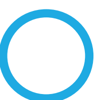
CONGRESO SAIB 2022

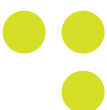
Resúmenes de Comunicaciones Orales y Posters

Abstracts of Oral Communications & Posters



SAIB

Sociedad Argentina de Investigaciones en Bioquímica y Biología Molecular



LVIII Annual Meeting of the Argentine Society for Biochemistry and Molecular Biology Research

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Cell Biology
Pablo Aguilar
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Lipids
Martín Oresti
CONICET - Universidad del Sur

Microbiology
Hebe Dionisi
CONICET

Plants
Elina Welchen
CONICET – Universidad Nacional del Litoral

Signal Transduction
Graciela Boccaccio
CONICET

PROGRAM AT A GLANCE

	Tuesday 8th	Wednesday 9th	Thursday 10th	Friday 11th
8:30 10:30		**Oral Communications** “Sala Magna” Microbiology "Sala Plumerillo" Plants "Sala Horcones" Lipids - Neurosciences	**Oral Communications** “Sala Magna” Plants "Sala Plumerillo" Microbiology "Sala Horcones" Cell Biology	**Oral Communications** “Sala Magna” Cell Biology "Sala Plumerillo" Plants - Biotechnology "Sala Horcones" Signal Transduction – Enzymes
10:30 11:00		Coffee break	Coffee break	Coffee break
11:00 12:30		*“Sala Magna”* Plenary Lecture **Dr. Ernesto Podesta**	*“Sala Magna”* Plenary Lecture **Dr. Mario Feldman**	*“Sala Magna”* Plenary Lecture “Cono Sur” **Dr. Rodrigo Gutierrez**
12:30 14:30	Registration	“Sala Magna” Round Table Surf your career Free time for lunch	Free time for lunch	Free time for lunch
14:30 16:30		**Symposium** “Sala Magna” Signal Transduction "Sala Plumerillo" Plants "Sala Horcones" Young Investigators	**Symposium** “Sala Magna” Lipids "Sala Plumerillo" Microbiology "Sala Horcones" Young Investigators	**Symposium** “Sala Magna” Cell Biology "Sala Plumerillo" Plants "Sala Horcones" Short Plenary Lectures
16:30 17:00		Coffee break	Coffee break	Coffee break
17:00 19:00	“Sala Magna” *Opening Ceremony* *Plenary Lecture Alberto Sols.* **Dra. Isabel Varela Nieto**	POSTERS (Central Hall)	POSTERS (Central Hall)	POSTERS (Central Hall)
19:00 20:30	“Sala Magna” Plenary Lecture **Dr. Craig Roy**	“Sala Magna” Plenary Lecture Hector Torres **Dra. Ana Belén Elgoyhen**	“Sala Magna” Plenary Lecture Ranwel Caputto **Dra. Alejandra del Carmen Alonso**	“Sala Magna” Plenary Lecture **Dr Maximiliano Gutierrez**
20:30 22:00	Welcome Cocktail *Central Hall*		SAIB Society Annual Meeting	Awards presentation and Closing Ceremony

THIS MEETING WAS SUPPORTED BY:

Biodynamics

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PROGRAM

TUESDAY, November 8, 2022

12:30-16:30 REGISTRATION

17:00-17:30 OPENING CEREMONY
Eduardo A. Ceccarelli
SAIB President
Room "Sala Magna"

17:30-19:00 PLENARY LECTURE ALBERTO SOLS
Dra. Isabel Varela-Nieto
Instituto de Investigaciones Biomédicas "Alberto Sols" (CSIC-UAM), CIBERER-ISCIII, Departamento de Fisiopatología Endocrina y del Sistema Nervioso Universidad Autónoma de Madrid, España.
Insights into age-related hearing loss: oxidative stress and neuroinflammation
Chairperson: Eduardo A. Ceccarelli
Room "Sala Magna"

19:00-20:30 PLENARY LECTURE
Dr. Craig Roy
Biological and Biomedical Sciences (BBS), Department of Microbial Pathogenesis, School of Medicine, Yale University, USA
Structural analysis of the Dot/Icm type IVB secretion machine
Chairperson: María Isabel Colombo
Room "Sala Magna"

WELCOME COCKTAIL

WEDNESDAY, November 9, 2022

8:30-10:30 ORAL COMMUNICATIONS

Room "Sala Magna". Microbiology.

Room "Sala Plumerillo". Plants.

Room "Sala Horcones". Lipids - Neurosciences.

10:30-11:00 COFFEE BREAK

11:00-12:30 PLENARY LECTURE

Dr. Ernesto Podesta

Instituto De Investigaciones Biomédicas – INBIOMED, Oficina De Coordinacion Administrativa Houssay - OCA – HOUSSAY, CONICET UBA, Argentina

Specific cellular micro-environments to spatially or temporally regulates a biological process. Foundation for new therapeutic targets

Chairperson: José Luis Bocco

Room "Sala Magna"

12:30-14:30 FREE TIME FOR LUNCH

12:30 -14:00. ROUND TABLE. SURF YOUR CAREER

Room "Sala Magna"

Chairperson: Mariano Polo

Speakers: Paula Casati, Ana Laxalt, Belen Elghoyen, Marisa Colombo, Luis Mayorga

14:30-16:30 SIGNAL TRANSDUCTION SYMPOSIUM

Room "Sala Magna"

Chairpersons: Graciela Boccaccio, Ricardo Biondi

Dr. Hernán Grecco

Departamento de Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina

Multiplexed imaging of caspase activation dynamics unravels the connectivity of the apoptotic network

Dra. María Vera Ugalde

Department of Biochemistry Faculty of Medicine and Health Sciences McGill University Canada.

Imaging single mRNA molecules to investigate neuronal protein homeostasis

Dr. Diego Presman

IFIBYNE – CONICET Departamento de Fisiología, Biología Molecular y Celular, FCEN-UBA, Argentina

Chasing transcription factors one molecule at a time

Dr. José Badano

Laboratorio de Genética Molecular Humana Institut Pasteur de Montevideo, Uruguay

The BBS4-cilia axis in adipogenesis: insights for obesity in Bardet-Biedl syndrome

14:30-16:30 PLANTS SYMPOSIUM I

Room "Sala Plumerillo"

Chairpersons: Nicolás M Cecchini, Elina Wechen

Dr. Gustavo MacIntosh

Roy J. Carver Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, USA

Colonization strategies and defense responses in soybean-soybean aphid interactions

Dr. Yariv Brotman

Department of Life Sciences, Ben Gurion University of the Negev, Beersheva, Israel

Mapping Crop Plants Metabolic Landscape by Untargeted Metabolomics at Different Environmental Conditions

Dra. Francisca Blanco

Centro de Biotecnología Vegetal, Universidad Andrés Bello, Chile
Involvement of the cell wall and its dynamic in plant defense against aphids

14:30-16:30 YOUNG INVESTIGATORS SYMPOSIUM 1
Room "Sala Horcones"
Chairpersons: Alejandra Mussi, Matias Asension Dies.
Dra. María Carla Martini
Instituto de Biotecnología y Biología Molecular. UNLP – CONICET
Loss of RNase J leads to multi-drug tolerance and accumulation of highly structured mRNA fragments in Mycobacterium tuberculosis.
Dra. Laura Emilce Navas
Instituto de Agrobiotecnología y Biología Molecular (IABIMO)- INTA Castelar
Bacterial transformation of compounds derived from residual lignin into bioproducts"
Dr. Ariel Waisman
LIAN-CONICET, FLENI Escobar
The transcription factor Oct6 regulates the dissolution of the pluripotent state by inhibiting Nanog and inducing differentiation-associated transcription factors

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16:30-17:00 COFFEE BREAK

17:00-19:00 POSTER SESSION
Central Hall

CB-15 to CB-30
PL-18 to PL-46
MI-16 to MI-31
BT-02 to BT-13
LI-01 to LI-07
EN-04 to EN-07

19:00-20:30 PLENARY LECTURE HECTOR TORRES

Dr. Ana Belén Elgoyhen

INGEBI CONICET, Facultad de Medicina, Universidad de Buenos Aires, Argentina

Argentina in the Auditory Landscape: the Catalytic Force of the Instituto "Héctor N. Torres"

Chairperson: Gabriela A. Salvador

THURSDAY, November 10, 2022

8:30-10:30 ORAL COMMUNICATIONS

Room "Sala Magna". Plants.

Room "Sala Plumerillo". Microbiology.

Room "Sala Horcones". Cellular Biology.

10:30-11:00 COFFEE BREAK

11:00-12:30 PLENARY LECTURE

Dr. Mario Feldman

Department of Molecular Microbiology, School of Medicine Washington University in St Louis, USA

Dissecting the virulence strategies of the superbug Acinetobacter baumannii

Chairperson: Eduardo A. Ceccarelli

Room "Sala Magna"

12:30-14:30 FREE TIME FOR LUNCH

14:30-16:30 LIPIDS SYMPOSIUM

Chairpersons: Silvia Alejandra Belmonte, Gerardo Martín Oresti

Room "Sala Magna"

Dr. Iván López Montero

Departamento de Química Física Facultad de Ciencias Químicas Madrid España

Self-assembly of rotating ATP synthases in lipid bilayers. A mechanical view

Dra. Dolores Busso

Centro de Investigación e Innovación Biomédica, Universidad de los Andes, Chile

Implications of HDL cholesterol metabolism for oocyte biology and female fertility

Dr. Ernesto Ambroggio

Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC) UNC, Argentina

The interaction of viral structural proteins with oligonucleotides and membranes

14:30-16:30 MICROBIOLOGY SYMPOSIUM

Chairpersons: Hebe Dionisi, Eleonora Campos

Room "Sala Plumerillo"

Dra. Rosalba Lagos

Facultad de Ciencias, Universidad de Chile, Chile

Antarctic Peninsula soils host a diverse microbiota with a variety of antibiotic resistance genes

Dr. Fabricio Cassan

Instituto De Investigaciones Agrobiotecnológicas, INIAB-CONICET, UNRC, Córdoba, Argentina

The importance of denitrification performed by rhizobia used as inoculants in South America

Dra. Paulina Fermani

Instituto de Biología de Organismos Marinos, IBIOMAR, CONICET, Puerto Madryn, Chubut, Argentina.

Effects of climate change on aquatic systems and their microbial communities

14:30-16:30 YOUNG INVESTIGATORS SYMPOSIUM 2

Room "Sala Horcones"

Chairpersons: Carlos Garcia Mata ,Georgina Fabro.

Dr. Ariel Herrera-Vásquez

Centro de Biotecnología Vegetal. Universidad Andrés Bello, Santiago - Chile.

The TGA transcription factors from clade II negatively regulate the Salicylic Acid accumulation in Arabidopsis"

Dr. Damián Alejandro Cambiagno

Unidad de Estudios Agropecuarios (UDEA). Instituto Nacional de Tecnología Agropecuaria (CONICET – INTA)

Small RNAs modulating systemic plant defenses

Dra. Laura Evangelina Garcia

IBAM -CONICET-U.N.Cuyo

Low efficiency in the splicing of foreign mitochondrial genes in Lophophytum

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16:30-17:00 COFFEE BREAK

17:00-19:00 POSTER SESSION

Central Hall

CB-31 to CB-46

PL-47 to PL-75

MI-32 to MI-47

BT-14 to BT-25

ST-05 to ST-10

LI-08 to LI-16

19:00-19:15 Tribute to Hugo J. Maccioni: Carlos Argaraña

19:15-20:45 RANWEL CAPUTTO CONFERENCE

Dra. Alejandra del Carmen Alonso

Department of Biology and Center for Developmental Neuroscience, College of Staten Island, and Biology Program, The Graduate Center, The City University of New York, Staten Island, NY, USA

Tau induced neurodegeneration in Alzheimer disease: structural and other functions

Chairperson: Marie Elena Alvarez

20:45-22:00 SAIB society meeting

FRIDAY, November 11, 2022

8:30-10:300 ORAL COMMUNICATIONS

Room "Sala Magna". Cellular Biology.

Room "Sala Plumerillo". Plants - Biotechnology.

Room "Sala Horcones". Signal Transduction - Enzymes.

10:30-11:00 COFFEE BREAK

11:00-12:30 PLENARY LECTURE "CONO SUR"

Dr. Rodrigo Gutierrez

Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas Pontificia Universidad Católica de Chile, Chile

Plant life at the extreme in the Atacama Desert

Chairperson: Ana María Laxalt

12:30-14:30 FREE TIME FOR LUNCH

14:30-16:30 CELULAR BIOLOGY SYMPOSIUM

Room "Sala Magna"

Chairpersons: Marisa Colombo, Pablo Aguilar

Dra. Gabriela Pagnussat

IIB-CONICET Universidad Nacional de Mar del Plata, Argentina

Homocastasterone biosynthesis in the female gametophyte is essential for early embryogenesis in Arabidopsis

Dra. Luciana Balboa

Instituto de Medicina Experimental, IMEX, CONICET, Buenos Aires, Argentina

Targeting immunometabolism in host defense against Mycobacterium tuberculosis

Dra. Natalia de Miguel

Instituto Tecnológico de Chascomús INTECH, Argentina

Role of extracellular vesicles in parasite: parasite communication in Trichomonas vaginalis

Dra. Laura Delgui

IHEM-CONICET, UN Cuyo, Mendoza

Birnaviral Hijacking Of Endosomal Membranes

14:30-16:30 PLANTS SYMPOSIUM II

Room "Sala Plumerillo"

Chairpersons: María Valeria Lara, Jose Estevez

Dr. Nir Sade

The George S. Wise Faculty of Life Sciences, Tel Aviv University, Israel

Setaria viridis plasma membrane aqps contribute to root hydraulics and root to shoot signaling of gas exchange

Dra. Daniela Sueldo

Department of Biology, Norwegian University of Science and Technology, Noruega

Role and suppression of plant extracellular enzymes during bacterial infection

14:30-16:30 SHORT PLENARY LECTURES SYMPOSIUM

Room "Sala Horcones"

Chairpersons: Anabella Lodeyro, Germán Rosano

Dra. Noelia Foresi

Instituto de Investigaciones Biológicas-CONICET, Facultad de Ciencias Exactas y Naturales, UNMDP, Argentina

Novel role for nitric oxide and nitric oxide synthase in primary nitrogen metabolism in photosynthetic organisms

Dr. Lisandro Otero

Instituto de Biotecnología Ambiental y Salud Leloir (INBIAS). UNRC. Centro de Rediseño e Ingeniería de Proteínas (CRIP). UNSAN, Argentina.

Long-range signaling mechanism of a bacteriophytochrome at the atomic level

Dr. Luciano Abriata

Protein Expression and Structure Facility, EPFL, Switzerland.

Towards Chemistry and Structural Biology in the Metaverse

16:30-17:00 COFFEE BREAK

17:00-19:00 POSTER SESSION

Central Hall

CB-47 to CB-65

PL-75 to PL-100

MI-48 to MI-63

ST-10 to ST-16

NS-03 to NS-07

SB-1 and SB-2

19:00-20:30 PLENARY LECTURE

Dr. Maximiliano Gutierrez

The Francis Crick Institute, United Kingdom

Mycobacterium tuberculosis infection of host cells in space and time

Chairperson: Federico Sisti

Room “Sala Magna”

20:30-21:30 AWARDS PRESENTATION and CLOSING CEREMONY

ORAL COMMUNICATIONS- Wednesday, November 12th

Room "Sala Plumerillo"

PLANTS

Chairpersons: Matías D. Hartman, Anabella Lodeyro

8:30 PL-01

MBD1, MBD2, MBD3 and MBD4 proteins participate in abiotic stress responses

Giustozzi, Marisol; Qüesta, Julia; Casati, Paula

8:45 PL-02

Unravelling the contribution of mesophyll and bundle sheath chloroplasts to increased stress tolerance

Demarchi, Mariana; Arce, Rocío Cecilia; Campi, Mabel; Chan, Raquel Lía; Carrillo, Néstor, Lodeyro, Anabella Fernanda

9.00 PL-03

A complex interplay between the transcription factors AtHB23, AtPHL1 and AtMYB68 fine tunes Arabidopsis lateral and primary root development and adaptation to salinity

Spies FP, Perotti MF, Jo CI, Hong JC, Chan RL

9:15 PL-04

Regulatory networks established by TCP and GLK transcription factors during Arabidopsis seedling development

Alem, Antonela; Gonzalez, Daniel; Viola, Ivana

9:30 PL-05

An Oomycete effector alters plant immunity and development by manipulating auxin signaling

Bogino, María Florencia; Wirthmueller, Lennart, Fabro, Georgina

9:45 PL-06

Identification of molecular mechanisms associated with increased yield caused by mechanical treatment in *Arabidopsis thaliana*

Raminger, Lorena; Chan, Raquel; Cabello, Julieta.

