CIRCADIAN CLOCK

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Light signaling pathways interact with the circadian clock to help organisms synchronize physiological and developmental processes to periodic environmental cycles. The plant photoreceptors responsible for clock resetting have been characterized, but signaling components that link the photoreceptors to the clock remain to be identified. In a previous work we reported a novel family of night light–inducible and clock-regulated genes (LNKs) that play a key role linking light regulation of gene expression to the control of daily and seasonal rhythms in Arabidopsis thaliana. Particularly, the homologous genes LNK1 and LNK2 were shown to control circadian rhythms, photomorphogenic responses, and photoperiodic dependent flowering. In the present work, we analyze the role of the whole LNK family, LNK1-LNK4, in these processes. We found that LNK3 and LNK4, individually as well as together, didn't affect circadian rhythms, photomorphogenic responses, and photoperiodic dependent flowering. Nevertheless, depletion of LNK3 and LNK4, or both, in a *lnk1;lnk2* mutant background gave rise to perturbations in some of the above mentioned phenotypes. This evidence suggests a complex interaction network among the LNK family members in the linkage between light signaling and the circadian clock.

PL-C14
ROLE OF MSH6 DURING DNA RECOMBINATION IN *Arabidopsis thaliana*

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The mismatch repair (MMR) pathway promotes genome stability by increasing the fidelity of DNA replication and recombination. The initial step of the pathway requires heterodimers of MSH proteins. The MSH6-MSH2 heterodimer recognizes base-base mismatches and small insertion/deletion loops. To further understand the role of *Arabidopsis* MSH6 *in vivo*, we first generated transgenic plants expressing the β-glucuronidase (GUS) reporter gene under the control of the MSH6 promoter. Histochemical staining demonstrated that MSH6 is preferentially expressed in proliferating tissues. We then investigated protein function during meiotic and somatic recombination using previously described reporter assays. The meiotic tester line contains two reporter genes that encode green (GFP) and red (RFP) fluorescent proteins located at 16 cM under the regulation of a seed-specific promoter. Seeds with exclusively green or red fluorescence indicate a meiotic recombination event between the markers. The somatic recombination assay construct contains two overlapping halves of the GUS reporter gene (namely GU and US). Recombination between identical (homologous recombination) or 1.6% divergent (homologous recombination) U repeats leads to the formation of an active GUS reporter gene. Disruption of MSH6 has no effect on the rate of meiotic recombination, but increased the frequency of homologous or homologous recombination by 1.3- or 4-fold, respectively, relative to wild-type plants. We conclude that MSH6 plays an important role during somatic recombination in plants.

PL-C15
MECHANISMS INVOLVED IN THE CELLULAR ENERGY HOMEOSTASIS IN PLANTS

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The evolutionarily conserved AMPK/Snf1/SnRK1 kinase complex is a master regulator of cellular metabolism in eukaryotes, involved in adjusting the cell function to the energy status. In photosynthetic organisms, SnRK1 plays this role by maintaining the cellular energy homeostasis through a balance of energy yielding and energy requiring processes, in response to fluctuating environmental conditions. To fulfil this function, SnRK1 deploys a wide variety of molecular mechanisms, e.g. wide-ranging transcriptional reprogramming, translational regulation and/or metabolic reprogramming. Together with the Target of Rapamycin (TOR) kinase complex, SnRK1 has also been recently proposed to be

a main regulator of developmental programs. While many mechanistic aspects of SNRK1 downstream functions related to energy management and developmental processes are relatively well characterized, how SnRK1 integrates cellular energy information to induce these processes is still unknown. This work is centered in revealing and understanding the factors that define SnRK1-mediated sensing of the cell energy status. Having as premise that SnRK1 might be receiving energy status information in a specific intracellular location, we conducted thoughtful localization study of this kinase. Our results identified and localized a non-nuclear fraction of SNRK1.1, the catalytically active subunit of SnRK1. Experiments of energy imbalance at the level of chloroplasts, the organelles of photosynthetic energy metabolism, revealed a link between cellular energy level and the intracellular distribution of SnRK1.1. These findings suggest that the non-nuclear SnRK1.1 fraction is responsive to cell energy fluctuations and hence might be directly gauging the cell energy status. The analysis of this dynamic behavior using photoconvertible probes of the DENDRA family, together with similar studies conducted on TOR provides novel evidence about the energy sensing mechanisms and the crosstalk between these two main regulatory pathways. Our work introduces a new model of the interaction of TOR and SnRK1 and the putative mechanisms connecting them with the energy production in photosynthetic organisms, with implications for eukaryotes in general.

PL-C16
LIGHT REGULATION OF ALTERNATIVELY SPLICED GENES DURING *Arabidopsis thaliana* SEED GERMINATION

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Light is one of the most important factors regulating seed germination, plant growth and development. Light-sensing photoreceptors tightly regulate gene expression to control photomorphogenic responses. Although many levels of gene expression are modulated by photoreceptors, it is still unknown the importance of alternative splicing (AS) in the promotion of seed germination by light. During the last years consecutive reports demonstrated a steadily increasing percentage of alternatively spliced genes in plants. AS can lead to different outcomes and can produce transcripts that code for proteins with altered or lost function. Several examples have demonstrated AS functional importance in various processes like photosynthesis, defense responses, the circadian clock, hormone signaling, flowering time and metabolism. The aim of this work is to study the transcriptome and alternatively spliced genes expressed during light induction of seed germination in *Arabidopsis thaliana*. We evaluated the effect of R/FR (red/far red) light on AS in light-induced Col-0 seeds using high-throughput RNA sequencing (RNA-seq). We found that a Rp (red pulse) compared to a FRp (far red pulse) given after cold stratification, affects the AS of 226 genes. Some of these AS events were associated with genes involved in mRNA processing, RNA splicing and mRNA metabolic processes. Moreover, we showed that some of these AS events are highly conserved across distinct developmental stages in plants. By using a combination of R/FR light and phytochrome mutants approach, we showed that light modulates the AS pattern of some of these genes, i.e.: U2AF65 (an auxiliary splicing factor) in a phytochrome B exclusive manner and RS31 (a splicing regulator), in a phytochrome independent manner. Our results demonstrate that in Arabidopsis seeds (1) R/FR light triggers AS changes in different genes and, in some cases, (2) light exerts its effects through the action of phytochrome B. We conclude that AS is a source of gene expression diversity for proteins involved in the promotion of seed germination by light

PL-C17
NON-THERMAL PLASMAS AFFECT SEED QUALITY, PLANT GROWTH AND DNA METHYLATION PATTERNS IN SOYBEAN

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Non-thermal plasmas are partially ionized gases, usually generated by low-current electrical discharges that can be sprayed onto biological tissues. They are a novel and promising technology that might be employed for seed treatment before sowing as they allow enhancing seed health while promoting germination and vigor in a fast, cost-effective and eco-friendly way. This work analyses the effects of two different cold plasma treatments (N_2 for 3 min and O_2 for 2 min) applied to soybean [Glycine max (L.) Merr.] seeds on seed quality and growth parameters of plants (6 and 20 days-old) grown from them. Comparing plasma treatments with the control (non-treated), the results showed improvements of 10% in the number of normal seedlings, 2% in the germination rate, 4% in the germination index, 10% and 20% in vigor index I and II respectively and a decreased of 5% in the electrical conductivity of seeds. Plants grown from treated seeds exhibited a promoted growth in both analyzed stages: root length, total length and fresh weight increased 20%, 16% and 9% (respectively) in plants of 6 days and 16%, 10% and 8% (respectively) in plants of 20 days; leaf area and chlorophyll content in leaves of 20 days were also improved (4 and 20 %, respectively). MSAP (Methylation Sensitive Amplified Polymorphism) markers were assayed in order to investigate if the evaluated treatments induced epigenetic changes. Preliminary results exhibited differential methylation patterns between plants grown from treated and non-treated seeds. The possible role of the epigenetic variability in the origin of the observed phenotypic differences will be discussed.

PL-C18

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SALICYLIC ACID SIGNALING PATHWAY AS KEY PLAYER IN THE EARLY ACTIVATION OF IMMUNE RESPONSES IN MAIZE

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The fungus *Fusarium verticillioides* is the main causal agent of maize ear rot and also produces massive amounts of the fumonisins, which have deleterious effects on humans and farm animals. One of the strategies to control this pathogen is biological control, through the use of non-pathogenic fungal species, such as members of *Trichoderma* genus. These fungi have been proved to be antagonist agents able to control fungal pathogens, to be plant growth promoters, improving and maintaining soil productivity, and can also stimulate local and distant immune responses (Induced Systemic Resistance, ISR) to prevent future pathogenic attacks. Thus, the focus of our research was to globally explore the early regulatory events modulated by ISR in silks from maize plants inoculated with *Trichoderma atroviride* and challenged with *F. verticillioides*, through a transcriptional and hormonal approach. For this, we carried out comparative tests of transcriptional expression using the RNA-seq technique and quantified the hormonal levels of salicylic acid (SA), abscisic acid and jasmonic acid through HPLC/MS in maize silks. Our transcriptome analysis, together with the hormone quantitative determinations performed, also shed some light on the role of hormones in the regulation of immune responses in maize silks after ISR activation. The results show a decrease in the SA levels during the pathogen infection in inoculated plants with *Trichoderma* and also an increase in the gene expression involved in the SA signaling pathway in maize silks. It is likely that, in maize silks, SA acts as a central signaling element to mediate local and systemic activation of defenses.

PL-C19
PAP-SAL1 CHLOROPLAST RETROGRADE PATHWAY MODULATES IRON DEFICIENCY RESPONSE IN ALKALINE SOILS

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Iron (Fe) is an essential micronutrient for plants and is present abundantly in the Earth's crust. However, its bioavailability in most soils is low, due to their alkaline pH that lowers the solubility of the metal. In previous communications, we have demonstrated the existence of a link between the organelle-nucleus retrograde signaling PAP-SAL1 pathway, which senses the internal Fe deficiency, and the iron uptake activity in roots. One of the objectives of this study was to characterize mutant plants in this signaling pathway with respect to their growth in alkaline soils. We measured phenotypic characteristics such as rosette area and chlorophyll content, and we observed that mutant lines presented an increment in both compared to Wild-Type Col-0 plants. Likewise, we studied genes implicated in the biosynthesis of secondary metabolites derived from phenylpropanoids, which, excreted to the rhizosphere, improve Fe availability, in two different pH conditions: 5.7 (control) and 7.5 (alkaline). The relative expressions of these genes were elevated in mutant lines compared with Wild-Type seedlings in both pH conditions. Finally, we quantified the phenolic compounds excreted to the rhizosphere. Results show an increase in these metabolites in the culture medium in mutant lines compared to Wild-Type plants. In summary, PAP-SAL1 mutants show alterations in the biosynthesis of Fe-mobilizing metabolites that improve their growth in alkaline soils. As we have mentioned previously, our results suggest that the PAP-SAL1 retrograde pathway has a connection with Fe deficiency sensing in *Arabidopsis thaliana*

SIGNAL TRANSDUCTION

ST-C01
IT TAKES TWO TO TANGO: YvfTU AND DesKR TWO COMPONENT SYSTEMS REGULATE ABC TRANSPORTER TRANSCRIPTION

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Two component systems (TCS) play a major role in signal transduction in prokaryotes for cellular adaptation to environmental conditions and stresses. In *Bacillus subtilis*, the DesKR TCS can detect changes in membrane fluidity upon a temperature downshift and induce the expression of a fatty acid desaturase, encoded by the *des* gene, allowing cell adaptation to cold shock. It has been previously demonstrated that overexpression of DesR in a *desK* null mutant results not only in the induction of the *des* gene, but also of the operon *yvfRSTU*, encoding a putative ABC transporter (YvfRS) and a TCS (YvfTU). Bioinformatic analysis of this operon revealed that YvfTU is highly similar to DesKR and the promoter region upstream of *yvfR* (P*yvfR*) to the regulatory region of *des*. We previously demonstrate that YvfTU can perceive temperature changes, inducing the expression of YvfRS only at 37°C, and that DesKR is also required for this induction.In this work we demonstrated by electrophoretic mobility shift assays that such phenotype was due to a joint regulation of YvfRS ABC transporter by both TCS. Only if DesR and YvfU act together, a slow migrating complex is formed. This result was confirmed by *in vivo* analysis of mutant strains, which also revealed that unphosphorylatedDesR was involved in the induction of the transporter. Finally, by molecular modeling we analyzed the formation of a heterodimer between both regulators, which was tested *in vitro* and *in vivo*.Our findings illustrate one of the few examples in which bacteria use two high homologous TCSs to adjust gene expression and adapt the organisms to a new environmental condition.

ST-C02

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TcAMPK: IDENTIFICATION AND CHARACTERIZATION OF AN ENERGY REGULATORY HUB IN *Trypanosoma cruzi*

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The AMP-activated protein kinase (AMPK) is a heterotrimeric enzyme involved in maintaining energy homeostasis in many organisms. *Trypanosoma cruzi*, the causative agent of Chagas disease, affects between 6 and 7 million people. During the transition between the mammal host and the insect vector, *T. cruzi* faces nutritional, oxidative, osmotic and other types of stress, all of which can prompt the parasite to remodel its metabolism and force it to re-establish their homeostasis. The ability to respond to stress, allows the parasite to differentiate and survive. It was shown that *T. brucei* AMPK is involved in the differentiation from the slender to stumpy stages and in surface protein expression changes in response to nutritional stress. We identified four candidates for the AMPK subunits of *T. cruzi* (α1 and α2 catalytic subunits, β and γ regulatory subunits). Its expression in *T. cruzi* epimastigotes was confirmed by RT-PCR, Western blot with a phospho-AMPKα specific antibody, mass spectrometry and by incorporation of ^{32}P to the specific AMPK substrate SAMS in a kinase activity assay. This last assay also allowed us to observe the upregulation of AMPK activity under epimastigote starvation, and the inhibition of this activity with dorsomorphin, a specific inhibitor. Also, each of these subunits can revert the 'glucose dependent' phenotype of *S. cerevisiae* conditional mutants alternatively lacking one subunit of the AMPK ortholog SNF1. Our results show, for the first time, the presence of a functional AMPK ortholog in *T. cruzi*. In the future, we aim to discover its role through the life cycle and stress responses of this parasite.

ST-C03
CALCIUM SIGNALING: THE COMMUNICATION BETWEEN INTRACELLULAR CA^{2+} STORES IN HUMAN SPERM

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Calcium (Ca2+) and cAMP signaling are essential in regulating processes required after sperm ejaculation that lead to fertilization, including the acrosome reaction (AR). This process is necessary for sperm to traverse the egg envelope and to expose a fusogenic membrane that allows egg-sperm fusion. Progesterone and ZP3, the most studied AR inducers, elicit an intracellular Ca2+ increase needed for AR in human sperm. This increase is mediated by a first Ca2+ influx but also the intracellular calcium stores are involved. It is know that intracellular Ca2+ stores play a central role in the regulation of [Ca2+]i and in the generation of complex Ca2+ signals such as oscillations and waves. In a previous report we have proposed that in human sperm the cAMP analogue 8-(p- chlorophenylthio)-2'-O-methyladenosine-3', 5'-cyclic monophosphate (8-pCPT-2'-O-Me-cAMP) elicits an intracellular Ca2+ release which is involved in AR. The aim of this study was investigate which Ca2+ stores and Ca2+ channels are involved in the 8-pCPT-2'-O-Me-cAMP pathway in human sperm. We performed real time dynamic assays in calcium-free medium -with high time and spatial resolution- using fluorescent Ca2+ sensors. We found that 8-pCPT-2'-O-Me-cAMP, induce a Ca2+ wave that starts in the posterior neck region and propagates to the acrosome region. This calcium response was sensitive to NED-19 and Thapsigargin, highlighting the participation of different Ca2+ stores, ionic channels (Two Pore Channels) and Ca2+-ATPasas (SPCA).

ST-C04
THE ROLE OF MITOCHONDRIA IN CALCIUM SIGNALING AND HUMAN SPERM PHYSIOLOGY ACTIVATED BY PROGESTERONE.

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The main function of mitochondria is the production of ATP through the electron transport chain and lipid oxidation. In addition, this organelle plays an important role in Ca2+ buffering and signaling, shaping and extending the kinetics of Ca2+ signals. Calcium signaling is a key regulatory mechanism in sperm functions such as capacitation, motility, hyperactivation, chemotaxis and acrosome reaction. Progesterone (Pg) has been associated with several processes of sperm physiology, since it directly activates membrane Ca2+ channels. Our group have previously observed that Pg induces an intracellular calcium increase in sperm in media with ≈100nM [Ca2+]. It is unclear which calcium reservoirs of the sperm are involved in this calcium increase. The aim of this study was to investigate the role of mitochondria in calcium signaling involved in the Pg pathway and others functions such as acrosomal reaction and motility parameters in human spermatozoa. To this end, we loaded capacitated human sperm with fluorescent calcium sensor Fluo3-AM, then treated with progesterone in absence or presence of mitochondrial inhibitors in medium containing different [Ca2+]. We used real time dynamic assays with high speed and spatial resolution, in single cells analysis, and computer software to analyze cellular motility parameters. We observed that Pg generated increases of intracellular Ca2+ with particular kinetics and patterns in media with different [Ca2+]. We also noticed that mitochondrial inhibitors altered the Ca2+ patterns and kinetics previously observed. These results suggest that mitochondria participates in calcium signaling in response to Pg.

ST-C05

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EXPRESSION REGULATION OF PROTEIN KINASE A SUBUNITS FROM *Saccharomyces cerevisiae*

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In *S.cerevisiae* the protein kinase A (PKA) is a tetramer composed of catalytic subunits, *TPK1*, *TPK2*, *TPK3* and a regulatory subunit, *BCY1*. PKA controls several cellular events in response to different stimuli. The specificity in cAMP-PKA signaling is maintained at different control levels and one of them is the regulation of the expression of PKA subunits. We study the expression of PKA subunits during thermal stress. The promoter of each subunit is differentially regulated during this stress. *TPK1*promoter is the only one upregulated, and accordingly, there is an increase in *TPK1* mRNA in response to stress. The *BCY1* promoter has low activity, but a high mRNA level is measured. The analysis of mRNA stabilities indicates that the half-life of *BCY1* mRNA is greater than that of *TPK1*, and both are stabilized upon thermal stress. *TPKs* and *BCY1* promoters and 5'UTRs sequences affect the stability of their mRNA. Tpk1 protein levels do not show a significant change during thermal stress but do increase when the cells undergo a second stress, however Bcy1 protein remains constant. Analyzing the *in vivo* localization of *TPK1* mRNA during thermal stress, it was visualized that this mRNA is localized in cytoplasmic granules resistant to cycloheximide. Overall, in response to thermal stress, the mRNAs of the PKA subunits are differentially expressed and more stable; *TPK1* mRNA is induced and forms granules and is not translated until the cell leaves the stress conditions

ST-C06
ROLE OF AKR1B1 IN TUMOR AGGRESSIVENESS AND ITS INTERPLAY WITH THE P53 PATHWAY IN BREAST CANCER

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AKR1B1 belongs to a superfamily of aldose reductases, and catalyzes conversion of aldehydes to alcohols. It has been proposed that through this activity AKR1B1 may affect different aspects of cell metabolism thereby conditioning tumor progression. However, its function in cancer is not fully understood, and some evidences are contradictory. For example, AKR1B1 was found to be hypermethylated in breast cancer patients, suggesting that it may play a tumor suppressive role. In contrast, it has been shown to promote migration and EMT in triple negative cell lines. To understand the effect of AKR1B1 in breast cancer we studied the consequences of its downregulation and overexpression on migration and invasion *in vitro*. We also analyzed the role of AKR1B1 on cell survival and EMT. By studying the effect of the enzyme on xylose reduction and prostaglandin F2a synthesis we explored the effects on cell metabolism. To analyze the effect of AKR1B1 on tumorigenesis and metastatic potential *in vivo*, we used a model of orthotopic transplantation in immunocompetent mice. By performing qPCR, luciferase assays and western blot we showed that different members of the p53 family affect AKR1B1 expression. In order to understand the clinical impact of our findings we analyzed breast cancer databases. We searched for correlations between AKR1B1 expression levels and p53 status. We also analyzed the impact of AK1B1 levels on clinical outcome. In summary, we provide novel data on the role of AK1B1 on tumor-associated phenotypes and we found a novel link between AKR1B1 expression and the p53 family that may help to understand the complex role of this enzyme in breast cancer.

ST-C07
ICMT COOPERATES WITH TUMOR AGGRESSIVENESS AND IT IS UNDER COMPLEX CONTROL BY p53 FAMILY MEMBERS

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ICMT plays a key role in the regulation of prenylated proteins by catalyzing carboxymethylation of the C-terminus. Despite growing evidences suggesting that alterations in the prenylated protein network may affect tumor progression, the regulation of this complex post-translational modification process and the specific role of ICMT are not completely understood. Our work unveils a link between post-prenylation processing and the p53 pathway. We found that p53 family members affect ICMT levels. By performing qPCR, luciferase assays, chromatin immunoprecipitation and western blot we characterized the effect of different p53 family members on ICMT expression. Our results suggest that ICMT is under precise regulation in normal cells but becomes overexpressed during tumor progression. We also showed that ICMT overexpression contributes affects tumor-associated phenotypes in vitro and tumor formation in vivo. To gain insight into the molecular mechanisms of these effects we studied the consequences of ICMT deregulation on RAS/MAPK pathway and actin cytoskeleton. Moreover, we found a correlation between p53 status and ICMT expression in breast and lung cancer patients. We also analyzed the impact of ICMT overexpression on clinical outcome and defined groups with differential behavior, conditioned by p53 status. Our results suggest that the functional interplay between p53 family members and p53 mutant forms will affect ICMT levels during tumorigenesis and this, in turn, will cooperate to drive mechanisms of tumor aggressiveness.

ST-C08

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DAL81 AND UGA3 TRANSCRIPTION FACTORS AND STRESS RESPONSE IN *Saccharomyces cerevisiae*

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*S. cerevisiae*is able to metabolize a wide variety of nitrogen compounds. For example, amino acids are used as nitrogen or carbon source, both in anabolic or catabolic processes. The γ-aminobutyric acid (GABA) is an amino acid widely distributed in nature and has several functions on different organisms. Particularly, yeast cells can use it as sole nitrogen source. GABA presence determines the activation of the pleiotropic (Dal81) and the specific (Uga3) transcription factors (TF), which induce the expression of the*UGA*genes. The proteins necessary for GABA catabolism are encoded by*UGA4*(GABA specific permease),*UGA1*(GABA transaminase) and*UGA2*(succinate semialdehyde dehydrogenase). In addition, some authors have proposed that the activity of the GABA metabolic pathway affects cells tolerance to stress. In this study we analyzed the effect of TF involved in GABA genes regulation on stress response. We found that Uga3 has a very important role on tolerance against both heat shock and oxidative stresses. Dal81 is also important for cell survival after stress, although at a lesser extent. However, GABA induction of the genes regulated by Uga3 and Dal81 does not produce changes in cell growth after heat shock and oxidative stress. Early life stress can have positive impacts on longevity and during the normal aging process global protein synthesis generally declines with increased organismal age. However,*UGA4*expression increases with the culture age independently on the presence of Uga3 and Dal81. These results suggested to us that Uga3 and Dal81 could have a physiological role on stress tolerance, besides GABA metabolism regulation.

ST-C09
CONTRIBUTIONS OF THE PMC1, VCX1 AND YVC1 PATHWAYS TO CYTOSOLIC CALCIUM IN RESPONSE TO PHEROMONE

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Saccharomyces cerevisiae haploid cells secrete mating pheromones that indicatethe presence of cells of one mating type to those of the opposite mating type. This initiates a sequence of events, which includes cellular arrest and growth polarization towards the secretory cell. Studies showed that the incorporation of calcium is necessary for cell survival and signal transduction to coordinate the genes involved. Studying the dynamics of calcium using microscopy and the fluorescent sensor GCaMP6f we found that pheromone does not generate a single elevation of cytosolic ca2+ levels but transient increases in the form of bursts. Our results suggest that the information transmitted by calcium is encoded in the temporal distribution of these bursts. Calcium uptake depends on at least two pathways, system HACS and system LACS. Through genetic mutations, we eliminated the activity of these pathways and we were able to observe the dependence with the temporal distributions of calcium bursts. We have proposed that the calcium response not only depends on transport pathways from the extracellular medium, but it can also depend on each of the different calcium flow pathways to and from each of the internal reservoirs. The aim of this work isto define the role of calcium transporters in internal reservoirs in response to pheromone. To address this, we monitored cells with mutated vacuolar Ca2+ ATPase (*pmc1Δ*), cell with mutated vacuolar membrane antiporter with Ca2+/H+ (*vcx1Δ*) and cell with mutated vacuolar channel (*yvc1Δ*). We are currently studying the variation of calcium burst distributions by eliminating the activity of these pathways through genetic mutations

ST-C10
INTERACTIONS BETWEEN PROTEIN TYROSINE PHOSPHATASE 1B (PTP1B), EGFR AND FAK IN INTACT CELLS

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Different cell behaviors, such as contractility, migration and proliferation, depend on specific spatiotemporal regulation of adhesion complexes and the cytoskeleton. Previous work from our laboratory revealed that protein PTP1B has a critical role in this regulation. Several substrates of PTP1B have been identified, such as the Epidermal Growth Factor Receptor (EGFR) and the Focal Adhesion Kinase (FAK), which also are able to interact with each other. However the functional relationships between these interactors and their spatial location remain unknown. We used Bimolecular Fluorescence Complementation (BiFC) for direct visualization and analysis of the interactions in intact CHO or PTPWT cells. This approach is based on complementation and restoration of fluorescence when two non-fluorescent fragments of a fluorescent protein are a few nanometers apart. N- and C-fragments of YFP were fused to PTP1B, FAK and EGFR. Confocal fluorescence sectioning and reflection microscopy revealed positive BiFC signal for PTP1B/FAK, PTP1B/EGFR and FAK/EGFR pairs at different cellular compartments. In absence of EGF stimulation PTP1B/EGFR BiFC occurred at the membrane/substrate interface, although not associated with adhesion complexes. In contrast, after stimulation with EGF, BiFC was in puncta with increasing size and density in internal locations. This pattern was altered depending on substrate, temperature and concentration of EGF. On the other hand, BiFC between FAK/PTP1B, and FAK/EGFR was observed at adhesions in the membrane/substrate interface. Our results indicate that PTP1B recognize components of cell-matrix adhesion complexes and EGFR in different membrane subcompartments. Supported by CONICET and ANPCyT.

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POSTERS

BIOTECHNOLOGY

BT-P01
BIOCONVERSION OF GLYCEROL INTO POLYHYDROXYALKANOATES BY AN INDIGENOUS STRAIN, *Halomonas titanicae* KHS3

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Halomonastitanicae KHS3 was isolated from hydrocarbon-contaminated water in Mar del Plata harbor. This strain is able to accumulate polyhydroxyalkanoates (PHAs), reserve polymers that can be used as raw material for the preparation of bioplastics. The aim of this work was to evaluate the ability of *H. titanicae* KHS3 to use glycerol as the only source of carbon and energy and convert it into PHAs. When grown in mineral salts medium with 0.25% commercial glycerol, PHAs synthesis was only moderate. However, when cells were harvested at mid-exponential phase and resuspended in medium depleted of nitrogen source, PHA accumulation was dramatically increased and reached up to 60% of dry cell weight. Such accumulation also occurred when cultures were fed with glycerol obtained from a biodiesel reactor, both in its crude form (contaminated with methanol and salts) and partially purified (technical grade), suggesting that this bioconversion potentially represents a way of adding value to the otherwise disposable glycerol.We show the kinetics of PHA accumulation after nitrogen deprivation under different conditions. Good PHA accumulation occurred in media containing between 2 and 10% NaCl. PHAs inside cells remained stable after long incubations in nitrogen-lacking medium, but decreased rapidly after re-addition of ammonium sulfate.The purified polymer is currently under RMN analysis to determine its precise chemical composition.

BT-P02
BIOREMEDIATION OF HEAVY METALS USING GENETICALLY MODIFIED *Chlamydomonas reinhardtii*

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Heavy metals are an important source of water pollution around the world. They are toxic at very low concentrations and cannot be degraded or destroyed. Cells have diverse strategies for handling heavy metals: all eukaryotic organisms synthesize small proteins called metallothioneins, which are the first response to high concentrations of metals. In addition, most organisms present frataxin, an essential protein involved in iron homeostasis and related to other metals too, such as copper. In this work we propose *C. reinhardtii* as a model for remediation of heavy metals in water and effluents. This unicellular green algae, that has a simple life cycle, allows us to isolate transgenic cells easily. Transgenic *Chlamydomonas* expressing a soybean metallothionein (GmMT3) or frataxins of *C. reinhardtii* (CrFH) and maize (ZmFH2) were obtained by electroporation and the presence of transcripts was confirmed using quantitative Real Time PCR. In liquid cultures supplemented with Cu, the GmMT3 lines and those that express frataxins grow faster than the wild type line. ICP-MS analysis of the recovered cells showed that transgenic lines have a higher capacity than the control line to incorporate metals such as Cu, Fe and Zn, both in their cell wall and intracellularly. Currently a practical application over a metallic sludge is being tested. Results are promising since transgenic lines resist better the stress generated by high amounts of heavy metals, developing larger amounts of biomass (with the ability to adsorb and absorb metals) in a shorter time than the wild type strain.

BT-P03
EFFECT OF PROBIOTIC BACTERIA ISOLATED FROM PATAGONIA ON ZEBRAFISH GUT MICROBIOTA AND GROWTH

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Probiotics are an interesting alternative for sustainable aquaculture. The aim of this study was to assess the effect of probiotic bacteria isolated from Patagonian fish (T4, H16, and TW34) on gut microbiota and growth performance using zebrafish as an experimental model. Assays included one recirculating system (3 tanks) for each probiotic treatment (commercial feed inoculated with one probiotic strain at 1x107 CFU/g) and a control system (only commercial feed). Each tank was randomly stocked with 18 fish, whose weight and length were determined at 0, 15, 30, 60, and 90 days during probiotic treatment. Fish specific growth rate (SGR), condition factor (K), and food conversion ratio (FCR) were also calculated. Total viable bacteria, lactic acid bacteria (LAB), enterobacteria, and *Vibrio* spp. were quantified by plate-counting to assess the intestinal microbiota at the end of the experiment. As the probiotic strains have antimicrobial activity against fish pathogens, their abundances were detected using the double layer agar method. After 90 days of treatment with T4, H16, or TW34, intestine LAB counts were higher, and *Vibrio* spp. and enterobacteria lower than those of the control group. Strains T4, H16, or TW34 were recovered from the intestinal microbiota of treated fish (6.8, 7.24, and 7.4 Log10CFU/g of intestine, respectively). At the same time, fish weight of groups fed with T4 or H16 was

significantly greater than that of the control. Particularly, fish treated with H16 showed the highest SGR and K, and the lowest FCR, thus its application in aquaculture could be promising.

BT-P04
NOVEL RECOMBINANT ANTIGENS OF *Leishmania* (*Viannia*) *braziliensis* FOR LEISHMANIASIS IMMUNODIAGNOSIS

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Leishmania (Viannia) braziliensis is the main causative agent of American tegumentary leishmaniasis (ATL) in Argentina. Despite of the development of molecular methods, nowadays its diagnosis remains as a challenge. In this work we applied seroproteomic approaches for the selection and identification of *L. (V.) braziliensis* antigen candidates, for sensitive and specific immunodiagnoses of this endemic disease. By two dimensional Western blots of amastigote extract of *L. (V). braziliensis* , three antigen candidates were selected for their differential reactivity against sera from patients with ATL and non-reactive with Chagas disease, which cross-reaction have been previously reported. They were identified by Mass Spectrometry and Fingerprinting analysis. One of them was overexpressed in *Escherichia coli*, purified and used for serological tests. To analyze their immunological performance, sera from ATL patients and 52 from non ATL cases were included in this study. The antigen selected was termed HAT-LbAg1 (50.2 kDa, IP 5.2). The sensitivity - specificity of this antigen immunoblotting and ELISA were 80.5 - 90.5% and 70.7- 72, 88% respectively. With this molecular methods of identification of new candidates to ATL diagnosis, the cross-reaction with Chagas disease was reduced, increasing the specificity values of the immunoblotting technique. On the other hand, the sensitivity percentage can be improved by the combination with other candidates to diagnose true positives cases of ATL. Further studies are necessary to know the performance of HAT-LbAg1 and the other candidates in their application in novel immunological techniques.