10:00 PL-07

Mitochondrial genome recombination and repair in plant somatic hybrids

Gandini, Carolina Lia; Garcia, Laura Evangelina, Sanchez-Puerta, M. Virginia

Room "Sala Magna"

MICROBIOLOGY

Chairpersons: María Corvi – Laura Delgui

8:30 MI-01

The antifungal peptide SmAPα1-21 derived from the α-core of plant defensin DefSm2-D induces cell-wall stress, membrane permeabilization, peroxisome biogenesis, oxidative stress and cell death in Fusarium graminearum conidia. Role of His 19 in peptide internalization

Fernández, Agustina; Malbrán, Ismael; Guzmán, Fanny; Bakás Laura; Vairo Cavalli Sandra

8:45 MI-02

Contrasting study among Trypanosomatids reveals conserved chromatin organization around trans splicing-acceptor site

Zambrano Siri, Romina Trinidad; Beati, Paula; Smircich, Pablo; Alonso, Guillermo Daniel; Ocampo, Josefina

9.00 MI-03

EVASION OF THE CELLULAR IMMUNE RESPONSE BY THE NS5 PROTEIN OF THE DENGUE VIRUS. IMPACT ON THE PATHOGENESIS OF THE FOUR SEROTYPES FOR A RATIONAL DESIGN OF TETRAVALENT VACCINES

Gonzalez Lopez Ledesma, María Mora; Costa Navarro, Guadalupe; Pallares, Horacio; De Maio, Federico; Iglesias, Gabriel; Gebhard, Leopoldo; Paletta, Ana; Giraldo Giraldo, María; Rajsbaum, Ricardo; Ceballos, Ana; Gamarnik, Andrea

9:15 MI-04

Analysis of histones epigenetic marks across the Giardia lamblia cell cycle

Díaz-Perez Luciano , Patolsky Rocío , Segovia Caterina, Touz María Carolina and Rópolo Andrea

9:30 MI-05

Characterization of Cysteine-rich protein families of Giardia lamblia and their role during antigenic variation

Molina, Cecilia Rita; Luján Lucas Agustín; Rodríguez-Walker, Macarena; Saura Alicia; Fernández, Elmer; Luján, Hugo Daniel.

9:45 MI-06

Antibodies to protozoan variable surface antigens induce antigenic variation

Luján Lucas Agustín; Molina, Cecilia Rita; Iribarren, Paula; Saura, Alicia; Midlej, Victor; De Sousa, Wanderley; Álvarez, Vanina; Luján, Hugo Daniel.

10:00 MI-07

The SARS-CoV-2 mutation landscape is shaped by niche environmental factors

Masone, Diego; Ruis, Christopher; Hinrichs, Angie S.; Alvarez, Maria Soledad; Polo, Luis Mariano

10:15 MI-64

Nanobodies: a versatile and low-cost tool for Hepatitis E virus research, diagnostics and therapeutics

Arce, Lorena Paola (1,2); Pavan, María Florencia (2); Bok, Marina (3); Parreño, Viviana (3); Vizoso Pinto, María Guadalupe (1); Ibañez, Lorena Itatí (2)

Room "Sala Horcones"

LIPIDS - NEUROSCIENCES

Chairpersons: Lucila Gisele Pescio - Gerardo Martín Oresti

8:30 LI-01

The pair ceramide-1-phosphate/ceramide kinase regulates intracellular calcium concentration during sperm exocytosis

Vaquer, Cintia Celina; Suhaiman, Laila; Pavarotti, Martín; De Blas, Gerardo; Belmonte, Silvia Alejandra.

8:45 LI-02

The biosynthesis of sphingolipids with very-long-chain PUFA is enhanced by testosterone in spermatogenic cells

Santiago Valtierra, Florencia Ximena; Paternolli, Aldana; Oresti, Gerardo Martín

9:00 LI-03

Fusion of micron-size vesicles: interplay between the mitochondrial Mfn2 protein and lipids

Peñalva, Daniel A.; Monnappa, Ajay K[2]; Natale, Paolo; López-Montero, Iván

9:15 LI-04

Cytosolic phospholipase A2 modulates triacylglycerides accumulation in renal cells under osmotic stress through arachidonic acid-PPARg axis

Parra, Leandro; Zerpa, Andrea; Erjavec, Luciana; Casali Cecilia; Setton-Avruj, Patricia; Fernández María del Carmen

9:30 LI-05

Resveratrol effect on renal osmoprotection: Modulation of COX-2 expression

Erjavec, Luciana Cecilia; Casali, Cecilia Irene; Parra, Leandro Gastón; Recabarren, Manuel; Fernández, María del Carmen

9:45 LI-06

FPR2/ALX signaling and their lipid mediator pathways: pleiotropic roles in neurotoxicity.

Benzi Juncos, Oriana Nicole; Alza, Natalia Paola; Salvador, Gabriela Alejandra

10:00 NS-01

Assembly of mitochondrial complexes in the brain of a rat model of Alzheimer

Novack GV, Galeano P, Castaño EM, Cuello AC, Morelli L

10:15 NS-02

Addressing the molecular signatures of selective vulnerability to Parkinson's disease using cell-type specific α-syn expression and single-nucleus RNA-seq

Arcuschin, Camila D.1,2; Lantheaume, Alexia3; Salehi, Saeede3; Zare, Abdolhossein3; Briese, Michael3; Tovote, Philip3; Espósito, María Soledad4 and Schor, Ignacio E.1,2**

ORAL COMMUNICATIONS- Thursday, November 13th

Room "Sala Magna"

PLANTS

Chairpersons: Damian Cambiagno - María Virginia Sanchez Puerta

8:30 PL-08

Contribution of proline metabolism to the control of cellular redox homeostasis under biotic stress in Arabidopsis

Cislaghi, Ana Paula; Álvarez María Elena

8:45 PL-09

IDENTIFICATION AND CHARACTERIZATION OF ZMS5H: ELUCIDATION OF ITS ROLE IN BIOTIC STRESS RESPONSE.

Serra, P., Righini Aramburu, S., Dillon, F., Grotewold, E., Falcone Ferreyra, M.L., Casati, P.

9.00 PL-10

Elucidating the molecular mechanisms of the temperature short-term memory in Arabidopsis plants

Murcia, Germán; Nieto, Cristina; Sellaro, Romina; Prat, Salomé; Casal, Jorge

9:15 Pl-11

Connection between CYTOCHROME c and the TOR pathway to regulate growth in Arabidopsis

Canal, María Victoria; Mansilla, Natanael; Gras, Diana; González, Daniel; Welchen, Elina

9:30 PL-12

Autophagy modulates growth and development in the moss *Physcomitrium patens*

Pettinari, Georgina; Finello, Juan; Plaza Rojas, Macarena; Liberatore, Franco; Robert, Germán;Otaiza-González, Santiago; Velez, Pilar; Theumer, Martin; Agudelo-Romero, Patricia; Enet, Alejandro; Gonzalez, Claudio, Lascano, Ramiro; Saavedra, Laura

9:45 PL-13

Chloroplast redox status modulates the cell expansion phase of leaf development associated to changes in proteasome activity and endoreduplication index

Rocío C. Arce1, Martín L. Mayta, Michael Melzer, Mohammad-Reza Hajirezaei, Anabella F. Lodeyro, Néstor Carrillo

Room "Sala Plumerillo"

MICROBIOLOGY

Chairpersons: Alejandra Mussi y Matías Asención Diez

8:30 MI-08

MliR, a novel MerR-like regulator of iron uptake, impacts metabolism, membrane remodeling and cell adhesion in the marine Bacteroidetes Bizionia argentinensis

*Pellizza, Leonardo *, Bialer, Magalí Graciela *,Sieira, Rodrigo,Arán, Martín,* Pellizza and Bialer equally contributed to this work*

8:45 MI-09

The polysaccharides degrading mechanisms of two soil bacterial isolates

Topalian, Juliana; Ontañon, Ornela; Navas, Laura; Valacco, M. Pía; Campos Eleonora

9.00 MI-10

Characterization of the protective response to FtsA imbalance-induced lysis in Streptococcus pneumoniae

Olivero NB, Cortes PR, Hernández Morfa M, Zappia VE, Jaime A, Echenique J.

9:15 MI-11

IMPACT OF DNA POLYMERASE IV ON GENOME EVOLUTION IN THE OPPORTUNISTIC PATHOGEN PSEUDOMONAS AERUGINOSA

Castell, Sofía Daiana; Tumas, Ignacio Nicolás; Miserendino, María Clara; Pezza, Roberto Jose; Ceschin, Danilo Guillermo, Monti, Mariela Roxana.

9:30 MI-12

INFLUENZA-INFECTED CELLS INCREASE LEVOFLOXACIN TOLERANCE OF STREPTOCOCCUS PNEUMONIAE

Hernández-Morfa M., Reinoso-Vizcaíno N., Olivero N., Zappia V., Cortes P., Jaime A;J. Echenique.

9:45 MI-13

Rate and molecular structure of DNA direct repeats rearrangements in P. aeruginosa.

Moro, Camila; Monti, Mariela; Pezza, Roberto; Argaraña, Carlos 1.

10:00 MI-14

PSEUDOMONAS AERUGINOSA ADHERES TO APOPTOTIC CELLS THROUGH A FUNCTIONAL TYPE IV PILI FORMING STABLE CLUSTERS

Dea, Celeste; Pepe, María Victoria; Roset, Mara; Peruani, Fernando; Kierbel, Arlinet

Room "Sala Horcones"

CELULAR BIOLOGY

Chairpersons: Laura Delgui – Luciana Balboa

8:30 CB-02

FOXO-mediated Repression of DICER-1 Regulates Metabolism and Stress Resistance in Drosophila

Ingaramo, Ma. Clara; Sanchez, Juan Andrés; Gerve, Paula; Dekanty, Andrés

8:45 CB-04

SPECIALIZED POLYMERASE IOTA MAINTAINS GENOMIC STABILITY BY PREVENTING EXCESS PRIMPOL-DRIVEN DNA REPLICATION

Venerus Arbilla, Sofía [1]; Mansilla, Sabrina Florencia [1]; Bertolin, Agostina [2]; Siri, Sebastián Omar [1]; De la Vega, María Belén [1]; Wiesmüller, Lisa [3]; Gottifredi, Vanesa [1]

9.00 CB-06

INHIBITORS OF ROCK KINASES INDUCE MULTIPLE MITOTIC DEFECTS AND SYNTHETIC LETHALITY IN BRCA2-DEFICIENT CELLS

Siri, Sebastian; Mares Ahlers, Candelaria*; Gottifredi, Vanesa*

9:15 CB-08

Use of Translesion DNA synthesis inhibition as a sensitizer of tumor cells

Okraine, Yiovana Verónica; de la Vega, María Belén; Mansilla, Sabrina; Gottifredi, Vanesa

9:30 CB-10

SHORT NUCLEOTIDE VARIANTS ON G-QUADRUPLEXES AS A CAUSE OF ONCOGENES TRANSCRIPTIONAL EXPRESSION VARIATIONS.

Piga, Ernesto.; Bezzi, Georgina.; Lorenzatti, Agustin.; Binolfi, Andres.; Calcaterra, Nora.; Armas, Pablo.

9:45 CB-12

ACYL-COA SYNTHETASE 4 MODULATES MITOCHONDRIAL FUNCTION AND METABOLISM IN BREAST CANCER CELLS.

Benzo, Yanina ; Prada, Jesica G.; Dattilo Melina A.; López, Paula F; Castillo, Ana F; Mori Sequeiros García, M. Mercedes; Ringelnman Sanchez, Paola ; Poderoso, Cecilia; Maloberti, Paula M.

10:00 CB-14

Role of nuclear O-GalNAc glycosylation in CHO cells

Garay, Yohana ; Irazoqui, Fernando

ORAL COMMUNICATIONS- Friday, November 14th

Room "Sala Plumerillo"

PLANTS - BIOTECHNOLOGY

Chairpersons: Laura Lucía Saavedra Borelli

8:30 PL-14

Circular foreign mitochondrial DNA in the mitochondria of parasitic plants

Roulet, Maria Emilia; Ceriotti, Luis Federico; Sanchez-Puerta, Maria Virginia

8:45 PL-15

The immune receptor BNT1 is involved in *Myzus persicae* infestation of Arabidopsis

Peppino Margutti, Micaela; Silva-Sanzana, Christian, Palomeque, Julieta, Herrera Vasquez, Ariel; Blanco-Herrera Francisca; Cecchini Nicolas.

9.00 PL-16

AtHB40 AND AtHB53 PLAY A ROLE IN ROOT DEVELOPMENT AND NaCl RESPONSE

Mora, Catia Celeste; Chan, Raquel Lía

9:15 PL-17

A de novo diploid genome assembly allows dissecting the transcriptomic differences underlying the clonal phenotypic diversity in cultivar 'Malbec'

Calderón, Luciano; Carbonell-Bejerano, Pablo; Muñoz, Claudio; Bree, Laura; Sola, Cristobal; Bergamin, Daniel; Gomez-Talquenca, Sebastian; Ibañez, Javier; Martínez-Zapater, José Miguel; Weigel, Detlef , Lijavetzky, Diego

9:30 BT-01

BIOSENSING PERFORMANCE OF THREE DIFFERENT WHOLE CELL BIOSENSOR BASED ON LEAD-RESPONSIVE TRANSCRIPTION FACTORS

Gándola Yamila, Alvarez Macarena, Gasulla Javier, Nadra Alejandro D.

Room "Sala Magna"

CELULAR BIOLOGY

Chairpersons: Natalia de Miguel – Andres Dekanty

8:30 CB-01

Soluble factors from radiation-induced senescent tumor cells reduces growth of non-irradiated tumor cells

Salvarredi, Leonardo. Agüero, Hector, Buscema, Vanesa Callegari, Eduardo Castro, Claudia, Lopez, Luis

8:45 CB-03

CHARACTERIZATION AND BIOLOGICAL ACTION OF POLYETHYLENE GLYCOL-COATED MAGNETITE NANOPARTICLES IN A CELLULAR MODEL OF VIRAL ONCOGENESIS

Principe, G; Lezcano, V; Tiburzi, S; Miravalles, AB; Rivero, S; Montiel Schneider, MG; Lassalle, V; González Pardo, V

9.00 CB-05

Pharmacological modulation of the circadian clock as a novel strategy to treat glioblastoma

Wagner Paula M; Guido Mario E

9:15 CB-07

Tubulin as a regulator of Phosphatidylserine exposure in red blood cells and its impact on haemorrheology.

Balach, Melisa Micaela; Etcheverry, Micaela; Monesterolo, Noelia Edith; Santander, Verónica Silvina, Rivelli Antonelli, Juan Franco; Ureta Miralla, Lucia Belen; Casale, César Horacio; Campetelli Alexis Nazareno.

9:30 CB-09

URSOLIC ACID NEGATIVELY MODULATES THE LIPID DROPLET METABOLISM, A KEY COMPONENT OF THE ROTAVIRUS INFECTION

Tohmé, M. Julieta; Converti, Ayelen; Caruso, Benjamín; Wilke, Natalia; Colombo, M. Isabel; Delgui, Laura R.

9:45 CB-11

EFFECT OF OLIGODEOXYNUCLEOTIDE IMT504 ON MURINE β-CELLS

Converti, Ayelén; Bianchi, M. Silvia; Montaner, Alejandro; Lux-Lantos, Victoria; Bonaventura, M. Marta; Delgui, Laura

10:00 CB-13

Characterization of the immune response against recombinant Tacaribe arenaviruses as new vaccine candidates to prevent Argentinean hemorrhagic fever

Gallo, Giovanna; Chiale, Carolina; Sepúlveda, Claudia; Brignone, Julia; Gamboa, Laura; Saavedra, María del Carmen; Zúñiga, Elina; López, Nora

Room "Sala Horcones"

SIGNAL TRANSDUCTION - ENZIMES

Chairpersons: Graciela Lidia Boccaccio – Eleonora Campos

8:30 ST-O1

2-arachydonoyl glycerol (2-AG) drives TRPV-dependent sensory signals that increase the intracellular cholesterol trafficking in *Caenorhabditis elegans.*

Bruno Hernandez-Cravero, Sofia Gallino, Jeremy Florman, Cecilia Vranych, Philippe Diaz, Ana Belén Elgoyhen, Mark J. Alkema; Diego de Mendoza

8:45 ST-O2

Xrn1 regulates cAMP-PKA specificity during thermotolerance.

Ortolá Martínez, María Clara; Galello, Fiorella; Pautasso, Constanza; Portela, Paula; Rossi, Silvia.

9.00 ST-O3

Quantitative proteomics reveals possible mechanisms of regulation of adipogenic differentiation in 3T3-L1 cells.

Del Veliz, Samanta; Müller Sergio; Rivera, Lautaro; Uhart Marina; Bustos Diego Martin.

9:15 ST-O4

Analysis of cytosolic calcium burst of Saccharomyces cerevisiae during the response to the sexual pheromone.

Tarkowski, Nahuel; Ponce Dawson, Silvina; Aguilar, Pablo S.

9:30 EN-01

COMPARATIVE STRUCTURAL, BIOCHEMICAL AND KINETIC STUDIES BETWEEN THERMOSTABLE AND COLD-ADAPTED GLUCOAMYLASES

Wayllace, Natael Maximiliano; Busi, María Victoria; Gomez-Casati, Diego Fabián

9:45 EN-02

Biochemical and structural characterization of two phosphoenolpyruvate carboxykinases fromthe green alga Chlamydomonas reinhardtii

Torresi, Florencia; Rodriguez, Fernanda; Gomez-Casati Diego; Martín, Mariana

10:00 EN-03

Cell-free glycobiology: enzymatic modification of α-glucans with glucosamine moieties

Iglesias, María Josefina; Iglesias, Alberto; Asencion Diez, Matías D.

ABSTRACTS

ORAL COMMUNICATIONS

Cell Biology

CB-1

Soluble factors from radiation-induced senescent tumor cells reduces growth of non-irradiated tumor cells

Salvarredi, Leonardo. [1, 2, 3] *Agüero, Hector* [1] *Buscema, Vanesa* [4] *Callegari, Eduardo* [5]

Castro, Claudia [6, 7,] *Lopez, Luis* [8]

1 Fundación Escuela de Medicina Nuclear; 2 Comisión Nacional de Energía Atómica; 3 Instituto Balseiro; 4 FCEN-UNCuyo; 5 Sanford School of Medicine, University of South Dakota; 6 Facultad de Ciencias Medicas - UNCuyo; 7 IMBECU UNCuyo-CONICET; 8 IHEM UNCuyo-CONICET.

Radiation therapy is one of the most important options in treating cancer. Based on DNA damage, ionizing radiation (IR) activates different cell death programs such as apoptosis, autophagy and senescence. The latter is characterized by an irreversible cell cycle arrest and the secretion of a protein profile known as the senescence-associated secretory phenotype (SASP). SASP can induce multiple effects on neighboring cells not exposed to radiation (naïve cells), a phenomenon that in radiotherapy is part of the so-called bystander effects (BE). The aim of this work was to evaluate whether SASP from radiation induced senescent cells produces BE on naive tumor cells. Murine melanoma B16F0 cell line cultures were exposed to 0 or 10 Gy doses of IR (NI-B16F0 and IR-B16F0 cells) and cell growth and clonogenic capacity were reduced. To explore the mechanism involved, apoptosis and senescence were analyzed. Apoptosis was not observed but cells became senescent 3 days after radiation. To explore the biological activity of SASP from B16F0 senescent cells, conditioned media from IR or NI cells were harvested on day 3 after radiation (IR-CM and NI-CM). Naive B16F0 cells were incubated with NI/IR-CM and cell growth and

migration were analyzed. IRCM reduced cell growth but not migration. To further explore if IR-CMs affected non-cancer cells, NIH 3T3 cell line was incubated with CMs. No significant changes on cell growth were observed. During the growth studies, it became apparent that B16F0 cells exposed to IR-CM exhibited morphological features indicative of senescence, which led us to histochemically stain for the senescence marker SA-β-gal. As predicted, B16F0 cells treated with IR-CM exhibited pronounced SA-β-gal positivity. The protein profile present in the CMs was characterized. Proteins from IR-CMs and NI-CMs were separated in large polyacrylamide gels. IR-CMs presented a greater number of bands compared to NI-CMs indicating an increased protein diversity. Furthermore, aliquots from the same CMs were analyzed by means of two-dimensional nano-liquid chromatography-mass spectrometry (2DnanoLC-MS/MS) with the aim of identifying specific proteins. The in silico analysis confirmed that IR-CMs presented higher protein diversity than NI-CMs. Using bioinformatics tools, biological processes and pathways of protein profiles contained in CMs were analyzed. IR-CMs expressed more proteins related to positive regulation of cellular senescence and less proteins related to positive regulation of cell growth and cell migration. In addition, IR-CMs were enriched in proteins related to oxidative stress induced senescence. In conclusion, cells undergoing radiation induced senescence reduces growth of not exposed tumor cells by secretion of soluble factors associated with senescence induction and maintenance. The identification of these factors might help identifying new targets towards manipulating them for therapeutic benefit

CB-2

FOXO-mediated Repression of DICER-1 Regulates Metabolism and Stress Resistance in Drosophila

Ingaramo, Ma. Clara; Sanchez, Juan Andrés; Gerve, Paula; Dekanty, Andrés

Instituto de Agrobiotecnologia del Litoral, CONICET-UNL

The adipose tissue plays an essential role in metabolism and physiology which impacts on animal lifespan and disease susceptibility. Dicer-1, a conserved type III endoribonuclease involved in miRNA processing, has been shown to be crucial in this organ for the adaptation to nutrient deprivation. However, mechanisms underlying Dcr-1 regulation in response to nutrient and metabolic challenges and the precise role of Dcr-1 in modulating metabolism, stress responses and aging remain unknown. Here we provide evidence that Dcr-1 plays a key role in the Drosophila fat body, a functional analog of vertebrate adipose and hepatic tissues, in the regulation of metabolism, stress resistance and longevity. We showed

that Dcr-1 expression is tightly regulated in the fat body under different stress types and physiological conditions including starvation, oxidative stress and aging. Fat body specific depletion of Dcr-1 resulted in altered lipid metabolism and increased resistance to oxidative and nutritional stress, while a substantial extension in lifespan was observed in Dcr-1 heterozygous mutants. We also provide mechanistic evidence showing that the transcription factor FOXO regulates Dcr-1 expression upon nutrient deprivation. Under these conditions, JNK-dependent activation of FOXO in the fat body is required for the repression of Dcr-1 expression and miRNA biogenesis. Chromatin immunoprecipitation (ChIP) assays revealed that FOXO binds to a conserved DNA binding site in Dcr-1 promoter, thus directly repressing its transcription under starvation. The mechanism described here coupling FOXO activation in the adipose tissue to the repression of Dcr-1 implicates a novel and previously uncharacterized function for JNK-FOXO axis integrating nutrient status with miRNA biogenesis and physiological responses at the organismal level.

CB-03

CHARACTERIZATION AND BIOLOGICAL ACTION OF POLYETHYLENE GLYCOL-COATED MAGNETITE NANOPARTICLES IN A CELLULAR MODEL OF VIRAL ONCOGENESIS

Principe, G [1,2]; Lezcano, V [1,2]; Tiburzi, S [1,2]; Miravalles, AB [1]; Rivero, S [3,4]; Montiel Schneider, MG [3,4]; Lassalle, V [3,4]; González Pardo, V [1,2].

1 Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur (UNS), San Juan 670, Bahía Blanca, Argentina. 2 Instituto de Ciencias Biológicas y Biomédicas del Sur (INBIOSUR); UNS-Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Bahía Blanca, Argentina. 3 Departamento de Química, Universidad Nacional del Sur (UNS), Avda. Alem 1253, Bahía Blanca, Argentina. 4 Instituto de Química del Sur (INQUISUR); UNS-Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Bahía Blanca, Argentina.

Magnetic nanotheranostics represent promising alternatives to the traditional diagnostic and treatment procedures available for different pathologies. The goal of this work was to analyze the biological action of polyethylene glycol-coated iron oxide nanoparticles (MAG.PEG) to generate a non-toxic carrier to optimize the delivery of drugs for Kaposi's sarcoma treatment. The MAG.PEG were synthesized by the hydrothermal method displaying a hydrodynamic diameter of 204 nm and a zeta potential of -22.1 mV.

Firstly, MAG.PEG effects on cytotoxicity and cell viability were evaluated on endothelial cells expressing vGPCR, which prompts Kaposi's sarcoma. By Trypan blue technique, we found that the incubation of these cells neither with MAG nor MAG.PEG (1-150 μg/ml) provoked significant changes in the number of living cells. In addition, cell viability analyzes (MTS and neutral red) revealed that a significant increase in metabolic and lysosomal activity was detected at higher concentrations of MAG or MAG.PEG (100-150 μg/ml) after 48 h of incubation. Secondly, the localization and accumulation of MAG.PEG (1-150 μg/ml) towards the cells was observed directly under a phase contrast microscope. In addition, MAG.PEG assembled within or nearby the cells and cell morphology remained unchanged regardless of the nanoparticles concentration. Furthermore, the presence of vesicles containing MAG.PEG inside vGPCR cells was confirmed by transmission electron microscopy. Thirdly, the iron content quantified by Prussian blue staining showed that the degree of accumulation of MAG.PEG depends on the concentration used. Finally, to steer the MAG.PEG to certain localization in the cell culture, a magnetic field generated by a moderated power magnet was used. The results indicated that the magnetic stimuli induced MAG.PEG accumulation in the zone where the magnet was placed. In conclusion, concentrations between 1 and 50 μg/ml of MAG.PEG would be suitable as drug carriers in this cellular model since no alterations in cell proliferation and viability were observed under the tested conditions. In addition, targeting MAG.PEG to a tumor with a magnet would avoid adverse effects on normal tissues.

CB-04

SPECIALIZED POLYMERASE IOTA MAINTAINS GENOMIC STABILITY BY PREVENTING EXCESS PRIMPOL-DRIVEN DNA REPLICATION

Venerus Arbilla, Sofía [1]; Mansilla, Sabrina Florencia [1]; Bertolin, Agostina [2]; Siri, Sebastián Omar [1]; De la Vega, María Belén [1]; Wiesmüller, Lisa [3]; Gottifredi, Vanesa [1]

1: Fundación Instituto Leloir - Instituto de Investigaciones Bioquímicas de Buenos Aires, Buenos Aires, Argentina; 2: Chromosome Replication Laboratory, The Francis Crick Institute, 1 Midland Road, London NW1 1AT, UK; 3: Department of Obstetrics and Gynecology, Ulm University, 89075 Ulm, Germany

DNA replication must adapt to imperfect templates which are frequent even in genotoxin-free conditions. Replicative polymerases come to a halt when encountering damaged DNA, causing the activation of a

signaling network known as DDR (DNA Damage Response). DDR includes mechanisms of DNA damage tolerance, such as TLS (Translesion DNA Synthesis), which engages specialized polymerases (TLS-Pols) into the replication of imperfect DNA templates. There is a surprising redundancy among TLS-Pols. To study the differential contribution of these polymerases to the DDR, we explored the levels of replication stress triggered by the downregulation of different specialized DNA polymerases under CDDP-treated conditions. As expected, replication stress increased after depletion of almost all TLS-Pols, with the surprising exception of polymerase Iota (Pol ι), whose downregulation caused reduced replication stress, faster DNA replication rates, increased frequency of origin firing and diminished S phase length. Such a unique contribution of Pol ι to the optimization of the DNA Damage Response was linked to the capacity of Pol ι to limit nascent DNA elongation under both stressed and unstressed conditions. In the absence of Pol ι, the faster DNA replication rates diminished the length of S phase with no apparent upregulation of replication stress signals. Yet, these at first sight harmless effects on the DNA replication dynamics were accompanied by augmented levels of under-replicated DNA in M phase, chromosome instability and micronuclei accumulation. M phase abnormalities were rescued when the excess nascent DNA elongation triggered by Pol ι depletion was restrained by simultaneously depleting the repriming polymerase PrimPol. Hence, our results reveal that Pol ι contributes to the maintenance of genomic stability by preventing excess PrimPol-driven DNA replication. In the future, we plan to characterize the mechanism by which Pol ι contributes to the DDR by testing the relevance of each of its domains; as well as which PrimPol domains are critical for driving genomic instability in Pol ι deficient backgrounds.

CB-05

Pharmacological modulation of the circadian clock as a novel strategy to treat glioblastoma

Wagner Paula M; Guido Mario E

CIQUIBIC-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba; Departamento de Química Biológica Ranwel Caputto, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba 5000, Argentina

All living organisms have adapted through evolution to the day/night cycles and most mammals have developed a circadian timing system to adjust their physiology and behavior to the 24 h light/dark cycle. This biological timekeeping regulates diverse cellular processes in organs, tissues, and even in individual

cells, including tumor cells. Nowadays, it is known that the cellular clock is composed of the transcripcional machine and the metabolic/cytosolic oscillator. On the whole, these oscillators work together to maintain the cellular homeostasis. However, modern life with hypocaloric diets, nocturnal shift work, prolonged artificial illumination, etc. have severely altered the temporal organization of behavior and physiological processes and can cause metabolic disorders and an increased risk of cancer. In particular, glioblastoma (GBM) is the most common and aggressive type of brain tumor accounting for 80% of primary malignant tumors of the central nervous system. Due to its great resistance to conventional therapies, new chemotherapeutic approaches are necessary considering the impact of the circadian clock on tumor biology. Previous results from our laboratory evidenced metabolic oscillations in T98G cells from GBM in ROS levels and glycerophospholipids metabolism as well as a marked temporal drug susceptibility when treated with Bortezomib (proteasome inhibitor). In addition, a strong interaction between the metabolic oscillator and the transcriptional circadian machinery was observed in T98G cells that had decreased expression of the molecular activator Bmal1. Here, we investigate how the metabolic/cytosolic oscillator disruption can be used as a novel therapeutic strategy for the treatment of GBM using selective pharmacological inhibitors of casein kinases 1δ and 1ϵ (PF670462) and glycogen synthase kinase 3α/β (CHIR99021). These kinases determined the speed at which the cellular circadian clock runs due to their role in phosphorylation of clock proteins. The results showed a cytotoxic effect of these inhibitors in T98G with an IC50 of 1.5 uM and 8.6 uM when cells were treated with PF670462 or CHIR99021, respectively. Moreover, wound healing assays revealed that the wound closure was delayed in CHIR-treated cells as compared with control cells. Alterations on redox oscillations and temporal susceptibility to Bortezomib were also observed when cells were treated with these inhibitors. On the other hand, disruption of the molecular clock also evidences cytotoxic effect on tumor survival when T98G cells were treated with SR9009 (REV-ERB agonist) or KL001 (inhibitor of CRY degradation).

Overall, our observations suggest that the pharmacological modulation of the cellular circadian clock can be postulated as a novel therapeutic strategy for the treatment of GBM. Understanding and delving into tumor regulation from a chronobiological viewpoint will further help to design new treatments that maximize therapeutic benefits.

CB-06

INHIBITORS OF ROCK KINASES INDUCE MULTIPLE MITOTIC DEFECTS AND SYNTHETIC LETHALITY IN BRCA2-DEFICIENT CELLS

Siri, Sebastian; Mares Ahlers, Candelaria; Gottifredi, Vanesa

Fundación Instituto Leloir, Instituto de Investigaciones Bioquímicas de Buenos Aires- CONICET

Hereditary breast and ovarian cancers are autosomal dominant diseases frequently caused by mutations in the breast cancer susceptibility genes BRCA1 and BRCA2. BRCA1 and BRCA2 are DNA repair genes and their protein products regulate homologous recombination (HR), a repair pathway that processes highly toxic DNA double-strand breaks (DSBs). BRCA2-deficient cancer cells are highly sensitive to poly-ADP-ribose polymerase inhibitors (PARPi) due to the trapping of PARP in DNA. The persistence of those adducts augments double strand break formation, which are selectively toxic in BRCA2-deficient cells. Thus, it is broadly assumed that DNA damage is a prerequisite for BRCA2 synthetic lethality (SL). Challenging such a notion, here we show that inhibiting ROCK kinases in BRCA2 deficient cells triggers SL independently from acute replication stress. Such SL is preceded by enhanced M-phase defects such as anaphase bridges, and abnormal mitotic figures, which were associated with multipolar spindles, supernumerary centrosomes and multinucleation. SL in BRCA2 deficient cells was also triggered by inhibiting Citron Rho-interacting kinase, another enzyme that, similarly to ROCK kinases, regulates cytokinesis. The SL by ROCK and Citron Kinase suggest that BRCA2-deficient cells are sensitive to M phase inhibitors. Supporting such hypothesis, we identified four other druggable protein targets with a function in M phase and whose inhibition also generates SL in a BRCA2 deficient context. Further supporting the link between M phase and the killing of BRCA2-deficient cells, the prevention of mitotic entry by Early mitotic inhibitor 1 (EMI1) depletion promoted survival of BRCA2 deficient cells treated with inhibitors of ROCK kinases. These results suggest that BRCA2 deficient cells are addicted to proteins that control the M phase, revealing a potential therapeutic approach to kill BRCA2-deficient tumors independently from PARPi.

CB-07

Tubulin as a regulator of Phosphatidylserine exposure in red blood cells and its impact on haemorrheology.

Balach, Melisa Micaela; Etcheverry, Micaela; Monesterolo, Noelia Edith; Santander, Verónica Silvina, Rivelli Antonelli, Juan Franco; Ureta Miralla, Lucia Belen; Casale, César Horacio; Campetelli Alexis Nazareno.

INBIAS-CONICET - Departamento de Biología Molecular, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto

Previously, our group showed that in erythrocytes from diabetic and hypertensive patients, plasma membrane tubulin is increased when compared with erythrocytes from normal subjects. At the plasma membrane, tubulin inhibits several P-ATPases, including lipid flippases which results in increased phosphatidylserine (PS) exposure. Since in normal erythrocytes, the progressive PS exposure becomes a senescence signal that leads to macrophage-mediated phagocytosis during eryptosis, we set out to determine whether the increase in membrane tubulin influences erythrocytes lifespan and whether this effect contributes to the development of anemia. For this, we performed an experiment with rats where the content of erythrocytes membrane tubulin was naturally altered ((Wistar Kyoto strain (WK, normotensive), diabeticized WK (streptozotocin treatment) and SHR (spontaneously hypertensive rats)), or modified pharmacologically by Taxol or Tyrosine treatment. During a period of 7 weeks, we monitored the erythrocyte half-life time, erythrocyte PS exposure and the reticulocyte count in peripheral blood, among other parameters. We found that in erythrocytes from SHR, diabetic, as well as in Taxol-treated WK animals, the PS exposure was higher than in WK and Tyrosine-treated SHR rats. Accordingly, the erythrocyte half-life was shorter in SHR, diabetics and Taxol-treated WK than erythrocytes from WK rats, showing an inverse correlation between PS exposure and erythrocyte half-life time. Furthermore, those animal groups with increased PS exposure and shortened erythrocyte half-life time displayed increased reticulocyte count. The blood count displayed a slight decrease in RBC count, hematocrit and haemoglobin values, for Taxol-treated WK, suggesting a mild anemia only in this animal group. The case of Tyrosine-treated SHR rats deserve a separate analysis. For this animal group we found a massive PS exposure peak at week 11 post birth, and this peak was time correlated with an increased death rate and an increase in reticulocyte count, suggesting that Tyrosine accelerates the clearance of a population of red blood cells. Our hypothesis is that this population could be related to erythrocyte age and experiments are underway to probe it.

As a consequence of the accelerated red blood cells clearance, the spleen can increase its size. We looked at the spleen/body weight ratio and found a marked increase in the size of the spleen in Taxol-treated animals and a slight increase in SHR rats; while in Tyrosine-treated SHR rats, the spleen size was much similar to that of WK rats, which confirm the spleen involvement in the erythrocyte clearance. These results suggest that, in red blood cells, plasma membrane tubulin can regulate the lifespan of these cells through a mechanism that involves the regulation of lipid flippases.