BT-P05
IMMOBILIZATION AND CHARACTERIZATION OF G51 KERATINOLYTIC ENZYMES WITH POTENTIAL FOR WOOL PROCESSING

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Bacillus sp. G51 produces extracellular keratinases with potential for shrink-proofing of wool. Keratinases are proteases with autolytic activity which are restringing their industrial application in free form. Immobilization could contribute to a better control of their catalytic activity. Our aim was to immobilize and characterize G51 extracellular enzymes by cross-linking of enzyme aggregates (CLEA). G51 culture supernatant was used for CLEA with glutaraldehyde as cross-linking agent. G51 enzyme units (EU)/glutaraldehyde ratio was optimized, obtaining the best recovery of the proteolytic activity with the lowest ratio tested (8.4% with 3.5 EU/mlglu25%). CLEA-G51 thermal stability was higher (91 and 71% of residual activity after 1 h at 50 and 60°C, respectively) than that of free enzymes (40 and 5% residual activity under the same conditions). After 4 month-storage at room temperature, the free and immobilized enzymes kept 20 and 80% of residual proteolytic activity, respectively. This improvement of storage stability suggests that immobilization could prevent G51-keratinase autolysis and loss of activity. More than 60% of the proteolytic activity was preserved in the 3rd use, and it gradually diminished to 30% after seven re-uses. CLEA-G51 enzymes retained its wool keratinolytic activity (0.06 EU/ml), which is essential for wool shrink-proofing. CLEA-G51 operational and storage advantages could be valuable for industrial applications. Particularly, increased molecular size of immobilized G51 keratinases could avoid their diffusion into the wool fiber, allowing wool treatments with higher enzyme concentrations and without excessive degradation.

BT-P06
ENGINEERED BACTERIAL OUTER MEMBRANE VESICLES AS AN EXPERIMENTAL VACCINE AGAINST CHAGAS DISEASE

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Outer membrane vesicles (OMVs) are nanoparticles released from bacteria. Three of the most promising characteristics of OMVs are their high adjuvant capacity, their safety and the possibility of generating genetically engineered vesicles. Therefore, the utilization of OMVs as vaccines offers promising potential against a wide range of diseases. With this in mind we proposed to evaluate the potential role of engineered OMVs carrying different *Trypanosoma cruzi* antigens as an experimental immunogen against Chagas disease. We selected two antigens which have been extensively evaluated in vaccination models against *T. cruzi*, Tc24 and Tc52. The rational of selecting these antigens is that as a first step we propose to elucidate the advantage of using OMVs as carriers of parasite antigens and evaluate their adjuvant properties. As the first time reported, we were able to obtain recombinant OMVs with the selected *T. cruzi* antigens expressed on the outside of the vesicles as well as packaged within their lumen. These rOMVs were preliminarily evaluated in a murine prime-boot-challenge scheme for Chagas disease. During the vaccination stage, a slight increase in IFN-γ production was detected in immunized animals. In the challenge phase, a mild decrease in parasite load in vaccinated animals versus control groups could be detected. Several factors still need to be tested in order to optimize the use of rOMVs as a possible vaccine. In summary, the results so far obtained indicate that genetically designed OMVs could be a possible path for the development of novel strategies for trypanosomatids immunization. Funding: Fundación Bunge y Born y Fundación Fiorini.

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BT-P07
NEW CULTIVARS OF GLOBE ARTICHOKE WITH POTENTIAL APPLICATION IN CHEMOPREVENTION AND INFLAMMATORY PROCESSES

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Extracts of three regional cultivars Gauchito, Gurí and Oro Verde of *Cynara cardunculus var. scolymus* were analyzed by liquid chromatography coupled to electrospray ionization quadrupole-time-of-flight mass spectrometry (HPLC-ESI-QTOF-MS). Since irrigation plus fertilization and irrigation plus gibberellic acid application were the treatments with more effect on plant agronomical performance, fresh leaves and edible bracts of those plants were used to chemical characterization. The richest diversity in caffeoylquinic acids and flavonoids was found in Gauchito and Gurí leaf extracts. However, the highest content of caffeoylquinic acids was determined in Gauchito bract extracts (3608.0 ± 286.0 mg kg^{-1} of mono-caffeoylquinic acids and 10064.5 ± 378.3 mg kg^{-1} of di-caffeoylquinic acids). Both bract and leaf extracts were assessed by their radical scavenging capacity showing antioxidant activity. The leaf extracts of Gauchito and Gurí cultivars demonstrated the potential to inhibit the viability of neuroblastoma cells (SH-SY5Y), according to the results published for mesothelioma cells with extracts from Italian artichoke cultivars. Therefore, both Gauchito and Gurí artichoke cultivars could be good candidates for chemoprevention. Moreover, the effect of both cultivars over the type I interferon (rhIFN-I) biological activity was studied using a reporter gene assay. The incubation of Gauchito and Gurí leaf extracts with WISH-Mx2/EGFP cells for only 24h was enough to register a clear inhibition of the rhIFN-I activity, suggesting a role as modulators of its biological functions. Even though further research is needed, our results suggest that the Gauchito and Gurí artichoke crop waste may be exploited in pharmaceutical formulations to prevent pathological human signals related with cancer or chronic inflammation.

BT-P08
OPPORTUNITIES BEHIND *Geobacter*AU RESPIRATION. HYBRID NANOSTRUCTURES ISOLATION AND CHARACTERIZATION

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Geobactersulfurreducens is an anaerobic gram(–) bacteria capable of using a wide range of electron acceptor including insoluble Fe(III) (hydr)oxides and anode electrodes, thus expelling electrons beyond cells limits. C-type cytochromes named Omc (Outer membrane cytochromes) are the main redox proteins involved in external electron transfer. We have previously described the capacity of *Geobacter*to use Au(III) ions as electron acceptor, thus forming AuNPs that help improve current production in electrogenic biofilms. In this work we go forward on the isolation and characterization of nanostructures synthetized by *Geobacter* cultures grown in batch. Au respiration was performed at standard growing conditions for 1 week period. Soluble fraction containing gold NPs as well as biological material was then obtained and applied to a sucrose discontinuous gradient to achieve nanoparticle size separation. TEM microscopy from different fractions showed that NPs separation from cellular debris was achieved finding smaller NPs (2-10 nm) in 30% sucrose fraction and larger NPs in 60% sucrose fraction mostly of spherical shape. Performing SDS polyacrylamide electrophoresis of these fractions we detected several proteins together with NPs, including C-type cytochromes as observed by TMBZ stain. Of 5 cytochromes detected in the initial material, four of MR 78, 65, 50 and 45 kDa were also found in the sucrose fractions. Conjugates formed of cytochromes-AuNPs, hybrid nanostructures with particular electrochemical properties, were analyzed with electrochemical techniques. These results may also explain Au(III) respiration mechanism.

BT-P09
A GENETIC SYSTEM TO EXPRESS RECOMBINANT ANTIGENS IN *Bacillus* FOR LEISHMANIASIS VACCINES DEVELOPMENT

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American tegumentary leishmaniasis (ATL) is an endemic disease in Argentina and there are no vaccines for human application. Heterologous expression of specific antigens in generally recognized as safe bacteria (GRAS) could be a valid alternative for vaccine formulations. In order to obtain a system for protein exposure on *Bacillus subtilis*, a genetic construction was developed. For that, a transcriptional fusion was created between CotB, a protein expressed in spores, and LbAg1, an immunogenic protein of *Leishmania (V.) braziliensis*. The structural gene of CotB was amplified by PCR from *B. subtilis* 168, subsequently reamplified using specific primers to incorporate a multiple cloning site towards the 3' extreme and cloned into the pRSETa plasmid. Structural gene encoding LbAg1 was amplified from *L. (V.) braziliensis* and subsequently re-amplified adding to the 3' extreme: i) the *Xma*I restriction site, ii) a sequence encoding for six histidine residues and; iii) the *Bam*HI restriction site. This construction was cloned downstream of cotB. Finally, the hybrid gene obtained *cotB-polylinker-lbAg1-6His* was digested with *Hind*III and *Bam*HI and cloned into the integration vector pDG364 obtaining the plasmid called pSPOK. This plasmid may be used for cloning any gene of interest allowing its expression on outer spore surface of *B. subtilis*. The utility of *B. subtilis* spores for the delivery and *in-vivo* LbAg1 presentation is under assay. The safety and easy handling of *B. subtilis* make this expression system useful for the expression on the surface of bioactive molecules such as recombinant antigens capable of triggering a protective immune response.

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BT-P10
NEW BIOLOGIC SENSORS FOR DETECTION OF BIOAVAILABLE TOXIC HEAVY METALS

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Contamination with heavy toxic metals is a worldwide concern, affecting not only human health but also biodiversity. The use of whole-cell bacterial biosensors (WCB) emerges as a simple and cost-effective alternative to conventional detection procedures. Although poorly sensitive, these engineered bacteria report only the bioavailable fraction, being more appropriate to evaluate risk. Our group focuses on the study of metalloregulators, the key component of WCB and the main determinant of sensitivity. Previously, based on non-selective variants of GolS -the *Salmonella* Au(I) sensor/transcriptional regulator- we developed WCB for the simultaneous and high sensitivity detection of a broad-spectrum of metals including mercury (Hg), lead (Pb) and cadmium (Cd), which are among the most hazardous contaminants in the environment. Taking advantage of the unusual plasticity exhibited by GolS and its functional paralogue CueR, we applied site-directed mutagenesis on the metal binding region of these sensors to privilege recognition of Hg(II), Pb(II) or Cd(II). The functionality of the mutant sensors was evaluated by the activation of specific reporter genes in response to different metal ions. We obtained a set of GolS- or CueR-derived mutant sensors with altered metal recognition. GolS variants improved selectivity toward Hg(II) while analogous CueR-derivatives privileged recognition of Pb and Cd in detriment of other metals, highlighting differences on the metalloregulator scaffolds. Our results indicate that it is possible to improve selectivity of GolS or CueR to allow the development of specific WCB for reporting bioavailable Hg, Pb or Cd on the environment.

BT-P11
USE OFSspDnaB MINI-INTEIN FOR THE PURIFICATION OF RECOMBINANT PHARMACEUTICAL PROTEINS

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Inteins are self-splicing polypeptides with ability to excise themselves from flanking protein regions with remarkable precision. The aim of this work was to implement a purification methodology using the *Synechocystis*sp. DnaB mini-intein (SspDnaB) for the production of recombinant human growth hormone (rhGH) in *Escherichia coli*. We designed an expression vector to produce rhGH N-terminal fused to the CBD-SspDnaB chimeric protein. The CBD (Chitin Binding Domain) tag allows purification of proteins by affinity chromatography while SspDnaB mini-intein undergoes a self-cleavage reaction enabling the elution of rhGH without the affinity tag. Two rhGH variants were studied, the natural hGH whose first amino acid is phenylalanine (Phe-hGH) and a variant with an additional methionine at its N-terminal end (Met-hGH). The hGH coding sequences were *E. coli* codon optimized and synthesized with the first aminoacid (Met or Phe according to the rhGH variant) right after cleavage site of SspDnaB mini-intein. Optimization of growth and induction conditions allowed the expression of large quantities of both rhGH variants. Then, the recombinant proteins were extracted from *E. coli* cells and purified by affinity chromatography. Both rhGH variants were efficiently bound to the chitin column by the CBD. However, Phe-hGH could not be recovered suggesting that Phe is not a recommended aminoacid at the N-terminal end of the target protein for the self-cleavage of the SspDnaB mini-intein. On the other hand, Met-hGH was efficiently recovered with high purity obtaining a yield of approximately 12 g/L.

BT-P12
CHARACTERIZATION OF MILK-CLOTTING ACTIVITY OF BREWERS' SPENT GRAIN PROTEASES

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Study and characterization of the residues of agri-food industries has generated great interest in the scientific community in the last thirty years, given that they can constitute an intermediate raw material for the manufacture of value-added products. Brewers' spent grain (BSG) is a rich source of protein that is currently only used to supplement livestock feed or discarded. However, enzymes present in BSG could be isolated and used to carry out another process, thus reducing the environmental impact of direct discarding. The aim of this work was to analyze the milk-clotting activity (MCA) of BSG proteases. From BSG, the remaining proteins were extracted with Tris-HCl buffer pH 8 100 mM and precipitated with $(NH_4)_2SO_4$ at a final concentration of 80% w/v. Proteolytic activity was determined against azocasein. Activity was observed in the supernatant (SN). SN proteolytic activity was optimized, finding the optimum values at pH 7 and 40ºC. MCA of SN was determined by Arima method, using different SN: milk ratios. Incubations were at 37ºC overnight. The 1:1 ratio was found to be more suitable for obtaining the best clot. Caseinolytic activity of SN on α-, β- and κ-casein subunits was also evaluated to determine the degree of hydrolysis. Incubations were performed for 6 h at 37ºC and the percentage of hydrolysis was evaluated by SDS-PAGE. Positive proteolytic activity was observed on the κ-casein subunit, and little or no activity on α and β- subunits. These results suggest that BSG may be a possible source of enzymes that can be used for the production of spreadable cheeses.

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BT-P13
βγ-CRYSTALLIN DOMAIN OF *Lysinibacillussphaericus*PI-PLC PLAYS A CENTRAL ROLE IN PROTEIN STABILITY

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βγ-crystallins have emerged as a superfamily of structurally homologous proteins with representatives across all the domains of life. A major portion of this superfamily is constituted by members from microorganisms. This superfamily has also been recognized as a novel group of Ca^{2+}-binding proteins with huge diversity and variable properties in Ca^{2+}-binding, stability and association with other domains.
We have recently described the development of a new phosphatidylinositol (PI) phospholipase C from *Lysinobacillussphaericus* (LS-PI-PLC) to be used for oil degumming which was shown to efficiently remove PI from crude oil. Here, the role of the C-terminal βγ-crystallin domain of LS-PI-PLC is analyzed in the context of the whole protein. A truncated protein in which the C-terminal βγ-crystallin domain was deleted (LS-PI-$PLC_{\Delta CRY}$) is catalytically as efficient as the full-length protein (LS-PI-PLC). However, the thermal and chemical stability of LS-PI$PLC_{\Delta CRY}$ are highly affected, demonstrating a stabilizing role for this domain. It is also shown that the presence of Ca^{2+} increases the thermal and chemical stability of the protein both in aqueous media and in oil, making LS-PI-PLC an excellent candidate for use in industrial soybean oil degumming.

BT-P14
ENZYMATIC OIL DEGUMMING USING A NOVEL GLYCEROPHOSPHOLIPID:CHOLESTEROL ACYL TRANSFERASE

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In the last decades, the need for oils to be used as food and for the production of fuels has been in constant increase. Crude vegetable oils are a complex mix of triglycerides, phospholipids (or gums), sterols, glycolipids, tocopherols, free fatty acids, metallic traces and other minor compounds. The refining of oil involves many steps, including the removal of phospholipids (also known as oil "degumming"), which causes the major losses in the industrial process of oil refining. Thus, degumming is an important issue that needs to be addressed with cost effective methods. Traditionally, physical and chemical methods have been used. More recently, developments were made to use enzymatic degumming, which possess several advantages over chemical and physical processes. Enzymatic degumming has been employed using a wide variety of enzymes to hydrolyze phospholipids, generating products that are more easily removed by centrifugation. The most common enzymes used are phospholipase C (PLC) and phospholipase A (PLA), which reduce the phospholipid content and leave less oil trapped by the gums. Thus, upon enzymatic treatment the overall yield increases, which represents a significant economic benefit for the oil industry.Glycerophospholipid:cholesterol acyl tranferases (GCAT) are enzymes that attack acyl groups from phospholipids just like PLA enzymes. However, they additionally transfer the acyl group to a free sterol, reducing the amount of free fatty acids in treated oil.In this work, 6 different GCAT candidates obtained from an *in silico* analysis were evaluated in terms of their expression, thermal stability and ability to remove phospholipids using oils with different phospholipid contents, expecting to formulate a new enzyme for oil degumming. A GCAT from *Aeromonas enteropelogenes* was selected, due to its ability to work at temperatures up to 60 ºC, and to efficiently remove all phospholipids from crude oils (1200 ppm of phosphate). These and other features from this GCAT from *A. enteropelogenes* are described herein.

BT-P15
DESIGN OF *Escherichia coli*COCULTURE SYSTEM TO PRODUCE MULTI-METHYL-BRANCHED ESTERS

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Microbial lipid production represents a potential alternative feedstock for oleochemical industries. In a previous work, we engineered into *E. coli* a polyketide synthase (PKS)-based biosynthetic pathway from *Mycobacterium tuberculosis* and redefined its biological role towards the production of a variety of multi-methyl-branched esters (MBE). The biosynthetic pathway comprises the enzymes: FadD28; the PKS Mas, which synthetizes multi-methyl-branched fatty acids; and PapA5, that transfers this acyl group to an alcohol. The alcohol that acts as acceptor molecule to form the corresponding MBE was supplemented into the culture media.With the aim to develop a whole *de novo* bioprocess (i.e. without using exogenous alcohol), an *E. coli* co-culture system was engineered to modularize the "alcohol biosynthetic pathway" and the "PKS-based biosynthetic pathway".Thus, the MBE producing strain (RQ5 pMB22) was cultivated with an isobutanol producing *E. coli* strain. The last one, was obtained by transforming *E. coli* BL21 with pIAA11 and pIAA12 plasmids, encoding the enzymes necessary for the biosynthesis of isobutanol from glucose. The genes encoded in pIAA12 were later integrated into the chromosome, generating a new strain, CB1, that produced 18.75±0.45 mM of isobutanol after 24h of induction, an increase of 1800% with respect to BL21 original strain. When CB1 was cocultivated with RQ5 pMB22 in a minimal M9 medium, the consortium CB1/RQ5 at the best inoculation ratio of 1:2, produced 30.48±2.02 µg MBE ml^{-1}. This yield was comparable to that exhibited by a monoculture based on the single-cell RQ5 pMB22 with the external addition of isobutanol into the growth media.

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BT-P16
MORPHOLOGY, NUTRITIONAL COMPOSITION AND ACCUMULATION OF ASTAXANTIN IN*Oedocladimcirratum*

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The objective of the present work is to evaluate the morphological characteristics, nutritional composition and accumulation of carotenoids with special emphasis on astaxanthin, product of great commercial interest, due to the effect of nitrogen limitation on the algae *O. cirratum* (UTEX LB 1532) of terrestrial habit (humid soils). As an adaptation to face conditions of rapid desiccation of soils and high intensities of sunlight, the vegetative cells can develop thick cell walls and accumulate a high amount of carotenoids, differentiating into acinetas. Cultures for vegetative growth were performed in 1-liter bioreactors of standard mineralized medium, with aeration and continuous light at 23 ±1 °C and then this culture was subjected to nutritional stress conditions in standard mineralized medium without N source for production of acinetas rich in astaxanthin. This work presents the observations under growing and stress conditions and the nutritional composition of vegetative cells: total lipids. (5.27 ± 0.77%); proteins (44.6 ± 5.1%); carbohydrates (48.1 ± 4.4%); chlorophyll a and b (2.42 ±0.66 and 4.49 ± 0.39 mg / g) and acinetas: total lipids (23.7 ± 3.16%); proteins (28.2 ± 3.16%); carbohydrates (19.3 ±2.25%); chlorophyll a and b (1.61 ±0.43 and 1.49 ± 0.48 mg / g); total carotenoids (8.19 ± .46 mg / g). *O. cirratum* has a high potential for the production of astaxanthin since it is possible to induce the accumulation by modifying the culture conditions obtaining high levels compared, mainly, with *H. pluvialis*.

CELL BIOLOGY

BC-P01
A SINGLE NMT IS RELEVANT FOR *Toxoplasma gondii*LYTIC CYCLE

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Toxoplasma gondii is the causative agent of toxoplasmosis. This disease affects almost one third of the world's population with devastating effects. Despite the significant progress that has been made in order to develop new compounds to treat toxoplasmosis, the current therapeutic agents frequently used have toxic side effects. As such, scientists are in real need of finding new targets of intervention.Protein myristoylation is a post- and co-translational modification that affects a variety of proteins in many cells including parasites. It is catalyzed by N-myristoyltranferase (NMT), a conserved enzyme that has been described to be essential in many protozoan pathogens. However, up to date, there is scarce information on NMT and the extent of this modification in *T. gondii*. In this work *T. gondii* NMT (TgNMT) was identified and characterized. Structural analyses suggest that there are differences between human and *T. gondii* NMTs, which could be of importance to design specific inhibitors. Furthermore, this protein presents NMT activity *in vitro*, is expressed in both intra- and extracellular parasites and interacts with predicted TgNMT substrates. Additionally, TgNMT activity seems to be important for the lytic cycle. An *in silico*myristoylome predicts 157 proteins to be targeted by this modification with some of them being critical for the life cycle of this parasite. This analysis suggests that myristoylation could be regulating calcium homeostasis which is critical for *T.gondii* pathogenesis. Together, these data indicate that TgNMT could be an interesting target of intervention for the treatment of toxoplasmosis**.**

BC-P02
ANALYSIS OF EPITHELIAL-MESENCHYMAL TRANSITION (EMT) PROCESS IN RENAL COLLECTING DUCTS OF AGING RATS

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Renal function declines progressively with age. The EMT process has been suggested as a mechanism that drives fibrosis, with the consequent loss of tissue functions. In previous works, we demonstrated that inhibition of sphingomyelin synthase I (SMS1) activity induces collecting duct (CD) cells to lose their epithelial phenotype and to undergo an EMT process. Now we investigated the occurrence of EMT in renal papilla CD cells of aging rats (6 month). Taking advantage of the fact that CD in primary culture retains many characteristics of their behavior in intact tissue, primary cultures of CD cells from young (70 days) and aging rats were performed. We analyzed the expression of epithelial (α- and β-catenin) and mesenchymal (vimentin and α-smooth muscle actin, α-SMA) cell markers, cell proliferation, and the presence of primary cilia by immunocytochemistry. Contrary to what was observed in young rats, CD cells from aging rats exhibited impairment of cell-cell adhesion, a high expression of vimentin and α-SMA, and a significant increased number of isolated cells with fibroblastoid-like morphology expressing both proteins. We also observed greater proliferation and a decreased number of cells with primary cilium. These features are consistent with the alterations reported in tubular epithelial cells during renal fibrosis. The increased proliferation probably represents a mechanism to restore the integrity of the tubular epithelium. Our results suggest that as aging occurs, the balance between the EMT-MET processes in renal papilla is altered, but the link between these alterations and the impairment of SMS1 activity requires further studies.

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BC-P03
IMPLICATION OF SPHINGOSINE KINASE (SK) ACTIVITY IN OSMOTIC STRESS DURING POSTNATAL RENAL DEVELOPMENT

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Sphingosine 1 phosphate (S1P), the product of SK activity, regulates cell proliferation and survival. In neonatal rats, the renal papilla osmolality is much lower than in adults and increases dramatically after weaning. Previously, we demonstrated that the developmental regulation of SK expression and activity leads sphingolipid metabolism to the formation of S1P in the neonatal period, consistent with the immature-proliferative stage of neonatal renal papilla. Here, we evaluated the implication of SK activity in renal papilla collecting duct (CD) cells of 10-day-old rats subjected to osmotic stress. Taking advantage of the fact that CD cells in primary cultures retain many characteristics of their behavior in intact tissue, primary cultures of neonatal CD cells in hypertonic medium (HT) were performed. D,L-threo-dihydrosphingosine (tDHS) was used as an SK activity inhibitor. We analyzed by immunofluorescence the DBA lectin staining, a CD marker, and the cell morphology and proliferation. While cultured cells displayed an elongated morphology with regular intensity for DBA staining in isotonic medium, osmotic stress induced a more mature phenotype, reflected by a morphological change from an elongated to a hexagonal shape, and a greater positive DBA signal. When cells were subjected to HT in the presence of tDHS, cell-cell adhesion was impaired and a decreased intensity of DBA staining was observed. The proliferation decreased in cells subjected to HT, even in the absence of tDHS, with respect to the isotonic situation. These results suggest that SK activity is essential to maintain the epithelial phenotype in CD cells subjected to osmotic stress

BC-P04
HEXOSAMINE BIOSYNTHETIC PATHWAY (HBP) REGULATES STARD7 EXPRESSION IN JEG-3 CELLS

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StarD7 is a lipid binding protein that transfers phosphatidylcholine to the mitochondria. Our studies indicated that StarD7 protein levels are important to maintain JEG-3 trophoblast cellular homeostasis. It is well-known that changes in lipid metabolism and glucose concentration modulate several physiological processes. Moreover, glucose flux through the HBP leads to modification of various intracellular proteins with O-linked GlcNAc. Here, we explored the influence of elevated glucose levels on the StarD7 expression in JEG-3 cells. Results showed an increase in StarD7 as well as β-catenin expression when cells were incubated with 5.5 or 25 mM glucose at 2, 4, or 24 h (compared with 0.5 mM glucose) and these effects were reduced by the HBP inhibitors: azaserine and 6-Diazo-5-oxo-L-norleucine. In addition, the main markers of unfolded protein response (UPR) were assessed. In starvation conditions (0.5 mM glucose, over night) GRP78 and Ire1α levels were significantly elevated, whereas StarD7 and β-catenin were decreased. When cells were incubated with high glucose concentration, an early induction of GRP78 levels was observed, decreasing at 24 h. Related to Ire1α protein, a significant increased expression was detected with no changes in calnexin protein expression. The phosphorylation of eIF2α at Ser 51 decreased at all time assayed. These results indicate that glucose modulates StarD7 levels through HBP and also, that changes in glucose concentration induce activation of the UPR in trophoblast cells. Supported by FONCyT and SECyT-UNC.

BC-P05
TL(I) AND TL(III) INDUCE A NOVEL TYPE OF CELL DEATH (PARAPTOSIS) IN PROLIFERATING 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 through still poorly understood mechanisms, the kidney being a main target of Tl toxicity. Tl has two oxidation states, the monovalent (Tl(I)) and trivalent (Tl(III)) cations. We demonstrated previously that both Tl(I) and Tl(III) alter lipid metabolism in proliferating renal epithelial (MDCK) cells, increasing the content of phospholipids, cholesterol and triglycerides, as well as the number of lipid droplets. In this work we investigated further the possible mechanisms underlying those alterations. Confluent MDCK cells were incubated for 24 or 48 h in the absence or presence of Tl(I) or Tl(III) (10 or 100 μM). Phase-contrast and transmission electron microscopy evidenced cytoplasmic vacuolation that could be related to non-apoptotic death. RT-PCR analysis indicated that both Tl(I) and Tl(III) increased prohibitin (positive paraptosis modulator) expression without changing phosphatidylethanolamine-binding protein (negative paraptosis modulator) expression. Expression levels of proteins involved in autophagy and ER stress were analyzed by Western blot. Tl(I) and Tl(III) increased the expression of IRE-1, ATF-6, beclin-1 and sequestrin-1. Together, obtained results indicate that morphological and lipid metabolism alterations could be mediated by autophagy, ER stress and paraptosis, the latter explaining the increased contents of phospholipid and cholesterol in Tl-exposed MDCK cells.
**Both must be considered as last authors.*

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BC-P06
ACTIVATION OF LIPIN BY C-FOS: UNDERSTANDING THE INTERACTION THROUGH LIPIN

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c-Fos is a proto-oncoprotein that associates with components of the endoplasmic reticulum (ER) and activates the synthesis of phospholipids and glycolipids by a mechanism independent of it's genomic activity. c-Fos activates different enzymes involved in the synthesis of phospholipids and glycolipids that do not share similar structures or homology in their sequence, among them the enzyme phosphatidic acid phosphatase (Lipin). Lipin uses Phosphatidic acid (PA) as substrate and produces Diacylglycerol that will be used in the synthesis of phospholipids or phosphatidylinositides. Enzymes of the Lipin family are present in most tissues and are distributed both at the nucleus and in the cytoplasm; they are not integral membrane proteins but translocate from the cytoplasm to ER membranes to participate in the synthesis processes. It has been shown that Lipin interacts with the N-terminal domain of c-Fos and is activated through the basic domain of this protein (BD). The aim of our work is to study the Lipin domains involved in the interaction with c-Fos in order to understand the biology of the interaction between these enzymes and c-Fos. To this end, four enzyme deletion mutants fused to GFP were generated and its subcellular localization was observed in T98G cells (human glioblastoma multiforme), the subcellular localization and Lipin expression levels in synchronized cultures were also analyzed, comparing their expression to that observed for c-Fos. We are currently studying the interaction between these deletion mutants and c-Fos by FRET microscopy.

BC-P07
EFFECT OF RESVERATROL ON RENAL EPITHELIAL CELL ADAPTATION TO HYPERTONICITY

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Resveratrol (trans-3,4′,5-trihydroxystilbene, Rsv) is a small polyphenol molecule present in a large variety of plants such as mulberries, peanuts and grapes. Rsv was widely used by Asian medicine for fungal, inflammation, hypertension, allergy, cancer, and lipid diseases. In occident, it is widely used by population as dietary supplement due to its antioxidant property. Among the molecular effects of Rsv, the activation of AMPK and SIRT1 has been reported. Rsv-induced SIRT1 activation is responsible for NF-kB transcriptional activity downregulation and COX-2 expression decrease. It was also reported that Rsv has renoprotective actions. These observations are contradictory to our previous results showing that COX-2 expression is a key factor for renal cell survival and adaptation to changes in environmental osmolality. In the present work, we evaluated whether Rsv affects the adaptation of renal cells to hyperosmolarity. To do this, MDCK cells were subjected to high-NaCl media (512 mOsm/kg H_2O) for 24h in the absence or presence of different concentrations of Rsv (12.5, 25, 50, 100 µM). After treatment, cells were collected, counted and viability determined. COX-2, and other osmoprotective genes (BGT1, SMIT and AR) expression were determined. Rsv decreased the number of cells recovered after treatment in a concentration-dependent way. Rsv also affected cell morphology hindering the formation of the typical monolayer. We also found that Rsv decreased COX-2 expression and modulated osmoprotective gene. These results clearly indicate that Rsv impedes renal cells adaptation and survival to hyperosmolality by blocking key genes expression.

BC-P08
INVOLVEMENT OF SUMO CONJUGATION IN SMALL NUCLEAR RNA BIOGENESIS

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In addition to protein-coding genes, RNA polymerase II (Pol II) transcribes numerous genes that correspond to non-coding RNAs, including those of small nuclear RNAs (snRNAs). snRNAs are not only a fundamental component of the spliceosome but also some of them are necessary for the transcriptional activity of Pol II, as well as for maintaining cellular homeostasis.Although the functions of snRNAs are well understood, the regulation of their biogenesis is still not fully characterized. snRNA genes share common features with protein-coding genes, including the relative positioning of elements that control transcription and RNA processing. However, there are important differences in the set of proteins required for the proper expression and metabolism of these two gene types. SUMOylation is a reversible post-translational modification consisting in the conjugation of SUMO (small ubiquitin-related modifier) to different target proteins. It mainly regulates intra- and inter-molecular interactions and consequently the function of a great variety of cellular proteins. We are currently studying the involvement of SUMO conjugation in snRNA biogenesis. So far, we have observed that modifying the levels of global SUMOylation in cultured mammalian cells alters the levels of nascent/immature snRNAs measured by RT-qPCR. Furthermore, we have identified that several regulatory factors involved in transcription, elongation and 3'-end processing of the snRNAs are modified by SUMO and we have determined the target lysine residues in some of them. We are currently exploring the functional consequences of abrogating this modification within these regulatory factors.

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BC-P09
USING ENHANCER ACTIVITY PROFILES TO EXPLORE TRANSCRIPTIONAL PERTURBATIONS IN BREAST CANCER

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The identity and function of a cell are determined by gene regulatory networks, the de-regulation of which can lead to pathologies such as cancer. Enhancers are key genetic elements in these networks and the alteration of their function can lead to de-regulation of genes involved in oncogenic and/or tumor suppressor pathways. Furthermore, it is known that there are highly active regulatory regions, or super-enhancers (SE), with important participation in tumorigenic processes. The objective of this project is to find new relevant cellular pathways in different breast tumor types. For this, we analyzed the enhancer activity and transcriptional profiles in 17 breast cancer cell lines. First, we used ChIP-seq data for the H3K27ac histone modification to call peaks in each line, which were then combined into a unified set of regulatory regions. By merging nearby enhancers and ranking them according to their signal strength, we similarly defined a common SE set. Subsequently, we quantified the H3K27ac signal for each line across the common set of regulatory regions. We grouped the lines according to their histological subtype, and obtained differentially active regions for each group, from where we extracted the enriched motifs and their corresponding transcription factors. In parallel, using RNA-seq data in the context of breast cancer gene regulatory networks, we identified the master regulators that are differentially active in each group. The integration of these approaches would point to regulatory pathways super- and sub-active in the different breast cancer types, opening the possibilities for new lines of research.

BC-P10
WINE POMACE PRODUCTS ON MDCK DIFFERENTIATION

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Polyphenols are natural compounds that play an important role in the prevention and treatment of different diseases (cancer, cardiovascular). In the present study, a natural red (rWPP) and white (wWPP) wine pomace product whose capacity of NF-kB and Nrf2 pathways regulation has been suggested in previous studies, were used to evaluate the effect on the differentiation of renal cells. Before the addition of hypertonic medium, MDCK cells were treated with 2.5 µg GAE/mL of different rWPP and wWPP bioavailable fractions: gastrointestinal digestion (G) and colonic fermentation (F). After 48-h of NaCl treatment cells were fixed and morphological changes and polarized phenotype was assessed by inmunofluorescence microscopy. The hypertonic treatment reduces the cell number and increases the nuclear size of the control and WPP-treated cells. The cellular cortex of actin is maintained by the fractions F of rWPP and the wWPP showing morphology similar to endothelial phenotype. However, the fraction G of WPP alters the morphology and increases the stress fibers. Further, the differentiation by NaCl seems to generate a maturation of the cellular junctions increasing the localization of E-cadherin and ZO-1 in the cellular periphery that is maintained for the treatments with the WPP. These results support the potential benefits of colonic fermentation fraction from WPP in the differentiation of cells associated to cancer progression.

BC-P11
ALKALOIDS WITH SYNTHETIC LETHAL ACTIVITY AGAINST *BRCA*-DEFICIENT CELLS ISOLATED FROM *Zanthoxylum coco*

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The design of drug discovery strategies for cancer research is one of the most challenging efforts to develop novel targeted therapies. Synthetic lethality (SL) is a state-of-the-art precision strategy for cancer treatment that is based on the induction of selective toxicity against cancerous cells with certain genetic defects. Since mutations or epigenetic down-regulation of *BRCA* genes are known drivers of hereditary types of breast and ovarian cancers, they can be used as tools in phenotypic screenings to identify novel synthetic lethality inducers.Due to their broad chemical diversity, natural products are an important source of lead molecules for drug discovery. In this work we evaluated pure compounds and extracts from ≈200 plant species from Argentina using an innovative screening platform based on automated flow cytometry. We found that the alkaline extract from *Zanthoxilum coco* induce SL in *BRCA*-deficient cells. To identify the active metabolite/s, we performed bio-guided isolation following a sub-fractionation process and semi-preparative HPLC. Using spectroscopic methods (NMR) we were able to identify a set of benzophenantridine alkaloids as the responsible compounds of this activity. Early validation experiments using *BRCA*-KO cell lines and commercially available alkaloids from the same family confirmed our findings. In conclusion, this work put forward the benzophenantridine alkaloids from *Zanthoxylum coco* as promising molecules for target identification and drug development to treat *BRCA*-deficient cancers.

BC-P12
WINE POMACE PRODUCTS ON EPITHELIAL MESENCHYMAL TRANSITION

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The epithelial mesenchymal transition (EMT) is associated with renal fibrogenesis and kidney disease. During this transition, the epithelial cell loses its phenotype and gains the characteristics of a mesenchymal cell. This state is one of the key events in the development of cancer

metastasis. Polyphenols have been reported to have antimetastatic effects increasing the levels of epithelial markers and altering the expression of proteins associated to mesenchymal characteristics. The objective of this study was to evaluate the effect of a red and white wine pomace product (rWPP and wWPP) on EMT. After differentiation (72h NaCl-hypertonicity), MDCK cells were treated with 1.5 mM of oxalate and with the gastrointestinal and colonic fractions of rWPP and wWPP. Morphological changes and biomarkers of epithelial (E-cadherin, beta-catenin) and mesenchymal (vimentin) were assessed by inmunofluorescence microscopy. The treatment with WPP conserve the epithelial phenotype by not inducing changes in morphology observed when the cells were treated with oxalate along. The treatment with oxalate resulted in a loss of the peripheral E-cadherin and beta-catenin distribution and acquisition of vimentin fibers. However, the presence of WPP in the medium partially prevents the changes observed by the oxalate. In conclusion these results support the potential effects of wine pomace product (red and white) on metastasis to impair the invasion and migration of tumor cells through modulation of regulators of epithelial-mesenchymal transition. The characteristics of polyphenols render those promising candidates for effective adjuvant therapy against metastatic cancer.

BC-P13
DEVELOPMENT OF ISOGENIC CELLULAR MODELS TO VALIDATE SL INDUCERS IN *BRCA1*-DEFICIENT TUMOR CELLS

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Homologous recombination (HR) deficiency is a well-established driver of tumorigenesis, which is mediated by the aberrant activation of error-prone repair mechanisms. This deficiency is linked to somatic or germline mutations in the *BRCA* genes, as well as other genes that participate directly or indirectly in the HR pathway. This has led to a remarkable interest in developing therapies that take advantage of this tumor feature, including the outstanding case of PARP inhibitors recently approved by the FDA.In our laboratory we have performed a phenotypic screening using flow cytometry to identify synthetic lethality (SL) inducers in *BRCA1* and *BRCA2*-deficient cells. Using an open-source library of 680 kinase inhibitors we identified Polo-Like Kinase 1 (PLK1) as a molecular target for the induction of synthetic lethality in *BRCA1* deficient cells. In this work we focus in the development of isogenic cellular validation models that differ only in the expression levels of *BRCA1*, using breast and ovarian parental cell lines. To achieve this goal, we used a lentiviral system of shRNA expression to downregulate *BRCA1* in T47D and SKOV3 cell lines. The stable cell lines generated were then used to validate the SL-activity of the PLK1 inhibitors identified in the screening, as well as the activity of commercial PLK1 inhibitors that are currently in clinical trials.Conclusion: since PLK1 is a central kinase for mitotic progression that is currently under clinical investigation, our results suggest that PLK1 inhibitors could be used to treat patients' cohorts characterized by alterations in *BRCA1* expression.

BC-P14
LIGHT EFFECT ON *Acinetobacter*VIRULENCE

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In our group we have found that many bacterial pathogens such as *Acinetobacter baumannii* sense and respond to light integrating also a temperature signal. We have extensively characterized photoregulation at moderate temperatures such as 24ºC in this microorganism, showing that light exerts a global effect on its physiology modulating aspects related to persistence in the environment as well as virulence. We have shown that many of these processes depend on the blue light using FAD (BLUF) photoreceptor BlsA, which is a global regulator able to bind and antagonize the functioning of transcriptional regulators such as Fur in a light-dependent manner.Regulation by light occurs not only in *A. baumannii*, but is also widely distributed within the *Acinetobacter genus*. Most non-*baumannii*species harbor between two and six BlsA homologs, and photoregulation occurs not only at 24 but also at 37ºC. Many of these species are environmental, while others like *Acinetobacter* A47 and *nosocomialis* are important human pathogens. *A. nosocomialis* harbors three BLUF photoreceptors while A47 harbors two, modulating susceptibility to antibiotics, motility, iron uptake and hemolysis in the last case. Regulation by light at 37ºC in important pathogens could affect bacterial infections in humans. This situation could be particularly relevant for these pathogens given that they produce infections in skin wounds surface-exposed and soft tissue.In this work, we evaluate the effect of light in infections by A47, *A. nosocomialis* and *A. baumannii* towards a human keratinocyte cell line in culture, HaCaT. The model resembles skin wound infections, in which the stratum corneum has lost integrity and the bacteria come in direct contact with the cells. In this context, this model reflects situations in real infections, given that these species of *Acinetobacter* are recognized as natural colonizers of human skin. In this work, we have optimized infections using this model, varying the multiplicity of infection (MOI), time for adhesion or internalization, as well as antibiotic treatment. Our results so far show that light exerts an effect on *A. nosocomialis* virulence towards HaCaT cells, becoming the bacteria more virulent when in the dark rather than under blue light. In non-*baumannii*species, some processes such as biofilm formation, iron uptake or motility are regulated by light inversely than in *A. baumannii*, and in this work we show that virulence is also affected. Our results also pose the possibility that light could be regarded as a tool to control infections by these microorganisms.

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BC-P15
STUDYING THE FUNCTIONAL INTERACTION BETWEEN SRSF1 AND PIAS1 ON THE REGULATION OF SUMO CONJUGATION

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The RNA-binding protein SRSF1, a member of the SR family of proteins, has been deeply characterized as a regulator of the splicing process. After the discovery of its involvement in a wide variety of functions at several levels of gene expression regulation, this factor has become a paradigm of multitasking protein. Few years ago, our laboratory has revealed that SRSF1 modulates SUMO conjugation to different target proteins both in cultured cells and in vitro, displaying characteristics of a SUMO E3 ligase. A well-known member of this latter group is PIAS1, with which we have shown SRSF1 interacts. As part of the characterization of the role of SRSF1 in the SUMO conjugation pathway, we have generated a series of SRSF1 mutants, which not only display differential RNA binding capacities but also differential SUMOylation enhancing effects. Recently, we have observed that over-expression of wild type SRSF1 in HEK 293T cells, enhances PIAS1 protein levels. Working with the above-mentioned SRSF1 mutants, we are currently exploring whether the regulation of SUMO conjugation is due to the observed changes on PIAS1 levels. Furthermore, we expect to unravel whether SRSF1 affects PIAS1 by controlling its mRNA or protein stability and/or its translation rate.

BC-P016
STUDYING THE ROLE OF EISOSOMAL MEMBRANE DOMAINS IN CELLULAR AGING

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The plasma membrane (PM) of eukaryotic cells is compartmentalized into domains enriched in specific lipids and proteins. *Saccharomyces cerevisiae* contains at least a dozen of different nanodomains that exhibit different morphologies and dynamic behaviors. Particularly, eisosomes are nanoscale PM invaginations structured by scaffolds composed mainly by two cytoplasmic proteins Pil1 and Lsp1. More than 25 proteins including transporters, signaling molecules and proteins of unknown function have been localized in eisosomes, however, much remains to be learned about the mechanisms and biological roles of this lateral segregation. We are interested in understanding eisosomes´ role in aging using *S. cerevisiae* aging model. Performing replicative yeast aging assays we found that knockout strains for *PIL1* (eisosomes´ dissasembled) have significantly enhanced longevity. No effect was observed when *LSP1* was deleted (eisosomes assembled), suggesting that eisosomes´ structure plays a key role in yeast aging. Chronological aging assays were performed using the standard CFU method and propidium iodide cell-staining followed by flow cytometry to determine cell survival in two different genetic backgrounds. In order to determine if the longevity of *PIL1* mutant is associated with a limitation in the availability of nutrients, an uptake assay was set up enabling the challenge of a specific hypothesis: activity of an aminoacideisosomal permease is affected by eisosomes´ disassembly increasing lifespan. Understanding eisosomes´ role in aging will likely contribute to further describing complex *S. cerevisiae* aging process and nanoscale PM domains function.

BC-P017
STUDIES ON EISOSOMES DYNAMIC STRUCTURE IN *Saccharomyces cerevisiae*

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Eisosomes are protein and lipids complexes assembled onto the Plasmatic Membrane (PM) with sizes about 200nm long and 50nm wide. These structures are distributed as elongated invaginations all over the *Saccharomyces cerevisiae* PM. The roles played by nanodomains in the cellular physiology are known, however, the mechanism leading to their formation and maintenance is not completely comprehended. The evidence collected by our group, and others too, allow us to postulate that eisosomes are shaped and maintained through the agency of scaffolding consisting in autoassembled proteins with BAR domains (bin-amphiphiysin-rvs) Pil1 and Lsp1. As the matter of fact, we know that Pil1 and Seg1 proteins are main scaffolding organizers while Lsp1 plays a secondary role. On the other hand, unpublished results hint that eisosomes´ presence and shape are factors associated with the cell´s replicative lifespan. Considering this, we decided to study the eisosomes´ formation and maintenance molecular dynamics on young cells while ageing. Our work on fluorescence Autocorrelation Function on eisosomes´ proteins in conjunction with design, fabrication and use of microfluidic devices will be key in order to study individual cells throughout its lifespan.

BC-P18
EXTRACELLULAR VESICLES REGULATE SURVIVAL, CA^{2+}, AND TYROSINE PHOSPHORYLATION IN BOVINE SPERMATOZOA

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It is known that secretions present in the oviductal fluids affect sperm function, although the underlying mechanisms are unknown. Extracellular vesicles (EV) have been recently identified along the female reproductive tract, including the uterus and the oviduct. We have previously shown that endometrial EV stimulate human spermatozoa capacitation. Thus, since both capacitation and acrosome reaction (AR) depend on an increase in intracellular calcium concentration (Ca^{2+}i), we hypothesized that the regulation of Ca^{2+}i could be mediated by EV. Bovine oviductal EV isolated from isthmus and ampullary regions were composed of exosome and microvesicle populations of different size, determined by dynamic

light scattering, and presented the EV protein markers CD81 and HSP70. Highly motile sperm cells from cryopreserved bovine semen samples were selected, and incubated either in medium containing EV or in control medium. Cell survival at different time points, the level of protein tyrosine phosphorylation (p-Tyr), Ca^{2+}i, and AR were evaluated. We found that incubation with EV increased cell survival versus control conditions. Flow cytometry analysis of Ca^{2+}i using the calcium indicator Fura-4 AM showed that oviductal EV induce an immediate increase in Ca^{2+}i, and also showed that after a prolonged incubation time, a higher Ca^{2+}i was found only in the presence of EV. Moreover, EV induced sperm capacitation, determined by an increase in both protein p-Tyr and AR. Thus, our results contribute to elucidate the role of EV in the regulatory mechanism of the oviductal environment on different processes associated with sperm fertilizing capacity.

BC-P19
QUANTIFICATION OF FREE CYTOSOLIC Ca^{2+}CONCENTRATION IN *Saccharomyces cerevisiae*

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Saccharomyces cerevisiae haploid cells secrete mating pheromones (a & α) that bind to receptors in the plasma membrane of cells of the opposite sex. The recognition between pheromone and receptor triggers a signaling cascade, which includes a transient raise in cytosolic Ca^{2+} levels. In turn, this activates the calmodulin (CM), calcineurin (CN) and Crz1 transcription factor signaling pathway (Ca^{2+}-CN-Crz1 pathway). Activation of this pathway is necessary for maintaining cell viability during prolonged periods of time in the presence of high pheromone concentrations.
Currently it is accepted that an influx from extracellular calcium is induced in response to sexual pheromone, generating an elevation of cytosolic Ca^{2+} for 90 minutes. However, a recently published work indicates that there is not one single and continuous calcium elevation but several short duration discrete pulses (10-90 seconds). Bursts of Ca^{2+} are also observed in non pheromone treated cells, but in a sporadic way. These results suggest that the presence of pheromone results in an increase in the frequency of cytosolic Ca^{2+} pulses. Having a reliable estimate of cytosolic Ca^{2+} concentration is key to achieve a better understanding of calcium signaling during sexual pheromone response. In this work, we perform Fluorescence Correlation Spectroscopy (FCS) experiments in the cytosol of *S. cerevisiae* haploid cells using GCaMP6f as the Ca^{2+}dye to quantify the free concentration of this ion.

BC-P20
CHARACTERIZATION OF L-LEUCINE TRANSPORT IN *Saccharomyces cerevisiae* GROWN IN MEDIUM SUPPLEMENTED WITH *Moringa oleifera*

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Yeast model system is suitable to study the effect of different substances on the metabolism of eukaryotic cells. The system avoids effects present in cells of higher eukaryotes due to organ or tissues interaction. Tea extracts of *M.oleifera* ("moringa") have been considered beneficial to be incorporated into the diet of diabetic patients or with antioxidant properties. However, these beneficial effects have not been elucidated from the point of view of cellular biochemistry. The objective of the present work is to characterize the transport of the amino acid L-leucine in a culture medium supplemented with *M.oleifera*. Extracts of *C.sinensis* ("green") and *C.angustifolia* ("sen") were used for comparison. Results obtained show that the transport of the amino acid is increased in all media with respect to the control medium with yeast extract/peptone (YPD). The general amino acid system (GAP1) would be responsible for the observed phenotype. In the case of the sen extract it is found that it produces a phenotype highly sensitive to the presence of H_2O_2 in the solid culture that can be reversed by the addition of antioxidant N-acetyl cysteine. Both,sen and green tea increase the degradation of H_2O_2, which can be reasoned as to a greater activity of the catalase enzyme. Results can be interpreted considering that the extracts provide a poor source of nitrogen analogous to the amino acid L-proline. This amino acid requires greater activity of mitochondrial oxidative metabolism and concomitantly there is an increase in nonspecific amino acids transport systems activity.

BC-P21
ARGINYLTRANSFERASE (Ate1) REGULATES STRESS RESPONSE TO BORTEZOMIB OF HUMAN GLIOMA CELLS.

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The enzyme Ate1 mediates the post-translational addition of an Arg to proteins bearing acidic N-terminal amino acids that are mainly target to proteasomal degradation or macroautophagy. Previous studies reported that Ate1 downregulation suppresses cell death induced by different stressors. In addition, *Ate1*-knockout fibroblasts exhibit tumorigenic properties, including exacerbated contact-independent growth and chromosomal aberrations. We recently reported that increased cell membrane exposure of an Ate1 substrate (arginylated calreticulin) modulates the sensitivity to proteasomal inhibitor bortezomib (BT) of human oligodendroglioma (HOG) cells. Hence, further assessment is required to determine the Ate1 implication in the tumorigenic progression of BT treated cells. HOG cell death induced by BT comes through a mechanism that involves activation of the unfolded protein response (UPR) mediators, including the transcriptional activation of the spliced mRNA xbp1 and concomitant upregulation of DR5-R membrane expression. Moreover, we do not observed changes in autophagy flux at different time and drug doses. Strikingly, we found that Ate1 knockdown in HOG increases their sensitivity to BT in a macroautophagy independent way, suggesting that apoptosis of glioma cells induced by BT is strongly influenced by Ate1 expression. We postulates that Ate1 is an essential enzyme that regulates stress response and cell fate controlling the tumorigenic progress of cancer cells.

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BC-P22
SPECIFIC HAPLOTYPE ANALYSES OF COMPLEX MOLECULAR REARRANGEMENTS AT THE HUMAN CHROMOSOME REGION 4Q35

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A polymorphic tandem repeat of 3.3 Kb units (D4Z4) is present at the telomere region of human chromosome 4q. Distal to D4Z4 is another polymorphic sequence, named pLAM, which shows three possible haplotypes: 4qA, 4qB and 4qC. The shortening of the tandem D4Z4 to 1-10 units and the presence of the pLAM haplotype 4qA are key to the development of facioscapulohumeral muscular dystrophy, the second most frequent inherited human muscular dystrophy. In this report we present the molecular characterization of complex D4Z4 alleles using pulsed field gel electrophoresis (PFGE) followed by Southern blot hybridization with alternative specific probes from the 4q35 region. PFGE analyzes were performed on a BioRad CHEF DRII device using conditions described in our previous report1. Single and double digestion with the restriction enzymes EcoRI, AvrII and HindIII allowed us to determine the size of different D4Z4 alleles. Haplotype-specific PCR-labeled probes, prepared using the modified digoxigenin-11-Dutp nucleotide, were used on Southern blot assays using chemiluminescence detection. Alternative polymorphic D4Z4 alleles/pLAM haplotypes (4qA/4qB) were recognized in genomic DNA samples from a cohort of individuals having clinical diagnosis of FSHD. This study represents the first molecular analyses of D4Z4 alleles/pLAM haplotypes at the 4q35 human chromosome region in individuals from Argentina.

BC-P23
DUX4 NEGATIVELY REGULATES THE ACTIVITY OF THE HUMAN GLUCOCORTICOID NUCLEAR RECEPTOR

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The retro-transposed gene *DUX4*, at the human chromosome 4q35, encodes a transcription factor that regulates the expression of zygote activated genes in placental mammals. Our laboratory showed that DUX4 is a toxic pro-apoptotic protein underlying the pathogenesis of facioscapulohumeral muscular dystrophy (FSHD), the second most common form of inherited myopathy in humans. We have demonstrated that DUX4 is a negative regulator of the progesterone nuclear receptor1. In this report, we explored if DUX4 is a co-regulator of the glucocorticoid nuclear receptor (GNR). The activity of DUX4 on the GNR was studied in a reconstituted system on cultured T47D and HepG2 cells, which do not express endogenously GNR. In these studies, cells were co-transfected with a plasmid expressing the GNR plus a reporter plasmid MMTV-Luc and the potential co-repressor activity of DUX4 monitored using a plasmid expressing either wild-type or mutant versions of DUX4. Results of these studies showed that DUX4 dramatically inhibits the transcriptional activating function of GNR. DUX4 variants carrying mutations at the nuclear localization (NLS-1/2) or homeodomains (H1/H2-IWF) sequences lose their repressor activity on the GNR. Taken together, our results indicate that DUX4 is a strong co-repressor of the GNR and that its nuclear location and/or its N-terminal region contribute to this activity. Although DUX4 is mostly considered a transcriptional activator, our results show that this protein could indirectly modulate gene expression by repressing the activity of hormone NRs.

BC-P24
TUBULIN REGULATES TWO KEY ENZYMES IN DIABETES MELLITUS: ALDOSE REDUCTASE AND NA^+, K^+-ATPASE

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Among the main pathogenic pathways of diabetes was reported the activation of the enzyme aldose reductase (AR) and the inhibition of the enzyme Na^+,K^+-ATPase (NKA). In our laboratory, we describe a new mechanism of regulation of AR activity by tubulin (Tub) that leads to the regulation of NKA. Previously, we demonstrate that Tub forms a complex with AR increasing its activity when this Tub/AR complex is incorporated into a microtubule. In this work, we demonstrate that Tub/AR interaction and subsequent activation of AR is prevented in vitro by phenolic acid derivatives (CAFs). In cell cultures and human erythrocytes, exposed to high concentrations of glucose, we showed that glucose induces the activation of AR, increase in the polymerization of Tub and formation of Tub/AR complex. Because of these changes inhibition of NKA activity and decrease of erythrocyte deformability was observed. Similar results can be found in erythrocytes from diabetic subjects. On the other hand, in rat lenses exposed to high concentrations of glucose we observed the development of diabetic cataracts and the activation of the AR enzyme. All these effects of high glucose concentrations can be prevented in the presence of CAFs, because they prevent the Tub/AR interaction. Finally, in streptozotocin-induced diabetic rats we found that the CAFs can prevent the development of diabetic cataracts, the reduction of erythrocyte deformability and arterial hypertension. These results allow us to postulate a new mechanism of regulation of enzymatic activities AR and NKA by tubulin that explain the regulation of both enzymes associated with the development of secondary pathologies of diabetes.

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BC-P25
CELLS WITH PHAGOCYTIC ACTIVITY ON THE WALL OF SEMINIFEROUS TUBULES OF MOUSE TESTIS

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In the wall of the seminiferous tubules (TS) of rodent's testis, the peritubular myoid cells (MP) form a monolayer providing peristaltic action. Recently, macrophages located in the wall of mouse TS, known as peritubular macrophages (MacP), were described as cells capable of releasing colony stimulating factors and enzymes, involved in the biosynthesis of retinoic acid for the differentiation of spermatogonia A. There are no studies detailing the interaction of MacP with MP cells and whether they have phagocytic activity. We set out to locate, characterize and quantify MacPs in the wall of TS of mouse testis and determine if they present phagocytic activity. Microscopy images showed that MacPs have a cytoplasm with long projections that extend up to 100 µm long. They are adhered to the outer face of the MP cells and in all cases they are observed introducing their extensions between the spaces of the adjacent MP cells. The cell count revealed that in stage VII-VIII of the TS, there are 6.1 ± 0.62 MacP per 40,000 µm² of surface. Interestingly, consecutive confocal planes showed that 13.5 ± 2.74% of the MacPs contacted and enveloped undifferentiated spermatogonia A and 7.56 ± 3.58 % of MacP had cytoplasmic granules that were labeled with the antibody that identifies spermatogonia A (anti-Cad 1). By labeling with anti-Land 1 antibody, it was found that cytoplasmic granules corresponded to the phagocytic compartment pathway. These results indicate that in the TS wall,MacP are activated and phagocyte spermatogonia A. Tests are being carried out to determine if other cells of the TS germinal epithelium are also phagocyted byMacP.

BC-P26
ESSENTIAL OIL OF *Artemisia mendozana*INHIBITS PROLIFERATION OF TUMOR B16F0 CELLS AND INDUCES SENESCENCE

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The basic pathophysiology of cancer comprises aberrations at different points in the cell cycle. Due to the increasing incidence of cancer worldwide, there is an intense search for new therapeutic strategies to treat this disease. In this area, important efforts have been oriented to exploring the action of compounds of plant origin. *Artemisia mendozana* var mendozana (ajenjo), is a plant belonging to the Asteraceae family that growth in the piedmont of Mendoza province and it is used as a medicinal plant with antispasmodic and antifungal properties. The essential oil of ajenjo (EAO) contains 28 compounds of which the highest concentrated are: artemisia alcohol 4.8%, α-thujone 5.1%, borneol 11.2% and bornyl acetate 43.7%. In this project the effect of EAO was analyzed in in vitro proliferation of B16F0 murine melanoma cells. For the assay the cells were cultured with vehicle DMSO (control) or 13-45 µg/mL EAO dissolved in DMSO for 72 h. The growth rate (GI) ± SE was calculated from 3 independent experiments. At 72 h of culture, GI of the control was 8.4±0.33 and with EAO was: 5.8±0.56 (A); 3.1±0.03 (B); 2.4±0.47 (C); 2.1±0.20 (D) and 1.5±0.17 (E) for 13,18, 27, 36 and 45 µg/mL respectively. GI of D and E were significantly different to the control ($p \leq 0.001$). At 72 h of culture, the percentage of senescent cells of the control was 20.9±1.7 and with EAO 56.5±8.3, 51.2±3.0, 51.1±3.2, 31.8±5 and 35.8±9.6 for 13,18, 27, 36 and 45 µg/mL respectively. These results show that EO, at low concentrations, is a powerful inhibitor of the in vitro proliferation of B16 F0 cells

BC-P27
SNX17 IS A NOVEL REGULATOR OF ANTIGEN INTERNALIZATION AND CROSS-PRESENTATION BY DENDRITIC CELLS

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Sorting nexins (SNXs) are a family of effector proteins known to regulate several features of endocytosis and membrane trafficking. They are cytoplasmic and membrane associated proteins characterized by the presence of a particular type of phox-homology (PX) domain, the SNX-PX domain. As PX domains function mostly by binding phosphatidylinositol-3-monophosphate (PtdIns3P), SNXs are associated with PtdIns3P-enriched elements of the early endocytic network. From there, they function in diverse processes, including endocytosis, endosomal sorting, signaling and tubulation. In this study, we focus on the role of SNX17 in dendritic cells (DCs), which are the most potent antigen presenting cell type to achieve efficient cross-presentation. This immunological process is characterized by the presentation of exogenous antigens in association with MHC-I molecules to CD8+ T lymphocytes. Here, we identify SNX17 as a key regulator of antigen internalization and cross-presentation by DCs. By silencing the expression of SNX17 with shRNAs, we observe a significant defect in the uptake of soluble antigens (endocytosis) and 3 µm latex beads (phagocytosis), as compared to control cells. Accordingly, the cross-presentation of these same antigens is hampered in SNX17 knock-down DCs. Interestingly, the cell surface expression and intracellular recycling of MHC-I molecules is not affected in SNX17 deficient cells, as evaluated by flow cytometry analysis. Overall, our results provide compelling evidence that SNX17 plays an important role in the endocytic trafficking of DCs allowing the internalization and cross-presentation of exogenous antigens.

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BC-P28
INSULIN REGULATES THE LRP1 TRANSLOCATION TO THE CELL SURFACE IN MÜLLER GLIAL CELLS

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Low-density lipoprotein (LDL) receptor-related protein-1 (LRP1) is an endocytic and signaling receptor expressed in retinal Müller glial cells (MGCs). This receptor regulates the molecular activity of different membrane proteins, included insulin receptor (IR), which is involved with the MGC motility and metabolism. Moreover, insulin increases the LRP1 expression in the cell surface of neurons and hepatic cells, although the intracellular route of this LRP1 sorting in these cells and MGCs is not well established. Hence, in the present work we investigate the insulin-induced LRP1 translocation to the plasma membrane in human retinal MGC-derived cell line, MIO-M1. By electron microscopy we observed that LRP1 is stored in small vesicles (mean diameter range of 100–120 nm), which were positive for sortilin and VAMP2, and also incorporated GLUT4 when it was transiently transfected. Next, by biotin-labeling protein assay we observed that the LRP1 translocation to the plasma membrane was promoted by insulin-regulated exocytosis through intracellular activation of the IR/PI3K/Akt axis and Rab-GTPase proteins, such as Rab8A and Rab10. Moreover, these Rab-GTPases regulated both the constitutive and insulin-induced LRP1 translocation to the plasma membrane. Finally, we found that dominant-negative Rab8A and Rab10 mutants impaired insulin-induced intracellular signaling of the IR/PI3K/Akt axis, suggesting that these GTPase proteins as well as LRP1 cell surface level are involved in insulin-induced IR activation. We propose that insulin-induced LRP1 translocation to the plasma membrane is essential for IR activity, which might be relevant for the function of MGCs during pathological disorders of the retina associated with insulin resistance.

BC-P29
Chlamydia trachomatis PERTURBS ANTIGEN CROSS PRESENTATION BY INTERFERING RAB14-CONTROLLED TRANSPORT

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Chlamydia trachomatis (*Ct*) is the most common sexually transmitted bacterium that replicates inside a vesicle called inclusion. *Ct* manipulates Rab GTPases, master controllers of vesicular transport, to ensure its survival and replication. Dendritic cells (DC) are the most powerful antigen presenting cells and an essential link between innate and adaptive immunity. We have shown that *Ct* intercepts Rab14-vesicles to acquire host lipids necessary for its growth and multiplication. In addition, DC requires Insulin-regulated aminopeptidase (IRAP)-Rab14 endosomes for efficient antigen cross presentation. We hypothesised that *Ct* recruits Rab14 not only as a strategy for nourishment, but also to interfere with antigen presentation. By confocal microscopy, we observed that Rab14 is associated with the plasma membrane at the entry site of the bacterium. Later, Rab14 is recruited to the chlamydial inclusion membrane and remains there throughout the entire bacterial life cycle. Interestingly, we distinguished two populations of chlamydial inclusions in DC. On one hand, we found small *Ct*-containing vesicles that colocalize with EEA1 or LAMP1; and on the other hand, we observed larger ones that recruit Rab14 and exclude markers from the endocytic/degradative pathway. Chlamydial infection did not modify MHC-I expression, shown by western blot. However, infection as well as Rab14 silencing caused a redistribution of MHC-I molecules and interfered with their transport towards the plasma membrane, assessed by flow cytometry. Our results suggest that Rab14 is involved in the decrease of antigen cross presentation observed in Ct-infected cells.