CB-08

Use of Translesion DNA synthesis inhibition as a sensitizer of tumor cells

Okraine, Yiovana Verónica; de la Vega, María Belén; Mansilla, Sabrina; Gottifredi, Vanesa

Fundación Instituto Leloir-IIBBA-CONICET

DNA replication is continuously challenged by endogenous and exogenous damage. To ensure a fully duplicated genomic material, DNA replication relies on DNA damage auxiliary processes such as translesion DNA synthesis (TLS). TLS is a DNA damage tolerance mechanism that uses DNA specialized polymerases to replicate through damaged DNA, therefore promoting genomic stability and cell survival. While normal cells benefit from TLS activation, cancer cells exploit TLS to escape from cancer therapy (e.g. overexpression of specialized Pol eta (η) in tumors cells that resist genotoxins that cause augmentation of replication barriers has been reported). Hence, the identification of tools to down modulate TLS may provide valuable information for the improvement of therapies that challenge DNA replication. Our group has demonstrated that the cyclin kinase inhibitor p21 is a potent TLS inhibitor. Recently, we identified the minimal region of p21 (PIRp21) which binds to the proliferating nuclear antigen (PCNA) as the minimal region. We combined genotoxins that induce accumulation of replication barriers by different mechanisms (UV radiation, cisplatin and checkpoint inhibitors) with the expression of the PIRp21 in tumor cells. In such conditions, the PIRp21 efficiently impairs the recruitment of specialized pols to replication factories, increasing cell death and genomic instability. Given that the PIRp21 should act as a global inhibitor of all specialized polymerases, we compared the impact of the PIRp21 and the knock down (KD), of Pol η expecting to reveal more extreme phenotypes when using the PIRp21. Surprisingly, pol eta KD caused a stronger arrest in S phase and a replication stress, which was more extreme than the PIRp21. On the other hand, the genomic instability and cell death was higher after PIRp21 expression than after Pol eta KD. The complexity of the observations led us to consider the benefits of comparing the effect of the PIRp21 and the PIR-Pol η, a small version of Pol η that conserves the capability of binding to PCNA. Such an analysis will shed light into TLS inhibition strategies that may sensitize tumor cells to genotoxins.

CB-09

URSOLIC ACID NEGATIVELY MODULATES THE LIPID DROPLET METABOLISM, A KEY COMPONENT OF THE ROTAVIRUS INFECTION

Tohmé, M. Julieta [1, 2]; Converti, Ayelen [1]; Caruso, Benjamín [3]; Wilke, Natalia [4]; Colombo, M. Isabel [1]; Delgui, Laura R. [1, 5]

1- IHEM, CONICET- UNCuyo 2- FFyB, UJAM 3- IIByT- CONICET. FCEFyN, UNC 4- CIQUIBIQ- CONICET. FCQ, UNC. 5- FCEN, UNCuyo

Rotavirus (RV) is one of the leading causes of acute severe gastroenteritis, mainly affecting children under five years of age. Severe cases are associated with dehydration and can lead to the death of children. Even though there are RV vaccines available that have been demonstrated to be effective for RV immunization, the poor socioeconomic conditions and the limited access to public health, among other factors, are responsible for the lower immunization rates in low-income countries, which are also the most affected.

The treatment of RV gastroenteritis is based on avoiding dehydration; this is why the study of potential anti-RV is needed. We demonstrated that ursolic acid (UA), a natural triterpenoid, exerts anti-RV effect in vitro. Then, we observed that the anti-RV effect of UA was due to the negative modulation of the lipid content in the infected cells. Lipid droplets (LDs) are ubiquitous organelles composed of a neutral lipid core, surrounded by a phospholipid monolayer associated with several proteins that play a key role in the LDs metabolism. Also, LDs are crucial organelles involved in the RV replication cycle. When infected with RV, we observed a peak of accumulation of LDs at 2 hours post-infection (h p.i.). But, the treatment with UA significantly reduced the accumulation of LDs, and the peak at 2 h p.i. did not occur. Concomitantly, the number and size of RV viroplasms (VPs) formed in UA-treated conditions were significantly lower and the viral progeny abrogated.

To elucidate the mechanism of action of the UA-induced negative modulation of LDs, we approached several studies. We implemented biophysical techniques based on the formation of lipid lenses that resembled LDs, to determine the effect of UA on lens formation. Thereby, we demonstrated that UA interferes with LDs biogenesis. Also, intending to analyze LDs degradation, we observed that the treatment of the cells with UA reduced the number and size of LDs by inducing lipolysis.

LDs can be degraded by two interdependent types of autophagy sequentially acting: chaperone-mediated autophagy (CMA) and macroautophagy. The CMA is responsible for degrading the proteins that surround LDs, perilipin 2 (PLIN2) and 3, and then the macroautophagy degrades the lipid content of the LDs. We demonstrated that UA favours CMA, inducing the accumulation of the phosphorylated form of PLIN2, which also loses its distribution associated with LDs. Finally, we observed that LC3 II, a key marker of macroautophagy, was significantly accumulated in UA-treated cells at 4 h p.i. and that LDs and LC3 II co-colocalized, suggesting that UA induces CMA and macroautophagy, both processes involved in LDs degradation.

Altogether, our results indicate that UA negatively interferes with LDs biogenesis and favours LDs lipolysis, driving to a reduction in the number and size of LDs, crucial organelles for RV replication, then conducting to a reduction in VPs formation and finally lowering a new viral progeny production.

CB-10

SHORT NUCLEOTIDE VARIANTS ON G-QUADRUPLEXES AS A CAUSE OF ONCOGENES TRANSCRIPTIONAL EXPRESSION VARIATIONS.

Piga, Ernesto.; Bezzi, Georgina.; Lorenzatti, Agustin.; Binolfi, Andres.; Calcaterra, Nora.; Armas, Pablo.

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G-quadruplexes (G4s) have been proposed as transcriptional regulatory elements originally and majorly described for oncogenes. On the other hand, genomic scale association studies by massive DNA sequencing revealed that short nucleotide variants (SNVs) associated with human diseases are frequently found near transcription start sites, within proximal promoter regions (PPRs). The goal of this work was to identify SNVs overlapped with G4s forming sequences (sG4s) described as transcriptional regulators of oncogenes, that may affect G4 folding (hereafter called SNV-sG4s). First we performed a bioinformatics analysis using Ensembl database to identify the SNV-sG4s found in G4s described as transcriptional regulators of several oncogenes. For each reference sequence (WT) we generated a collection of sequences containing each variant and a mutant sequence (M) with no sG4 (unable to form G4). Then we used several G4 folding predictors to identify those SNV-sG4s that may affect G4 folding or stability. Results allowed us to choose some SNV-sG4s for further in vitro analyses. Circular Dichroism (CD) spectra, qPCR stop assays, CD melting assays and 1D 1H NMR spectra indicated that some SNV-sG4s cause quantitative or qualitative spectral changes probably related with variations in G4s stabilities. For the cases of SNV-sG4s that produced significant structural variations, we cloned the sG4 in their WT and variant versions into pGL3 promoter vector. Transfection into HEK293 cells revealed that SNVs alter luciferase reporter activity. Then, for c-MYC, one of the best described genes controlled by the G4s analyzed, we analyzed the effect of SNVs in the context of the promoter by cloning fragments of the promoter that contain the sG4 and their respective variant versions, controlling the expression of the firefly luciferase reporter gene in the pGL3-Basic plasmid, and transfected HEK293 cells. The evidence obtained allows us to infer that the presence of SNV-sG4s that alter G4 formation could change the

expression level of the reporter gene regulated by the c-MYC promoter. Moreover, to analyze the effect of SNVs in the context of the promoter of c-MYC, we cloned fragments of the promoter that contain the sG4 and their respective variant versions, controlling the expression of the firefly luciferase reporter gene in the pGL3-Basic plasmid, and transfected into HEK293 cells. The evidence obtained allows us to infer that the presence of SNV-sG4s that alter G4 formation could change the expression level of the reporter gene regulated by the c-MYC promoter. Finally, for c-MYC, we evaluated in vitro the differential interaction of SNV-sG4s with CNBP, a protein described binding to the sG4 within c-MYC PPR and to unfold G4s, and with pyridostatin (PDS), a G4 stabilizing ligand. For CNBP, we performed electrophoretic mobility shift assay (EMSA) and CD with interval scan spectra, inferring that the decrease in the stability and formation of the G4 generated by the SNV-sG4 could generate the differential binding and G4-unfolding activity of CNBP. For PDS, we carried out CD melting and qPCR stop assays, concluding that the destabilizing effect generated by SNV-sG4 could be reversed with the use of ligands that increase the stability and formation of G4s. Results suggest that SNV-sG4s that alter G4 folding may be the cause of differential expression of oncogenes and should be considered as a novel molecular etiology mechanism for the predisposition or establishment of cancer.

CB-11

EFFECT OF OLIGODEOXYNUCLEOTIDE IMT504 ON MURINE β-CELLS

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IMT504 is an oligodeoxynucleotide (ODN) that exerts regenerative properties. We demonstrated that the presence of IMT504 significantly promotes metabolic improvement in the diabetic condition in diverse animal models. Using diabetic IMT504-treated animals we showed a different gene expression profile and protective effects against apoptosis in pancreatic cells. Then, we investigated if these effects were due to its direct effects on those cells. In the same way, IMT504-treated in vitro β-cells showed a different gene expression profile of genes related to their functionality and a modification on glycogen synthase kinase-3 (GSK3) phosphorylation, a protein that participates in the regulation on β-cell function. Based

on these results, here we evaluated the ODN internalization and protective effects against apoptosis in a pancreatic β-cell line.

For internalization of the ODN, we analyzed the IMT504-Texas Red incorporation into Beta-TC-6 cells by immunofluorescence at different time points. At 5 and 15 min post-internalization (p.i.) the IM504 was found both, associated to the cytoplasmic membrane and in the cytoplasm. At 30 and 60 min p.i., when the percentage of cells with IMT504 reached the maximum, IMT504 was almost exclusively in the cytoplasm of the cells. Complementary, we used flow cytometry to monitor IMT504 internalization. We showed that 69% of the cells treated with IMT504-Texas Red for 60 min incorporated the ODN. The cytoplasmic destiny of the ODN was confirmed by the treatment of the cells with methanol, which promotes the removal of soluble proteins found in the cytoplasm enabling us to differentiate cytoplasmic proteins from those anchored to membranes. We demonstrated that this ODN remains as a soluble molecule in the cytoplasm.

On the other hand, the protective effects of IMT504 against H2O2-induced cell apoptosis was analyzed in MIN6B1 cells. We observed that the IMT504 reversed the H2O2-induced apoptosis in a concentration dependent manner. Apoptosis is regulated by Bcl-2 proteins, comprising pro-apoptotic and anti-apoptotic members. In an effort to better understand the effect of IMT504 in H2O2-induced apoptosis, we evaluated the expression of apoptosis regulator genes Bcl2 and Bax. We observed that Bcl2, an anti-apoptotic factor, was unaltered. In contrast, the pre-treatment of the cells with IMT504 attenuated the increase of Bax induced by the exposition of the cells to the apoptotic stimulus. Likewise, the Bax/Bcl2 ratio was significantly decreased after IMT504 treatment.

Our results indicate that IMT504 internalizes shortly after contacting the β-cells, reaching the cytosol of the cells. Furthermore, IMT504 lead to the inhibition of H2O2-induced β-cell apoptosis suggesting the IMT504 treatment as a promising strategy in the prevention/amelioration of diabetes.

CB-12

ACYL-COA SYNTHETASE 4 MODULATES MITOCHONDRIAL FUNCTION AND METABOLISM IN BREAST CANCER CELLS.

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Acyl- CoA synthetase 4 (ACSL4) is an enzyme that catalyzes acyl-CoA synthesis from long chain fatty acid, being arachidonic acid its preferred substrate. In breast cancer, ACSL4 promotes tumor aggressiveness by increasing migration, proliferation and invasion. In this context, there is evidence of dysregulation of mitochondrial function, mitochondrial mass and subcellular spatial organization in cancer. Also, it is known that alteration of energy metabolism allows tumor cells to survive and spread even in challenging conditions. In MCF-7 breast cancer cells, a previous bioinformatic analysis showed that ACSL4 overexpression could modulate mitochondrial master regulatory genes involved in energy and respiratory metabolism. The aim of this work is to determine whether mitochondrial function and metabolism are modulated by ACSL4 in breast cancer cells. For this purpose, mRNA levels of genes related to mitochondrial function such as NRF-1/2, UCP2 and ANT1 were evaluated in MCF-7 overexpressing ACSL4 cells by qPCR. mRNA levels of these genes were significantly increased by ACSL4 overexpression related to control cells. Moreover, protein levels of mitochondrial complex III, VDAC1 and nuclear NRF-1 were significantly increased by ACSL4 overexpression in MCF-7 cells with respect to control cells. Respiratory and glycolytic function were studied using the Seahorse XF Cell Mito Stress Test and GlycoStress Test respectively. We observed a significant increase in oxygen consumption rate and basal respiration, maximal respiration, proton leak and respiratory reserve capacity in MCF-7 cells overexpressing ACSL4. Glycolytic function analysis demonstrated a significant increase in several parameters induced by ACSL4 such as extra acidification rate, non-glycolytic acidification, glycolysis and glycolytic capacity. On the other hand, results showed a significant decrease in the percentage of glycolytic reserve in MCF-7 overexpressing ACSL4. These results are consistent with the increase in mitochondrial activity induced by ACSL4 in this cellular model observed by MitotrackerRed staining. Mitochondrial mass was measured by MitotrackerGreen staining and analyzed by flow cytometry. Results showed a significant decrease of this parameter in MCF-7 cells that overexpress ACSL4 related to control cells. These results suggest that ACSL4 stimulates a greater mitochondrial turnover and a better response to high energy demand situations which in turn provides to the cells a greater capacity for invasion migration and proliferation. Thus, ACSL4 could not only confer an adaptive advantage to tumor cells by inducing glycolytic metabolism that favors tumor development but also could protect mitochondria against oxidative stress increasing antioxidant factors and promoting the expression of genes involved in mitochondrial biogenesis. Altogether, the results of this work extend the knowledge about the role of ACSL4 in respiration and mitochondrial function in breast cancer cells.

CB-13

Characterization of the immune response against recombinant Tacaribe arenaviruses as new vaccine candidates to prevent Argentinean hemorrhagic fever

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Junín (JUNV) mammarenavirus is the causative agent of Argentine hemorrhagic fever (AHF), a severe human disease endemic to agricultural areas in Argentina. The current approved vaccine, live-attenuated JUNV strain Candid#1, is effective against AHF, nevertheless, part of the population at risk is excluded from vaccination. Tacaribe (TCRV) is a naturally attenuated mammarenavirus and, as it is phylogenetically and antigenically highly related to JUNV, it stands as a possible vaccine candidate against JUNV. We have previously developed a reverse genetic system that allowed us to successfully rescue infectious recombinant TCRV (rTCRV). Based on this, we generated mutations in the genomic 5' non coding region, expected to attenuate viral propagation (rTCRV-NCR), and in the Exonuclease domain (ExoN) of the viral nucleoprotein (NP), predicted to counteract the IFN-I cell response (rTCRV-NCR-ExoN). We infected human dendritic cells (hMoDCs), the earliest mammarenavirus cell target, and observed expression of CD86 and HLA-DR activation markers at 24 and 48 hours post infection (h.p.i) with wild type TCRV and rTCRVs. Notably, rTCRV-NCR-ExoN produced the highest degree of activation, similar to that of Candid#1. No IFN-I release was observed from supernatants of infected hMoDCs with wild type TCRV, rTCRV and rTCRV-NCR; nevertheless, it was significantly increased in rTCRV-NCR-ExoN infected cells, suggesting that, as expected, the ExoN domain of NP counteracts the innate immune response. There

were no differences in viral titers or in intracellular viral RNA accumulation, determined by plaque assay and RT-qPCR, respectively.

We also characterized the IFN-I response in lung epithelial cell line A549 and observed that, as for hMoCDs, only rTCRV-NCR-ExoN and Candid#1 vaccine strain produced a high IFN-I peak in cell supernatants at 24 h.p.i. The IFN-I pathway was also monitored intracellularly, by using a luciferase reporter plasmid under the control of an IFN-inducible promoter. We observed high levels of the reporter gene expression only in rTCRV-NCR-ExoN and Candid#1-infected cells. These findings corroborated that rTCRV-NCR-ExoN is unable to counteract the IFN-I signaling pathway.

Lastly, we evaluated virulence of these vaccine candidates in a mouse infection model. Groups of two-day old Balb/cJ mice were inoculated intracranially with serial dilutions of wild type TCRV and each of the rTCRVs, and mortality was monitored daily over a 21-day period. rTCRV and mutant rTCRV-NCR were highly virulent causing almost 100% mortality at a dose of 1 PFU. rTCRV-NCR-ExoN induced 50% mortality, indicating that the NCR and ExoN mutations associated with a considerable degree of viral attenuation in vivo. The control groups of mice inoculated with medium or left untreated survived through day 21. In conclusion, we developed a recombinant mutant TCRV that demonstrated to be attenuated both in vitro and in vivo. This work may contribute to the development of safe vaccines to prevent hemorrhagic fevers produced by mammarenaviruses.