BC-P30
FUNCTIONAL CHARACTERIZATION OF THE HIV-1 VPU ACCESSORY PROTEIN

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The chronic and persistent viral replication that characterizes the Human Immunodeficiency Virus 1 (HIV-1) infection reflects the complex interplay between the host defenses and a number of viral factors whose primary function is to facilitate the evasion of such immune responses. In order to infect a new host, replicate on it for many years and spread to new individuals, HIV-1 should avoid not only the innate defenses, including the so-called “antiviral restriction factors”, but also the humoral and cellular adaptive defenses. To date, many restriction factors that actively act against HIV-1 have been identified, including APOBEC3G, TRIM5alpha, cyclophilin A, BST-2/tetherin, SAMHD1 and SERINC3/5. HIV-1 has evolved a variety of mechanisms to evade these factors, by either acquiring mutations in the viral proteins susceptible to their action or encoding specific proteins that neutralize them. Known as "accessory proteins" (Vif, Vpr, Vpu and Nef), those viral factors act as molecular adapters that connect specific cellular targets with proteolytic or alternative intracellular trafficking pathways. Our lab is currently focusing on Vpu, one of the more attractive HIV-1 proteins from both clinical and pharmacological points of view. Among its many functions, Vpu promotes the downregulation of its specific targets, including the viral receptor CD4 and the restriction factor BST-2/tetherin, through cellular mechanisms that are not fully understood. We sought to study them at a molecular level by creating a complete proteomic profile of cellular proteins that interact specifically with wild-type and well-characterized Vpu mutants. This information allowed us to identify and analyze 1) new Vpu substrates; 2) key cellular proteins important for its multiple actions; and 3) cellular factors that regulate the trafficking pathways followed by Vpu. On the other hand, viruses generally “hijack” specific components of "normal" cellular pathways to facilitate the progression of their infectious cycle. Our analysis could eventually also define the participation of yet unknown factors in the complex cellular mechanisms that Vpu uses to perform each of its specific actions.

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NEUROSCIENCE

NS-P01
DIFFERENTIAL COCHLEAR HAIR CELL DEGENERATION IN MICE WITH IMPAIRED POTASSIUM RECYCLING

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Potassium ion (K^+) is essential for sound transduction in mammalian inner ear. KCNQ4, a voltage-gated K^+ channel, is expressed in cochlear hair cells (HCs) and in the central auditory pathway. KCNQ4 mutations lead to DFNA2, a progressive sensorineural deafness, due to chronic depolarization of HCs. Our aim is to analyze the progression of HC loss over time generated by absence of KCNQ4 channel in a mouse model lacking its expression (*Kcnq4-/-*). Quantitative PCR on wild-type mouse revealed the strongest *Kcnq4* mRNA level in basal cochlear turn, while it decreases ~50% in middle-apical turns. By using immunofluorescence on cochlear whole-mounts, we estimated cell number average and plotted cytocochleograms. We observed the highest outer hair cell (OHC) degeneration in basal turn starting early (3 weeksold(W)), which progresses to middle and apical turns in older mice (10-58W). Moreover, cell death progression correlated with different OHC stereociliar disarrangement patterns. Degeneration differed according to OHC row: the middle one exhibited the maximum decrease at 10W in *Kcnq4-/-* mice. Furthermore, inner HCs reached total loss in basal initial segment at 40W and cell loss also progresses to middle turn with age. Our results indicate that both HCs degenerate but starting at different ages, contributing to elucidate the mechanisms leading to profound hearing loss in DFNA2 patients.

NS-P02
AKT/FOXO3A PATHWAY: SIGNALING TARGET FOR α-SYNUCLEIN OVEREXPRESSION AND MANEB MEDIATED NEUROTOXICITY

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α-synuclein (α-syn) overexpression and manganese-based pesticides such as Maneb (Mb) have been both implicated as etiological factors of Parkinson's disease. We have previously reported the neuroprotective role of Akt/FoxO3a in amyloid β- and Fe-induced injury. In this work, we studied the role of the above-mentioned pathway in the effect of Mb and/or α-syn overexpression on IMR-32 human neuroblastoma cells. For this purpose, we exposed these neurons for different times (24-72 h) to increasing Mb concentrations (6-24 μM) and evaluated the redox status, Akt/FoxO3a subcellular localization and phosphorylation levels, and cell viability. The same parameters were evaluated in neurons stably overexpressing the wild type form of α-syn and exposed to either Mb or its vehicle.
Mb exposure provoked a time- and concentration-dependent decrease in neuronal viability. This cytotoxic effect was mediated by the increase in reactive oxygen species (ROS), lipid peroxides and membrane cell permeability (LDH release). Intriguingly, Mb exposure in α-syn-overexpressing neurons showed decreased ROS content and LDH release, with no changes in lipid peroxides. Mb was also found to induce changes in α-syn aggregation and phosphorylation, as measured with the intracellular probe Thioflavin S and by immunocytochemistry.
On the other hand, Mb exposure and α-syn overexpression unconnectedly triggered the increase in Akt and FoxO3a nuclear localization. However, Mb exposure in α-syn overexpressing neurons enhanced FoxO3a nuclear localization without increasing cell death. We hypothesize that FoxO3a might be an α-syn target related with its unexpected protective role.

NS-P03
ANALYSIS OF THE BEHAVIOR OF NEURAL STEM CELLS UNDER OXIDATIVE STRESS INDUCED BY IRON AND COPPER

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Traditionally, it was thought that the mammalian nervous system lacked the ability to self-repair after injuries or neurodegeneration. We now know that the adult brain does indeed hold the capacity to regenerate, albeit to a limited extent. Endogenous neural stem cells (NSCs) could be a regenerative source for the damaged neural cells but because their number and regenerative ability are limited, they cannot fully repair the damaged tissue. Factors present in the injured microenvironment (such as inflammatory mediators and reactive oxygen species (ROS)) influence survival, self-renewal, migration and neuronal differentiation of both endogenous NSCs and transplanted exogenous stem cells.
In order to test this hypothesis, we used the transition metals iron and copper to induce oxidative stress in NSCs. To determine the appropriate concentration and exposure time, the viability of the cultures treated with metals was assessed using the MTT assay and by Trypan Blue staining. To evaluate the extent of metal-induced effects on NSCs, cell morphology and generation of ROS (measured by using the probe DCFH-DA) were analyzed. Moreover, the type of cell death after the exposure to iron and copper was evaluated by differential nuclear staining with fluorescent dyes acridine orange and ethidium bromide. We demonstrate that both metals can stimulate the production of ROS in NSCs cultures an induce apoptosis or necrosis of the stem cells.
Additionally, we investigated the effects of iron and copper on the ability of NSCs to proliferate and generate new neurospheres or to differentiate into neurons. We have observed that both metals affect NSCs survival under proliferation conditions. Furthermore, by immunofluorescence and Western blot analysis using specific markers, we demonstrated that metals promote neuronal differentiation, perhaps, as part of an intrinsic mechanism of the central nervous system to repair the damaged tissue.

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ENZYMOLOGY

EN-P01
*Leptospirainterrogans*HAS GLUTATHIONE METABOLISM

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Glutathione (GSH) is the most abundant low molecular mass thiol in almost all eukaryotic cells, as well as in proteo and cyanobacteria. It is synthesized enzymatically in two ATP-Mg^{2+} dependent steps: first, glutamate cysteine ligase (GCL) establishes a peptide bond between cysteine and glutamate, forming γ-glutamylcysteine (γ-GC). Second, glutathione synthetase (GS, which is a member of ATP-Grasp superfamily) adds glycine residue to the carboxy terminus of γ-GC, producing GSH. Until now, there is not information available about the presence of GSH metabolism in *L. interrogans* (the causative agent of leptospirosis). In this work, we present the recombinant expression and functional characterization of LinGCL and LinATPGrasp, responsible for GSH synthesis in this pathogen bacterium. LinGCL showed higher substrate promiscuity than LinATPGrasp and its enzymatic activity was inhibited by γ-GC and GSH. GSH also inhibited LinATPGrasp but with a lower effect. Finally, we measured the activity of these enzymes and the GSH content in bacterial cell extract. These measurements indicated a higher GSH level in *L. interrogans* than in *L. biflexa* (free-living bacteria). The last result would help explain the virulence of *L. interrogans* and indicate the GSH role as part of its antioxidant metabolism.
Granted by ANPCyT (PICT2014-2103 and PICT2016-1778).

EN-P02
MONOTHIOL GRXS: SMALL PROTEINS INVOLVED IN MANY CELLULAR PROCESSES IN *Trypanosoma cruzi*

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Glutaredoxins (Grx) are small proteins linked to redox metabolism and intracellular iron homeostasis. *Trypanosoma cruzi* is the protozoan parasite and causal agent of Chagas Disease. In this work, the functional properties of monothiolicGrxs of T. cruzi are presented. The recombinant proteins were able to catalyze the GSSG reduction *in vitro,* using T(SH)2 as an electron donor. The Grxs were able to coordinate iron-sulfur cluster (ISC) by an *in vitro* reconstitution assay. Absorption spectra revealed two characteristic peaks of Grx-ISC complexes. In addition, gel filtration chromatography profiles indicated that the Grx-ISC complexes are formed by dimeric Grx species. On the other hand, yeast complementation experiments (with *Grx*mutants) demonstrated that the phenotype of the Δgrx5 and Δgrx4Δgrx5 mutants could be rescued by complementation with the heterologous Grxs, partially suppressing the cells sensitivity to exogenous oxidants. In parallel, *T. cruzi*Grx-overexpressing epimastigote cells showed increased oxidative stress tolerance and raise the mammalian cells infection capacity (of recombinant trypomastigotes). These results indicate that *T. cruzi*Grxs could participate in processes related to the infective capacity of this parasite. These results suggest that *T. cruzi*Grxs have important functions in the redox metabolism and biogenesis of ISC in the parasite.
Granted by ANPCyT (PICT2014-2103 and PICT2016-1778) and Fundación Bunge y Born (Chagas disease investigation grant, 2016).

EN-P03
FUNCTIONAL CHARACTERIZATION OF A GLUTAREDOXIN-LIKE PROTEIN FROM *Entamoeba histolytica*

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Entamoeba histolytica, an intestinal parasitic protozoan, is the causative agent of amoebiasis. The parasite usually lives and multiplies within the human gut, an environment of reduced oxygen pressure. During tissue invasion, *E. histolytica* is exposed to elevated amounts of exogenous reactive oxygen species (ROS), which are highly toxic for the parasite. The metabolic pathway for ROS detoxification in this organism is a matter of controversy. Because neither glutathione nor its associated enzymes were found to occur, it has been proposed the cysteine as a main intracellular thiol and one of the compounds responsible for maintaining the intracellular redox balance. Nevertheless, we report in this work the discovery of a glutaredoxin-like protein encoded in *E. histolytica* genome through bioinformatic techniques. We present the molecular cloning from *E. histolytica* genomic DNA of an encoding gene for monothiol glutaredoxin-like protein (EhGRX1). The cloned gene was expressed in *Escherichia coli*, and the corresponding recombinant protein was purified by chromatography and characterized. The recombinant protein catalyzed GSH-dependent low molecular disulfide reduction. No synergistic effect of EhGRX1 with thioredoxin system in low molecular mass disulfide reduction was observed. Treatment with different oxidants was carried out and its oligomeric state was revealed by non-reducing SDS-PAGE and gel filtration chromatography. The protein was detected in trophozoites by western blot experiments. To the best of our knowledge this is the first characterization of this type of protein in *E. histolytica.*
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EN-P04
STUDIES ON THE REGULATION, STRUCTURE AND FUNCTION OF A GLUCOAMYLASE FROM *Sacharophagus degradans*

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Saccharophagusdegradans is a gram negative marine bacterium. It is the most versatile bacterium, in terms of the degradation of complex polymers (CP) found to date. It is capable to degrade at least 10 complex polymers such as agar, alginate, chitin, cellulose, fucoidan, laminarin, pectin, pullulan, starch and xylan. The high rate of degradation of these polysaccharides makes this organism an excellent candidate to identify and investigate the biological properties of the degradative enzymes. Thus, we identify a gene coding for a putative glucoamylase from *Saccharophagusdegradans* (sdGA, accession number: ABD79864). The sdGA gene was synthesized by Genscript (USA) with codon optimization for the expression in *E. coli* cells and cloned into pRSF-DUET vector (named pRSFDsdGA). The plasmid pRSFDSdGA was successfully expressed in *Escherichia coli* BL21 Rosetta cells. Recombinant sd_GA is fused to a histidine tag at the N-terminal region of the protein. The full-length gene codes for a 803-amino acid polypeptide. The mature protein is a monomer with the molecular mass of 88 kDa. Recombinant sd_GA was purified using a nickel column. The recombinant enzyme showed maximum activity using maltose as substrate at 39°C and pH 6.0. Kinetic studies revealed that the Vmax and Km values of sd_GA are 0.0061 μmol/μg.min and 11,5 mM, respectively. Studies on the thermal stability of sd_GA and the effect of different metabolites on enzyme regulation were also performed. Our results show that both glycerol and acarbose are good stabilizers for sdGA.

EN-P05
N–TERMINAL LOOP OF ENDO β1,4 XYLANASE FROM *Ruminococcusalbus*8 IS INVOLVED IN ENZYME BINDING

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Xylans are major hemicellulose components of plant cell walls. The complete decomposition of xylan requires the action of a variety of enzymes. Endo β–1,4–D–xylanase (EC 3.2.1.8) is involved in main–chain hemicellulose breakdown. Xylanases often exhibit a multimodular structure consisting of catalytic domains (CDs) linked by one or more non–catalytic domains such as cellulose binding domains (CBDs), thermo–stabilizing domains (TSDs). *Ruminoccocusalbus* 8 is a bacterium endowed with multiple forms of plant cell wall–degrading enzymes. *Ruminococcusalbus* 8 endo β–1,4–D–xylanase (Xyn10A) is composed of a GH10 catalytic module together with an N–terminal loop without any function assigned as far as we now. From a homology model of Xyn10 we hypothesize that this loop could be important in enzyme stability and/or involved in substrate interaction. To afford evidence for this hypothesis, Xyn10A and its N–terminal truncated mutant (ΔNXyn10A) were heterologously expressed in *Escherichia coli* BL21 (DE3) cells and the recombinant enzymes were biochemically characterized. Removal of this region did not affect the catalytic activity against soluble xylan, but decrease its affinity, reducing the enzyme binding to the complex insoluble substrates. Results contribute to have further evidence in determining structure to function relationships that are useful for rational design of lignocellulolytic enzymes for application in biorefinery processes.

EN-P06
UDP-GLUCOSE, A KEY METABOLITE IN CARBOHYDRATE METABOLISM IN *Euglena gracilis*

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E. gracilis is a fresh water protist able to grow photosynthetic or heterotrophically using diverse organic compounds as 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, foods, paramylon and wax esters. When grown aerobically it produces an insoluble β–1,3–glucan (paramylon) as carbon storage. Paramylon and different oligo– and polysaccharides are synthesized by glycosyl transferases using UDP–glucose as substrate, being it generated in a reaction catalyzed by UDP–glucose pyrophosphorylase (EC 2.7.7.9; UDP–GlcPPase). In this sense, UDP–GlcPPase from *E. gracilis* was expressed in *E. coli*. Highly purified enzyme exhibited a monomeric structure, able to catalyze synthesis of UDP–Glc with a Vmax of 3350 U/mg. Glc–1P and UTP were the preferred substrates, although the enzyme also used (with lower catalytic efficiency) TTP, galactose–1P and mannose–1P. We identified redox modification of the enzyme that produces conformational changes, modifying the structure of the protein (monomers with different Stokes radii that interact forming oligomers). This change in the structure generates a loss of activity (also detected *in vivo*). RNAi assays were carried out against UDP–GlcPPase to analyze its functionality *in vivo* and to understand the importance of UDP–Glc in carbohydrate metabolism in *E. gracilis.*
Results suggest a key functionality of UDP–GlcPPase for carbohydrate metabolism as well a possible moonlighting role related with different redox conditions in the microalgae.
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EN-P07
STUDY OF PHYSIOLOGICAL COPPER NITRITE REDUCTASE MEDIATORS TOWARDS BIOSENSOR DEVELOPMENT

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Denitrifiers are a good source of enzymes that are useful for the development of biodevices for N-compounds sensing in environmental samples. Particularly, nitrite reduction is the way to enter into the denitrification pathway mediated by nitrite reductase enzyme. Two kinds of nitrite reductases can be found in nature: copper- and cytochrome cd1-containing nitrite reductases, NirK and NirS, respectively. NirKs receive the electrons from azurins, pseudoazurins and cytochrome c550, depending on the microorganism. Usually, green NirKs receive electrons from pseudoazurins while blue NirKs from azurins or c-type cytochromes. Nevertheless, NirKs rarely interact with electrodes directly. Hence, we need a physiological mediator that is able to interact with the electrode surface in a biosensor. Moreover, the best mediator-enzyme couple has to be experimentally determined for the design of the biosensor.We have cloned, expressed and purified several physiological mediators for NirKs: a green pseudoazurin from *Sinorhizobiummeliloti* 2011 (SmAzu2) and a cytochrome c550 from *Bradyrhizobium japonicum* USDA110 (BjCycA). We report here biochemical and electrochemical studies of the interaction of these mediators and several NirKs: a green NirK (SmNirK), a blue NirK (BjNirK) and a thermotolerant NirK from *Thermus scotoductus* SA-01 (TsNirK).The reoxidation of the mediator under argon atmosphere was spectrophotometrically monitored in a continuous assay in a sealed cuvette. All the electrochemical studies were performed on a 4-4´-dithiopyridine-sensibilized gold electrode. Cyclic voltammetry and chronoamperometry assays were performed.

EN-P08
INTRAMOLECULAR ELECTRON TRANSFER WITHIN BACTERIAL COPPER-CONTAINING NITRITE REDUCTASE NirK

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Cu-containing nitrite reductase (NirK) is a key enzyme of the biogeochemical N-cycle that catalyzes the conversion of nitrite (NO^{2-}) into nitric oxide (NO), a greenhouse gas that also degrades O_3 in the atmosphere. Understanding the inner working of this enzyme is fundamental. This work aims to characterize different site-directed variants that tune reduction potentials and/or the coordination environment of the Cu centers present in NirK, using kinetic and spectroscopic techniques. Site-directed variants (H171C y E315A) were obtained by standard methods. H171C was generated to directly modify the bridge that connect the two Cu ions of NirK, while E315A was produced to study how modulating the reduction potential of the active site affects electron transfer rates and the kinetics of nitrite reduction. Steady-state kinetics, UV-Vis and CW-EPR spectroscopy were used to achieve those goals.Both NirK variants were recombinantly produced. Molecular are similar to those of the wild-type NirK. UV-Vis and EPR spectroscopies showed the presence of Type-1 and Type-2 Cu^{2+} centers as in wild type NirK, although the T2 EPR signals were slightly different when compared with that of wild-type NirK. E315A yielded a Kcat identical to the wild-type enzyme (25 s^{-1}) but a KM 3-fold larger (60 μM). H171C showed no catalytic activity.Amino acids residues H171 and E315 do not affect quaternary structure and cofactor content. H171C variant is inactive. E315A shows a turnover number identical to that of wild-type NirK, but a KM 3-fold larger. This could mean that tuning the reduction potential of the Cu ion at the active site (T2) affects the rate of substrate binding.

EN-P09
*Euglena gracilis*EXPANSIN: PRODUCTION AND CHARACTERIZATION OF ITS BINDING TO INSOLUBLE SUBSTRATES

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Euglena gracilis is a fresh water protist with a large metabolic capacity because it is able to grow photosynthetically or heterotrophically. It is a source of products of interest such as wax esters, vitamins, amino acids and polyunsaturated fatty acids. We have identified in a transcriptomic work made in *E. gracilis* a transcript that codes for an expansin (EgExp). These proteins are relatively small (~ 25 kDa) with a "loosening" effect on cellulosic network lacking of hydrolytic action. They have been already found in plants and plant-pathogens such as bacteria and fungi. *E. gracilis* does not have a carbohydrate-based cell wall and is not reported to be a plant pathogen. However, it produces an insoluble and crystalline β–1,3 glucan called paramylon as storage polymer. Thus, the function of EgExp remains unknown in this microorganism.EgExp was expressed in *Escherichia coli* BL21 (DE3) cells as inclusion bodies. A solubilization protocol with urea 8 M was applied to denaturing the insoluble protein. After that, the chaotropic agent was removed through by dialysis. In order to characterize this protein, we evaluated the binding capacity to insoluble substrates. We observed it can bind to filter paper, sawdust and chitin, but not to paramylon. We performed the Langmuir isotherms as previously described to determine that the maximum binding capacity of EgExp is similar to that reported for other bacterial expansins. This result supports the right assignation structure- function to the polypeptide being necessary further work to in depth analyze the functionality of EgExp in *E. gracilis*.
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EN-P10
THE STARCH-BINDING DOMAINS OF SSIII-B FROM *Ostreococcustauri* ARE ESSENTIAL FOR CATALYTIC ACTIVITY

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Starch is the major energy storage carbohydrate in photosynthetic eukaryotes. Several enzymes are involved in building highly organized semi-crystalline starch granules, including starch-synthase III (SSIII), which is widely conserved in photosynthetic organisms. This enzyme catalyzes the extension of the α-1,4 glucan chain and plays a regulatory role in the synthesis of starch. Interestingly, unlike most plants, the unicellular green alga *Ostreococcustauri* has three SSIII isoforms. In the present study, we describe the structure and function of OsttaSSIII-B, which has a similar modular organization to SSIII in higher plants, comprising three putative starch-binding domains (SBDs) at the N-terminal region and a C-terminal catalytic domain (CD). Purified recombinant OsttaSSIII-B displayed a high affinity toward branched polysaccharides such as glycogen and amylopectin, and to ADP-glucose. Lower catalytic activity was detected for the CD lacking the associated SBDs, suggesting that they are necessary for enzyme function. Moreover, analysis of enzyme kinetic and polysaccharide-binding parameters of site-directed mutants with modified conserved aromatic amino acid residues W122, Y124, F138, Y147, W279, and W304, belonging to the SBDs, revealed their importance for polysaccharide binding and SS activity. Our results suggest that OT_ostta13g01200 encodes a functional SSIII comprising three SBD domains that are critical for enzyme function.

EN-P11
CHARACTERIZATION OF CrSEX4, A PHOSPHOGLUCAN PHOSPHATASE FROM *Chlamydomonas reinhardtii*

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Glucan phosphatases have emerged as essential enzymes for normal starch degradation in plants as well as glycogen metabolism in mammals. *Arabidopsis thaliana*phosphoglucan phosphatases starch excess 4 (SEX4) and like-SEX4 2 (LSF2) and human Laforin are the fundamental representatives of the atypical Dual Specificity Phosphatases (DSPs), which belong to the larger Protein Tyrosine Phosphatase superfamily. To understand the evolution of catalysis and regulation of these enzymes we are studying CrSEX4, a SEX4 homologous protein belonging to the unicellular green algae *C. reinhardtii*. We constructed a series of mutants by site directed mutagenesis and studied phosphatase activity of the recombinant enzymes as well as their ability to bind polysaccharides. The results led to confirm that C224 is the catalytic cysteine residue. Activity assays with the artificial substrate para-nitrophenylphosphate suggest the presence of a putative second substrate binding site in CrSEX4.

EN-P12
CATALYTIC MECHANISM AND REGULATION OF FERREDOXIN-NADP+ REDUCTASES FROM BACTERIAL PATHOGENS

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Ferredoxin-NADP+ reductases (FNR) constitute a family of proteins with a non-covalently bound FAD. They participate in redox metabolisms catalyzing the reversible electron transfer between NADP(H) and ferredoxin or flavodoxin. We identified that *Escherichia coli* FNR (EcFNR) is purified with the substrate/product NADP+ tightly bound. This binding produces an activity inhibition which is lost when NADP+ is released. This inhibition would implicate a different catalytic mechanism than the one reported for the plastidic enzymes. The crystallographic structure of EcFNR shows that NADP+ would interact with three arginine residues that could generate a site of very high affinity. These residues are conserved in other bacterial FNR but not in high efficiency plastidic enzymes from plants and cyanobacteria.We have studied the catalytic properties of FNR from different pathogenic bacteria: *E. coli*, *Pectobacteriumcarotovorum* (PcFNR) and *Brucella abortus* (BaFNR). We found that PcFNR is purified with NADP+ tightly bound as EcFNR. BaFNR presented a small proportion of the nucleotide bound. Catalytic efficiencies were determined noting that they were similar between EcFPR and PcFPR, but it was higher in BaFPR. All of them were lower than in plastidic FNR. Also, in EcFNR and PcFNR the absence of NADP+ decreased the stability of these proteins and the binding of NADP+ to the enzyme had a stabilizing effect on them.We propose that the high-affinity nucleotide binding is an essential catalytic and regulatory mechanism of these enzymes and it can be used as a differential target for the inactivation of metabolic pathways in which FNR participate in pathogenic bacteria.

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EN-P13
FLAVODOXIN REDUCTASES OF *Acinetobacter*. KINETIC CHARACTERIZACION AND RESPONSE TO REDOX STRESSORS

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Ferredoxin/flavodoxin NADP(H)-reductases (FPRs) are monomeric flavoenzymes carrying non-covalently bound FAD as cofactor, present in mitochondria, plastids and bacteria. Bacterial FPRs are classified in two groups that differ in the C-terminus structure. They are involved in reductive activation of enzymes and repair of Fe-S clusters after oxidative damage. Although most bacteria contain one of the two forms, some species carry both of them. The extremophile UV-resistant *Acinetobacter* sp. Ver3 conserved both FPR1 and FPR2 and a single flavodoxin. Here we report a biochemical characterization of the flavoenzymes and a study of their response to environmental factors as pro-oxidants or UV irradiation.Kinetic measurements under steady state conditions using flavodoxin as e- acceptor showed a faster electron transfer for FPR1 compared to FPR2 (kcat1=1.3 s^{-1} vs. kcat2=0.5 s^{-1}) with a higher Km for both flavodoxin (Km1=4.7 μM vs Km2=1.5 μM) and NADPH (Km1=85 μM vs Km2=34 μM).Challenge of *Acinetobacter* cells with superoxide propagators resulted in increased accumulation of FPR2 as revealed by immunostaining with specific antibodies. However, when cells were exposed to UV radiation, only FPR1 levels augmented. These data suggest that, although displaying similar catalytic efficiency, each FPR of *Acinetobacter* has a distinctive regulation mechanism in response to different stressors and redox stimuli.

LIPIDS

LI-P01
TESTOSTERONE AND SOLUBLE FACTORS FROM SERTOLI CELLS STIMULATE SPHINGOLIPID SYNTHESIS IN GERM CELLS

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Germ cells from male rodents require membrane sphingomyelins (SM) and ceramides (Cer) with very long chain polyunsaturated fatty acids (VLCPUFA), in non-hydroxylated (n-V) and 2-hydroxylated (h-V) versions, for normal spermatogenesis. We have previously shown that spermatogenic cells isolated from seminiferous tubules of adult rats, in culture, are able to synthesize de novo the sphingolipids (SL) Cer, SM, and Glucosyl-Cer (GlcCer). Here, we evaluated the effect on such biosynthesis of supplementing the cultures with testosterone (T), with the supernatant medium recovered from primary Sertoli cell cultures (SMSC), and with both. Germ cells from adult rats were isolated and cultured in a medium containing [3H]palmitate as SL precursor. Label from this marker was incorporated into species of Cer and SM that contain saturated fatty acids (S), monoenoic fatty acids (M) and also in those containing n-V and h-V. In germ cells supplemented with T, while the labeling of Cer with [3H]palmitate was unaffected that of SM was increased in all the molecular species. Interestingly, supplementation with SMSC increased the synthesis of molecular species of Cer and SM with S, M and h-V, but not those with n-V. Finally, supplementation with both, T and SMSC, resulted in increased incorporation of [3H]palmitate in all Cer and SM molecular species. The biosynthesis of GlcCer was not influenced by T or SMSC. Thus, the de novo biosynthesis of the SM species of spermatogenic cells is subjected to endocrine stimulation by T and to paracrine regulation by soluble factor(s) released from Sertoli cells that are yet to be identified.

LI-P02
NEW INSIGHTS INTO LIPID METABOLISM OF *Haematococcuspluvialis* IN RESPONSE TO HIGH-LIGHT STRESS

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The oleaginous unicellular microalga *Haematococcuspluvialis* is being intensely studied as a source of carotenoids and lipids with potential biotechnological and biomedical applications. The aim of this study was to analyze the effect of high-light stress on the regulation of triacylglyceride (TAG) and sterol synthesis in *H. pluvialis* UTEX 2505. After 24 hours of high-light exposure, both lipid classes significantly increased compared to the control condition. As expected, the use of inhibitors cerulenin, propranolol, xanthohumol and A922500 significantly decreased TAG synthesis under high-light stress, while mevastatin and 25-hydroxycholesterol reduced sterol content. These results together with qPCR and Western blot analyses revealed some of the enzymes of *H. pluvialis* involved in lipid synthesis in response to high-light. In addition, we observed that TAG inhibitors also reduced sterol content while sterol inhibitors diminished TAG production, denoting coordination between both synthesis pathways. Fosmidomycin, a specific inhibitor of the non-mevalonate pathway of isoprenoid biosynthesis, reduced total carotenoid content and antioxidant capacity together with TAG and sterol content. Finally, inhibition of carotenoid, TAG and sterol synthesis significantly reduced cell viability suggesting their participation in *H. pluvialis* survival and adaption to high irradiance. These findings reveal features of lipid synthesis and function of *H. pluvialis* under high-light stress, and points towards new possible molecular targets to enhance lipid production in microalgae

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LI-P03
MENADIONE MODULATES ADIPOGENIC DIFFERENTIATION BY INHIBITION OF PI3K/AKT PATHWAY IN 3T3-L1 CELLS

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We have previously demonstrated that menadione-induced oxidative stress significantly diminishes adipogenesis and is able to dephosphorylate and inactivate phosphatidylinositol 3-kinase (PI3K)/Akt pathway in 3T3-L1 cells (SAIB 2017). To investigate this unexpected behavior of this adipogenic key pathway against oxidative stress, we mimicked menadione effect by studying adipogenic differentiation in the presence of LY294002, a well known PI3K inhibitor. In the absence of menadione, we found that PI3K inhibition drastically decreased adipogenesis. At a molecular level, the expression of peroxisome proliferator activated receptor gamma (PPAR gamma), the master regulator of adipogenesis, was also found to be decreased. To investigate if PI3K/Akt pathway was responsible of menadione-caused adipogenesis inhibition, we used insulin in the presence of menadione as a PI3K gain-of-function strategy, both being present during the whole differentiation process. These experiments showed that insulin was sufficient to rescue PPAR gamma expression, without altering cell viability. These results show that PI3K/Akt is a key pathway in the antiadipogenic effect of menadione on 3T3-L1 cells.

LI-P04
MODULATION OF HEPATIC TRIACYLGLYCEROL IN RATS FED WITH A FUNCTIONAL MILK FAT

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A Functional Milk Fat (FMF) enriched with Conjugated Linoleic Acids (CLA) and trans-Vaccenic Acid (VA; t11-18:1) showed a potential health benefits on lipid regulation. The aim was to study some biochemical mechanisms involved in liver triacylglycerol (TAG) regulation of rats fed FMF at high fat levels. Male Wistar rats were fed (60-d) with S7 (soybean oil –SO- 7%), S30 (SO 30%), MF30 (SO 3%+ Milk Fat -MF- 27%) or FMF30 (SO 3%+ FMF 27%) diets. TAG levels, TAG secretion rate (TAG-SR), fatty acids (FA) composition, FA incorporation, lipogenesis and β-oxidation were determined in liver. Statistical analysis: Student's t test or ANOVA (1x3) and Tukey's test, *: $p<0.05$. Compared to S7, S30 diet increased liver TAG content and FA incorporation reflected by a higher FATP2, FATP5 and CD36 gene expression. FMF30 and MF30 reduced liver TAG accretion (-31.4* and -31.3%*), and the changes (%) vs S30 diet were: 1) TAG-SR, FMF30: +31.2* and MF30: +7.0; 2) liver FA incorporation: mRNA levels of FATP2, FMF30: -47* and MF30: -39*; FATP5, FMF30: -19 and MF30: -33*; CD36, FMF30: -51* and MF30: -82*; 3) lipogenesis: activities of: G6PDH, FMF30: -24* and MF30: -34* and FAS, FMF30: +6.3 and MF30: -31.7*, and mRNA levels of: ACC, FMF30: +59* and MF30: +24.7; FAS, FMF30: +80* and MF30: +89*; SCD1a, FMF30: +529* and MF30: -7.9; DGAT, FMF30: -26.4 and MF30: -46.3* and PPARγ, FMF30: +68* and MF30: +19.7, and SCD1a index, FMF30: +236* and MF30: +192* and 4) β-oxidation: CPT-Ia activity, FMF30: +32* and MF30: -31*; mRNA levels of: ACO, FMF30: -1.3 and MF30: -44.6* and PPARα, FMF30: +58* and MF30: -15. CLA and VA of FMF provided additional mechanisms of lowering liver TAG content

LI-P05
EFFECT OF A FUNCTIONAL MILK FAT ON OXIDATIVE STRESS AND INFLAMMATION IN RATS FED HIGH FAT LEVEL

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High intake of fat, includingMilk Fat (MF), has been associated with oxidative stress, inflammation and Non-Communicable Chronic Diseases (NCCD) risk. However, the MF can be modified through decrease of saturated fatty acids and increase bioactive components, leading to a Functional Milk Fat (FMF). Since, the analysis of biomarkers for oxidative stress and inflammatory state in liver is essential for the diagnostic and control of NCCD, the aim of this study was to investigate some of these biomarkers in rats fed diets containing MF and FMF at high levels. Male Wistar rats were fed (60-d) with S7 (soybean oil, 7%), S30 (soybean oil, 30%), MF30 (soybean oil, 3% + MF, 27%) or FMF30 (soybean oil, 3% + FMF, 27%) diets. The reduced glutathione to oxidized glutathione (GSH/GSSG) ratio by Capillary Electrophoresis, Lipoperoxidation (LPO) by TBARs and mRNA expression of Catalase (CAT), Glutatione Peroxidase(GSH-Px), Glutatione Reductase (GR), the nuclear factor (NFκβ), Nuclear Factor Erythroid-2 (Nrf2) and proinflammatory cytokines (TNF-α and IL6) by RT-PCR were assessed in liver. Compared withS7, S30 and MF30 increased LPO and expression of NFκβ, Nrf2 and CAT. The GSH/GSSG ratio decreased in both groups respect to S7. The expression of IL-6 and TNF-α increased in MF30 compared with S7. FMF30 decreased LPOand the mRNA expression of CAT, NFK-β, Nrf2, TNF-α, and IL6 reaching values similar to S7. In conclusion, the intake of FMF30 was able to attenuate the stress and inflammatory status caused by the consumption of high levels of fat, which could contribute to reduce the NCCD risk.

LI-P06
STUDY OF NITROLIPID IN MACROPHAGE MIGRATION, CHOLESTEROL EFFLUX AND IMPLICATION ON PLAQUE REGRESSION

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In atheroma plaque formation trapping of lipid-laden macrophages in the arteries intima is a critical step, and reversion of this process is important for the outcome of atherosclerosis disease. When modified-LDL in the intima is taken up by macrophages, it stimulates uncontrolled

lipid accumulation and inhibition of macrophages migration. Nitro-Fatty Acids (NO2-FA) have shown anti-atherogenic effects on ApoE-KO mouse. The aim of this study is to explore the action of nitrolipids on cellular components of the atherosclerotic plaque. In order to do this, RAW264.7 macrophages and L6 myoblast migration were evaluated in transwell and wound healing assays in presence of NO2-OA, oleic acid (OA) and curcumin. Results, expressed as cells migrated through the membrane and as percentage of wound closure, showed that NO2-OA increases not only RAW264.7 migration but also abolished the inhibitory effect of oxidized-LDL (oxLDL). L6 migration was not affected by NO2-OA. Plaque regression is characterized by increased macrophages egress from the plaque and cholesterol efflux. Therefore, efflux assays were carried out in RAW267.4 loaded with fluorescent labeled (DiI) oxLDL and efflux was stimulated with NO2-OA in presence of acceptor proteins (serum or HDL). NO2-OA increased the rate lipid efflux as was revealed by fluorescent microscopy but no significant changes were observed in ABCA1 and ABCG1 cholesterol transporters at transcriptional level. In summary, these results represent new contributions for the still unclear mechanisms of regression since NO2-OA may stimulate lipid efflux and modulate macrophages to emigrate from plaque and facilitate their regression

MICROBIOLOGY

MI-P01
REGULATION OF TAILOCINS PRODUCED BY *Pseudomonas fluorescens* SF4C

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Bacteriocins are antimicrobial proteins produced in bacteria to compete for nutrients and space. We previously reported that the synthesis of tailocins (phage-tail-like bacteriocins) in *P. fluorescens* SF4c is upregulated by mitomycin C, a SOS-response inducer. The aim of this work was to study the regulation of tailocins in *P. fluorescens* SF4c. Proteomics has been used as a tool to assist in understanding bacterial behavior upon exposure to different stimuli. In this report, a comparative proteomic analysis between bacteriocins extracted from cultures of strain SF4c treated with mitomycin C or left untreated was performed. When the cells were treated with mitomycin C, a greater abundance of bacteriocin-associated proteins was observed. Those findings confirmed the previous results. In *P. aeruginosa*, the bacteriocins expression is controlled by the PrtN activator and the PrtR repressor. Bacteriocin regulation, however, is unknown in *P. fluorescens*. An orthologue of *prtR* gene was identified in the tailocin cluster of strain SF4c, though no gene homologous to *prtN* was detected. Therefore, we constructed a *prtR* null-mutant. The production of tailocins was abolished in this mutant in both conditions (induced with mitomycin C or uninduced). Moreover, the tailocin expression was measured through the use of the transcriptional fusion to *gfp*. No expression was observed in the mutant whereas the strain SF4c displayed a significantly strong promoter activity. The promoter activity was partially restored in the complemented mutant. This suggested that, unlike in *P. aeruginosa*, PrtR regulates expression of bacteriocins in *P. fluorescens* in a positive fashion.

MI-P02
ISOLATION OF PULLULANASE-PRODUCING MICROORGANISMS OF BIOTECHNOLOGICAL INTEREST

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Microorganisms are essential for obtaining enzymes due to their low cost of production and genetic engineering possibilities. There is strong biotechnological interest in microbial enzymes applicable in food processing, agriculture, pharmaceutical industry and molecular biology. Bioprospecting describes the procedure of exploration, extraction, screening and trading of new products using biological diversity as source. Different enzymes are involved in processes related to the synthesis and degradation of α-glycosidic bonds. Pullulanases are a type of glycoside hydrolase debranching enzymes, which act on α-1,6 bonds of starch and other α-glucans like pullulan, consisting essentially of maltotriosyl units connected by α-1,6 bonds. Pullulanases are widely used in the saccharification process to obtain useful products such as maltose, amylose and glucose by debranching starch; so, they are highly employed in the production of high-fructose corn syrup and in the starch processing industry. The aim of this work was to explore, isolate and identify aerobic bacteria producing enzymes acting on pullulan. By bioprospecting samples of soil and high starch-containing vegetables, 40 bacterial isolates were obtained, of which 3 secreted pullulan-hydrolytic enzymes. Bacteria were identified as *Bacillus firmus*, *Alcaligenesfaecalis*and *Micrococcus luteus*. HPLC analysis of the products of hydrolysed pullulan with isolated enzymes showed maltotriose as the main product. Accordingly, these enzymes were classified as pullulanases. In particular, pullulanase isolated from *B. firmus* produced the highest debranching activity.

MI-P03
A TRYPOMASTIGOTE SURFACE GLYCOPROTEIN REGULON INVOLVED IN *Trypanosoma cruzi*INFECTIVITY

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In the absence of transcription initiation regulation, organized subsets of Trypanosoma cruzi RNAs must be post-transcriptionally co-regulated in response to extracellular signals. Hence regulons, functionally linked mRNAs modulated by trans-acting factors, regain importance. The RNA-

binding protein TcUBP1 binds a large variety of transcripts including trans-sialidase/trans-sialidaselike (TcS) superfamily, a polymorphic group of surface glycoproteins preferentially expressed in the infective forms of the parasite. In vitro RNA-binding assays showed that a 50-nt cis-element highly conserved in the 3'UTR of most TcS family members mediates the interaction with TcUBP1. When steady-state levels of these mRNAs were analyzed by qPCR in replicative non-infective parasites ectopically expressing TcUBP1-GFP, an average of 12-fold increase was observed in comparison to non-induced and GFP induced controls. Moreover, FISH assays revealed that RNA localization of TcS family transcripts change after induction of TcUBP1-GFP to a perinuclear localization, suggesting a subcellular distribution appropriate for RNA translation. Experiments are underway to quantitatively measure these transcript levels in polysomal fractions of both induced and non-induced samples. Finally, cell derived trypomastigotes obtained from epimastigotes expressing TcUBP1-GFP showed a 2-fold increased infectivity comparing to the non-induced controls. Altogether, our results point to a coordinately up-regulation effect of TcS proteins as a response to induction of TcUBP1, thus reflecting a switch towards trypomastigote-form mRNA expression patterns

MI-P04
FIRST REPORT OF *Xanthomonassacchari*ISOLATED FROM SUGARCANE WITH LEAF SCALD DISEASE IN TUCUMÁN

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Xanthomonassacchari is a closely related bacterium to *Xanthomonasalbilineans*, the causal agent of sugarcane leaf scald disease. It is unknown if X. sacchari is able to infect sugarcane. Recently, two strains of *X. sacchari* (named Xs14 and Xs15) were isolated from sugarcane with leaf scald disease. Our goal is to analyse key virulence factors and to assess pathogenicity of *X. sacchari*. First, exopolysaccharide (EPS) and biofilm characteristics were analysed in media with different carbon source (glucose: GLU; sucrose: SUC). EPS yield was determined as dry weight after KCl/ETOH precipitation. Relative EPS-Xs14 production in GLU and SUC was 100% and 70% respectively. On the other hand, EPS-Xs15 relative production was 38% in GLU and 34% in SUC. Low shear rate viscosity (LSRV) was calculated with a Brookfield viscometer. LSRV of EPS-Xs14 and EPS-Xs15 obtained in GLU were significantly higher ($P<0.05$) than LSRV of EPS produced in SUC medium. In vitro assays showed that bacterial adhesion in GLU medium was also significantly higher than adhesion measured in SUC for both strains ($P<0.05$). Biofilm formation was analysed at 48h, 72h, 96h, 120h and 144h. When grown in SUC, both strains showed defined channels and ordered cell organization. However, Xs15-biofilm was dissolved earlier than Xs14-biofilm. Finally, a preliminary in vitro assay using *Arabidopsisthaliana* epidermis measuring stomata opening, indicated that Xs14 could be more virulent than Xs15. Altogether these results suggest that natural nutrient environment favour biofilm development. Moreover, this biofilm characteristic could be associated with bacterium virulence.

MI-P05
GENE SEQUENCING OF SIX NEW S-LAYER PROTEINS FROM PROBIOTIC *Lactobacillus kefiri* STRAINS

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Surface layers are (glyco)-proteinaceous auto-assembled cell envelopes ubiquitously found in *Bacteria* and *Archaea*. *Archaea*. *Lactobacillus kefiri* is a probiotic microorganism carrying a glycosylated S-layer which showed to have interesting functional properties. Recently, we obtained the amino acid sequences of ten S-layer proteins (SLP) from different strains of *L. kefiri*. Regarding several structural differences, different groups of strains were distinguished. In this work, we show the most recent results obtained for the SLP from two aggregative and four non-aggregative new *L. kefiri* strains belonging to our collection. Primers located outsides and inside the SLP genes were used to amplify genomic DNA. The amino acid sequences were deduced using ExPASy Translate Tool, and the theoretical properties were predicted using ProtParam software. The mature proteins contain between 541 and 568 amino acids, the percentage of hydrophobic amino acids varies from 34.9 to 38.6%, and the calculated pI ranges between 9.49 and 9.67. All the SLP present the O-glycosylation site SASSAS. The major differences among strains are found in the C-terminal region, while N-terminal region is relatively conserved, which leads to cross-reactivity using a specific polyclonal mouse antiserum raised against one of the proteins (SLP8348). Each new sequenced SLP belongs to one of the groups previously defined, suggesting that this classification could be representative of the *L. kefiri* SLP family. Our results contribute to understand the functional properties of the SLPs not only as surface bacterial components, but also as unique macromolecules with high potential in nanobiotechnology.

MI-P06
BIOFILM FORMING PATHOGENIC *Klebsiella pneumoniae* AND *Escherichia coli* STRAINS: SIDEROPHORE PROFILE

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We have reported that the antagonism between uropathogenic strains of *K. pneumoniae* and *E. coli* in mixed biofilms in artificial urine medium (AUM) is due to nutrient competition with a greater ability of *K. pneumoniae* to utilize ferric iron. We aim to investigate the siderophore production profile of both strains and its role in inter-species competition. Genotypic characterization was performed by PCR using specific primers for siderophore genes related to its biosynthesis and its receptor. Bacteria were grown planktonically in modified M9 (MM9) for 2 d at 37°C and as biofilms in AUM for 5 d at 37°C. Culture supernatants were evaluated for both siderophore production by chrome azurol S (CAS) assay and chemical determination of catechol- and hydroxamate-containing molecules. Results showed that both strains contain enterobactin

(cathechol) related genes, but only *E. coli* also has salmochelin (cathecol), aerobactin (hidroxamate), and yersiniabactin (mixed) related genes. CAS assays evidenced the production of siderophores by both species when growing in MM9. *E. coli* produce both catechol- and hydroxamate-containing compounds (89±33 and 111±50 pmol/10^6 cells), whereas *K. pneumoniae* only displayed catechols (47±17 pmol/10^6 cells). Biofilms showed similar levels of catechols in all single-species *K. pneumoniae* and *E. coli* biofilms and mixed biofilms. Single-species *E. coli* biofilm express higher amounts of hydroxamates, compared to mixed biofilms. Even when *E. coli* produce a higher amount and variety of siderophores than *K. pneumoniae*, in mixed biofilms, a differential siderophore utilization might take place, allowing *K. pneumoniae* to outcompete *E. coli*.

MI-P07
TRANSCRIPTOMIC ANALYSIS IN BLV-EXPERIMENTALLY INFECTED CATTLE LEADING TO HIGH OR LOW PROVIRAL LOAD

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Bovine Leukemia Virus (BLV) is a highly prevalent pathogen causing a fatal lymphoproliferative disease in the bovine species. After experimental infection, proviral load peaks at 30 days post infection (dpi), then, cattle progress to two different phenotypes: one is characterized by high proviral load (HPL) in peripheral blood, while the other is identified by low proviral load (LPL) in peripheral blood. In LPL cattle a sharp decrease in proviral load is evidenced at 38 dpi. We studied the transcriptome in peripheral blood cells from 10 cattle (5 of each phenotype) infected with BLV at 38 dpi, to identify the host genes differentially expressed in those animals that progress to LPL, recognized as BLV-resistant. RNA seq experiments showed 499 genes differentially expressed ($p<0.05$) between both phenotypes: 281 upregulated and 218 downregulated in LPL compared to HPL cattle. Gene ontology analysis revealed that genes related to inflammatory response, humoral immune response and leukocyte migration were up regulated in cattle progressing to LPL compared to HPL ($p< 0.05$), while the regulation of homeostatic process, antigen receptor signaling pathway and phospholipid transport were downregulated in LPL compared to HPL cattle ($p< 0.05$). The huge difference in transcript expression found at 38 dpi, suggests that mechanisms used to control the proviral load are turned on early after the infection, although the phenotype of LPL or HPL is established only after 90 dpi.

MI-P08
DEPLETION OF TbRRM1 INDUCES RNA POL II TRANSCRIPTION-ELONGATION IMPAIRMENT AND R-LOOPS ACCUMULATION

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TbRRM1 is an essential SR-related RNA binding protein from *Trypanosoma brucei*, the causative agent of sleeping sickness. Previous studies from our lab indicate that TbRRM1 depletion leads to both decreased RNA Pol II transcription-elongation rate and compacted chromatin in a particular polycistronic transcription unit.In the present work we showed, by chromatin and RNA immunoprecipitation assays, that TbRRM1 is both recruited to chromatin and to specific RNAs. Given this association, we further characterized TbRRM1 binding properties in the presence of RNAse A, RNAse H and Actinomycin D. Interestingly, TbRRM1 recruitment to chromatin increased under these treatments, thus suggesting that RNA and chromatin compete for TbRRM1 binding. In addition, we showed by RTqPCR and chromatin fractionation, that the abundance of transcripts belonging to genes downregulated after TbRRM1 depletion increases in the chromatin-associated RNA fraction. Finally, as the RNAse H results suggested that TbRRM1 binds DNA-RNA hybrid, we studied whether *TbRRM1* knockdown induces the formation of R-loops. To this end, we performed indirect immunofluorescence assays with the S9.6 antibody. TbRRM1 depleted cells showed a significant increase in the number of positive intranuclear dots, thus suggesting that TbRRM1 prevents R-loops accumulation.Altogether, our results suggest that RNA Pol II transcription-elongation impairment, induced by TbRRM1 depletion, might be a consequence of RNA Pol II slowing down due to R-loops accumulation. Our hypothesis is that TbRRM1 helps to displace the nascent mRNAs from the site of transcription, which prevents the formation of R-loops.

MI-P09
DECONSTRUCTING COMPLEXES, CHARACTERIZING REDOX STRUCTURES OF *Geobacter*EXTRACELLULAR RESPIRATION

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Geobactersulfurreducens is an electrogenic bacteria capable of reducing insoluble electron acceptors. This ability is mainly due to a wide variety of c-type cytochromes (c-Cyts), many of which localized in the extracellular space (Omc c-Cyts), allowing electron transfer beyond the cell limits. Therefore, its study is of great interest: either to elucidate its complex extracellular electron transport chain or for its clear technological potential. In previous studies we observed Omc c-Cyts of G. sulfurreducens form supramolecular complexes. This motivated these work objectives to separate different complexes and evaluate direct electron transfer in and between redox complexes. For this, extracellular proteins were isolated from cultures grown in medium with acetate as electron donor and fumarate as final electron acceptor. Next, we performed a hydroxyapatite chromatography and retained proteins were eluted with increasing phosphate buffer concentrations. Protein and c-Cyts contents were estimated by spectrophotometry and SDS gel electrophoresis. We observed the presence of 2 to 7 c-Cyts per elution, which allowed us to infer that different elutions contained different c-Cyts complexes. Altogether, 10 c-Cyts were detected of MW between 80kDa and 15kDa that would include OmcB-C, OmcZ, OmcS and OmcE, previously reported to be involved in external electron transfer. Cyclic voltametry tests

enabled us to evaluate different complexes interactions with vitreous carbon electrodes and characterize its electrochemical response. These results allow us to move forward in the study of multi- c-Cyts complexes, their composition and manner of action.

MI-P10
ON THE CHARACTERIZATION OF ADP-GLUCOSE PYROPHOSPHORYLASE FROM NON-PHOTOSYNTHETIC CYANOBACTERIA

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Until recently, it was considered that all members of the cyanobacterial phylum were capable of performing oxygenic photosynthesis. This view dramatically changed after the discovery of a group of non-photosynthetic cyanobacteria named *Melainabacteria*. Using publicly available metagenomic data, we identified sequences encoding putative ADP-glucose pyrophosphorylases (EC 2.7.7.27, ADP-GlcPPase) from free-living and intestinal *Melainabacteria.* The genes coding for these proteins were de novo synthetized and overexpressed in *Escherichia coli*. Purified enzymes showed ADP-GlcPPase activity, with Vmax values of 2.3 and 7.1 U/mg for the free-living and the intestinal *Melainabacteria,* respectively. Both enzymes had similar affinities towards ATP (S0.5~ 0.3 mM), although the one from intestinal source displayed 6-fold higher affinity for glucose-1P. ADP-GlcPPases are allosteric enzymes regulated by metabolites from the main route of carbon utilization in the respective organism. Thus, the enzyme from photosynthetic organisms is activated by 3-phosphoglycerate and inhibited by inorganic phosphate. Interestingly, both ADP-GlcPPases from *Melainabacteria* were mainly activated by glucose-6P (A0.5~ 0.3 mM) but not by 3-phosphoglycerate, while ADP inhibited both enzymes with I0.5 values between 1 and 4 mM. To the best of our knowledge, this is the first biochemical characterization of a key metabolic protein from non-photosynthetic cyanobacteria. We consider this work will contribute to better understand the evolution of allosteric mechanisms in ADP-GlcPPases, a critical enzyme for the synthesis of glycogen in bacteria and starch in plants.

MI-P11
GENOMIC POSITION OF RPOBC LOCUS IMPACTS THE PHYSIOLOGY OF *Vibrio cholerae*

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Growth rate (GR) is a key parameter of bacterial physiology widely varying among microorganisms. The genetic basis of GR is not well understood. In fast-growing bacteria, the genes encoding RNA polymerase (RNAP) are close to the origin of replication (oriC). During exponential growth, fast growers perform multi-fork replication. Thus, genes near to oriC are benefit with higher dosage. Therefore, such positional bias can be a strategy to maximize RNAP expression. To experimentally test this, we used *Vibrio cholerae*, a fast-growing human pathogen. Its genome can be widely modified by natural transformation coupled to recombineering techniques based on lambda phage recombination sites. We aimed at modifying the genomic location of the rpoBC locus, encoding the sole bacterial RNAP. For this we flanked it with lambda phage attR and attL sites allowing its excision or inversion. We inserted attB' sites enabling site-specific re-integration in heterologous locations. Growth curves show that the insertion of att sites does not affect bacterial physiology. Next, Int and Xis recombinases were expressed transiently. Recombination reaction reconstitutes a marker that enables mutant selection. Two strains were built: one with an inverted rpoBC and a second where the locus was relocated far from the oriC. Locus inversion does not present any evident phenotype. Relocation of rpoBC produced colonies of smaller size and slower GR. These physiological alterations are probably due to the difference in gene dosage that occurs during the rapid growth phase by multi-fork replication.

MI-P12
REAL TIME PCR EXPRESSION IN *Streptococcus uberis* UNDER PLANKTONIC AND BIOFILM GROWTH CONDITIONS

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Bovine mastitis is an infectious disease that affects dairy cows causing reduction in milk production and low-quality milk. A wide variety of bacteria classified as contagious and environmental, can cause the disease. *Streptococcus uberis* is a versatile pathogen recovered from the natural environment of dairy cows able to form biofilm and secretes several virulence factors in milk facilitating establishment of infection. The aim of the present work was to study the expression of *sua* and *hasC* genes (encoding virulence factors) and *luxS* gene (associated to quorum sensing) of *S. uberis* strains at biofilm and planktonic growth by Real Time PCR. Two strains SU23 and SU50, categorized as moderate and strongly biofilm producers were used. Total RNA was isolated from both strains at 24, 48 and 72 h. cDNA synthesis and Real Time PCR was performed. Normalized expression levels genes were calculated with the threshold cycle method (2-ΔΔCT) with *gapdh* as control. Results obtained showed a significant increase in sua expression on biofilm growth (SU23 and SU50) (6,4 and 1,8 fold, respectively) compared to planktonic growth at 48 h. Moreover, no changes in expression levels of *hasC* gene were observed. However, an increase of*luxS* expression was observed in SU50 strain at 48 h (6,7 fold). Our results suggest that the genes assayed could be involved in mature biofilm state.

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MI-P13
RRNA OPERON COPY-NUMBER CORRELATES TO GROWTH RATE OF *Bradyrhizobium*

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Growth rate (GR) varies widely among bacterial species. Comparative genomics suggest that the structure of the chromosome contributes to determine GR. In particular, bacteria bearing a high number of ribosomal operons (rrn) display faster GRs. *Bradyrhizobium* speciesare capable of interacting symbiotically with soy, enabling the biological fixation of nitrogen by the plant increasing crop productivity. Bradyrhizobiaceae show a particularly slow GR which makes its study difficult and impacts on its biotechnological utility. Close examination of complete genomes within this group shows that this clade bears 1 or 2 rrn. We hypothesize that species with 2 rrn should grow faster. To test this, we used as model, the completely sequenced strains *B. diazoeficiens* USDA110 and *B. diazoefficiens* USDA122, with one rrn and *B. japonicum* E109 and *B. japonicum* USDA6T that have 2 rrns. Growth curves of this four strains allowed obtaining their generation time (GT) in YEM broth. *B. diazoefficiens* USDA110 and USDA122 displayed median GT of 17 and 20 hours respectively. Meanwhile, *B. japonicum* E109 and U6T displayed a significantly lower GTs of 15h and 9 hs respectively. These differences in GR remained across all media tested. In sum, we observed that strains bearing 2 rrn grow faster than those bearing a single operon. Our results suggest that the addition of additional rrn could allow the increase of the GR of *Bradyrhizobia*. We are cloning rrn to introduce additional copies in the strains to try to artificially modify the GR of these slow-growing microorganisms.

MI-P14
ABILITY OF LACTIC ACID BACTERIA TO AFFECT *Escherichia coli* O157:H7 BIOFILM FORMATION

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Enterohemorrhagic *Escherichia coli* (EHEC) is a human pathogen responsible for outbreaks of bloody diarrhea and hemolytic uremic syndrome worldwide. Cattle is the main reservoir, therefore fresh meat is considered vehicle of this pathogen. Besides of its different virulence factors, EHEC has the ability to form biofilm on food contact surfaces. On the other hand, some lactic acid bacteria (LAB) can inhibit the growth of certain pathogens so its use as bioprotective agents is usually applied. The objective of this study was to evaluate the ability of five LAB strains to affect EHEC biofilm formation by means of exclusion assay. Also, to complete the molecular characterization of the studied pathogenic strain, the detection of biofilm related genes in E. coli NCTC12900 was assessed and corroborated by PCR. Five LAB strains were selected for their better ability to form biofilm at 10 °C on polystyrene microplates. Pathogenic cells were seeded on a 48h-biofilm of each LAB and incubated for 24 h at 10 °C. Total biofilm cells were collected and enumerated on selective agar media. *Lactobacillus plantarum* CRL 1075, was able to reduce the pathogen biofilm in one logarithmic unit without affecting its own biofilm viability. *P. pentosaceus* CRL 908 and CRL 2145 did not affect *E. coli* sessile cells but their own biofilm was affected differently. *L. plantarum* CRL 683 and CRL 1482 produced a decrease in the pathogen counts but their own biofilm was negatively affected. This study is a first approximation oriented to found biological solutions to mitigate *E. coli* biofilm on food surfaces by using bioprotective LAB cultures.

MI-P15
HEXOSE-1P PARTITIONING IN *Rhodococcus*: A COMPARATIVE ANALYSIS BETWEEN DIFFERENT RHODOCOCCAL SPECIES

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The study of enzymes related to carbon partitioning between different storage compounds is critical for a better understanding of the physiology and metabolism of biotechnological microorganisms. The oleaginous *Rhodococcusjostii* and the phytopathogenic *R. fascians* are two actinobacteria capable of accumulate lipids and glycogen with differential metabolic features. Then, we comparatively analyzed the growth of both rhodococcal species in different carbon sources and the production of glycogen and TAGs. Results show different behavior regarding carbon source utilization between both species: while *R. fascians* reach the stationary phase in hours, R. jostii take almost 12 days when both are grown in glycerol; and the opposite occurs when growth on glucose. Furthermore, glycogen accumulation significantly differs depending on the carbon source, nitrogen availability and growth phase. The expression analysis of two key enzymes involved in hexose-1P partition, ADP-glucose pyrophosphorylase and UDP-glucose pyrophosphorylase, in the different culture conditions showed a differential expression pattern for the latter whilst ADP-GlcPPase remained constant. Then, UDP-GlcPPase could be subjected to protein turn-over regulatory mechanisms while the activity of ADP-GlcPPase (and consequently glycogen accumulation) is allosterically regulated, according to dynamic metabolic scenarios. In this regard, our results reinforce the importance of deepen the study of the carbon metabolism to identify the major physiologic differences between these rhodococcal species for future metabolic engineer and biotechnological applications.

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MI-P16
CHARACTERIZATION OF A VACCINE PLATFORM FOR MUCOSAL ADMINISTRATION BASED ON *Lactobacillus* AS CARRIER

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Since mucosal surfaces are the main route of entry for microbial pathogens, the induction of a protective immunity at this level emerges as an attractive field of study for vaccine development. It has been reported that oral route is capable to induce an immune response at level of other distal mucosal surfaces and eventually at systemic level. Different strategies have been postulated for antigen delivery to gut mucosal surface, here we have proposed to use a probiotic bacteria Lactobacillus acidophilus (LB) as a vaccine carrier for targeting antigens into gut mucosa. We selected *Escherichia coli* O157:H7 (STEC) as a experimental model. STEC is an emerging bacterial pathogen that has a great regional impact, responsible for the Hemolytic Uremic Syndrome. As antigen, three STEC proteins (EspA, Intimin, Tir) were fused-in-frame and combined also with the binding domain of the S-layer protein (SLAP domain) of LB (EITS). EITS binding to the lactobacilli surface was estimated by Quantitative Infrared Western Blots, determining that until 20 µg of EITS protein can be coated on 108 lactobacilli. BALB/c mice were orally immunized with EITS-coated lactobacilli (0, 2 and 4 weeks). Although no significant increase of fecal IgA titers against EIT was detected, mice EITS-immunized were able to control an oral challenging infection (1010 UFC of STEC) estimated by reduction of fecal shedding of STEC.

MI-P17
FLUORESCENT *Leishmania*PARASITES, A USEFUL METHOD FOR STUDYING THE EFFICACY OF NEW DRUGS

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The conventional methods for assessing the efficacy of a new drug in an animal model of cutaneous leishmaniasis (CL) are laborious, time consuming, and do no support automation for the parasite load quantification. The objective of this work was to standardize and validate a method based on parasites expressing tomato red fluorescent protein in order to quantify the parasite load in an animal model of CL.The tomato gene was subcloned into pIR1SAT plasmid, and before the electroporation of *Leishmania (Leishmania) amazonensis* (MHOM/BR/73/M2269), it was linearized with SwaI. The plasmid replaces one copy of the SSU rRNA gene, and the integration into *Leishmania* genome was confirmed by PCR. Parasites were selected in presence of nourseothricin and cloned in blood agar plate. Fluorescence was measured in intracellular amastigotes (am) obtained from cutaneous lesions of BALB/c mice in a plate reader. In addition, the infectivity of fluorescent parasites was compared with the wild ones. After that, the efficacy of a topical treatment with epigallocatechin gallate (EGCg) in mice infected with *Leishmania (L.) amazonensis* was determined with the method standardized and compared with the conventional technique. The positive control group was treated with of meglumine antimoniate.An excellent linear correlation was observed between the number of am and the fluorescence emitted by the parasites (r2 = 0.98). In vivo, the parasites fluorescence was stable after several months post-infection, and the parasites were infective as the wild type. No difference was observed in the parasite load quantified by the fluorescence method and the conventional one. On the other hand, EGCg showed leishmanicidal activity inhibiting the parasite load in lesion (64 %).The fluorescence emitted by the tomato red protein in transgenic parasites is a good indicator of parasites viability. The method was reproducible, cheap and useful for studying the efficacy of new leishmanicidal drugs in an animal model of cutaneous leishmaniasis.