CB-14

Role of nuclear O-GalNAc glycosylation in CHO cells

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Epigenetics represents chemical and covalent modifications that can take place on DNA and nuclear proteins without modifying the sequence of these molecules, which have a key role in the physiology of DNA, such as in regulation of gene transcription. Previously, we and other groups around the world have shown that O-GalNAc glycosylation, one of the most important post-translational modifications, is occurring in proteins of the cell nucleus, in addition to Golgi apparatus. Using CHO ldlD cells as an experimental model, which does not have the ability to synthesize UDP-GalNAc, we study the nuclear role

of O-GalNAc glycosylation in CHO cells. The presence of polypeptide GalNAc-transferase 3 isoform was observed in the cell nucleus by Z stack methodology (IF). Using VVL lectin, we demonstrated terminal Tn (GalNAcαSer/Thr) residues with nuclear localization. O-GalNAc glycan sites of CHO nuclear proteins were identified in key nuclear proteins of cell physiology. Through mass spectrometry (proteomics) we were able to detect that CHO ldlD with enhanced nuclear O-GalNAc glycosylation (as consequence of GalNAc in cell culture) modified the expression level of multiple cellular proteins. These results are a significant contribution on insight the nuclear O-GalNAc glycan roles in the regulation of protein expression. In addition, the nuclear O-GalNAc glycosylation affected the rate of cell proliferation. Nuclear proteins of CHO cells that are modified by terminal O-GalNAc residues (such as histones and transcription factors) have already been described before, so this change in protein expression may be due to control of gene transcription mediated by O-GalNAc glycosylation.

Plants

PL-01

MBD1, MBD2, MBD3 and MBD4 proteins participate in abiotic stress responses

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Plants are sessile organisms so they are exposed to different unfavorable environmental conditions, such as high levels of ultraviolet B radiation, salinity and drought conditions. These environmental conditions cause different damages to macromolecules such as proteins, RNA and DNA. In particular, DNA damage must be repaired for survival.

In eukaryotes, DNA is organized in a complex and dynamic structure, which is chromatin. The nucleosome is the basic unit of chromatin and is composed of a histone octamer wrapped by 147 base pairs of DNA. The chromatin structure is flexible to allow the processes of transcription, replication, recombination and DNA repair to take place. Therefore, histones are subject to multiple posttranslational modifications, which include acetylation, methylation, ubiquitination and phosphorylation. One of these regulations that alters chromatin affinity is DNA methylation; in plants and animals, the cytosines are modified by the addition

of a methyl group. DNA methylation is usually associated with a repressed chromatin state. There are specialized methyl cytosine binding domain (MBD) proteins that have the ability to interpret these modifications. MBD proteins act in concert with chromatin remodeling complexes, histone deacetylase proteins and histone methyltransferases to establish repressive chromatin and play a biological role in genomic stability. Previous studies from our laboratory showed that maize plants deficient in the expression of one MBD protein, MBD101, presented a laxer chromatin, showing a higher sensitivity to UV-B radiation with increased DNA damage after a treatment. In Arabidopsis there are 5 proteins homologous to MBD101: MBD1, MBD2, MBD3, MBD4 and MBD12. Apart from the conserved MBD domain, these proteins also carry a CW-type Zinc Finger (CW-Zf), and thus we refer to this subfamily as CW-MBDs. Therefore, the aim of our work was to study the role of MBD1, MBD2, MBD3, MBD4 proteins in the response to UV-B radiation and high salinity concentrations. We observed that *mbd1*, *mbd2*, *mbd3* and *mbd4* mutants and *mbd1 mbd2 mbd4* and *mbd2 mbd3 mbd4* triple mutants showed more DNA damage compared to WT Col-0 plants after exposure to UV-B radiation. However, plants deficient in MBD proteins showed a lower number of dead cells after exposure to UV-B radiation. In addition, a similar response was observed when *mbd1*, *mbd2*, *mbd3* and *mbd4* mutants and *mbd1 mbd2 mbd4* and *mbd2 mbd3 mbd4* triple mutants were exposed to salt stress. Hence, the mutants *mbd1, mbd2, mbd3, mbd4* and the triple mutants *mbd1 mbd2 mbd4* and *mbd2 mbd3 mbd4* showed increased resistance to UV-B radiation and salt stress conditions. Thus, MBD proteins under study act in response to UV-B and salt stress.

PL-02

Unravelling the contribution of mesophyll and bundle sheath chloroplasts to increased stress tolerance

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Maize is the crop with largest global production, and its products are important as food and energy supply, being a major source of economic development. However, it is extremely susceptible to stress,

which ultimately limits the corn yield. This species was domesticated in the tropical regions of southern Mexico and has been spread to habitats with dramatically different environmental conditions, in many cases, adverse to its development. Given its economic relevance, improving stress tolerance in maize could represent a major achievement in agricultural terms. In maize, the CO_2 fixation is divided into two cell types (C4 photosynthesis). The primary assimilation occurs in the mesophyll (M) cells, where the chloroplasts generate ATP and NADPH principally by the linear electron flow. On the other hand, the classic C3 photosynthesis takes place in the chloroplasts of the bundle sheath (V) cells, performing only cyclic electron transport that produces only ATP, nor NADPH, the latter being generated by alternate enzymatic ways. Previous research from our laboratory has demonstrated that the introduction of a constitutively expressed cyanobacterial flavodoxin (Fld) directed to chloroplasts resulted in the generation of tobacco plants with increased tolerance to multiple stress sources. The protective function of Fld has been associated to its interaction with the photosynthetic electron transport chain at photosystem I level, sharing redox properties with isofunctional ferredoxin. Application of Fld technology to a C4 species is still uncharted territory. In order to elucidate the contribution of each cell type to the stress tolerance and to determine if Fld can productively interact with cyclic and/or linear electron transport as it does in C3 chloroplasts, we generated transgenic maize plants expressing Fld specifically in the chloroplasts of V (Zmv-pfld, for Zea mays V plastidic Fld) or M cells (Zmm-pfld, for Zea mays M plastidic Fld).

We had already set up the conditions for the isolation of maize fractions enriched in V and M chloroplasts, demonstrating the presence of Fld expression in V and M chloroplasts in *Zmv-pfld* and *Zmm-pfld* genotypes, respectively, confirming tissue-specific location. Besides, application of oxidative conditions by paraquat, which act as an alternative electron acceptor from photosystem I generating superoxide, showed lower electrolyte leakage for *Zmm-pfld* in comparison to control and *Zmv-pfld* genotypes. Furthermore, *Zmm-pfld* plants subjected to extreme drought in soil also exhibited an improved tolerance compared to *Zmv-pfld* and null segregants lines. To sum up, our results indicate that presence of Fld in M chloroplasts provides an advantage when facing adverse situations. We expect to determine if the lack of Fld effect when expressed in V chloroplasts is due to a null interaction with the cyclic electron transport, and in general to thoroughly establish the features of the conferred tolerance.

PL-03

A complex interplay between the transcription factors AtHB23, AtPHL1 and AtMYB68 fine tunes Arabidopsis lateral and primary root development and adaptation to salinity

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Plant adaptation to the environment is a fine-regulated process involving different biomolecules and modulation levels. Roots are the organs that firstly sense changes in the soil and accordingly accelerate or arrest their primary or lateral growth and development. Among the biomolecules participating in root development and adaptation, transcription factors (TF) play crucial roles, usually activating or repressing their targets and concomitantly entire signal transduction pathways. AtHB23 is a member of the HD Zip I family, described as a negative regulator of lateral root initiation and an activator of the salinity response. A yeast- two-hybrid (Y2H) screening using an Arabidopsis TF library as prey and AtHB23ΔAHA as bait revealed the interaction with two TFs belonging to the large MYB family: AtMYB68 and AtPHL1. In this work, we investigated the interplay between AtHB23, AtPHL1, and AtMYB68 and their impact on root development. Firstly, we corroborated the interaction between AtHB23 and both MYB TFs in independent yeast assays and plants by BiFC. Notably, both partners also interact between them. By using transgenic plants carrying the promoters driving the GUS reporter gene, we analyzed their expression patterns. The three TFs coincided in specific cell groups and developmental stages in the root system. AtMYB68 and AtHB23 exhibited a similar expression pattern in secondary and tertiary LRs and the base of the secondary LR primordium (LRP), whereas AtPHL1 was expressed in the primary root vascular system and tip, more precisely in the columella cells. MYB68 silencing did not affect primary root length but significantly diminished LRP density, the opposite phenotype to that shown by *amiR23* plants. The *phl1* mutants had longer primary roots and PHL1 overexpressors the opposite. On the other hand, *phl1* mutants did not show significant differences in LR neither in LR and a similar scenario was observed in MYB68 overexpressors. Relative total LR length diminished in a*miR68* plants and significantly augmented in *phl1* mutants, like in *amiR23* plants. Under salinity conditions, *myb68* mutants and AtPHL1 overexpressors had fewer starch granules, the same characteristic shown by *amiR23* plants, while *phl1* mutant and AtMYB68 overexpressor plants did not exhibit significant differences compared to WT. In agreement with this scenario, testing salinity survival, *phl1* mutant and AtMYB68 overexpressor plants tolerated the salt condition just as WT, whereas PHL1 overexpressors and *amiR68* silenced plants were more sensitive to this stress, the same phenotype seen in *amiR23* plants. These behaviors involved the modulation of the auxin transporter AUX1. Altogether, the results suggested that AtPHL1 interacts with AtHB23 avoiding the latter to fulfill its function. In contrary, AtMYB68 may be helping AtHB23 to better respond to salinity conditions. The evaluation of the phenotype of crossed AtHB23, AtPHL1, and AtMYB68 mutant and overexpressor plants supported this complex model.

PL-04

Regulatory networks established by TCP and GLK transcription factors during Arabidopsis seedling development

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After germination, light exposure promotes cotyledon opening and expansion and chloroplast development in a process called de-etiolation. Golden2-like (GLK) proteins are members of the GARP family of MYB transcription factors involved in chloroplast development, immunity and senescence. TEOSINTE-BRANCHED1/CYCLOIDEA/PCF (TCP) proteins constitute a family of transcription factors that control various processes of plant development such as embryogenesis, germination, and morphogenesis of leaves and flowers. They also function in plant immunity and hormonal signaling. They can be grouped into two major classes, I and II. In Arabidopsis, the TCP family consists of 24 members, 13 belonging to class I and 11 to class II, whereas GLK genes exist as pairs, GLK1 and GLK2. In previous studies, we found that GLK1 interacts with several class I TCPs. Moreover, we reported that the class I protein TCP15 and GLK1 are jointly required for cotyledon opening and the induction of cell expansion and photosynthesis associated genes during de-etiolation of Arabidopsis seedlings. In this study, we analyzed the relationship between GLK transcription factors and class II TCPs during de-etiolation. We found that mutants in a class II TCP from Arabidopsis shows a delay in the opening of cotyledons when exposed to light and decreased expression of genes involved in cotyledon opening and chloroplast development, similarly to observations made in GLK1 and TCP15 mutant plants. However, protein-protein interaction between GLK1 and class II TCP members was not detected in yeast-two-hybrid assays. Further molecular and genetic analyses of loss-of-function mutants and gain-of-function constitutive overexpression transgenic lines for these genes revealed that they act by regulating the expression of similar groups of light-regulated genes. Moreover, we identified a regulatory network where these transcription factors interconnect to promote the opening of the cotyledons and the establishment of the photosynthetic apparatus. Taken together, this study provides new evidence of the concerted function of the TCP and GLK families during Arabidopsis seedling development and suggests that class I and class II TCPs have incorporated into the regulatory network affected by GLK transcription factors acting at different levels.

PL-05

An oomycete effector alters plant immunity and development by manipulating auxin signaling

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The biotrophic oomycete *Hyaloperonospora arabidopsidis* has co-evolved with its host, *Arabidopsis thaliana*. The study of their interaction contributed to postulate the "arms race" hypothesis between plants and pathogens. According to it, pathogens evolutionarily develop effectors that can suppress the first layer of plant immune responses induced by their conserved molecules or PAMPs, which is known as PAMP-Triggered Immunity (PTI). Similarly, plants co-evolved resistance genes/proteins that recognize these effectors, thus triggering Effector-Triggered Immunity (ETI). Interestingly, some effectors are also able to suppress ETI, but can become recognized by the plant and so on. Plant proteins involved in growth and development are among the host targets of pathogen's effectors. It has been proposed that certain pathogens can manipulate the growth-defense trade-off on their own benefit, tampering hormonal pathways to suppress PTI and maintain compatibility with the host. We have previously observed an activation of the auxin responsive *DR5:GUS* reporter gene in *Hpa* infected plants, suggesting that the auxin signaling pathway was induced during a compatible interaction. Thus, we set up to investigate the role of an *Hpa* effector (HaRxL106) that, when expressed in an estradiol-inducible manner in the *DR5:GUS* background, partially recapitulated the phenotype observed with the whole pathogen. Here we describe our findings regarding this effector: 1) It interacts with a component of the Arabidopsis auxin-signaling pathway (IAA11) as we could confirm via BiFC and yeast-two-hybrid assays; 2) The interaction of IAA11 is stronger with the C-terminal part, than with the N-terminal of HaRxL106; 3) Upon *Hpa* compatible interaction, auxin responsive genes are induced; 4) The overexpression of the effector in wild type background, as well as in *iaa11* mutant plants, generated an altered growth phenotype with elongated hypocotyls and petioles and a curved narrow leaf lamina, reminiscent of the shade-avoidance syndrome displayed by plants experiencing persistent shadow; 5) *iaa11* mutant plants are more susceptible to *Hpa* and to the bacteria *Pseudomonas syringae* DC3000 than wild type plants. Based on the above-mentioned results, we propose a working model where HaRxL106 negatively influences the repressor activity of IAA11, probably releasing one or more ARF transcription factors that would then induce the

transcription of auxin responsive genes. This activation of auxin signaling apparently contributes to the enhancement of the susceptibility to *Hpa*.

PL-06

Identification of molecular mechanisms associated with increased yield caused by mechanical treatment in *Arabidopsis thaliana*

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Arabidopsis thaliana genotypes that yield significantly more than others exhibit large stem diameters and an increased number of vascular bundles. These characteristics can be obtained by adding a 1.5 g hanging weight placed at the apex of a 4 cm height Arabidopsis floral rod for 48 h. The increase in the number of vascular bundles happens by duplication of the existing ones from the apex through the base, like auxin flow, similarly to branching from the floral stem. Auxin and its carriers are essential for the response to the hanging weight treatment and new bundle generation.

To investigate the putative role of brassinosteroids (BS) in this process, we initially evaluated mutants in genes involved in the BS signaling pathways. The *bes1* and *bzr1* did not respond to the weight treatment, and in agreement, the exogenous addition of brassinazole (an inhibitor of BS biosynthesis) to WT plants interrupted the response.

BCR1 is a negative regulator of branching and a positive regulator of the CLE44 peptide expression, which participates in the proliferation and differentiation of procambium cells. BCR1 is regulated by auxin, BS, and strigolactones (SL). The weight treatment on *brc1*, *cle44*, and *max4* mutants did not cause any effect. The same scenario was observed when the sparsely branched ecotype of Arabidopsis, Zurich (Zu-0), was weight treated. In agreement, none of these genotypes increased seed production; on the contrary, CLE44 mutant showed a decrease in seed yield. The better performance showed by WT plants after weight treatment can be explained by an increase in photosynthates transport from source to sink tissues. The expression of genes encoding enzymes participating in starch synthesis and degradation, as well as sucrose transporters, was induced after treatment. In addition, treated plants had a higher concentration

of lipids in seeds, while the opposite occurred in *cle44* mutants. Roots were also affected by weight treatment, increasing the primary root diameter and the number of vascular bundles, the total biomass, and the sucrose transport by phloem tissue. Altogether the results indicate that the weight treatment triggers a complex process involving AUX, BS, and SA phytohormones. Moreover, we can conclude that molecular mechanisms involving branching play a crucial role in this event, and CLE44 has a critical role in this response which needs further investigation.

PL-07

Mitochondrial genome recombination and repair in plant somatic hybrids

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Recombination and repair of plant mitochondrial genomes are not well understood. Several re-combination pathways have been inferred in studies of Arabidopsis mutants of nuclear factors involved in mitochondrial recombination surveillance. Somatic hybrids between phylogenetically distant species offer a remarkable model to study mitochondrial genome recombination in wild type plants. Previously, we reported highly chimeric mitogenomes in two somatic hybrids between the Solanaceae *Nicotiana tabacum* and *Hyoscyamus niger* unrevealing that most rearrangements occurred by homologous re-combination and that similar regions contributed by both parents are preferentially lost. However, the number of recombination events inferred from assembled plant mitogenomes is underestimated be-cause only a subset of the co-existing genomic and subgenomic arrangements are represented. In this study, we developed a bioinformatic pipeline that infers recombination events by analyzing mapping patterns and genotype switches using paired-end read information without assembling the mitogenome. We analyzed the recombination map of a somatic hybrid produced by chemical protoplast fusion be-tween *N. tabacum* and another Solanaceae, *Physochlaina orientalis* and re-analyzed the two somatic hybrids between tobacco and *H. niger.* We identified between 45 and 107 homologous recombination (HR) events in the hybrids. We were also able to infer the molecular mechanism of HR and found that the majority involved the Break-induced Replication (BIR) pathway. Interestingly, the information offered by the paired end reads showed that independent events frequently occur in the same regions of the recombining tracts within and across somatic hybrids, suggesting the existence of

recombination hotspots in plant mitogenomes. In addition, three non-homologous recombination events were also detected in two of the hybrid mitochondria. In conclusion, BIR is the main pathway of mitogenomes replication/recombination following protoplast fusion in somatic hybrids. In addition, the somatic hybrid model presumably mimics the process after foreign mitochondria enter the host cell, suggesting that foreign DNA is integrated in plant mitogenomes through this pathway.