MI-P18
EVALUATION OF ANTIMICROBIAL ACTIVITY OF HYBRID BATERIOCINS IN PATHOGENIC STRAINS

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Bacteriocins are bacterial antimicrobial peptides. Enterocin CRL35 and microcin V are produced by *Enterococcus mundtii* and *Escherichia coli*, respectively. They are active on microorganisms phylogenetically related to the producer strain. Ent35-GGG-MccV is a hybrid bacteriocin obtained by fusion of the genes of Enterocin CRL35 and Microcin V with a sequence encoding for a hinge region of three glycine between them. New peptides derived from Ent35-GGG-MccV with improved bioactivity were obtained by saturation mutagenesis focused on the hinge region. The aim of this work is to compare the antimicrobial potency and the spectrum of action of the different variants against indicator and pathogenic strains related to food-borne diseases. To compare the activity of the different variants, determinations were made of the minimum inhibitory concentration and a count of viable cells of bacterial suspensions treated with the peptides. Moreover, the bacteriocins were tested against *Shigella spp*. isolates from fecal sample in child with GI disease. As a conclusion, all the hybrid peptides were able to decrease the viability of the indicator strains and two variants (Ent35-GYG-MccV and Ent35-GNG-MccV) showed higher antimicrobial activity than the original bacteriocin, on *E. coli* and *Listeria monocytogenes*. In addition, the peptides exhibited antimicrobial activity against all the strains of *Shigellasonnei*tested. The approach was highly successful to obtain mutants with improved bioactivity with respect to the original hybrid.

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MI-P19
NEW STRUCTURES OF MUTS BOUND TO DNA REPLICATION STRUCTURES: A CHANGE TO GAIN ACCESS TO THE REPLISOME

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MutS contributes to the DNA replication fidelity by recognizing mispairs (MMs) and recruiting factors involved in the Mismatch Repair (MMR). We have revealed a non-canonical function of MutS in the replication machinery: the regulation of the access of the mutagenic DNA polymerase (Pol) IV to replication sites. Our previous results indicated that DNA substrates (single-stranded, homoduplex, heteroduplex, primed and GT-primed DNAs) modulate the ability of MutS to regulate the Pol IV action. Here, we examined the effect of these DNA substrates on the structure and biochemical properties of MutS. The replication substrate GT-primed DNA, but not the other DNA structures, specifically induced a compaction of MutS. When we tested primed-DNAs containing the 12 different types of MMs, the CC, GG and GA produced also the structural MutS compaction. This agrees with the fact that Pol IV predominantly generates these MMs when copying DNA. We also characterized the properties important for the repair pathway initiated by MutS, namely the ATPase activity, MutL interaction and stimulation the MutL-endonuclease activity, in the presence of the DNA substrates. In addition, we are testing if the structural change induced by the replication substrate GT-primed DNA is necessary for MutS gaining access to the replisome. Thus, this work reveals that complex protein-DNA interactions could dictate the function of MutS in a particular pathway, repair or regulation of the access of Pol IV.

MI-P20
FROM BIOINFORMATICS TO REGULATORY LINK DISCOVERY: METAL-DEPENDENT BtaE GENE EXPRESSION IN *Brucella*

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VjbR is a LuxR-type transcriptional regulator that plays an essential role in the pathogenesis of facultative intracellular bacteria of the genus *Brucella*. Previous analyses from our group revealed that the VjbRtransciptionalnetwok is highly complex and provided data suggesting that in specific promoters this regulator interacts with possible competitor and/or coactivator transcription factors. To examine this hypothesis, we recently developed a bioinformatic method to identify conserved sequences adjacent to VjbR-binding motifs, which could act as binding sites for competitors or coactivators. Using this tool we identified four different highly conserved motifs, one of which showed similarity with binding sites for transcription factors of the ferric uptake regulator (Fur) family. In order to analyze the possible functionality of this sequence, we examined the expression of *btaE*, a VjbR target gene encoding a trimeric autotransporter adhesin, whose promoter region contains four repeats of the identified motif. Using either *lacZ* transcriptional fusions or Western blot, we observed that under low nutrient, acidic conditions mimicking the intracellular environment that *Brucella* encounters within the host, expression of *btaE* was negatively regulated by ferric iron in a dose-dependent manner. Experiments performed in different mutant backgrounds including the Fur-type regulator *irr* gene and the Rrf2-type iron-responsive transcription factor *rirA* provided further insights into both metal specificity and the relationship between metal homeostasis and BtaEadhesin expression under harsh environmental conditions

MI-P21
EPIDEMIOLOGY OF BOVINE LEUKEMIA VIRUS IN BEEF CATTLE IN SOUTHEAST REGION OF THE PROVINCE OF BUENOS AIRES.

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The bovine leukemia virus affects cattle and causes enzootic bovine leukosis, with high economic losses in dairy cattle production systems. The current epidemiological situation of BLV in beef cattle herds is unknown. This justifies the study of prevalence of infection in beef cattle herds and the detection of potential risk factors in order to implement prevention and control measures according to BLV prevalence levels. The aim of this work was to evaluate the rate of positivity to BLV in beef cattle herds in the south-eastern region of the province of Buenos Aires. An analytical study was carried out using a non-probabilistic sampling for convenience in 16 establishments of the region. 15 animals per establishment were sampled, with a total of 240 sera collected. In addition, a survey was conducted to collect epidemiological information, with emphasis on potential risk factors. The cattle were diagnosed by BLVgp51 antibody detection by means of indirect ELISA method. The seropositivity rate was 0.41% (95% CI: 0.011-2.300). The positive animal was a bull Aberdeen Angus, in reproductive state. Given the found preliminary results, special considerations should be taken at the male reproducers purchase, since they may introduce the infectious agent in this area. A preventive measure could be their negative BLV diagnosis at the purchase moment and a quarantine period prior to get the herd

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MI-P22
ELIMINATION OF BACTERIAL BIOFILMS WITH AN AEROSOL FORMULATION OF SILVER SULFADIAZINE

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The healing processes of wounds in skin and soft tissues are permanently challenged by the risk of microbial infection, particularly in chronic wounds which are extremely susceptible of being colonized by gram-negative as well as gram-positive pathogens growing in a biofilm life-style. Dressings such as gauze, ointment or gels containing silver sulfadiazine have been developed to avoid infection and biofilm formation on wounds. In this study we test in vitro an aerosol formulation of silver sulfadiazine (Platsul-$A_{AEROSOL}$, SoubeiranChobet SRL) for spray administration against biofilms of *Pseudomonas aeruginosa* and against biofilms of two strains of *Staphylococcus aureus* being resistant (MRSA) or sensible (MSSA) to methicillin. The spray allows the administration of the silver formulation without the direct contact with the wound, thereby avoiding possible cross-infection and trauma to the patient. Furthermore, the sealed container and the positive pressure by which the product is expelled avoids contamination of the product after reiterative usage. Thus, we evaluated this formulation on biofilms by carrying out biomass quantification by crystal violet staining assays, as well as viability measurements and live/dead confocal microscopy. This formulation displayed a strong antibiofilm effect, showing total bactericidal activity on biofilms of *P. aeruginosa* even after an 800-fold dilution of the product, and after a 100-fold and 10-fold dilution for MRSA and MSSA, respectively. Considering the benefits of a spray administration, our results support this kind of aerosol formulation as a potential improvement over conventional dressings of silver sulfadiazine

MI-P23
CHARACTERIZATION OF A NEW TRANSCRIPTION FACTOR OF THE MERR FAMILY OF*Bizioniaargentinensis*

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The marine genera belonging to the Flavobacteriaceae family are largely responsible for the remineralization of organic matter in the oceans and have a great environmental importance in aquatic ecosystems. However, there is still scarce information about their biological cycles and the molecular mechanisms that regulate them. In this scenario, the bacterium *Bizioniaargentinensis*, isolated in the National Antarctic Territory, constitutes an interesting model for the study of these marine bacteria since it has suitable characteristics for its management in the laboratory and its genome was sequenced. In this context, from a structural genomic study of *B. argentinensis* a new transcription factor belonging to the superfamily of the transcription factors MerR, named BA40, has been identified and characterized. Our preliminary studies showed that the protein level of BA40 is increased after exposing the bacteria to a medium lacking of nutrients, acting as a shock protein related to stress by oligotrophy. In order to elucidate a consensus DNA sequence to which BA40 was specifically bound, a high affinity selection method was used from random DNA sequences (SELEX). Using this technique, we were able to select a consensus sequence of 8 bp for which BA40 showed 21 times more affinity than a random sequence of the same length. Finally, we optimized molecular biology tools to generate mutants of *B. argentinensis* lacking the MerR gene in order to complement our results and identify the genetics targets of the transcription factor using ChIP-seq

MI-P24
CHARACTERIZATION OF *Tritrichomonasfoetus* EXCRETION/SECRETION ANTIGENS

Abdala ME1; Rivero MB1; Luque ME1; Carranza PG1; Prucca CG2; Rivero FD1
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Bovine trichomonosis, a disease of the bovine urogenital tract, is caused by the protozoan *Tritrichomonasfoetus* (Tf). It causes endometritis, infertility and premature death of the embryo, which generates considerable economic losses. Have a worldwide distribution and in Argentina it´s considered endemic. Excretion/secretion antigens (AES) have been shown to be virulence factors in both *Trichomonas vaginalis* (Tv), Tf homolog in humans, and in other organisms and constitute a target for the development of diagnostic methods and vaccines. The objective was to determine the AES repertoire of Tf. Six isolates were incubated with buffer TIB to perform AES purification and then were filtered and concentrated by ultracentrifugation. An SDS-PAGE was performed and then sent for analysis by MALDI-TOF. The data obtained were analyzed and compared against the Tf and Tv genomes. The results showed that there is a low homology between AES of Tf vs Tv, however, a high homology was observed between the different Tf isolates. The proteins present in AES of Tf were classified functionally, showing among others the following groups: posttranslational modification proteins (14%), binding proteins (12%), vesicular transport proteins (11%) and unknown function proteins (18%). In conclusion: a) There are great similarities between the AES of different Tf isolates, which allows to identify the profile of excretion/secretion. b) The differences with Tv allow us to infer certain specificity of the AES of Tf c) According to the diverse proteins found, different trials can be approached to evaluate new targets for diagnosis and treatment of this disease.

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MI-P25
ROLE OF MUTAGENIC DNA POLYMERASES IN THE MUTAGENESIS OF LasRIN *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa (PA) causes chronic airway infections (CAI) in cystic fibrosis (CF) patients by unique strains, which persist during their total life-span. PA bases its strategy on a process of genetic adaptation that underlies the characteristic phenotypic diversification process that favors its marked persistence. This diversification usually involves point mutations in specific genes, so the control of the mutation rate constitutes a key factor associated with the persistence of the infection. Under environmental stress conditions, such as chronic infections, stress response mechanisms are induced in order to survive. Part of this response involves the induction of mutagenic DNA polymerases, which lack prof-reading activity and consequently increase mutation rates. PA possesses at least three of these DNA polymerases: Pol IV, ImuB and DnaE2, however little is known about their participation in PA diversification processes. Here, we evaluate the involvement of these Pols in the mutagenesis of a key gene for the adaptation of PA during CAI, *lasR*. By performing *in vitro* diversification assays using Pols mutant strains we determined that neither of the Pols were involved in *lasR* mutagenesis since the emergence of *lasR*variants were similar to the parental strain. It has been reported that the action of this Pols could be DNA strand specific. We investigated this possibility by measuring the mutagenesis in a gene that was in a opposite location to *lasR*: *rpoB*. Results obtained showed that Pol IV was involved in *rpoB* but not *lasR*, suggesting that the action of this Pol is gene specific.

MI-P26
DEVELOPMENT OF A MOLECULAR METHODOLOGY FOR *Yersinia enterocolitica*DETECTION IN FOODS

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The rapid detection of pathogenic microorganisms in foods is an essential part of the quality control to ensure the consumer health. *Yersinia enterocolitica* (*Ye*) is anenteropathogen that causes enterocolitis and extraintestinal symptoms. Its transmission is by oral route, commonly through contaminated foods. This species includes 6 biotypes, and its pathogenicity is attributed to plasmid and chromosomal virulence factors. The objective of this work was to develop a sensitive and specific methodology to detect all *Ye* biotypes by a one-step molecular technique. For this purpose, a conventional PCR targeted to the *yst* chromosomal gene, which encodes a thermostable enterotoxin associated with diarrhea in clinical cases of yersiniosis, was designed. Previous results in our laboratory demonstrated that the *Ye* detection limit (DL) in culture corresponded to 45 cfu/ml, while the DL obtained by PCR targeted to *yst* gene corresponded to 6 cfu/ml. Moreover, the results obtained in the specificity tests revealed that 19 strains of different *Ye* biotypes were yst^+, while 10 strains of other *Yersinia* species and 14 strains belonging to other enterobacteria were yst^-. Our findings indicate that the PCR based on the detection of the *Yyst*gene is a sensitive and specific technique which allows accelerate the detection times in comparison with the culture techniques, and might be used for the rapid detection of this microorganism in foods. It would be a very valuable tool to be included in a systematic surveillance of *Ye* in foods aimed to monitor the spread of this enteropathogen and to prevent the risk of infection in humans

MI-P27
SPECIFICITY AND STRUCTURAL BASIS FOR LINOLEIC ACID MODULATION OF THE *Salmonella* PHOP/PHOQ SYSTEM

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PhoP/PhoQ is a two-component system (TCS) distributed amongst several Gram negative bacteria, consisting of the histidine kinase PhoQ, and the transcriptional regulator PhoP. The PhoP/PhoQ system regulates the adaptation to Mg^{2+}-limiting environments, and controls virulent phenotypes in pathogenic bacteria. In*S.* Typhimurium, PhoP/PhoQ controls the invasion and proliferation within host cells. We have previously reported that long chain unsaturated fatty acids (LCUFAs) are input signals of the*S.* TyphimuriumPhoPQ TCS, repressing the transcription of PhoP regulated genes by inhibiting PhoQautokinase activity. In addition, NMR data obtained by our group pointed that LCUFAs act as ligands for PhoQ periplasmic domain (PhoQp). The aims of this work were to determine if LCUFAs repression upon PhoPQ signaling also occurs in other Enterobacteriaceae; and to identify specific PhoQp amino acid residues involved in LCUFAs-sensing. We constructed a series of*lacZ*-transcriptional fusions to previously characterized PhoP-activated genes in*Escherichia coli*and*Serratia marcescens*. We also performed site-directed mutagenesis over*S.* Typhimurium*phoQ*, obtaining a number of PhoQ mutants with substitutions or deletions in PhoQp domain. β-galactosidase assays showed that there was no significant PhoP/PhoQ modulation by growth in LB medium supplemented with linoleic acid either in*E. coli*or*S. marcescens*. In addition, only two of the engineered mutant variants of PhoQp showed altered linoleic acid repression. Taken together, our results suggest that LCUFAs modulation is*Salmonella*-exclusive, and can be impaired by structural modifications of β-strands 3 and 7

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MI-P28
KUP AND KIMA: TWO SECONDARY TRANSPORTERS INVOLVED IN THE HOMEOSTASIS OF K+ IN *Enterococcus faecalis*

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E. faecalis are facultative anaerobic Gram-positive bacteria and constitute part of the normal intestinal flora of humans. In recent years however, it has emerged as a clinical important opportunistic pathogen. The robust physiology of these bacteria facilitates tolerance to various stresses. Despite its controversial profile, *E. faecalis* are part of food products, either due to contamination or as part of starter, adjunct or non-starter cultures. Besides, ion homeostasis is a key factor for all living cells. Particularly, potassium (K+) is the most abundant cation in the cytosol, and its uptake is tightly regulated. Intracellular K+ is important for cellular metabolic processes such as gene expression, pH homeostasis, osmotic adaptation and enzymatic activity. A search for genes present in *E. faecalis* genome coding for proteins with high homology for K+ transporters and a subsequent topology analysis, revealed the presence of a Kup as well as a KimA homologue. These proteins are regulated by the secondary messenger c-di-AMP in *L. lactis* and *B. subtilis*, respectively. To study the functional properties of the proteins encoded by both genes we used *E.coli* LB650 (Δ*kdpABC5* Δ*trkH* Δ*trkG*). This strain is a triple mutant for the main K+ transporter systems and, hence, it is unable to grow at low K+ concentrations. *E. faecalis* JH2-2 *kup* and *kimA* genes were individually cloned in plasmid pWH844 using *E. coli* LB650 as host, to check if their expression could restore growth in minimal salt media when no KCl is added. Both evidence growth with or without K+ supplementation. These results suggest that Kup and KimA are involved in the K+ transport in. *E. faecalis*.

MI-P29
PARTICIPATION OF THE HPV ONCOPROTEINS IN THE REGULATION OF DISC LARGE 1 DURING HPV ONCOGENESIS

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The tumor processes are related to the deregulation of cellular polarity proteins which assure the correct division, morphology and cell proliferation. High-risk oncogenic human papillomaviruses (HPV) are related to the development of cervical cancer. The HPV E6 viral oncoprotein is able to interact with the human Disc large polarity protein (DLG1), located at the adherent junctions. DLG1 expression in organotypic cultures expressing E6/E7 HPV oncoproteins results in a redistribution of DLG1 from the cell contacts to the cytoplasm, as well as an increase in its levels. This is in agreement with studies using biopsies of cervical lesions. In order to understand the molecular mechanisms involved in this deregulation of DLG1, we performed a series of analyses in cultured cells.We studied the expression of DLG1 in the presence of HPV E6 by immunofluorescence, being able to detect the relocalization of DLG1. We also found that an overexpression of DLG1 causes an impact in the localization of E6. To corroborate the binding between DLG1 and E6 we performed FRET experiments and we detected for the first time a direct interaction between the viral and the polarity protein within the cell.We also studied the contribution of E7 on the expression of DLG1. We observed that the dual expression of E6 and E7 induces a delocalization and an increase in DLG1 levels in the insoluble cell fraction. These results suggest that viral oncoproteins promote the stabilization of DLG1 in the cytoplasm with probable changes in its oncosuppressive functions. These data contribute to the molecular understanding of the alteration of cell polarity during the oncogenesis.

MI-P30
CHARACTERIZATION OF A CueR VARIANT THAT RESPONDS TO +1 AND +2 HEAVY METAL IONS

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Resistance to toxic heavy metals in bacteria is controlled by cytoplasmic metalloregulators of the MerR family. These transcriptional activators bind metals ions with high affinity at the dimer interface, using residues from both monomers. According to the array of available ligands at the metal-coordination environment, MerR sensors are group in three categories: the CueR-like Cu(I)/Ag(I)/Au(I) sensors, the archetypal MerR Hg(II) sensors and the ZntR-like Zn(II)/Pb(II)/Cd(II) sensors. CueR coordinates +1 ions using two conserved cysteine residues (C112 and C120) from one monomer. MerR and ZntR-like sensors use, in addition, a third C residue from the other monomer to bind +2 ions that require a high number of coordination ligands. Interestingly, CueR and its orthologues have a conserved serine (S77) in place of the third cysteine, but its relevance is still unknown. We previously shown that a *Salmonella* Typhimurium strain carrying a cueR-S77C mutant allele activates CueR-controlled genes in response to Cu(I), Ag(I), Au(I), and also to Hg(II). Furthermore, this mutant sensor also responds to Pb(II), Cd(II) and Co(II) in a strain lacking the Zn(II)/Pb(II)/Cd(II) transporter ZntA. Here, we reproduced the S77C mutation in the *Escherichiacoli*CueRortholog from which structural data is available. Using specific reporter genes, we validated the response of ECCueR-S77C to +1 and +2 metal ions. We also analyzed and compared the interaction of ECCueR-S77C and ECCueR with Pb(II) or Co(II) by recording the UV−vis spectra. Our results contribute to understand the importance of S77 in CueR-like proteins for the exclusion of +2 ions from the metal binding site.

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MI-P31
Serratia marcescens SHLA HEMOLYSIN IS INHIBITED BY NI2+

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Serratia marcescens is an opportunistic human pathogen that represents a growing problem for public health. We have previously reported that *S. marcescens* is able to invade, survive and proliferate in non-phagocytic cells. After proliferation, the ShlAhemolysin is responsible for an increase in the cytosolic Ca2+ concentration, remodeling the actin cytoskeleton and promoting an exocytic-like egress. It has been reported that ShlA is necessary for cytotoxicity in different cells lines. At sub-lytic concentrations, it induces ATP depletion and K+ efflux. The aim of this work is to find tools that allow us to understand the function of ShlA. Performing invasion assays, co-localization with a calcium fluorophore and autophagy assays, we determined that in presence of Ni2+ wild-type *Serratia* behaves like a *shlBA* mutant. These assays were done in sub-lytic conditions and the phenotypes are blocked by the addition of the calcium chelator BAPTA-AM. Next, we performed hemolytic and cytotoxicity assays and we determined that the addition of Ni2+ to the wild-type strain prevented the lysis of the eukaryotic cells, obtaining a phenotype similar to the observed in the *shlBA* mutant. The addition of BAPTA-AM did not inhibit the eukaryotic cells lysis. We conclude that in presence of Ni2+ wild-type *Serratia* behaves like a *shlBA* mutant both in lytic and in non-lytic conditions. Moreover, we hypothesize that ShlA is responsible for two distinct phenotypes. The lytic phenotype is independent of the ShlA-mediated intracellular Ca2+ concentration fluctuations. In contrast, the ones that are induced in sub-lytic conditions of ShlA are dependent of a Ca2+ mobilization.

MI-P32
RECRUITMENT OF THE OXA-58 CARBAPENEMASE IN OUTER MEMBRANE VESICLES OF*Acinetobacter baumannii*

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Acinetobacter baumannii constitutes a worrisome problem in health-care institutions, displaying carbapenem resistance due to the overproduction of OXA-type class-D carbapenemases (CHDLs) (1). Part of this resistance is mediated by the production of outer membrane vesicles (OMV), with postulated roles in cell protection, pathogenicity, and polymicrobial co-infections. However, the detailed mechanisms of OMV production and selection of cargo proteins remains largely unknown. We studied here the biogenesis and secretion of OXA-58 carbapenemase in *A. baumannii*, using as a model ATCC 17978 (Ab17978) cells transformed with plasmid pOXA-58 which directs high-level expression of the blaOXA-58 gene. OXA-58 production was evaluated by immunoblot and carbapenemase activity in different subcellular fractions of wild-type Ab17978 or Δ*carO*mutants lacking the outer membrane protein (OMP) CarO. We found that OXA-58 is a membrane-associated lipoprotein, and that this post-translational modification is essential for its recruitment into OMV since mutant OXA-58 lacking the Cys residue subjected to lipidation was excluded from the OMV. The absence of the OMP CarO in the cells disrupted the selection process of recruitment of OXA-58, allowing the secretion of the non-lipidatedcarbapenemase. Moreover, CarO also interacts with OmpA, another OMP having pivotal roles in OMV composition. Overall these results indicated that CarO represents an important regulatory component in the selection of proteins recruited for secretion in OMV and in the biogenesis of these entities in *A. baumannii*.

MI-P33
CHARACTERIZATION OF *Trypanosoma brucei*OCTANOYLTRANSFERASE HARNESSING *Bacillus subtilis* MUTANTS

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Lipoic acid (LA) is a universally conserved sulfur-containing cofactor involved in one-carbon and oxidative metabolism. Interfering with LA synthesis would be a potential chemotherapeutic target against parasites like *Trypanosoma cruzi* and *T. brucei*, due to its essentiality to cell viability. However, while the ways by which proteins become lipoylated are very well characterized in prokaryotes, information concerning eukaryotes is scarce. It was previously found by in silico analyses that *T. brucei* Tb11.01.1160 gene product (TbLipT) is similar to bacterial and yeast octanoyltransferases. It was also demonstrated that it functionally complements a *lip2* mutant in *Saccharomyces cerevisiae*. These results strongly suggested that TbLipT is the *T. brucei*octanoyltransferase. In order to deeply characterize the substrate specificity of the octanoytransfer reaction we expressed this protein in *B. subtilis* mutants defective in different steps of the lipoylation pathway, and observed the functional complementation and protein lipoylation patterns of the transformed mutants. The fact that the *B. subtilis*Δ*gcvH* and Δ*lipL* mutants were not complemented, suggests that TbLipT transfers octanoate specifically to the H protein, in a similar way as LipM in *B. subtilis* and Lip2 in yeasts. By site-directed mutagenesis it could also be confirmed the importance of Lys161 and Cys195 for the octanoyltransferase activity of the trypanosomal protein.

MI-P34
ARSENIC METABOLISM IN HIGH-ALTITUDE ANDEAN LAKES

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The High-Altitude Andean Lakes (HAAL) consists of several shallow lakes located in a high-altitude desert of Central Andes region. In these lakes elevated concentrations of arsenic (As) were found in water, attributed mainly to volcanic activity. Diamante Lake is one of the most

extreme environments within HAAL, due to its alkalinity (pH 11) and high As concentrations (234 mg/L). Given the high As content, arsenic metabolism was studied by metagenomics analysis of the Diamante Lake Red Haloarquea Biofilms (DLRB). All the genes necessary for the arsenic detoxifying mechanism (*arsABC*), arsenate respiration (*arrBA*) and arsenite oxidation (*aioBA*) were found in this study. To verify the importance of arsenic as a bioenergetic component, in this work we carried out physiological and molecular assays in the haloarquea*Halorubrum sp*. DM2 (*Euryarchaeota phylum*) isolated from DLRB. The anaerobic and aerobic growth of this strain was determined by adding arsenic to the culture medium, arsenite (As III), as electron donor and arsenate (As V) as acceptor. We observed that in the presence of arsenate (As V) the anaerobic growth of *Halorubrum*sp. DM2 was greater with respect to the control. However, no growth differences were observed in aerobiosis. Moreover, results performed by RT-qPCR assays showed an increment in the transcriptional levels of the arsenic-related genes when arsenic was added to the growth medium. Although it is not yet possible to assign the underlying molecular mechanism, these results suggest that *Halorubrum*sp. DM2 is able to use arsenic as a bioenergetics substrate to maintain its growth.

MI-P35
*Acinetobacter baumannii*CarO OUTER MEMBRANE PROTEINS ARE INVOLVED IN BASIC AMINO ACIDS UPTAKE

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The family of outer membrane (OM) proteins CarO is restricted to the family Moraxellaceae, where *Acinetobacter baumannii* belongs. We previously described four polymorphic *carO* variants with the corresponding CarO proteins exhibiting distinctive properties such as differential migrations in gels, antigenicity, formation of oligomeric structures, and L-ornithine permeation abilities. To further characterize the different *A. baumannii*CarO variants we used *A. baylyi* ADP1 as model. For this purpose we constructed ADP1 recombinant cells harboring genetic replacements for each of the *A. baumanniicarO* variants. We evaluated the physiology of the mutants concerning growth in rich and minimal medium, susceptibility to external aggressors, and CarO production. All of the replacement mutants showed similar growth rates comparable to that of the wild-type ADP1, comparable susceptibility to ionic detergents and NaCl stress, and same levels of CarO accumulation in the OM. Motility assays indicated that restitution of CarO to the OM restored the motility of the recombinants in semisolid medium. Nutrient utilization assays conducted in minimal media supplemented with different compounds, including amino acids, sugars, and different phenolic compounds, indicated differential growth of the mutants only on the basic amino acid arginine. Mutants carrying CarO variants III and IV were impeded to grow in arginine, while cells carrying variants I and II could grow similarly to wild-type cells. The overall results indicate that CarO participates in the selective uptake of basic amino acids and suggest roles in the interaction with abiotic and biotic surfaces.

MI-P36
CONTROL OF MEMBRANE LIPID HOMEOSTASIS IN BACTERIA

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Bacteria strictly control the synthesis of their membrane phospholipids in response to different nutritional conditions but the underlying regulatory mechanisms are obscure. In *Bacillus subtilis* fatty acids (FA) are produced by a type II synthase (FASII) consisting of a repeated cycle of reactions. In FASII, all fatty acyl intermediates are covalently linked to acyl carrier protein (ACP) and shuttled from one enzyme to another. Malonyl-CoA, which is synthesized by acetyl-CoA carboxylase (ACC), is an essential lipid intermediate in FA biosynthesis in all living cells. When the acyl-ACPs reach the proper length, they become substrates for the enzymes involved in phospholipid synthesis (PLS): PlsX (acyl-ACP:PO4 acyltransferase), PlsY (acyl-PO4:glycerol-PO4 acyltransferase) and PlsC (acyl-ACP:1-acylglycerol-PO4 acyltransferase). In our lab, it was shown that FASII and PLS are coupled at the PlsX step in *B. subtilis*. In the absence of PlsX, both PLS and FA synthesis are arrested. Here, we show that a Δ*plsX* strain accumulates acyl-ACPs and malonyl-CoA, indicating that ACC activity is not repressed. Moreover, acyl-ACPs hydrolysis by heterologous expression of a thioesterase (TesA) does not fully release inhibition of FASII in a plsX mutant. Furthermore, we determined that *plsY* and *plsC* mutants also accumulate high levels of long chain acyl-ACPs while exhibiting an active FASII. These findings suggest that in *B. subtilis*, in contrast to other bacteria, long chain acyl-ACPs are not feedback inhibitors of ACC and FASII. Our results shed new light into the control of lipid homeostasis in bacteria

MI-P37
SCREENING AND CHARACTERIZATION OF *Mycobacterium tuberculosis*ACYL-COA CARBOXYLASE INHIBITORS

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Tuberculosis (TB) represents a major public health problem worldwide. This disease remains today one of the leading causes of death from an infectious agent. The emergence of multidrug-resistant and extensively drug-resistant *Mycobacterium tuberculosis* (Mtb) strains has challenged TB control. Therefore, the discovery of novel pharmacological targets for the development of a new generation of drugs is urgently needed. Acyl-CoA carboxylases (ACCases) are key enzymes providing acyl-CoAs, substrates for biosynthesis of all the unique lipids present in Mtb cell envelope. Bioinformatic, biochemical and structural analysis of MtbACCase 5 complex indicated that the main catalytic activity of this enzyme corresponds to that of a propionyl-CoA/acetyl-CoA carboxylase. This complex is formed by the biotinylated α subunit AccA3, the carboxyltransferase β subunit AccD5 and the small ε subunit AccE5. Studies carried out by high-density mutagenesis in Mtb and directed mutagenesis in *M. smegmatis* indicate that this enzyme complex is essential for the viability of mycobacteria, inferring that the ACCase 5

complex has an essential activity for this bacterium. Also the analysis of a conditional mutant demonstrated that AccD5 and AccE5 are part of an essential ACCase involved in lipid biosynthesis, and proposed ACCase 5 as an attractive target for tuberculosis drug discovery. In this work, we used two different enzyme-based assays to identify inhibitors of ACCase 5. High throughput screening assays were implemented to test millons of compounds belonging to Glaxo SmithKline and Novartis, and found several molecules that inhibited more that 85% of the ACCase 5 PCC activity. We further analyzed these candidates by conventional enzymatic methods and found five compounds that inhibited ACCase 5 at low µM concentrations. For these compounds IC50 and MIC on Mtb H37Ra were obtained, and their influence in the biosynthesis of fatty and mycolic acids was evaluated. These results validated the high throughput screening assay as a powerful tool for identifying novel enzyme inhibitors that could be developed as anti-tuberculosis drugs.

MI-P38
RamA IS INVOLVED IN REGULATION OF THE LPS MODIFICATIONS AND RESISTANCE ANTIBIOTICS IN *Serratia marcescens*

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The lipopolysaccharide (LPS) lipid A is recognized by the innate system. The gram-negative bacteria modify the LPS to avoid host immune system and to resist killing by antimicrobial peptide. In many bacterial species, modifications in the LPS that confer resistance to antimicrobial peptides are regulated by the PhoP/PhoQ system. It was previously reported that, in *Klebsiella pneumoniae*, the RamA transcriptional regulator, functions as an alternate regulator of genes involved in lipid A biosynthesis. *S. marcescens* is an enteric bacterium that can function as an opportunistic pathogen within immunocompromised hosts. We found a *ramA* homolog gene in *Serratia marcescens* RM66262, and its promoter region a putative recognition site for the PhoP regulator. In this work, we analyzed the regulation of *ramA* and the genes *lpxO1* and *lpxO2* required for the modification of LPS in *Serratia*. The contribution of PhoP and RamA in the regulation of *lpxO1* and *lpxO2* was analyzed using *gfp*-containing reporter plasmids and electrophoretic mobility shift assay. Our results show that, in *S. marcescens*, PhoP is involved in the regulation of RamA expression and that both PhoP and RamA modulate the expression of the *lpxO1* and *lpxO2* genes. In addition, we showed that overproduction of RamA in *S. marcescens* reduces susceptibility to a tetracyclines, quinolones. The results suggest that RamA of *S. marcescens* participates in the regulation of LPS modifications and antibiotic resistance.

MI-P039
Lactobacillus SWITCHES THE PRODUCTION OF PSEUDO-COBALAMIN TO VITAMIN-B12 IN THE ABSENCE OF PURINES

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Lactobacillus coryniformis CRL 1001 and L. reuteri CRL 1098 have the complete gene machinery and synthesize pseudo-cobalamin as final product in a vitamin B12 free commercial medium. Unlike vitamin-B12 (the most biologically active form), the pseudo-cobalamin contains adenine instead of 5,6-dimethlbenzimidazole (DMB) in the Coα-ligand. Considering the vitamin B12-gene clusters of these bacteria, the aim of this work was to analyzed the production of corrinoids with DMB (vitamin B12) instead of adenine (pseudo-B12) as lower ligand base in a vitamin B12 free chemically defined medium (CDM) without purines. Genome-wide screening of genes related to purine metabolism showed that both strains possess all pur genes necessary for the synthesis of inositol monophosphate, the main precursor for purine biosynthesis. Accordingly, both strains were able to grow in B12 free CDM without purines, with the supplementation of different synthetic intermediaries. Isolated compounds with positive B12 activity were quantified and characterized by LC/MS-MS. Total corrinoids values were higher for both strains in comparison to those obtained in vitamin B12 free commercial medium. Interestingly, CRL 1001 strain synthesized cobalamin, suggesting that this strain is able to activate DMB as nitrogenous base instead adenine when it is in excess in a purine-free medium. This is the first demonstration of a metabolic shift to produce vitamin B12 in *Lactobacillus*.
*Both authors contributed equally

PLANT

PL-P01
TRANSCRIPTOME-BASED IDENTIFICATION OF *N. benthamiana*-BACTERIA INTERACTION RT-QPCR REFERENCE GENES

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RT-qPCR is a widely used technique for the analysis of gene expression. Accurate estimation of transcript abundance relies strongly on a normalization that requires the use of reference genes that are stably expressed in the conditions analyzed. Initially, they were adopted from those used in Northern blot experiments, but an increasing number of publications highlight the need to find and validate alternative reference genes for the particular system under study. The development of high-throughput sequencing techniques has facilitated the identification of such stably expressed genes. *Nicotianabenthamiana* has been extensively used as a model in the plant research field. In spite of this, there is scarce

information regarding suitable RT-qPCR reference genes for this species. Employing RNA-seq data previously generated from tomato plants, combined with newly generated data from *N. benthamiana* leaves infiltrated with Pseudomonas fluorescens, we identified and tested a set of 9 candidate reference genes. Using three different algorithms, we found that *NbUbe35*, *NbNQO* and *NbErpA*exhibit less variable gene expression in our pathosystem than previously used genes. Furthermore, the combined use of the first two is sufficient for robust gene expression analysis. We encourage employing these novel reference genes in future RT-qPCR experiments involving *N. benthamiana* and *Pseudomonas spp.*

PL-P02
PEACH PPZAT12 AND *Arabidopsis* ATZAT12 ORTHOLOG TRANSCRIPTION FACTORS HAVE CONSERVED MOLECULAR ROLES

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Cold storage (CS) is needed to prevent the rapid decay of peaches at ambient temperature. However, low temperature can generate chilling injury (CI), a group of physiological disorders which severely affects fruit quality. Many post-harvest approaches were developed to prevent CI, being heat treatment (HT) a proven efficient strategy. C2H2 transcription factor-encoding PpZAT12 was HT- and CS-induced in CI-tolerant cultivars, as A. thaliana AtZAT12. Both 35S::AtZAT12 and 35S::PpZAT12/KO-AtZAT12 *A. thaliana* transgenic lines showed impaired growth and development, rendering smaller plants (compared to Wild Type Col-0) with flowering time delay, curved leaves and down-pointing siliques. Known targets of AtZAT12 were assessed by RT-qPCR and a label-free proteomic approach was performed on adult leaves, finding many molecules commonly modified in gain-of-function genotypes. These results suggest that ZAT12 orthologs could have similar molecular mechanisms to *in vivo* regulate phytohormones metabolism, stress responses, RNA processing, retrograde signaling and photosynthesis. *In silico* analyses of putative targets not only pointed out their relationship with observed phenotypes but also with temperature stress protection. Moreover, their fruit orthologs present conserved temperature-driven expression. Overall, ZAT12 orthologs have similar genomic features, temperature sensitivity and molecular mechanisms to allow plant cells to tolerate undesired agronomic disorders, such as CI.

PL-P03
DEVELOPMENTAL CHANGES IN LEAF ANATOMY BRINGS NEW INSIGHTS IN GRASS PHOTOSYNTHESIS EVOLUTION

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Kranz syndrome is a specific suite of leaf functional properties in plants using C4 photosynthesis. These include the existence of discrete compartments: one more closely connected to the atmosphere, where the CO_2 is captured; and a larger one, where the Calvin cycle takes place. The current model of C4 photosynthesis evolution from a C3 ancestor proposes a series of gradual leaf anatomical changes followed by biochemical adaptation of the enzymatic machinery. This transition, which occurred several times in a wide range of angiosperms lineages, is particularly interesting in grasses. In this work, we performed qualitative and quantitative measures in leaf cross sections of close related C3, C4, and intermediates grass species to comparatively study anatomical changes that characterized each type of leaf. We also combine this information with leaf physiology measurements (gas exchange assays and chlorophyll fluorescence) to correctly interpret leaf anatomy. We determine that the grade of differentiation in photosynthetic structures increases towards the distal extreme of all type of leaves analyzed. Likewise, we observed qualitative and quantitative differences between species in vascular bundle density, chloroplast distribution and bundle-sheath size. Altogether, this analysis allows us to increased knowledge on the evolutionary path that lead to Kranz anatomy in grasses.

PL-P04
bHLH-TT8 TRANSCRIPTION FACTOR FROM *Sorghum* RESTORES BROWN SEED COAT IN *TT8 Arabidopsis* MUTANTS

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Sorghum [*Sorghum bicolor* (L) Moench.] is one of the most important cereals cultivated in the world. The pre-harvest sprouting, due to an inadequate establishment or maintenance of seed dormancy, is responsible for high yield losses. Flavonoids are secondary metabolites with wide biological functions described in plants. Different evidences suggest that their accumulation in the seed coat could be involved in the establishment and maintenance of dormancy. Thus, this research aims to elucidate the biological roles of the putative bHLH-SbTT8 transcription factor (TF) from sorghum and its contribution in the flavonoid biosynthesis. We generated *Arabidopsis* transgenic plants expressing this transcription factor under the control of CaMV35S in the background *tt8* (mutants in *transparent testa 8*, the orthologous gene of SbTT8, 35Spro:SbTT8). SbTT8 TF partially complemented the flavonoid deficiency of *tt8* mutants in seed coats. By vanillin assay, transgenic plants showed proanthocyanidins accumulation in immature stages of transgenic seeds. Moreover, when dormancy was studied by calculating the germination index and the mean germination time, 35Spro:SbTT8 plants exhibited an intermediate behavior between *tt8* mutants and the Wild Type Col-0 (higher levels than *tt8* mutants, but lower than WT plants). In addition, in root tips of *Arabidopsis* plants expressing SbTT8 fused to GFP, SbTT8 localized primarily in the nucleus, although faint fluorescence was also detected in the cytoplasm, consistent with its predicted role as a transcription factor.

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PL-P05
THE ROLE OF A NOVEL MADS BOX PROTEIN DURING FLOWER DEVELOPMENT

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Grasses differ from the angiosperm family because of their modified flowers. Grass flowers lack of the conventional sepals and petals, having instead two bract-like organs called lemma and palea. The homology of the lemma and palea is yet a theme of discussion. To begin to understand the identity of these novel organs we study the molecular evolution and perform the functional characterization of genes which codes for MIKC type MADS BOX transcription factors. These proteins are characterized by having a MADS BOX domain with binding-DNA function; an I domain that allows the selective formation of DNA-binding dimmers; a K domain that, due to its hydrophobic residues, is implicated in protein dimerization and a C-terminus domain less preserved among the different proteins, which is involved in transcriptional activation or the formation of transcription factor complexes. In this work we characterized the overexpression of MADS BOX candidate genes on the model species *Arabidopsis*. For that, genes were amplified and cloned in vector construction for gene overexpression. The Wild Type Col-0 plants were transformed. The phenotype was characterized exhaustively, analyzing the whole plant with special emphasis in the flower development.

PL-P06
MAYSIN IN LEAVES: A KEY ANTIFUNGAL AGENT ACCUMULATED AFTER TRICHODERMA-ACTIVATION OF ISR

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The genus *Trichoderma* includes a group of filamentous soil-borne fungi with positive effects on plants. The beneficial strains belonging to this genus act as antagonists of phytopathogenic organisms and stimulate plant immune system to reduce the later incidence of several plant pathogens. One of the most striking phenomena during *Trichoderma sp*. host root colonization is the priming of plant immunity in distant tissues. When plant defenses are primed, a faster and more effective response is triggered to a subsequent attack by pathogens reducing disease incidence and severity. However, the regulatory processes that mediate the priming of plant defense mediated by *Trichoderma sp*. is still unknown. Thus, the ability of four different strains of *Trichoderma (T. atroviride, T. reesei, T. harzianum* and *T. virens*) to stimulate secondary metabolism in distant leaves is evaluated. In this work, we study the role of secondary metabolites and their connection with plant defense induced by *Trichoderma spp*. Flavonoid metabolism was analyzed and maysin metabolism was found differentially regulated after priming of plant defenses in maize plants. Fungal growth inhibition and gene expression analysis confirmed the role of maysin as a key antimicrobial compound to control spore germination and pathogen infection in maize leaves

PL-P07
CONTRIBUTION OF RPL10 PROTEINS IN*Arabidopsis* ABA SIGNALING

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Ribosomal protein L10 (RPL10) is an integral component of eukaryotic ribosomes large subunit. The protein is best known for its role in protein translation, but also has extra-ribosomal functions in different organisms. *Arabidopsis thaliana* contains three genes coding for RPL10 (*RPL10A-B-C*). Previously, we demonstrated that these three genes are not functionally equivalent and are differentially involved in plant development and abiotic stress. Considering that abscisic acid (ABA) regulates plant growth, development and stress responses, we here investigated the participation of RPL10 proteins in ABA signaling pathways. We first analyzed seed germination and early development in simple *rpl10s Arabidopsis* mutant plants grown in the presence or absence of exogenous ABA. The results indicate that *rpl10A* mutant plants showed less sensitivity, whereas *rpl10B* and *rpl10C* mutants showed hypersensitivity to the hormone with respect to WT plants. Then, we evaluated the inhibition of primary root elongation by ABA by transferring 7-day-old seedlings germinated in the absence of ABA to medium supplemented with the hormone.While *rpl10A* mutant plants exhibited less inhibition, *rpl10B* and *rpl10C* mutants showed a more pronounced inhibition of root growth by ABA treatment than Wild Type Col-0 plants. A similar ABA sensitivity phenotype was observed in *rpl10* mutants with regard to petiole length and leaf yellowing. Surprisingly, the expression of the three *RPL10* genes was induced both during seed germination and after post-germinative growth by exogenous ABA treatment. Taken together, these data provide evidence that RPL10 proteins may play different roles during ABA signaling.

PL-P08
A NOVEL GENETIC FUNCTIONAL UNIT RELATED WITH STRESS-RESPONSE OLIGOSACCHARIDES

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The accumulation of compatible solutes is one of the physiological responses for abiotic stress adaptation of cyanobacteria. Those are low-molecular mass solutes that do not interfere with cell metabolism. In filamentous-heterocystic cyanobacteria, in addition to sucrose we have previously described the synthesis of sucroglucans (polymer series derived from sucrose) in response to salt stress and elucidated the structures.

In the present study we analyzed the phenotype of *Nostoc* mutants in an *orf* with high similarity with the sucrose synthase gene, and the occurrence of sucrose and sucroglucans in cells subjected to abiotic stresses (e.g. desiccation, high temperature, and salt). Less stress tolerance was shown in cells of mutants lacking the protein product of that *orf*, which paralleled the absence of oligosaccharide accumulation. Surprisingly, our results revealed a polar effect in one of the mutants that could involve an adjacent gene in the synthesis of the oligosaccharides. RT-PCR analyses revealed that both genes increased their expression under stress treatments. Subsequently, we investigated the presence of this gene arrangement (putative operon) in different cyanobacterial genomes, concluding that it was restricted to filamentous strains belonging to Nostocales and Stigonematales. The two *orf*s were expressed in *Escherichia coli* for functional characterization, as part of the elucidation of the metabolic pathway leading to the oligosaccharide biosynthesis in *Nostoc sp*. Supported by UNMdP EXA 841/17, CIC and FIBA

PL-P09
TRANSCRIPTION FACTORS PHL1 AND ATHB23 INTERACT IN *Arabidopsis* MODULATING FRUIT DEHISCENCE AND YIELD

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Plants are continuously subjected to stress situations and they have evolved molecular and physiological responses allowing them to deal with such conditions. Molecular responses mainly occur at the transcriptional level mediated by transcription factors (TFs). TFs are able to interact with other proteins generating a more complex gene expression regulatory scenario. Plant TFs are classified in different families and among them the MYB-CC and the HD-Zip I ones have been associated to development in response to environmental or nutritional changes. AtHB23 belongs to the HD-Zip I family and PHL1 to the MYB-CC one. A yeast two hybrid assay indicated that these TFs interact in this system. We were able to validate such interaction by Bimolecular Fluorescent Complementation assay (BiFC) in plants. The expression pattern of both encoding genes was determined analyzing transgenic *Arabidopsis* plants carrying prPHL1:GUS and prAtHB23:GUS constructs. GUS expression was evident for both genotypes in two tissues: pedicel-silique node and silique funiculus. Phl1 plants opened earlier the siliques and showed increased yield, but no differences were detected in the number of seeds per silique neither in the number of total siliques when plants were grown on soil in normal conditions. Notably, *athb23* silenced plants also exhibited higher yield than controls but not more opened siliques than Wild Type Col-0 at the same developmental stage. Increased yield in silenced and mutant plants was particularly detected in plants grown on sand watered with Hoagland solution. Altogether, the results suggest a joint and cooperative function of PHL1 and AtHB23 related to fruit dehiscence and seeds abscission, two important agronomic traits

PL-P10
THE *Arabidopsis* TRANSCRIPTION FACTOR ATHB40 NEGATIVELY REGULATES ROOT ELONGATION

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AtHB40 is an *Arabidopsi*s transcription factor (TF) belonging to the HD-Zip I (homeodomain-leucine zipper I) family. These TFs have been associated to plant development in response to abiotic stress factors. In particular, AtHB40 and its paralogs, AtHB21 and AtHB53, were reported as essential for the transcription of *NCED3* in axillary buds. NCED3 encodes a key enzyme in abscisic acid biosynthesis. Aiming at knowing the role of AtHB40, we applied different experimental strategies including the study of the expression pattern using transgenic plants carrying the promoters of *AtHB40* (Pr40), *AtHB21* (Pr21) and *AtHB53* (Pr53) fused to the reporter GUS gene. Moreover, we characterized three insertional mutants and an estradiol induced AtHB40 overexpressor line. Pr40 seedlings exhibited GUS expression in the tips of the main and lateral roots, mainly in the quiescent center and columella cells, and in root vascular system ABA induced *AtHB40* expression in the root tip. Pr53 seedlings showed expression in lateral roots whereas no GUS was detected in roots of Pr21 plants. Moreover, *athb40* mutant lines displayed longer main roots whereas overexpressor line presented shorter ones both compared with Wild Type Col-0 plants. A deeper exploration of *athb40* mutant roots by confocal microscopy indicated an increase of the number of cells in the transition zone in the tip of the main root. This latter differential phenotype disappears with fluoridone treatment, a repressor of ABA biosynthesis. Altogether, these results suggest that AtHB40 has a functional role as a repressor of main root elongation and that this role is not redundant with that of its paralog genes AtHB53 and AtHB21

PL-P11
CHARACTERIZATION OF ENZYMES INVOLVED IN RAFFINOSE BIOSYNTHESIS IN *Brachypodiumdistachyon*

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Raffinose (Raf, an α-1,6-galactosyl extension of sucrose) is important for membrane stabilization during seed desiccation. Raf also plays a key role as osmoprotectant in leaves of plants exposed to heat, cold, salinity and drought. To better understand the molecular mechanisms regulating Raf synthesis in plants, we cloned the genes coding for UDP-sugar pyrophosphorylase, galactinol synthase, and Raf synthase from *Brachypodiumdistachyon*, a model grass closely related to rice and wheat. All proteins were successfully expressed in *Escherichia coli* and purified to electrophoretic homogeneity by IMAC and gel filtration chromatography. UDP-sugar pyrophosphorylase (which synthesizes UDP-galactose) displayed a Vmax of 1000 U mg-1 and S0.5 values of 0.25 and 0.06 mM for galactose-1P and UTP, respectively. Galactinol synthase (which produces galactinol) showed a Vmax of 8 U mg-1 and S0.5 values of 0.09 and 1.0 mM for UDP-galactose and myo-inositol, respectively. These results are in good agreement with those previously reported for enzymes from dicotyledonous species. To the best of our knowledge, this

is the first report showing kinetic parameters for UDP-sugar pyrophosphorylase and galactinol synthase from a grass species. We consider this information will be valuable to better understand the reaction mechanisms and regulatory properties of these enzymes, a key step to obtain genetically modified organisms with improved abiotic stress resistance.

PL-P12
BIOLOGICAL CONTROL FOR THE MANAGEMENT OF SUDDEN DEATH SYNDROME OF SOYBEAN

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Sudden death syndrome (SDS) is one of the most yield-limiting diseases of soybean. The disease is caused by at least four distinct Fusarium species, with *Fusarium tucumaniae* being the main causal agent in Argentina. Currently, there is no effective treatment for SDS. Therefore, the use of biological control agents could be an effective strategy for disease management. Three bacteria previously isolated from the soybean rhizosphere, *Pseudomonas fluorescens* BNM 297 and BNM 296 *and Bacillus amyloliquefaciens* BNM 340, were examined for their *in vitro* antagonism tests against F. tucumaniae and for their capability to increase plant growth. Results indicated that BNM 297 inhibited mycelial growth of the pathogen by 40% and the other two strains by 35%. Plant growth promotion was evaluated by germination efficiency and vigor of healthy soybean seeds inoculated with the bacteria. These studies revealed that BNM 297 and BNM 296 showed a modest inhibitory activity (< 30% inhibition) while BNM 340 did not affect germination and vigor compared to control plants. These results suggest that *B. amyloliquefaciens* BNM 340 could be a potential biocontrol agent against *F. tucumaniae*, although this hypothesis has to be tested *in vivo*. Thus, phenotypic parameters such as plant weight, leaf pigment contents and root isoflavone accumulation will be scored. We have observed that plant weight and photosynthetic pigment contents were reduced by 25% and 30%, respectively, while isoflavones accumulated in soybean seedlings infected with *F. tucumaniae* by 10 days post-inoculation compared to the uninoculated cultivar.

PL-P13
METABOLIC ADAPTATION OF *Chlorella* spp.: A LOOK CENTERED IN THE METABOLISM OF Ga3P

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Chlorella is a unicellular green alga with a great potential for application in biodiesel production, nutritional food for animals (including humans) and cosmetics treatment. One special characteristic of *Chlorella* is its ability to growth autotrophically through photosynthesis and also heterotrophically using organic carbon sources. When the organism switches from autotrophism to heterotrophism, the chloroplast is reduced and lipid bodies accumulating oil increase. Changes in the culture conditions are directly linked to cellular metabolic changes; however how carbon partition and metabolic pathways are modified is not completely understood. With focus on glyceraldehyde-3-phosphate (Ga3P), a key glycolytic precursor being partitioned inside the cell, we studied in this work the cytosolic (NAD+ dependent) Ga3P dehydrogenase (Ga3PDHase; EC 1.2.1.12) that catalyzes the reversible oxidation of Ga3P to 1,3 bisphosphoglycerate. The dynamics of carbon partition and Ga3PDHase activity was analyzed in different *Chlorella* species (Chlorella sp., *C protothecoides* and *C. saccharophila*) growing under variable trophic conditions. In these cells, oil and starch contents varied and Ga3PDHase protein and activity changed. Also, we expressed in a heterologous system two genes coding for Ga3PDHase in *Chlorella variabilis* and characterized the kinetic properties of the purified recombinant enzymes. Results suggest that metabolism involving Ga3PDHase is of great plasticity in *Chlorella* and Ga3P oxidation is highly increased under heterotrophic growing conditions that promote high oil accumulation.

PL-P14
COMPARATIVE PROTEOMICS OF *Prunuspersica* LEAVES EXHIBITING LEAF PEACH CURL DISEASE.

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Prunuspersica (L.) Batsch is a species of great economic importance. Several conditions affects its production; among them, the biotrophic fungus *Taphrina deformans* (Berk.) Tul. causes the leaf peach curl disease. Symptoms include chlorosis, pink pigmentation and leaf curling. In order to study the effect of the infection in leaves proteome, symptomatic leaves exhibiting the disease (50% of area thickening and curving) were collected from naturally infected trees. The leaves were processed by separating the symptomatic area (C) and the asymptomatic (N) region. Asymptomatic healthy leaves (G0) were also collected. Quantitative proteomics were performed using total extracted proteins and liquid chromatography/mass spectrometry. Differential proteins were identified using the Perseus software platform. A total of 1725 proteins were identified in leaves. Comparison of N with G0 identified only ninety proteins, while C against G0 or N, rendered 664 and 232 proteins, respectively. Data obtained revealed a repression of the protein synthesis machinery, a repression of the primary metabolism and photosynthesis, plus a turnover and reorganization of the cell wall, alterations in the secondary metabolism and an increase in the content of pathogen response proteins in G7C in comparison with G0. Complementary biochemical analysis including quantification of total protein content, Western blot of Rubisco and proteins involved in primary metabolism, and chlorophyll, anthocyanins and flavonoids quantitation, were conducted in order to validate the results obtained.

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PL-P15
FRATAXIN AS THE SOURCE OF FERROCHELATASE ACTIVITY IN PLANT MITOCHONDRIA

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Frataxin plays a key role in cellular iron homeostasis of different organisms. It is engaged in several activities at the Fe-S cluster assembly machinery and it is also involved in heme biosynthesis. In plants, two genes encoding ferrochelatases (FC1 and FC2) catalyze the incorporation of iron into protoporphyrin IX in the last stage of heme synthesis in chloroplasts. Despite ferrochelatases are absent from other cell compartments, ferrochelatese activity has been observed in plant mitochondria. Here we analyze the possibility that frataxin acts as the iron donor to protoporphyrin IX for the synthesis of heme groups in plant mitochondria as an alternative to the regular chloroplastic pathway. Our findings show that frataxin catalyzes the formation of heme *in vitro* when it is incubated with iron and protoporphyrin IX. When frataxin is combined with AtNFS1 and AtISD11 the ferrochelatse activity is increased suggesting that a protein complex is formed that regulates the activity of both AtNFS1 and frataxin. These results suggest that frataxin could be the iron donor in the final step of heme synthesis in plant mitochondria, and constitutes an important advance in the elucidation of the mechanisms of heme synthesis in plants.

PL-P16
ANALYSIS OF TREHALOSE 6-PHOSPHATE METABOLISM IN C4 PLANTS

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Trehalose 6-phosphate (Tre6P) is a signal metabolite that coordinates carbon metabolism with plant growth and development. Our current understanding of Tre6P metabolism and signaling in plants is based almost entirely on studies of *Arabidopsis*, a C3 eudicot species. The metabolism and growth of grasses that perform C4 photosynthesis (such as maize, sorghum and sugarcane) are very different from *Arabidopsis*. The aim of this project is to understand the regulation of carbon metabolism by Tre6P in the grass *Setariaviridis*, which has been widely adopted as a model for the major C4 crop species. By means of phylogenetic analysis we identified 21 genes encoding Tre6P-related enzymes: 10 Tre6P synthases (TPS), 10 Tre6P phosphatases (TPP) and 1 trehalase. Using publicly available RNAseq data for *S. viridis* we found that TPS1 (the isoform responsible for Tre6P synthesis) and two TPP isoforms are preferentially expressed in bundle sheath cells. To investigate the distribution of Tre6P in *S. viridis* leaves, we enriched mesophyll and bundle sheath cells by sequential sieving of finely ground leaf tissue (suspended in liquid nitrogen) through a series of nylon meshes with different pore sizes. Interestingly, we observed an asymmetric distribution of Tre6P (71-84% in bundle sheath cells). These results strongly suggest that Tre6P metabolism mainly occurs in bundle sheath cells. We envisage that knowledge acquired from study of *S. viridis* will be useful to better understand photoassimilate partitioning and source-sink relations in the major C4 crop species.

PL-P17
DELLA PROTEINS DO NOT HAVE A KEY REGULATORY ROLE IN FOLIAR PROANTHOCYANIN ACCUMULATION IN *Lotus* spp.

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The MYB-bHLH-WD40 (MBW) complex is considered the master regulator of anthocyanin and proanthocyanin (PA) biosynthesis in different plant species. In *A. thaliana*, DELLA proteins promote transcriptional activity of the MBW complex by sequestering MYBL2, resulting in increased anthocyanin levels. However, the role of DELLAs in transcriptional regulation of PA biosynthesis has not been evaluated yet. With this objective, we evaluated the effect of paclobutrazol (PBZ) and gibberellic acid (GA3) treatments on foliar PA accumulation in *Lotus corniculatus* (a species with high levels of PA) and *L. tenuis* (low levels of PA). Also, we evaluated the expression of DELLA orthologues in both species, and tested protein-protein interaction by Y2H assays between DELLAs and members of the MBW complex that preferentially regulate PA synthesis. Our results showed that PBZ and GA3 treatments affected the growth of *Lotus spp*.; however, foliar PA levels were not modified in any treatment x genotype (p-value>0.05). Curiously, anthocyanin accumulation increased in leaves of both genotypes treated with PBZ, despite the absence of a MYBL2 orthologue in *Lotus*, suggesting that DELLAs regulate anthocyanin synthesis by an alternative mechanism in this species. On the other hand, none of the two *Lotus* DELLAs (orthologous to CRY and LA from *Pisum sativum*) interacted with the *Lotus* TT8, TT2b, TT2d, MYBPA2 or MYB5 in Y2H assays. Our results strongly suggest that DELLA proteins do not have a key role in PA biosynthesis regulation in *Lotus spp*.

PL-P18
FURTHER STUDIES ON THE ROLE OF PLANT ADP-GLUCOSE PYROPHOSPHORYLASE SUBUNITS

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Starch is a highly important source of nutritional energy for humans and numerous animals. ADP-glucose pyrophosphorylase (ADPGlcPPase, EC 2.7.7.27) is the key enzyme in starch biosynthesis, producing the glucosyl-donor (ADPGlc) to elongate the glucan α-1,4-glucosidic chains. Plant ADPGlcPPases are heterotetramers comprising small (S) and large (L) subunits with unequal catalytic and regulatory roles. These are allosteric enzymes regulated by 3-phosphoglycerate (3PGA, activator) and inorganic orthophosphate (Pi, inhibitor). Amongst them, the

ADPGlcPPase from wheat endosperm has unique regulatory properties compared to others, since it is modulated by the ratio activator/inhibitor: 3PGA activation occurs after partial inhibition by Pi. We expressed the wheat endosperm ADPGlcPPase S and L subunit genes in Escherichia coli and also, we co-expressed them with well characterized subunits from the potato tuber ADPGlcPPase to obtain S/L hybrids. To further understand their distinctive roles, we characterized the recombinant proteins and analyzed its sensitiveness towards classic effectors (3PGA and Pi) as well as fructose-6P, phosphoenolpyruvate and fructose 1,6-bisphosphate. After kinetic analysis with one or a combination of two effectors, results show that ADPGlcPPases with the wheat endosperm L subunit (independently of the partner S subunit) are insensitive to assayed metabolites; whereas those versions harboring potato L subunits were highly activated. This approach offers new molecular tools to understand the regulatory mechanisms governing plant ADPGlcPPases activity and starch synthesis

PL-P19
DYNAMICS OF THE BRASSINOSTEROID TRANSCRIPTION FACTOR BES1 IN PLANT RESPONSES TO TEMPERATURE CUES

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Plant growth is coordinately regulated by environmental and hormonal signals, including a preeminent growth of brassinosteroids (BR). In this work we studied temperature effects on the dynamics of the BES1 transcription factor a key component of the signaling pathway downstream BR perception. In *Arabidopsis thaliana* seedlings, the promotion of hypocotyl growth induced by warm temperature (29ºC) compared to control conditions (20ºC) is enhanced in the *bes1-1D* gain-of-function mutant, compared to the wild type. The de-phosphorylated form of BES1 is considered to be active in the control of gene expression. We used plants bearing the *pBES1:BES-GFP* transgene and measured nuclear fluorescence levels by confocal microscopy. The plants exposed to high temperature showed increased nuclear protein in the hypocotyl and decrease levels in the cotyledons. We also measured *BES1* gene expression, by qRT-PCR, independently in the hypocotyl and in the cotyledons. We observed a deduction in expression levels in response to warm temperature, particularly in the cotyledons. We conclude that a differential balance of the transcriptional and posttranscriptional control of BES1 by temperature leads to opposite responses in the hypocotyl and cotyledons, which in turn correlate with the growth response.

PL-P20
INFLORESCENCE MERISTEM REGULATION IN *Brachypodiumdistachyon*

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In higher plants, a phase transition from vegetative to reproductive development is tightly coordinated through networks that integrate internal and external stimuli. PANICLE PHYTOMER2 (PAP2) is a SEPALLATA-like protein that plays an important role in regulating rice meristems and floral organ identity. PAP2 promotes the phase changes of the shoot apical meristem from vegetative to inflorescence meristem, acting together with AP1-like proteins to negatively regulate *RCN4*, a *TFL1* orthologue, in a conserved pathway between *Arabidopsis thaliana* and rice. Here we show functional analyses of BdPAP2, homologue to PAP2 in *Brachypodiumdistachyon*. *A. thaliana* plants expressing the 35SCaMV::BdPAP2 flowered earlier than control plants, which correlates with the higher expression levels of floral integrator genes *SOC1* y *FT* found in the over-expressing plants. Accordingly, *TFL1* expression levels were significantly reduced in these plants, suggesting that BdPAP2 function in regulating inflorescence meristem identity is highly conserved. Even more, considering that *SEP4* over-expression produce plants with a terminal flower similar to those of *tfl1*mutants, we can speculate that the negative regulation of *TFL1-like* genes is an ancestral function of the SEP proteins conserved among angiosperms.