PL-08

Contribution of proline metabolism to the control of cellular redox homeostasis under biotic stress in Arabidopsis

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To survive under stress conditions, plants must activate stress-specific signaling pathways that generate biochemical, physiological and morphological changes allowing adaptation. Proline (Pro) metabolism influences the cellular redox homeostasis in different ways. Pro dehydrogenase (ProDH) is a mitochondrial enzyme regulating the limiting step in Pro catabolism. In animals, this enzyme affects lifespan extension, apoptosis, tumor suppression, and cell survival. In Arabidopsis, ProDH promotes ROS production during activation of the hypersensitive response. Arabidopsis ProDH is encoded by two genes, *AtProDH1* and *AtProDH2* that are induced during plant-pathogen interactions. Both isoforms potentiate the activity of the plant plasma membrane NADPH oxidase, also known as respiratory burst oxidase homolog (RBOH). Under biotic stress conditions, RBOHD is responsible for apoplastic ROS (aROS) accumulation triggered by flg22 (22 amino acid peptide derived from bacterial flagellin) treatment. We are investigating how mitochondrial ProDH affects the activity of plasma membrane RBOHD. In particular, we are studying whether this is mediated by alterations of cytosolic redox homeostasis. We used a sensitive fluorimetry method to detect variations in the Grx1-roGFP2 protein acting as a glutathione redox potential sensor in Arabidopsis leaf discs. We detected clear changes in cytoplasmic glutathione redox dynamics upon flg22 perception. This involves a sustained oxidation of Grx1-roGFP2 that succeeds the transient aROS burst measured by luminol assay. Inhibition of ProDH reduced flg22-induced Grx1-roGFP2 oxidation in cytosol. As GSH/GSSG homeostasis is sensitive to the NADPH/NADP changes, we are evaluating how mitochondrial

ProDH affects the cytoplasmic NADPH/NADP ratio. Interestingly, *AtProDH1* and *AtProDH2* would mediate this effect by acting in different leaf tissues.

PL-09

IDENTIFICATION AND CHARACTERIZATION OF ZMS5H: ELUCIDATION OF ITS ROLE IN BIOTIC STRESS RESPONSE.

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Salicylic acid (SA) is a phytohormone that regulates multiple aspects of plant life, including disease resistance, leaf senescence, flowering, and thermogenesis. Levels of salicylic acid are regulated not only by activation of its biosynthetic pathway, but also through its modification by metabolic modifications, such as glycosylation, methylation, amino acid conjugation and hydroxylation. SA can be hydroxylated to 2,3-dihydroxybenzoic acid (2,3-DHBA) or 2,5-dihydroxybenzoic acid (2,5-DHBA), being these hydroxylated acids the major degradation products of SA. The reaction is catalyzed by enzymes known as SALICILYC ACID HYDROXYLASES (SHs). In this study, we report the characterization of the first SA hydroxylase in maize plants: salicylic acid 5-hydroxylase (ZmS5H), a 2-oxoglutarate dependent dioxygenase that catalyzes the formation of 2,5-DHBA by hydroxylating SA at the C5 position of its phenyl ring. Unlike other ZmS5Hs reported in rice and Arabidopsis thaliana, this enzyme does not show substrate inhibition. We also demonstrate that maize mutant plants in S5H accumulate SA. Next, we studied the role of S5H in the susceptibility against the infection with the fungal pathogen *Colletotrichum graminiciola*. *s5h* maize mutant plants exhibited less susceptibility against the pathogen attack compared to wild type plants (W22 ecotype) without showing any growth penalty. Moreover, the expression of genes associated with the development of the immune response mediated by SA, including *PATHOGENESIS RELATED 1* and *5* and *MITOGEN-ACTIVATED KINASE PROTEIN 1* was induced in *s5h* plants after the infection. We also performed infection experiments in transgenic Arabidopsis thaliana lines expressing *ZmS5H* in both *dmr6* and *s3h* mutant backgrounds. DOWNY MILDEW RESISTANCE 6 (DMR6) and SALICYLIC ACID HYDROXYLASE 3 (S3H) catalyze the conversion of the flavanone naringenin to the flavone apigenin and SA to 2,3-DHBA, respectively. It is also reported that AtDMR6 has also salicylic acid 5-hydroxylase activity. *dmr6* and *s3h* mutant plants accumulate SA, displaying less susceptibility against pathogen attack. However, when ZmS5H is expressed in either of the Arabidopsis mutants, susceptibility against *Pseudomonas syringae*

was restored. Together, these results provide evidence that ZmS5H has an important role during defense responses in maize plants.

PL-10

Elucidating the molecular mechanisms of the temperature short-term memory in Arabidopsis plants

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Plants modify their growth in response to temperature. Warm temperatures enhance stem growth, while cold temperatures inhibit it. This environmental cue is sensed by three plant thermosensors identified so far: PHYTOCHROME B (phyB), PHYTOCHROME INTERACTING FACTOR 7 (PIF7) and EARLY FLOWERING 3 (ELF3). The photo-sensory receptor phyB, represses the activity of CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1). In turn, COP1 represses the activity of the growth repressor ELONGATED HYPOCOTYL 5 (HY5). Warm temperatures inhibit this pathway to promote growth. Also, elevated temperatures induce changes in the RNA hairpin present at the 5´-untranslated region of the transcription factor PIF7, increasing its translation. High levels of PIF7 protein promote stem growth. ELF3, a component of the evening complex, represses the expression of PHYTOCHROME INTERACTING FACTOR 4 (PIF4), encoding a protein that promotes stem growth. In turn, warmth decreases the activity of ELF3 via a process that exhibit liquid-liquid phase separation, increasing the levels of PIF4 to enhance growth. In nature, plants experience fluctuating temperatures. Thus, we wanted to investigate the molecular mechanisms that store information about daytime temperatures to control stem growth during the night. We observed that daytime temperatures affected the nighttime growth of the stem (hypocotyl). We analyzed the molecular dynamics of the key components of the signaling network and hypocotyl growth in mutants under different day/night temperatures. The short-term growth memory required ELF3, PIF4, COP1, and HY5, which carried daytime temperature information into the night. In response to increasing temperatures, ELF3 forms nuclear condensates associated with reduced activity. However, these nuclear speckles showed poor sensitivity to subsequent cooling, representing a typical hysteretic effect. PIF4, controlled by ELF3, followed the same

pattern. We conclude that ELF3 achieves hysteresis and drives the PIF4 promoter into the same behavior, enabling a short-term memory of daytime temperature conditions.

PL-11

Connection between CYTOCHROME *c* and the TOR pathway to regulate growth in Arabidopsis

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Mitochondrial respiration is essential for energy production in most living organisms. Cytochrome *c* (CYT*c*) is a soluble heme protein of the mitochondrial intermembrane space, delivering electrons to complex IV during the last step of aerobic cellular respiration. We previously reported that *Arabidopsis thaliana* plants with lower CYT*c* levels (*cytc* mutants) exhibit decreased biomass and alterations in carbohydrate metabolism, explained in part by differential regulation in gibberellin homeostasis. In this work, we used plants with changes in CYT*c* levels to explore possible connections between CYT*c* and the growth regulatory pathway represented by the Target of Rapamycin (TOR) kinase. Detailed analysis of *cytc* mutants revealed phenotypic similarities with mutants in RAPTOR, a central component of the TOR complex. These comprise delayed germination, decreased root and hypocotyl growth, and delayed vegetative development. We focused on studying the growth of *cytc* seedlings, to understand how these plants administrate their energy resources for growth. We analyzed non-phosphorylated and phosphorylated ribosomal S6 kinase protein (S6K and P-S6K) levels as a readout of the activation of the TOR pathway in *cytc* mutants. We observed that the levels of both versions of S6K are lower than in wild-type plants, suggesting that TOR activity is affected in *cytc* plants. In agreement, autophagy, a process that is typically inhibited by the TOR pathway, is increased in *cytc* mutants during normal growth and in the presence of external sugar supplementation. Related to this process, the total amino acids content is increased in *cytc* mutants, probably as an energy source for growth. Our results allowed us to speculate that, in addition to their central role in ATP production, plant mitochondria also act as signalling organelles, orchestrating growth and development through different regulatory pathways, in part represented by CYT*c*.

PL-12

Autophagy modulates growth and development in the moss *Physcomitrium patens*

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Autophagy is an essential catabolic pathway for eukaryotic cell homeodynamics that mediates cellular recycling during growth, development and stress conditions, involving the coordinated interaction among more than 30 highly conserved autophagy-related (ATG) proteins and components of the secretory system. During this process, a wide range of intracellular material is engulfed into double-membrane structures termed autophagosomes, and transported this autophagic cargo to vacuoles in plant and yeast cells (or lysosomes in mammalian cells), to be degraded and recycled for different cellular purposes or remobilized to other parts of the organism. Most knowledge in the field of plant autophagy arises from studies in Angiosperms, mainly in the model *Arabidopsis thaliana*, or crops such as *Zea mays*, *Oryza sativa*, and *Glycine max*, pointing out that autophagy is virtually involved in all aspects of plant physiology. Studies of autophagy in the moss *Physcomitrium patens* have started to emerge, providing progress on evolutionary aspects of plant autophagy as well as species-specific roles of this process. *P. patens* apical growing protonemal cells have the singularity that they continue to undergo cell divisions as the plant develops. This feature provides a valuable tool to study autophagy in the context of a multicellular apical growing tissue coupled to development. Herein, we showed that the core autophagy machinery is present in the moss *P. patens*, and characterized the 2D and 3D growth and development of *atg5* and *atg7* loss-of-function mutants under optimal and nutrient-deprived conditions. Our results showed that 2D growth of the different morphological and functional protonemata apical growing cells, chloronema and caulonema, is differentially modulated by this process. These differences depend on the protonema cell type and position along the protonemal filament, and growth condition.

As a global plant response, the absence of autophagy triggers the spread of the colony through protonemata growth at the expense of a reduction of the 3D growth, such as the buds and gametophore development, and thus the adult gametophytic and reproductive phases. Altogether this study provides valuable information suggesting that autophagy has roles during apical growth with differential responses within the cell types of the same tissue and contributes to life cycle progression and thus the growth and development of the 2D and 3D tissues of *P. patens*.

PL-13

Chloroplast redox status modulates the cell expansion phase of leaf development associated to changes in proteasome activity and endoreduplication index

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Leaf growth is sustained by successive phases of cell proliferation and expansion that determine the final organ size. The rate and extension of these phases are modulated by several developmental and environmental cues. We show herein that changes in the redox status of tobacco chloroplasts caused by introduction of the alternative electron shuttle flavodoxin led to decreased leaf size. Flavodoxin activity as electron sink prevented over-reduction of the photosynthetic electron transport chain and propagation of reactive oxygen species. These effects correlated with significantly lower rates of cell expansion in leaves of flavodoxin-expressing plants. Neither the duration of the expansion phase nor the rate and extension of the proliferative stage were affected by the presence of the alternative electron carrier, resulting in smaller leaf cells without significant differences in their numbers. Cells from transformed plants contained fewer chloroplasts of wild-type size, but their cellular coverage was increased due to cell size reduction. Chloroplast redox modulation of leaf development was associated to increased proteasomal activity and lower endoreduplication. The results identify a new player in the global

regulation of plant organ growth, as represented by plastid-generated redox signals, and underscore the value of alternative electron shuttles to investigate the signaling role of chloroplast oxido-reductive biochemistry in plant developmental pathways.

PL-14

Circular foreign mitochondrial DNA in the mitochondria of parasitic plants

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Parasitic plants are characterized by their ability to feed directly from other plants, invading the roots or stems of their hosts through a specialized root called haustorium. This vascular and intimate contact allows the passage of water, nutrients, pathogens, and nucleic acids enabling the exchange of genetic information between unrelated species, a process known as Horizontal Gene Transfer (HGT). Most cases of plant-to-plant HGT involve the mitochondrial genome (mtDNA). Recently, widespread HGTs have been described in the mtDNA of two holoparasitic species of Balanophoraceae (Santalales): *Lophophytum mirabile* and *Ombrophytum subterraneum*. We deciphered the mtDNA of the close relative *L. pyramidale* and performed a comparative and evolutionary assessment to evaluate the incidence, mechanisms, and impact of transfer events from their mimosoid hosts (Fabaceae) to *Lophophytum spp*. The mtDNA of *L. pyramidal*e is 806,114 bp long and assembles into 79 circular-mapping chromosomes. This multichromosomal structure is likely an ancestral character of the family Balanophoraceae, in which the number and characteristics of the chromosomes are highly variable. Comprehensive phylogenetic analyses showed that *L. pyramidale* harbors six foreign, 13 chimeric, and 18 native protein-coding genes as a result of ancestral and recent HGT events from its hosts. Most foreign and chimeric genes in *Lophophytum spp*. were integrated by homologous recombination into the native locus and are likely functional. A genus-wide analysis revealed that 54% and 67% of the mtDNA of *L. pyramidale* and *L. mirabile*, respectively, was acquired from mimosoid hosts. This foreign DNA enlarged the mtDNA of *Lophophytum spp*. by a scale factor of 2.18-3.1 impacting also the coding content. A total of 42 circular chromosomes are mostly foreign in *Lophophytum spp*. mtDNAs, and are maintained as plasmid-like molecules. We propose a circle-mediated HGT model in which transferred foreign DNA circularizes

becoming a stable and perpetuating molecule that can eventually integrate into the resident mtDNA. This HGT mechanism is unprecedented in plant mitochondria or any other organelle across eukaryotes.

PL-15

The immune receptor BNT1 is involved in *Myzus persicae* infestation of Arabidopsis

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Nucleotide-binding leucine-rich repeat receptors (NLRs) are key sensors of the plant immune system, conferring resistance to diseases caused by diverse pathogens. NLRs have been found to act in several compartments inside the cell. However, NLRs have never been described as functioning from plastids, which are essential organelles for a successful defense against pathogens. Interestingly, we have recently found that one splice variant of the Arabidopsis NLR receptor BNT1 displays a functional plastid targeting signal. The BNT1.1 variant localizes to the cytoplasm while BNT1.2 is targeted to the plastid envelope. Thus, the differential activation of the BNT1 isoforms could play distinctive roles in plant defense responses against pathogens. Here, we analyzed the level of the BNT1.1 and BNT1.2 transcripts in different tissues and under multiple biotic interactions. We found that BNT1.2 shows the highest expression in most of the leaf tissues and conditions tested. However, in response to the aphid *Myzus persicae* infestation the BNT1.1 variant was specifically induced in vascular tissue. Moreover, aphid-responsive marker gene expression was altered in BNT1 loss-of-function plants. Taking this into account, we tested the performance of *M. persicae* in WT and *bnt1* mutant plants. Remarkably, the Electrical Penetrations Graphs Assays showed that, among the 120 feeding activities tested, the aphid spent more time ingesting sap from the phloem of *bnt1* mutant. This strongly suggests that the aphid's performance is higher in the *bnt1* null-mutant than in WT plants. However, the Choice Assays revealed that aphids preferred WT over *bnt1* plants. Together, our results might indicate a specific requirement for the cytoplasm or chloroplast located BNT1 at different stages of the aphid infestation. Future experiments complementing *bnt1* mutant with each of the variants will shed light into this attractive possibility.

PL-16

AtHB40 AND AtHB53 PLAY A ROLE IN ROOT DEVELOPMENT AND NaCl RESPONSE.

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AtHB40 and its paralog AtHB53 are Arabidopsis transcription factors (FT) belonging to the homeodomain-leucine zipper (HD-Zip) I family. Numerous reports show that several members of this TF subfamily are associated with developmental processes in response to abiotic stress factors. AtHB40, AtHB53, and AtHB21 were resolved in the same clade in phylogenetic trees and described as crucial for the transcription of NCED3 in axillary buds, a gene encoding a key enzyme for abscisic acid biosynthesis. Our previous work showed that AtHB40 is a repressor of primary root growth and also alters root gravitropic response, involving CYCLIN B and auxin transporters. Root plasticity allows plants to grow in soils with different compositions, since the root system can readapt its architecture depending on the environment. Given that HD-Zip paralogs were reported as having redundant functions, we decided to analyze the triplet at the same time using transgenic plants transformed with *prAtHB53:GUS* and *prAtHB21:GUS* constructs. Histochemical assays show that AtHB53 is expressed in lateral roots primordia, vascular system, and the root tip of lateral emerged roots. However, no expression was detected in primary roots. On the other side, AtHB21 was not detectable in any tissue of roots, and consequently, we put it aside from the analysis. We obtained *athb53* mutant plants and evaluated root phenotype in control growth conditions. Such mutants showed longer primary roots than Col-0 plants, similar to the observations done in the analysis of the athb40 genotype. To answer whether AtHB40 and AtHB53 could have redundant functions in roots and considering that AtHB40 modulates auxin carriers we treated *prAtHB53:GUS* 7-day-old seedlings with auxin (IAA) for different periods. GUS activity was evidenced in columella cells. Notably, this scenario was similar to the showed by AtHB40, indicating that in these conditions both genes are co-expressed. Then, we obtained crossed plants between *athb53* and *prAtHB40:GUS.* In 7-day-old plants, GUS activity was repressed both in control conditions and after IAA treatment, suggesting that AtHB53 modulates AtHB40 expression in primary roots and mediates its induction by IAA. Given that athb40 seedlings are tolerant to 150 mM NaCl treatment, we evaluated athb53 behavior in salinity conditions and observed that these mutants exhibit enhanced tolerance to NaCl compared with Col-0. Considering that AtHB53 is strongly expressed in lateral roots in normal conditions, we quantified lateral root density in *athb53* mutants. Although we did not observe significant differences between *athb53* and Col-0 plants in this trait, total lateral root length in *athb53* 8-day-old

seedlings was significantly increased compared with that of Col-0 seedlings in control conditions. Altogether, our results suggest that AtHB53 modulates AtHB40 expression in the primary root tip, and this regulation is related to the auxin effect. Furthermore, AtHB53 plays a role in primary and lateral emerged root growth.

PL-17

A de novo diploid genome assembly allows dissecting the transcriptomic differences underlying the clonal phenotypic diversity in cultivar 'Malbec'

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Most grapevine cultivars employed in the wine industry were originated from outcrossing two genetically divergent cultivars and then clonally propagated. Therefore, grapevine cultivars characterize by possessing diploid and highly heterozygous genomes. Despite the vegetative propagation system, intra-cultivar phenotypic and genetic diversity has been widely reported. Malbec is appreciated for producing high-quality red wines and recognized as the signature cultivar of the Argentine viticulture. Previous analyses of our group demonstrated notorious phenotypic differences among Malbec clones. Also, significant genetic differences were reported, mostly based on SNPs occurring across the intergenic regions. Here, aiming to dissect the transcriptomic bases of the clonal phenotypic diversity, we firstly assembled a truly-phased reference genome for Malbec. To perform the de novo assembly, we used a trio-binning approach implemented by the software Canu. We generated Illumina short-reads for Malbec parental cultivars (Magdeleine and Prunelard), that were employed to sort the PacBio long-reads generated for Malbec into its two component haplotypes. Finally, we obtained two assemblies: "Malbec-Prunelard" and "Malbec-Magdeleine", each one showing the total length and gene content expected for a haploid grapevine genome. On the other hand, we studied 27 clonal accessions grown under the same

conditions at Vivero Mercier experimental vineyard. Analytical and biochemical measurements were performed on mature berries (23° Brix), during two consecutive seasons (2017-2019). Afterwards, we chose the six clones exhibiting the greatest differences for the evaluated features. Whole RNA extractions were performed from berries to conduct RNA-seq experiments. More precisely, Illumina stranded paired-end reads were obtained for the six clones with their three biological replicates (18 samples). RNA-seq data was aligned to each haplotype of Malbec reference genome, to perform differential gene expression (DEG) and gene ontology (GO) enrichment analyses. After performing a multivariate discriminant analysis including all samples, clone 595 exhibited the most significant phenotypic and transcriptomic differences. In particular, 595 showed the highest total polyphenols concentration. At the same time, the DEG and GO enrichment analyses consistently showed that 595 exhibited significantly up-regulated genes involved in the anthocyanins biosynthesis pathways, when compared to the other clones. Furthermore, using a diploid assembly as reference enabled us to detect different sets of DEG and GO terms with each haplotype, and to observe differences in the intensity of the detected processes. Overall, these results highlight the importance of using a truly-phased assembly to analyze RNA-seq data and suggest that the clonal phenotypic diversity observed in Malbec could be explained by differences in the transcriptome.

Microbiology

MI-01

The antifungal peptide SmAPα1-21 derived from the α-core of plant defensin DefSm2-D induces cell-wall stress, membrane permeabilization, peroxisome biogenesis, oxidative stress and cell death in *Fusarium graminearum* conidia. Role of His 19 in peptide internalization

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Plant defensins are small cationic proteins ubiquitously expressed in the plant kingdom involved in innate immune defense. The primary structures are variable, but they all share the same tertiary structure, α/β structure stabilized by disulfide bridges: the so-called CSα/β fold. Two highly conserved regions have been identified in these proteins: the γ-core (GXCX3–9C), a well known determinant of the antimicrobial activity among disulphide-containing antimicrobial peptides and the α-core (GXCX3–5C), a less studied motif only present in plant defensins. The relevance of these motifs relies on the presence of positive residues which would allow the interaction with negative charges on the pathogen membrane and/or cell wall. In previous studies, we have demonstrated that synthetic peptides derived from these regions are active at micromolar concentrations against conidia from the phytopathogenic fungus Fusarium graminearum and we have characterized their action. Here, we continue the study of SmAPα1-21 mode of action (sequence: KLCEKPSKTWFGNCGNPRHCG; Minimum Inhibitory Concentration, MIC: 32 µM) and explore the correlation between the biological activity and primary structure of the α-core of DefSm2-D flower defensin focusing on the relevance of histidine 19. New peptides were designed by modifying the parent peptide (SmAPH19R and SmAPH19A, where His19 was replaced by Arg or Ala, respectively) and synthesized by the Fmoc solid phase method. Antifungal activity was determined against F. graminearum. Conidia membrane permeability was assessed by visualizing the influx of the membrane impermeant fluorescent red dye propidium iodide by confocal laser scanning microscopy (CLSM) after challenging conidia with each peptide. Reactive oxygen species (ROS) production was monitored on conidia with H2DCF-DA probe by fluorescence spectroscopy and CLSM. The peptides were derivatized with fluorescein and rhodamine B and subcellular localization in conidia was studied by CLSM by colocalization with the cell wall marker Trypan Blue. Transmission electron microscopy (TEM) was used to study the ultrastructural effects of SmAPα1-21 in conidial cells. SmAPα1-21 induced morphological changes in the cell wall and peroxisome biogenesis in F. graminearum conidia. SmAP2H19A and SmAP2H19R were found to be active against F. graminearum (MIC SmAPH19R: 40 µM and SmAPH19: 100 µM). The replacement of His19 by Ala produced a decrease in the net charge of one unit at pH 5.5 with a significant increase in MIC, thus evidencing the importance of the positive charge in position 19 of the antifungal peptide. All three peptides produced permeabilization of the conidia membrane and induced oxidative stress through ROS production. However, the replacement by Ala turned all the processes slower. SmAPH19R and SmAPH19A were localized in the conidia cell wall whereas SmAPα1-21 was internalized, first entering through the basal and apical cells of the macroconidia. As the incubation times were prolonged, SmAPα1-21 localized in all the cells of the spores with a non homogeneous distribution in the cell cytoplasm. SmAPα1-21 has a multi-step mechanism of action against F. graminearum conidia that involves at least alteration of the fungal cell wall, membrane permeabilization, peroxisome biogenesis, and induction of oxidative stress. The extracellular localization of peptides SmAPH19R and SmAPH19A highlights the role of the His 19 residue in the internalization.

MI-02

Contrasting study among Trypanosomatids reveals conserved chromatin organization around trans splicing-acceptor site

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Trypanosoma cruzi, *Trypanosoma brucei* and *Leishmania major*, usually known as TriTryps, are the causal agents of animal and human sickness. TriTryps are characterized by having complex life cycles, alternating between a mammal host and an insect vector. One peculiarity of these organisms is that their genes are organized in long transcriptional units that give rise to polycistronic transcripts, which maturate into mRNA by a process known as trans-splicing. Even though gene expression regulation occurs mainly post-transcriptionally, it has been recently shown that chromatin plays a role in modulation. In this work, we made a comparative analysis of genome-wide chromatin organization and its potential impact on gene expression for the parasite stage present in the insect vector for the TriTryps using MNase-seq and RNAse-seq data, publicly available or generated by our laboratory. To compare average nucleosome positioning and mRNA patterns, we predicted the most likely trans-splicing acceptor sites and used them as reference points to plot average nucleosome or RNA-seq signals. By representing MNase-seq data, we corroborated the presence of a mild nucleosome depleted region (NDR) around trans-splicing acceptor sites (TASs) in *T. cruzi* and *L. major*, but not in *T. brucei*, as previously reported. However, when analyzing H3 ChIP-seq data, we uphold that TAS protection in *T. brucei* is due to a non-histone complex instead of a well-position nucleosome, as previously claimed. Moreover, we showed that this nucleosome organization around TASs is not just an average, since the same layout is conserved in most of the genome. Furthermore, the strand-specific analysis revealed that the NDR is not exactly at the TAS but a

few base pairs upstream of that point. We corroborated that this trough, is coincident with a footprint of DNA-RNA duplex, as previously observed.

Additionally, it was previously shown that average nucleosome density around TAS correlates with average RNA-seq signals. To test how strong is that correlation, we performed gene clustering using k-means with either nucleosome occupancy or mRNA signals relative to TAS as predictor variables. From the MNase-seq clustering, we observed a homogenous distribution of average nucleosome density in the three organisms except for a subset of genes with unusually high nucleosome density in *T. cruzi*. As opposed, from RNA-seq analysis, we obtained well-defined gene clusters for the three organisms supported by high silhouette values. Particularly, we observed that there is a subset of genes with markedly high mRNA levels compared to the rest, but the correspondence between nucleosome density and mRNA signal is only partial. To have a better understanding of the role and conservation of those subsets of genes with unusual characteristics among TriTryps, we are currently performing GO and Metabolic pathway analysisanalyses.

MI-03

EVASION OF THE CELLULAR IMMUNE RESPONSE BY THE NS5 PROTEIN OF THE DENGUE VIRUS. IMPACT ON THE PATHOGENESIS OF THE FOUR SEROTYPES FOR A RATIONAL DESIGN OF TETRAVALENT VACCINES

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Dengue virus (DENV) is the most important mosquito-borne virus, with an estimated infection rate of 390 million people per year. Despite its global burden, there are no antiviral agents or universal vaccines available to cope with this infection. Vaccine development has been a great challenge due to the

presence of four serotypes (DENV1-4). A previous exposure to any of the serotypes can lead to a more severe clinical manifestation upon infection with a heterologous one. This feature, along with the rapid expansion of co-circulation of DENV serotypes and the increasing infection incidence, highlights the importance to understand differences among serotypes and their interaction with the host cell.

DENV is a single-stranded RNA virus that relays on ten viral proteins to carry out its replication cycle and counteract the immune response. NS5 is the largest and most conserved protein across serotypes. It comprises two domains, the N-terminal methyltransferase (MTase) domain, which is essential for viral RNA capping, and the C-terminal RNA-dependent RNA-polymerase in charge of RNA synthesis. NS5 is also a potent antagonist of type I interferon signaling pathway through STAT2 degradation. In this study we demonstrate that NS5 further interferes with nuclear factor-κB (NF-κB) activation cascade by degrading the host protein ERC1 in the course of infection.

ERC1 is a cellular protein with multiple functions. It is a regulatory subunit of the IKK complex involved in NF-κB activation pathway, it participates in the docking and/or fusion of Rab6-positive vesicles at the cell cortex and it forms a complex that drives cell motility. We found that during DENV2 infection, ERC1 is degraded in a proteasome-dependent manner, mechanism that requires UBR4 as E3 ubiquitin ligase. We determined that the MTase domain is the viral counterpart essential and sufficient for ERC1 degradation and that not all NS5 from the four serotypes are capable of ERC1 degradation. Based on this difference between the MTase domain of DENV1, 2, 3 and DENV4, we mapped the amino acid residues responsible for ERC1 degradation. We generated a recombinant DENV2 that exchanges serotype properties with DENV4 by a single amino acid substitution. Infection with this virus led to higher levels of cytokine expression and secretion, resembling that reported for DENV4, and increased cell motility. NF-κB regulates the expression of genes related to immune responses. Viruses have thus developed a variety of strategies to modulate this pathway. In this regard, although DENV infection triggers the production of large amounts of pro-inflammatory cytokines, infected cells block further NF-κB activation, albeit the underlying mechanism has not been fully described. In the present study, we provide a novel viral mechanism for modulating NF-kB activation and cell migration by a DENV serotype-dependent ERC1 degradation. Importantly, these findings are relevant for the rational design of DENV tetravalent vaccines.

MI-04

Analysis of histones epigenetic marks across the *Giardia lamblia* cell cycle

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Giardia lamblia is a parasitic protozoan that inhabits the upper intestine of humans and animals causing a disease called giardiasis. This parasite switches between the vegetative form (trophozoite) and the resistant one (cyst). The change from one form to the other is bi-directional, depends on the microenvironment surrounding the parasite, and must be fast and efficient to ensure infection from host to host. In the process of cell differentiation into cyst, it was shown that some epigenetic mechanisms involved in the modulation of gene expression occur. In this sense, our work is focused on studying the localization of different histone post-translational modifications, particularly in the expression and/or localization changes, the study of the localization of different histone post-translational modifications, particularly in the changes in the expression and/or localization of point histone 3 (H3) modifications. We found that during the growth of the parasite, the mono-, di- and trimethylation marks of the lysine 4 were localized in the nuclei, but during encystation, there was a switch towards the periphery of the cells, and a decreased expression in the nuclei. On the other hand, the dimethylation of the lysine 36 showed a heterogeneous nuclear signal between both nuclei of the same trophozoite, while trimethylation of the same lysine was homogenously distributed in each nucleus. Both marks showed an increase in the nuclei signal during early encystation, which decreased at the end of the process. Related to the acetylation of lysine 9, we found an evident nuclear mark during growth that slowly decreases as the encystation progress. At the same time, a cell peripheral mark in the encysting trophozoite appears. Finally, although we found a nuclear mark of acetylation on the lysine 27 during the growth of the parasite, this mark disappeared from the nuclei at very early time points after the beginning of the encystation process. From these results, a new question arises related to the final destination of histones during the encystation process, which goes from disappearing some marks to localization probably in endo-lysosomal peripheral vacuoles or the disappearing of some marks to localization probably in endo-lysosomal peripheral vacuoles or in exosomes. Our results contribute to previous findings that correlate methylation and acetylation of histones as crucial events during the differentiation of *Giardia lamblia*.

MI-05

Characterization of Cysteine-rich protein families of *Giardia lamblia* and their role during antigenic variation

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Giardia lamblia is an intestinal parasite known to encode several families of cysteine-rich proteins. Among these families are the "Variant-specific Surface Proteins (VSPs)", which are unquestionably involved in the process of antigenic variation. In addition to the VSPs, other families of cysteine-rich proteins have been described in Giardia, such as the “High Cysteine Membrane Proteins (HCMP)”, “High Cysteine Proteins (HCP)” and “Tenascin-like Proteins (TLP)”. However, these proteins are less well known and there is no consensus on their function, subcellular location and relationship with VSPs. Although numerous efforts have been made to determine the distinguishing features of VSPs and how many VSP genes exist in the *G. lamblia* genome, a clear profile of the VSP repertoire is still lacking. In this work, we perform a comprehensive analysis of Cys-rich families’ genes and proteins in the recently updated version of the Giardia genome, including their organization, characteristic features, potential evolution, and expression levels, combining pattern searches and predictions with massive sequencing techniques, integrating and reanalyzing as much omics data as possible. Our findings allow us to propose a new classification for genes and pseudogenes encoding Cys-rich proteins that better describe their involvement in Giardia biology and suggest that HCMPs play a protective role on the parasite during the turnover of one VSP by another. In addition, we design bioinformatic tools that allow comparing transcriptomes and proteomes obtained under different experimental strategies to study the biology of this and other pathogens of interest in public health.

MI-06

Antibodies to protozoan variable surface antigens induce antigenic variation

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The genomes of most protozoa encode families of variant surface antigens, whose mutually exclusive changes in expression allow parasitic microorganisms to evade the host immune response. It is widely assumed that antigenic variation in protozoan parasites is accomplished by the spontaneous appearance within the population of cells expressing antigenic variants that escape antibody-mediated cytotoxicity. Here we show, both *in vitro* and in animal infections, that antibodies to Variant-specific Surface Proteins (VSPs) of the intestinal parasite *Giardia lamblia* are not cytotoxic, inducing instead VSP clustering into liquid-ordered phase membrane microdomains that trigger a massive release of microvesicles carrying the original VSP and switch in expression to different VSPs by a calcium-dependent mechanism. Surface microvesiculization and antigenic switching are also stimulated when *Trypanosoma brucei* and *Tetrahymena thermophila* are confronted to antibodies directed to their GPI-anchored variable surface glycoproteins. This novel mechanism of surface antigen clearance throughout its release into microvesicles coupled to the stochastic induction of new phenotypic variants not only changes the current paradigm of spontaneous antigenic switching but also provides a new framework for understanding the course of protozoan infections as a host/parasite adaptive process.

MI-07

The SARS-CoV-2 mutation landscape is shaped by niche environmental factors

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Mutation landscapes and signatures have been thoroughly studied in a wide variety of species, including cancer and SARS-CoV-2. There is a good understanding of the mechanisms behind some pattern changes observed in different types of cancer. However, for SARS-CoV-2, knowledge about the processes behind the observed changes in the mutation landscape is limited. Fortunately, the massive amount of information available about this virus allows us to study modifications in their genomic sequences under different circumstances and correlate them with clinical data. Thus, it was described that different SARS-CoV-2 lineages favour infecting different tissues, with Omicron replicating in the upper respiratory tract (URT), compared to other lineages, which replicate in both URT and lower respiratory tracts (LRT). Here, we present results that link the viral replication niche to specific changes in mutational patterns and explain them by a particular mechanism. Surprisingly, those patterns look to be also modified with events occurring in the patient, such as vaccination. Hence, deductive reasoning allowed us to identify the steps of the coronavirus infection cycle in which those mutations initiate.

MI-08

MliR, a novel MerR-like regulator of iron uptake, impacts metabolism, membrane remodeling and cell adhesion in the marine Bacteroidetes *Bizionia argentinens*is

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The MerR family is a group of transcriptional activators with conserved N-terminal helix-turn-helix DNA binding domains and variable C-terminal effector binding regions. In most MerR proteins, the effector binding domain (EBD) contains a cysteine center that is suitable for metal binding and mediates the response to environmental stimuli such as oxidative stress, heavy metals or antibiotics. Here, we present a novel transcriptional regulator classified in the MerR superfamily that lacks an EBD domain and lacks conserved metal-binding sites and cysteine residues. This regulator of the psychrotolerant Bacteroidetes *Bizionia argentinensis* JUB59 is involved in iron homeostasis and has been named MliR (MerR-like iron responsive regulator). *In silico* analyzes analyses have shown that homologs of the MliR protein are widespread among different bacterial species. Deletion of the *mliR* gene resulted in reduced cell growth, increased cell adhesion and filament formation. Genome-wide transcriptome analysis revealed that genes associated with iron homeostasis were down-regulated in the *mliR* deletion mutant. Using NMR-based metabolomics, ICP-MS, fluorescence microscopy and biochemical analysis, we assessed metabolic and phenotypic changes associated with *mliR* deletion. This work provides the first evidence for a MerR family regulator involved in iron homeostasis and contributes to expanding our current knowledge of the relevant metabolic pathways and cellular remodeling mechanisms involved in the adaptive response to iron availability in bacteria.