PL-P21
THE CONTROL OF MERISTEM FATE BY GRASS C2H2-TYPE TRANSCRIPTION FACTORS

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The grass family includes species among which are outstanding cereals that constitute a very important part of human intake. The final form of the plant depends on the number, localization and fate of meristems. In grasses, the Cys2-His2-type (C2H2) zinc finger transcription factors seem to be important in controlling the fate of meristems. The aim of this work is to investigate the function of some of the grass C2H2-type transcription factors during early stages of plant development. To begin the characterization, we investigated the expression preference of the coding gene throughout the root, stem, leaf and inflorescence of the *Setariaviridis* (Sv) plant. In particular, *SvC2H2* is preferentially expressed in inflorescence with developing branches. The expression decays at late stages of inflorescence development. In order to generate knowledge about its function, plants of A. thaliana that overexpressed this gene were characterized. The phenotype suggests that *SvC2H2* is involved in meristems development regulation, hormones biosynthesis and signaling pathway.

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PL-P22
COPPER STRESS INDUCES HORMONAL CHANGES AND PROTEIN MODIFICATIONS IN THE ROOTS OF MAIZE SEEDLINGS

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The excess of Cu interferes with numerous biological functions that can affect plant growth. In the current study we present the impact of Cu stress on root of *Zea mays* L. (maize) seedlings regarding growth and hormone homeostasis. Maize seeds were germinated on filter paper in plastic dishes containing distillate water. Seedlings of a comparable size with roots of 1-2 cm length were selected and transferred to hydroponic culture containing diluted (1/10) Hoagland solution without (control, C) or with 50 or 100 µM of $CuCl_2$. Plants were grown in a controlled climate room at temperature of 24 ± 2 °C in darkness and, after 72 h of treatment, determinations were performed. The root apex (5 mm) segment and the rest of the root tissue were processed separately. The decrease in root length and biomass by Cu were accompanied by drastic reductions of absicic acid, indoleacetic acid, jasmonic acid (JA), isoleucine conjugate of JA, and giberellins GA4 and GA3 levels in the root apex. This was accompanied by an increase in protein carbonylation, ubiquitylation and SUMOylation in both parts of the root. Our results show that root hormonal imbalance produced by Cu exposure is -at least in part- responsible of the root growth inhibition. Also, severe protein post-translational modifications upon Cu-exposure occurred that took place in the entire root and are probably important to trigger and/or to sustain defence mechanisms against heavy metal toxicity.

PL-P23
IDENTIFICATION AND CHARACTERIZATION OF AGO, DCL AND RDR PROTEIN FAMILIES IN SWEET ORANGE

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Sweet orange (*Citrus sinensis*) is one of the most important species for fruit consumption cultivated worldwide. Its nutritional attributes for human health are well known since it is an excellent source of easy access vitamin C, besides the immense economic importance of this species. Nutritional quality, sensory appeal, defense against pathogens and many other physiological processes are regulated by small RNAs. The biogenesis and action of these regulatory molecules have been characterized in several species, being ARGONAUTE (AGO), DICER-LIKE (DCL) and RNA-DEPENDENT RNA POLYMERASE (RDR) the three main protein families involved these processes. Several studies have shown variation in the number and tissue specificity in the expression of the members of these families across species. We performed a genome-wide analysis using genomic data in order to characterize these important protein families in *Citrus sinensis*. Using a phylogenetic approach, we identified a total of 10 AGO, 5 DCL and 7 RDR proteins. Analyzing transcriptomic data from 5 different tissues, we obtained the expression profiles of these newly characterized proteins. A detailed analysis of these protein families in *Citrus sinensis* is presented in this work.

PL-P24
THE *Arabidopsis* TRANSCRIPTION FACTOR ATçtHB22 IS A POSITIVE REGULATOR OF SEED GERMINATION

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Transcription factors (TFs) play key roles in the regulation of plant growth and development. AtHB22 is a TF belonging to the homeodomain-leucine zipper I family which members were associated to developmental events in response to environmental factors. AtHB22 has not been resolved in any of the six phylogenetic clades in which members of this subfamily have been classified but has a paralog gene, AtHB51. Like AtHB21, AtHB40, AtHB53, and AtHB51it has only one intron downstream the codon for the fourth leucine in the LZ domain. There are not reports about the function of this TF in the literature but RNA-Seq databases indicate that it is expressed in root endodermis, procambium and hairs and is repressed in rosette leaves after different abiotic stress factors.Aiming at characterizing this TF we obtained insertional mutant plants. Such plants exhibited significant shorter roots 2, 4 and 8 days after sawing on Petri dishes. Stating the hypothesis that this differential phenotype could be caused by a delay in germination, seed germination was assessed indicating a significant delay in *athb22* mutant plants similar to that showed by abi4 mutants. Since the phytohormone ABA has a key role in germination rate, mutant and WT seeds were sowed in plates containing 0.25, 0.50 and 1.0 µM ABA during 12 days. Germination rate was clearly slower for *athb22* lines compared with WT and abi4 mutants, which exhibited an intermediate behavior and the effect was stronger at higher ABA concentrations. At the day 12 all the genotypes reached 100% germinated seeds.Altogether, these results suggest that AtHB22 is a positive regulator of germination lowering ABA sensitiveness.

PL-P25
LIGHT REGULATES PLANT ALTERNATIVE SPLICING THROUGH THE CONTROL OF TRANSCRIPTIONAL ELONGATION

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Light makes carbon fixation possible allowing plant and animal life on Earth. We have previously shown that light regulates alternative splicing in plants. Light initiates a chloroplast retrograde signaling that regulates nuclear alternative splicing of a subset of *Arabidopsis thaliana*

transcripts. Here we show that light promotes RNAPII elongation in the affected genes, while in darkness elongation is lower. These changes in transcriptional elongation are causative of the observed changes in alternative splicing, as revealed by different drug treatments and genetic evidence. The light control of splicing and elongation is abolished in an *Arabidopsis* mutant defective in the elongation factor TFIIS. We report that the chloroplast control of nuclear alternative splicing in plants responds to the kinetic coupling mechanism found in mammalian cells, providing unique evidence that coupling is important for a whole organism to respond to environmental cues.

PL-P26
IQD30 FROM *Arabidopsis thaliana* ACTS AS A LINK AMONG DIFFERENT CELLULAR PROCESSES

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Plant-specific IQ67 domain (IQD) members are part of an emerging family of calmodulin binding proteins. There are 33 IQD members distributed in four different classes in Arabidopsis thaliana. In this study, we made progresses in the characterization of one class IV protein of Arabidopsis, IQD30. Previously, in order to identify putative ligands of IQD30 protein, we carried out pull down assays followed by mass spectrometry. Using this methodology, it was possible to detect AXR4, a protein that controls the polar localization of auxin importers, AUX1. To assess whether IQD30 gene product is involved in auxin signaling, we studied the transcriptional profile of IQD30 in Arabidopsis treated roots with indol acetic acid (IAA). We also measured the growth of primary roots of a knockout IQD30 mutant and wild type lines under exogenous IAA treatments. qRT-PCR experiments showed that IQD30 transcript levels are significantly repressed in early post-treatment stages. On the other hand, phenotypic analysis revealed that under high concentrations of IAA, primary roots of iqd30 line exhibit lower growth than Col-0 line. These observations suggest that the mutant line has greater sensitivity to auxin than the wild genotype indicating that IQD30 could be involved in the signaling of this plant hormone. At the same time, the role of IQD30 gene product in plant development was evaluated. We observed that iqd30 seeds show lower viability and germination speed than the wild type seeds after being exposed to normal light conditions. These results indicate that IQD30 is not an effector molecule per se but it would act as a link among different cellular processes.

PL-P27
AtHB5 IS INVOLVED IN SOURCE-TO-SINK TRANSPORT OF SUGAR IN *Arabidopsis thaliana*

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AtHB5 is a HD-Zip I transcription factor (TF) from *Arabidopsis thaliana*. It was previously reported that a group of HD-Zip I TFs modified its expression during lignification process in Arabidopsis stems. Interestingly, while *AtHB7* and *AtHB12* increased its expression during stem maturation, *AtHB5* decreased it and it was the only HD-Zip I that exhibited this behavior. To investigate its biological function, mutants and overexpressing lines (OE) were obtained. In *athb5*, *AtHB7*, *AtHB12*, *AtHB6* and *AtHB16*were down-regulated, while in OE they were up-regulated. A phenotypic analysis showed that *athb5* had wider and more lignified stems, while OE had narrow and less lignified stems than WT. Furthermore, athb5 stems exhibit more phloem and xylem vessels than WT as well as in vascular tissue from leaves and pedicel. Also OE had more glucose and sucrose on rosette leaves than WT but less on cauline leaves. Moreover, aniline blue staining showed that OE had more callose in rosette leaves than WT, indicating a defective sugar transport. Regarding to root system, under normal conditions athb5 had larger roots while OE had shorter roots than WT. Furthermore, under complete dark conditions there were no differences between genotypes, but when they were supplemented with sucrose, athb5 exhibited larger roots than WT and OE shorter.
Arabidopsis plants were transformed with *AtHB5* promoter fused to *GUS* reporter, and preliminary results indicated expression in hypocotyl andvascular tissue.Altogether these results suggest that *AtHB5* is a negative regulator of some HD–Zip TFs and it is implicated in source-to-sink transport of sugar in Arabidopsis.

PL-P28
THE 5´-3´ EXORIBONUCLEASE XRN4 REGULATES CIRCADIAN RHYTHMS IN *Arabidopsis thaliana*.

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Circadian rhythms allow organisms to adjust biological processes to occur at the most appropriate time of day. They are controlled by a complex gene network —called the central oscillator—, in which genes interact mainly by transcriptional feedback loops, keeping an approximate 24h rhythm.Circadian rhythms are subject to many layers of regulation, such as transcriptional, epigenetic, post- transcriptional, and post-translational.Little is known about the mechanisms that act in the post-transcriptional layer. Among the different post-transcriptional mechanisms, in this work we focused on RNA stability and decay using the plant model organism *Arabidopsis thaliana*. We studied XRN4, the only 5´-3´ cytoplasmic exoribonuclease described in Arabidopsis.We characterized different outputs of the clock in the loss-of-function *xrn4-3* mutant. *xrn4* showed a longer circadian period of leaf movement, a late flowering time phenotype, and no appreciable change in hypocotyl elongation. We also observed a deregulation of mRNA levels from some core oscillator genes. Finally, we studied the decay rates of the core oscillator genes by inhibiting transcription using cordycepin.This is the first exoribonuclease reported to be involved in circadian rhythm regulation. RNA decay stands out as a key component in the control of circadian rhythms.

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PL-P29
THE FAMILY OF SUBTILISIN-LIKE SERINE PROTEASES IN BARLEY AND *Brachypodiumdistachyon*

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Proteases play a crucial role in nitrogen remobilization during plant senescence. Free amino acids produced by protein degradation in senescent tissues are translocated to new developing leaves and reproductive organs allowing an efficient nutrient recycling. Among other protease families, subtilisins (family S8A) have been strongly associated to leaf senescence in wheat but are still scarcely studied in other plant species. The aim of this work was to identify the members of subtilisin family in barley and the model *plant Brachypodiumdistachyon*, and perform the phylogenetic analysis in both species. By using conserved sequences of wheat subtilisins, we were able to identified 40 complete genes in barley and 60 in *B. distachyon* databases. Phylogenetic trees were constructed and the identification of conserved protein domains was performed. Although all sequences contain the typical domain PA (PA_subtilisin_like), a few of them lack domain I9 thought to act both as internal chaperon and protease inhibitor in the zymogen. Previous studies allowed us to identified sequence AK365933 as one of the highest expressed subtilisin gene in senescent barley leaves. Here, cloning of the gene and expression of the recombinant protease in Pichia pastoris was intended. Two constructions were tested for cloning, one complete and one without domain I9. Primers were designed and both sequences were amplified by PCR, ligated to pGEMT-easy cloning vector and used for E. coli transformation for sequence amplification prior to subcloning in P. pastoris expression vector pPIC9. Construction containing I9 domain did not gave positive clones in E. coli, possibly due to lethality of basal expression of active subtilisin. Construction without domain I9 was subcloned in P. pastoris expression vector and used for transformation of GS115 yeast strain. Preliminary analysis of transformants showed production of the recombinant protein but no protease activity was observed. Currently, work is in progress in order to clone the complete version containing the I9 activation domain in P. pastoris for the subsequent characterization of the recombinant protein.

PL-P30
GETTING OUT OF THE SHADE: CHARACTERIZATION OF NOVEL BHLH/HLH THAT MODULATE SHADE AVOIDANCE RESPONSES

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Plants grown at high crop densities receive reduced light irradiance as a result of mutual shading. Some plants have developed the ability to avoid shade by readjusting phenotypical traits such as growth rates of the stems, internodes and petioles, among others. Taken together, these responses are known as Shade Avoidance Syndrome (SAS). Whereas these responses may be useful in nature to improve photosynthesis, in agronomical practices they are detrimental because resources that should be used for the filling of the harvested organs are diverted to sustain elongation. At the molecular level, many of these responses are mediated by a family of typical and atypical basic helix loop helix transcription factors, bHLHs and HLH respectively. These transcription factors orchestrate a complex signaling network were some bHLH/HLHs act as activators of the SAS while others act as a brake of the responses. Using a high throughput transcriptome analysis of shaded *Glycine max*(Soybean) plants, we found a novel bHLH/HLH that could modulate SAS responses in this legume of agronomic interest. Using a reverse genetics approach, we set to study the role of the orthologs of this gene in *Arabidopsis thaliana* in the regulation of the shade avoidance response. Our findings suggest that only subsets of these orthologues are involved in the shade responses. This work contributes to the characterization of novel bHLH/HLHs that participate in the intricate signaling network of shade avoidance responses.

PL-P31
STRUCTURE AND ACTIVITY OF N-TERMINAL TRUNCATED VERSION OF *Arabidopsis* PROLINE DEHYDROGENASE ISOFORMS

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Proline dehydrogenase (ProDH) is a mitochondrial enzyme that catalyses the oxidation of proline (Pro) to Δ1-pyrroline-5-carboxylate (P5C). Arabidopsis contains two active ProDH isoforms AtProDH1 and AtProDH2.Modelling of *A. thaliana* ProDH isoforms over the structure of *E. coli* ProDH showed that these proteins adopt the shape of a distorted $(\beta\alpha)_{13}$ TIM-barrel fold. The 3D structure begins with 3 α-helixes located on top of the barrel simulating a "cap". Below this cap is buried the last α-helix of the protein carrying conserved active site aminoacids contacting Pro and the cofactor FAD. The N-terminal primary sequence of ProDHs from all kindoms of life is poorly conserved. To investigate the functional impact of this "cap" in catalysis and oligomerization of ProDH, we constructed deletion variants of AtProDHs. Both protein isoforms were expressed as recombinants in *E. coli*, using MBP as affinity purification tag. Proteins were tested for their activity *in vitro*, revealing that the "cap" is essential for ProDH activity. Oligomerization status was addressed by non-denaturing gel electrophoresis. Results of these assays will be discussed.

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PL-P32
IT´S A MATTER OF TIME: CIRCADIAN CLOCK INFLUENCE OVER DEFENSE RESPONSES IN *Arabidopsis thaliana*

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In an ever-changing world, possessing the capability to adapt and even predict daily and seasonal environmental changes presents as a valuable feature for living organisms. Subsequently, in most organisms multiple physiological and developmental processes are driven by an internal time keeping mechanism known as circadian clock, which accurately tuning contributes to an enhanced fitness. Given that plants do not have any specialized immune cells, each individual cell must regulate and balance the highly energy consuming stress responses with other cellular functions, such as growth. To that end, the circadian clock seems to temporally regulate immune responses leading to different degrees of susceptibility throughout the day in a process referred as circadian gating. Even though there is now a general consensus that stablishes biotic stress responses as circadian outputs, little is yet known about the actors and mechanism involved in this process. In order to shed some light regarding this intricate interplay, we set to explore the circadian function in previously known Arabidopsis thaliana immune response mutants. By this means, we identified an enhanced disease susceptibility mutant which displays altered clock outputs. In the current job we present a molecular and physiological characterization of this mutant, showing that a miss regulation of core clock genes is partially responsible for the impaired phenotype. Thus, these results comprehend a novel example of the relevance regarding correct circadian function in biotic stress responses.

STRUCTURAL BIOLOGY

SB-P01
STRUCTURAL AND FUNCTIONAL ANALYSIS OF FasR, A TRANSCRIPTIONAL REGULATOR OF LIPID BIOSYNTHESIS IN MTB

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The complex lipids present in the cell envelope of *Mycobacterium tuberculosis* (Mtb) constitute a unique structure critical to the virulence of this pathogen. The environmental signals and regulatory network involved in the global regulation of lipid metabolism in this bacterium is largely unknown; although examples of transcriptional and post-translational mechanism of regulation have recently been described. One of the key transcriptional regulators is FasR, a protein that binds to the fas-acpS promoter region and controls the *de novo* fatty acid biosynthesis becoming a central component involved in maintaining lipid homeostasis in mycobacteria. In this work, we present the X-ray structures of MtbFasR and of FasRin complex with C20-acyl-CoA, which corresponds to the pool of long-chain acyl-CoAs, the effector molecules that coordinate the expression of the two fatty acid synthase (FAS) systems present in mycobacteria. The effector molecule was found seated within a hydrophobic tunnel open at both ends and that extends all along the core of the ellipsoidal C-terminal effector-binding domain of FasR. Electrophoretic mobility shift assays with FasR mutants generated to prevent the binding of the FasR-ligand or to uncouple the transmission of the signal from the effector to the HTH domain of the regulator, gave further insights into the structural bases involved in the FasR-ligand recognition and FasR-DNA interaction. The structural characterization of this novel transcriptional regulator provides new insights into the regulatory mechanisms that maintain lipid homeostasis in Mtb and open the opportunity to identify molecules that could work as antimycobacterial compounds by inhibiting the activity of the transcriptional regulator.

SB-P02
STUDY OF PHOTOSTATE-STABILIZING MUTANTS OF THE BACTERIOPHYTOCHROME FROM *Xanthomonas campestris*

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Bacteriophytochromes (BphPs) are part of the phytochrome superfamily of photoreceptors. BphPs are proteins that bind biliverdin (BV) as their chromophore and typically present two photostates: a primarily red-absorbing form (Pr) and a far-red-absorbing form (Pfr).We have recently shown that the BphP from the plant pathogen bacteria *Xanthomonas campestris* (XccBphP) modulates the interaction with its host, and performed several biophysical studies showing that XccBphP is a bathy-like phytochrome (Pfr-enriched equilibrium as ground-state). Moreover, we have resolved the 3D crystal structure of the full-length protein in the Pr state (the first in photobiology).In order to evaluate the biological role of XccBphP*in vivo* and resolve its structure in Pfr form, we have designed punctual and randomized mutants of XccBphP in conserved residues linked to the photochemical behavior. By means of a UV-VIS spectroscopy protocol designed for a rapid and precise characterization of these mutants, we have characterized several mutants with different photochemical behavior, including one that stabilizes the Pfr.Several biophysical studies have been made with this mutant, including crystallogenesis and X-ray diffraction. The latter allowed us to obtain the first Pfr structure of a full-length phytochrome to compare it with the already solved Pr. Here, we show results of all these experiments, as well as some preliminary work done *in vivo*. We provide new insights on the structural mechanisms involved in the light-induced signal transduction of phytochromes.

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SB-P03
NAD^+ INHIBITS GAPDH AGGREGATION BY PREVENTING THE NITROSATIVE STRESS-INDUCED CONFORMATIONAL CHANGES

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Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is a multifunctional protein involved in cell death processes frequently associated with oxidative/nitrosative stress. S-nitrosylation of GAPDH facilitates its binding to the E3-ubiquitin-ligase Siah1, which has a nuclear localization signal that promotes the entrance of the protein complex to the nucleus causing apoptosis. GOSPEL (GAPDH's Competitor Of Siah1 Protein Enhances Life) protein interacts with GADPH and interferes with the binding between GAPDH and Siah1, inhibiting their apoptotic effect. Oxidative/nitrosative stress also induces the aggregation of GAPDH *in vitro*, which is in accordance with the presence of the enzyme in insoluble aggregates found in some neurodegenerative diseases. Evidence provided by our laboratory indicates that in the presence of nitric oxide (NO) GOSPEL co-aggregates with GAPDH increasing its aggregation rate. GAPDH Cys^{152} plays an essential role in this process since their S-nitrosylation initiates the oxidative modification that triggers the formation of disulfide-bonded aggregates. Both GAPDH aggregation and GAPDH-GOSPEL co-aggregation were inhibited by $NAD^{\cdot}$ Here we present preliminary circular dichroism studies indicating that NAD^+ inhibits the conformational changes induced by the NO donor NOR3. We also report the X-ray structure of GAPDH treated with NOR3 in the presence of NAD^+. The analysis shows that in addition to the NAD^+ density the difference map exhibits a positive density connected to the SH of Cys^{152} that could only be attributed to NO. These results suggest that NAD^+ could be inhibiting the NOR3-induced aggregation by stabilizing NO-GAPDH conformation

SIGNAL TRANSDUCTION

ST-P01
AGING AND EXPRESSION OF PERMEASE GENES IN *Saccharomyces cerevisiae*

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Amino acids serve as precursors for synthesis of proteins and other metabolites and also as carbon and nitrogen sources. Yeast cells are able to internalize amino acids since they possess permeases that facilitate their transport. Amino acid utilization is subjected to regulatory pathways such as GAAC (General Amino Acid Control), TORC1 and amino acid sensor SPS. These pathways operate in parallel and in a coordinated way to generate the correct transcriptional gene response to nutrient availability. The aim of this work was to study the expression levels of two permeases during aging and to analyse if these nutrient-sensitive pathways are involved in their transcriptional regulation. In particular, we focused on the Uga4 and Bap2 permeases, responsible for the uptake of the gamma-aminobutyric acid (GABA) and the branched-chain amino acids, respectively. We used *S. cerevisiae* mutant strains grown under two dietary conditions (with and without all amino acids), and transcriptional activity was measured. We found that in wild type cells *UGA4* expression increases along with aging in the growth conditions used, even in the absence of its inducer, GABA, and the GAAC pathway is partially responsible in maintaining these elevated levels. In contrast, *BAP2* expression does not vary with aging in wild type cells grown without amino acids, but it is higher in cells grown with amino acids at the beginning of the aging process and decreases after cells reach stationary phase. This behavior depends on GAAC. Further research must be done to understand the differential transcriptional profiles of the permeases studied and the influence they have on lifespan.

ST-P02
INFLUENCE OF THE CARBON SOURCE QUALITY ON AGE-RELATED PROCESSES IN YEAST

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Glucose is the primary source of carbon and energy for yeast. Glucose induces glycolytic enzymes and represses the utilization of alternative carbon sources. The Snf1 kinase is a key component of the cellular response to fluctuations in the levels and quality of the carbon source. Its activity is stimulated by glucose limitation, and induces genes that regulate the metabolism of alternative carbon sources, gluconeogenesis and respiration. Taking into account that one of the best known interventions to prolong lifespan is the caloric restriction and that Snf1 is considered a global energy regulator, the aim of this work was to study the participation of Snf1 in cellular longevity. We used strains deficient in the kinases Snf1 and Tor1 and minimal media with carbon sources of different quality. We found that mitochondrial activity of cells lacking these two kinases is higher than in wild type cells grown on non-fermentable carbon sources. All mutant strains grown on a respiratory carbon source are tolerant to oxidative stress. Cells lacking Snf1 and/or Tor1 from a fermentable carbon source at stationary phase are more tolerant to oxidative stress than wild type cells. We found that wild type cells from a strictly respiratory carbon source present a longer chronological lifespan than those from a fermentable carbon source. On caloric restriction growth media, strain is more long-lived than wild type and strains. On restricted carbon sources, there is more autophagy in wild type and cells than in cells. These results indicated that the age-related process, autophagy, is inversely associated with lifespan.

ST-P03

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AMINO ACID–SENSITIVE PATHWAYS DIFFERENTIALLY AFFECT LIFESPAN IN YEASTS

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Dietary regimens have proven to promote longevity in several eukaryotic model organisms including the budding yeast *Saccharomyces cerevisiae.* These interventions are effective strategies for preventing aging and diseases and many of them are linked to amino acid and protein levels. The internalization of amino acids is mediated by the Ssy1–Ptr3–Ssy5 (SPS) sensing pathway, their assimilation is regulated in the NCR supra–pathway by the TORC1 kinase, and during amino acid starvation the GAAC pathway is activated. The aim of this work was to study how these amino acid–sensitive pathways affect lifespan. We used wild type cells and cells deficient in genes participating in these metabolic pathways. Chronological lifespan (CLS) was measured using the colony forming unit spot assay in cells grown in the absence and in the presence of all amino acids. Lifespan decreases in wild type cells grown with amino acids and in cells deficient in genes of the GAAC pathway; whereas the TOR1 deficiency extends longevity. When tolerance against thermal stress was analysed during the aging process, we found that cells lacking Leu3, a transcription factor involved in the GAAC pathway, are less resistant and cells lacking Tor1 and Gln3 are more resistant than the other strains used. The amino acid presence has a protective effect in all cases assayed. We also determined that the SPS and GAAC pathways participate in both autophagy and the unfolded protein response (UPR) pathways. Altogether these results allow us to conclude that the pathways modulated by amino acids regulate lifespan and other age–associated processes in opposite ways.

ST-P04
IN VIVO RELEVANCE OF THE OLIGOMERIC STATE OF BCY1, THE REGULATORY SUBUNIT OF PKA IN *Saccharomyces cerevisiae*

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PKA is a tetramer formed by a dimer of the regulatory subunit (R), and two catalytic subunits. R subunits have a dimerization and docking (DD) domain at its N-t. We have reported that Bcy1 (R subunit from *S. cerevisiae*) forms a tetramer in solution, unlike its dimeric mammalian counterparts. A structural analysis by SAXS and crystallization of DD domain (1-50) showed that it forms a tetramer *in vitro*. A key residue for tetramerization is Arg45. Upon deletion of Gln44, the DDΔQ no longer forms tetramers but dimers. In order to study whether tetramerization is relevant *in vivo*, a wt strain overexpressing either DDWT or DDΔQ was used to evaluate several phenotypes commonly used as read-outs of PKA activity. The rationale for this approach was that a difference in phenotypic effects of the two constructs would reflect a differential capacity of competition with endogenous Bcy1, with its degree of oligomerization and therefore result in an alteration of PKA activity and/or localization. We analyzed growth, glycogen accumulation, heat stress and nuclear localization of the transcription factor Msn2. In most cases the difference observed between the control strain and the one expressing DDWT was not significant; however, the expression of DDΔQ produced a higher glucose accumulation, an increase in growth, and lower resistance to heat stress than the DDWT. The nuclear localization of Msn2-GFP was completely impaired in the presence of either DDWT or DDΔQ. The phenotype analysis indicates that PKA function is differentially altered by expression of DDΔQ or DDWT and suggests that the oligomeric state of Bcy1 *in vivo* is relevant for yeast physiology.

ST-P05
SIMULTANEOUS SINGLE-CELL QUANTITATIVE ANALYSIS OF THE UPR PATHWAYS BY MEANS OF FLUORESCENT REPORTERS

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The Unfolded Protein Response (UPR) is a cellular stress signaling cascade essentially triggered by the accumulation of misfolded proteins in the Endoplasmic Reticulum (ER). Three mechanistically distinct pathways (IRE1, PERK and ATF6) make up this collective response aimed at restoring homeostasis. Evidence has been reported about the UPR being linked with the development of malignant tumors and neurodegenerative diseases.In order to characterize the UPR dynamics, we employed a set of fluorescent reporters that allows us to monitor the activation of the UPR in human single cells and in real time. We have previously characterized novel reporters for the ATF6 and IRE1 pathways, and we describe here the design of a complementary 5'uORF ATF4-mCherry reporter for the PERK pathway. We have also developed a protocol for automated imaging, segmentation and tracking of single cells, which allowed us to perform a simultaneous quantitative analysis of the activation of the three UPR pathways.Interestingly, activation of the UPR in patients suffering from frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS) has been proposed to be linked to the toxicity of Tar DNA binding Protein-43 (TDP-43), the main component of intracellular inclusions related to these diseases. Here we show, using our reporters, that TDP-43 regulates the unfolded protein response. These findings will contribute to understand the etiology of TDP-43 proteinopathies.

ST-P06

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CHOLESTEROL UPTAKE IN *Tetrahymenathermophila*

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The ciliate *T. thermophila* incorporates exogenous cholesterol and converts it into 7,22 bisdehydrocholesterol, replacing tetrahymanol (a cholesterol surrogate) on the cell membranes. This process is accompanied by repression of tetrahymanol synthesis, and induction of genes involved in sterol bioconversion. By fluorescent microscopy, it was previously found that cholesteryl esters were mainly incorporated by phagocytosis. In a preliminary study, using a mutant strain defective in phagocytosis (II8GIA), we observed by GC-MS a reduction of cholesterol uptake and a negligible bioconversion, compared to a wild type strain (CU428). Nonetheless, II8GIA treated with cholesterol showed a significant upregulation of genes involved in bioconversion, indicating that these cells certainly respond to the presence of cholesterol. We followed the uptake of radiolabeled cholesterol over time in both strains and traced its modification to cholesteryl esters by TLC. Consistently with previous results, the kinetics of sterol incorporation indicated a rapid and massive intake in CU428 cells but it was slower in II8GIA. In addition, treatment with Cytochalasin D, which impairs phagocytosis, resulted in a delayed uptake of cholesterol in CU428 but also inhibited its incorporation in II8GIA, suggesting the involvement of several actin-dependent and independent mechanisms. Our results corroborate the hypothesis that cholesterol uptake is mainly due to phagocytosis, but also indicate that, at least, a secondary path of internalization exists and it has an associated signaling that affects gene expression.

ST-P07
LIGHT MODULATED ENZYMATIC ACTIVITIES OF THE LOV HISTIDINE KINASE PHOTORECEPTOR IN *Brucella abortus*

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Brucella abortus virulence increases when bacteria are previously illuminated with blue light, and this effect is mediated by LOV-HK. This enzyme is the sensor element of a two component system together with its response regulator, PhyR. This work is focused on understanding how light modulates *B. abortus* LOV-HK's enzymatic activities. Firstly, we determined in vitro autokinase activity by evaluating the phosphorylation level of LOV-HK in the presence of ATP[γ32P] and Mg2+. Our results showed that the phosphorylation level is greater when the enzyme is previously illuminated than when it is kept in the dark. Furthermore, a blind mutant does not show light activation. Secondly, we evaluated in vitro phosphotransferase activity by incubating LOV-HK with ATP, Mg2+ and PhyR. Samples were run in a SDS-PAGE containing Phostag, a reagent that delays the migration of phosphorylated proteins. Gel quantification revealed a greater level of phosphotransferase activity when illuminated. Additionally, in vitro phosphatase activity was assessed with previously phosphorylated PhyR, ADP and Mg2+. We found that LOV-HK has phosphatase activity independent of light. Finally, we analyzed in vivo phosphotransferase activity. To this end, we used B. abortuswt, lovhk::km and the complemented strains with lovhk and lovhk C69S. After SDS-PAGE-Phostag and subsequent WB analysis using anti-PhyR antibodies, we observed a greater level of phosphorylation when bacteria were previously illuminated. In conclusion, we found that LOV-HK is capable of acting as a phosphatase and that both autophosphorylation and phosphotransferase activities are increased upon light exposure

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