MI-09

The polysaccharides degrading mechanisms of two soil bacterial isolates

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The production of efficient enzymatic extracts for deconstruction of the structural polysaccharides that make up the plant cell wall, cellulose and hemicellulose, is an important process for the valorization of residual biomass. *Paenibacillus xylanivorans* A59 and *Cellulomonas* sp. B6 are bacterial strains with

capacity for degrading polysaccharides, isolated from the soil of pristine environments. Under appropriate culture conditions, both strains can secrete different carbohydrate active enzymes (CAZymes).

In this work, we studied the extracellular enzymatic activity and the exo-proteome of *P. xylanivorans* A59 and *Cellulomonas* sp. B6 strains when grown on different carbon sources, including sugar cane residue (SCR) and wheat bran (WB), as well as sucrose (SAC) as negative control. Both strains presented mainly extracellular xylanase activity when cultured on SCR or WB, determined as xylose equivalents of reducing sugars. The highest activity was achieved by culture on SCR for *P. xylanivorans* (11.06 IU/mL, 72 h) and on WB for *Cellulomonas* sp. B6 (3.06 IU/mL, 48 h). In all cases, cellulose degrading activity was low. By mass spectrometry analysis of the culture supernatants (E) we identified the main enzymes responsible for the observed activity. *P. xylanivorans* secreted 97 proteins when grown on SCR (E-SCR) from which 17 corresponded to CAZymes: 15 glycoside hydrolases (GHs), 1 with auxiliary activity (AA) and 1 pectate lyase (PL). In the case of *Cellulomonas* sp. B6, 195 proteins were identified in E-WB, from which 37 were CAZymes: 28 GHs, 1 AA, 2 glycosltransferases (GTs), 3 carbohydrate esterases (CEs) and 2 carbohydrate-binding proteins (CBMs). While in *P. xylanivorans* the most abundant CAZyme was a GH10 β-xylanase, in *Cellulomonas* sp. B6 several enzymes presented a similar high abundance, including endo- and exo-glucanases, xylanases and debranching enzymes. In both cases, extracellular components of ABC sugar transport systems were identified, which could be involved in the transport of mono- and small oligosaccharides into the cell.

Most CAZymes were encoded in distant regions of the genome, with few exceptions. A common feature was the presence of coding sequences for sugar transporters (mainly ABC family) and transcriptional regulators along with genes encoding CAZymes. In summary, both bacteria can secrete GHs with different relative abundance when cultured in lignocellulosic substrates, though the number and type of enzymes varied. *Cellulomonas* sp. B6 secretes a garter number of proteins and a more diverse repertory of CAZymes. Nevertheless, *P. xylanivorans* culture supernatant has higher xylanase activity, mainly due to a single GH10 xylanase. These results allowed us to build a model for polysaccharide utilization of each bacterium.

MI-10

Characterization of the protective response to FtsA imbalance-induced lysis in *Streptococcus pneumoniae*

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Cell division is a central process in bacteria and a prerequisite for pathogenicity. FtsZ, a tubulin homolog, and FtsA, an actin homolog, have fundamental roles in the bacterial division machinery. Division starts with the polymerization of FtsZ at the midcell and these protofilaments form a ring-like structure known as Z-ring. FtsZ assembly recruits other members of the divisome, such as FtsA that anchors the Z Ring to the membrane and recruits late-assemble proteins to complete the division. It has been demonstrated that a proper FtsA/FtsZ ratio is needed for cell division to occur. In *Streptococcus pneumoniae*, the relative amount of FtsA/FtsZ is 1:1.5. This relative abundance of FtsZ suggests that no-FtsA bound FtsZ is required during cell division. Here, we displayed that imbalances in the FtsA/FtsZ ratio due to overexpression of FtsA induced growth defects and bacterial lysis, while the FtsZ overproduction does not induce any morphological or growth defect. To identify putative protein-protein interaction, we performed a pull-down assay using GFP-Trap technology to compare the related proteins detected under native FtsA expression levels and a slight FtsA overexpression one without evidential morphological changes. Under native expression levels, the main hits were divisome proteins, such as FtsZ, while under overproduced FtsA conditions, we found an enrichment in stress response proteins. Among them, DnaK, a heat shock chaperone that binds to unfolded proteins, was the most relevant hit. Furthermore, it has been demonstrated that the two-component system CiaRH is important to maintain cell integrity helping *S. pneumoniae* to cope with lysis-inducing conditions. This protective effect involves the regulation of the major pneumococcal autolysin, LytA. We observed that autolysis induced by FtsA overexpression was abolished in the Δ*lytA* mutant, indicating the LytA dependence of this lytic event under these conditions. In addition, a ΔciaR mutation aggravates the lytic phenotype, which strengthens its protective role. The overexpression of FtsA produced cell wall alterations, evidenced by an increased propidium iodide uptake of the pneumococci, which might be the signal for the activation of LytA. The contrasting results obtained by overexpression of either FtsA or FtsZ support the idea that free FtsZ is needed for the division to occur and help to gain insight into how bacteria counter protein imbalances. In the case of an imbalance in the FtsA level, the CiaRH system is essential for the cell integrity of *S. pneumoniae*.

MI-11

Impact of DNA polymerase IV on genome evolution in the opportunistic pathogen *pseudomonas Pseudomonas aeruginosa*

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The human pathogen, *Pseudomonas aeruginosa* (PA), is a major etiologic agent in a number of acute and chronic infections. During infection development, PA gradually shifts from an acute virulent pathogen of early infection to a host-adapted pathogen of chronic infection. This adaptive process is mainly mediated by inactivating mutations that turn off acute virulence factors (i.e., motility appendages and pigments) and augment traits associated with chronic infection (i.e., antibiotic resistance). PA undergoes these evolutionary changes in response to selective forces, like the highly oxidative environment, during the chronic infection process. Identification of key players involved in this adaptation process may help to design more effective antimicrobial treatments. In this sense, the mutagenic DNA polymerase (Pol) IV catalyzes the error-prone bypass or incorporation of oxidized nucleotides. We previously reported that the Mismatch Repair protein MutS regulates the access of Pol IV to replication sites in PA by controlling Pol IV interaction with β clamp, which localizes Pol IV to sites of DNA synthesis. In the present work, we evaluated the involvement of Pol IV in the PA genome evolution under oxidative stress. With this aim, we analyzed a *mutT* deficient strain (T), where prevention of nucleotide oxidation is impaired and a *mutT mutSβ* (Tβ) strain, where *MutS* does not avoid Pol IV mutagenesis. It was also included the Pol IV-deficient strains *mutT dinB* (TD) and *mutT mutSβ dinB* (TβD). In order to study the role of Pol IV in the mutagenesis of the PA entire genome, we performed mutation accumulation (MA) experiments in which *de novo* spontaneous mutations accumulate across the genome randomly as selection is expected to be dramatically reduced. MA lines were initiated by creating replicates of each of the four founder strains and propagating lines for 2650 generations through repeated bottlenecks of a single, randomly chosen individual colony, thereby greatly reducing the effectiveness of selection. The whole genome of the founder strains and each MA line were then analyzed by next generation sequencing to evaluate the mutational events that occurred over the time frame of the MA experiment. All MA lines exhibited similar genome mutation rates (4 10-9 per nucleotide) and mutation spectra were dominated by base substitutions characteristic of oxidative DNA damage (AT>CG). However, Tβ MA lines showed increased mutations in particular cellular pathways that are inactivated in acute to chronic switch of PA infection, such us motility and pigmentation. The mutation preference for these pathways was not observed in the MA lines derived from T, TD and TDβ. Phenotypic analysis of the MA lines showed that mutations introduced by Pol IV effectively inactivated the target genes. A proportion of Tβ MA lines evolved to a hipopigmentation phenotype whereas T, TD and TDβ MA lines showed an increased production of

pyocyanin and pyoverdine pigments. Also, flagellar motility was decreased in some Tβ MA lines, an effect not shown by T, TD and TDβ MA lines. This analysis is currently being extended to other central phenotypes in the PA adaptation. In conclusion, our work reveals that Pol IV activity and its regulation by MutS might have an essential role in the acquisition of inactivating mutations important for the acute-chronic switch in PA infection.

MI-12

Influenza-infected cells increase levofloxacin tolerance of *Streptococcus pneumoniae*

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Streptococcus pneumoniae is a major bacterial pathogen that usually colonizes the upper respiratory tract and causes pneumonia, bacteremia, and meningitis in humans. Pneumococcal infections are generally treated with beta-lactams and fluoroquinolones (FQs), but FQ resistance has been reported in this pathogen. In our lab, we have reported that host cell oxidative stress induces FQ tolerance, an unusual ability to tolerate the antibiotic effects. We have also demonstrated that intracellular survival of *S. pneumoniae* increases in host cells that are coinfected with the influenza A virus (IAV). This synergistic mechanism depends on the SirRH two-component system, which controls the expression of certain stress genes needed for bacterial survival in host cells. The purpose of this work was to elucidate the role of SirRH in the mechanism of FQ tolerance and the impact of influenza A infection. To determine the contribution of SirR-regulated genes in the formation of FQ-tolerant pneumococci, we mutated genes encoding for enzymes involved in the oxidative stress response, such as *psaB* (encodes for a subunit of a manganese ABC transporter), *nrdH* (encodes for a glutaredoxin-like protein), and *sirR*. The three mutants showed a decreased FQ-tolerance induced by hydrogen peroxide in bacterial cultures, demonstrating that *psaB*, *nrdH*, and *sirR* play an important role in the mechanism of FQ-tolerance induced by oxidative stress. To analyze the effect of IAV infection on FQ tolerance induced by host cell oxidative stress, A549

pneumocytes were coinfected with the IAV and *S. pneumoniae*. We found a significant increase in FQ tolerance compared to A549 cells infected only with pneumococci. We determined that the cytoplasmic ROS levels were increased in IAV-infected cells, indicating that the IAV-induced respiratory burst is probably involved in this mechanism. When A549 cells were previously infected with IAV for 24 h, and then treated with NAC (a known ROS inhibitor) for 1 h before bacterial infection, we found a decrease in FQ tolerance of *S. pneumoniae*. To analyze the putative contribution of autophagy to the IAV-induced FQ tolerance in host cells, we coinfected MEF and MEF-atg5-KO (deficient in autophagy) cells with IAV and *S. pneumoniae*. We observed a similar FQ tolerance level to that observed in A549 cells, however, in MEF-atg5-KO we observed a decreased FQ tolerance related to MEF wt. These results suggest that oxidative stress genes are involved in the FQ tolerance mechanism in *S. pneumoniae*. Importantly, FQ tolerance is developed in coinfected cells in a ROS-dependent manner, and it occurs in autophagy-proficient cells only. We propose that our findings about FQ tolerance in *S. pneumoniae* are clinically relevant, due to this mechanism may cause complications in the antimicrobial treatment of pneumococcal infections.

MI-13

Rate and molecular structure of DNA direct repeats rearrangements in *Pseudomonas aeruginosa*.

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Genetic rearrangement of DNA repeated sequences has been proven important for the adaptation of different bacterial species, due to its capacity to alter the chromosomal structure. Long direct repeats can be deleted or expanded by different pathways depending on the level of homology and presence of different factors involved in DNA recombination, replication and repair. Homologous recombination is catalyzed by the RecA recombinase, which searches for homology between two DNA sequences and then promotes the exchange between DNA strands. It is also known that components of the Mismatch Repair System (MRS) have an inhibitory function over the recombination of divergent sequences.

We used a genetic reporter system previously generated in our laboratory, that consists of a plasmid containing two direct repeats (1268 bp) separated by a spacer, to determine the *in vivo* recombination

(deletion of one repeat and the spacer) rate for several mutant strains. We also investigated the influence of sequence homology between repeats using two versions of the reporter system; one containing repeats with perfect homology (HO) and another called homeologous (HE) which contains repeats with 5% of divergence.

Previous results from our laboratory indicate the coexistence of both RecA dependent and independent recombination mechanisms in this species, both resulting in the deletion of one of the repeats.

We found that in the WT strain, HO recombination (HOR) rate ($1.23x10^{-4}$) was 60 fold higher than the HER rate. In the Δ*recA* strain the HOR rate was 4 fold higher than the HER rate. More importantly, the HOR rate represents 1% of the rate found in the wt WT strain, whereas the HER represents 14%. This differs from our results in *E. scherichia coli*, where HER rate in the Δ*recA* strain represents 5% of the rate of the wt WT strain.

We also found that the HER rates in strains lacking factors involved in the Mismatch Repair System (Δ*mutS* and Δ*mutL*) were 6 and 2 fold higher than in the WT strain. Interestingly, deletion of the *MutS* protein had a much higher effect on a Δ*recA* background; with an HER rate 30 fold higher than the one found in the RecA deficient strain.

The homeologous repeat system contains 64 polymorphisms that can be used as molecular markers to identify the approximate recombination sites in recombined clones. These polymorphisms are not distributed evenly in the repeats, creating regions with different densities. We obtained the DNA sequences for multiple clones (46 - 59) of each strain and determined the approximate recombination sites. We found that the distribution of the recombination sites varied in the different mutants. In the absence of MutS, recombination occurs in a region with high mismatch density in 30% of the clones, in contrast with only 7% observed in the WT strain and 5% in the Δ*recA* strain. The double mutant strain Δ*mutS*/Δ*recA* however, showed 11% of recombination sites in this area.

Different to the observed in *E. coli*, our results indicate that there is a significant percentage of HER that occurs independently of the RecA recombinase in *P. aeruginosa*. Also we found that the MMR system has an inhibitory effect in the deletion of direct repeats, both dependent and independent of RecA, moreover, the effect of *mutS* seems to be stronger on the independent pathway. Additionally, in the absence of MutS, RecA seems to prefer not a region with a perfect homology but regions that contain a certain percentage of mismatches.

MI-14

Pseudomonas aeruginosa adheres to apoptotic cells through a functional type IV pili forming stable clusters

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The opportunistic pathogen *Pseudomonas aeruginosa* causes a wide range of acute and chronic infections. In cystic fibrosis (CF) airways, it forms multicellular aggregates called biofilms that are thought to contribute to chronic infection. Understanding the early steps of the transition from a free-swimming to a multicellular and sessile state is critical for developing strategies against chronic infections. We have reported that free-swimming *P. aeruginosa* attaches to apoptotic cells extruded from the epithelium, forming stable bacterial clusters. We further established that although clusters are permanent, individual adhesion is reversible and mediated by type IV pili (T4P). T4P are retractable filaments located at the poles of the bacterial cell and responsible for critical functions such as twitching motility (a form of surface-associated bacterial movement), adhesion to biotic and abiotic surfaces, virulence, and biofilm formation. The semi-flexible polymers of T4P are formed majorly by pilin (PilA) subunits. Pilus extension and retraction are mediated by PilB and PilT ATPases, respectively. There is another ATPase called PilU, which also contributes to pilus retraction, although its role is less clear. *pilT* mutants are typically hyperpiliated but totally impaired for twitching motility. We have now found that *pilTpilU* or *pilT* deletion mutants do not adhere to apoptotic cells for prolonged times and are, therefore, unable to cluster. These results indicate a role for retraction in adhesion not previously reported in *P. aeruginosa*. T4P are also involved in surface sensing, a process whereby surface engaged bacteria, through the Chp putative chemosensory system, up-regulate synthesis of the second messenger cyclic AMP (cAMP) and initiate physiological changes required for surface-associated lifestyles. PilH is one of the response regulators of the Chp system and it has been proposed to limit downstream signaling and/or to control the function of the retraction ATPases PilT/PilU. Deletion of pilH results in high levels of cAMP, diminished retraction function and hyperpiliation. We found that Δ*pilH* clusters on apoptotic cells but to a lesser extent than the wild type. This goes in line with our finding showing retraction is involved in adhesion. To rule out the possibility that an alternative adhesin stimulated upon T4P-mediated surface sensing was involved in cluster formation, we tested the Δ*pilH*Δ*pilB* mutant, which is constitutively activated for surface sensing but lacks surface pili. We found this mutant does not adhere to apoptotic cells for prolonged times either, and as so, is unable to cluster. Δ*pilH*Δ*pilT*ΔpilU, a mutant that is also constitutively activated for surface sensing, has surface pili but is unable to retract them, showed the same result. We conclude that a functional T4 Pilus is the main adhesin that mediates cluster formation. We are currently setting up a recently described pilin cysteine-labeling method.

Nanobodies: a versatile and low-cost tool for Hepatitis E virus research, diagnostics and therapeutics

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Hepatitis E virus (HEV) is an RNA virus responsible for the hepatitis E, a global emerging disease. Camelids have heavy chain only antibodies from which the variable domain (VHH or nanobody) that specifically binds to antigens can be isolated. Different expression systems can be used to produce VHH with high yield; VHH are easily modified and have superior physicochemical properties. In this work we obtained, selected, and modified nanobodies to detect the HEV capsid protein.

Method: HEV-3 ORF2 protein was expressed and purified using NiNTA under native conditions. A llama was immunized following a protocol of five successive subcutaneous injections with 150 g ORF2 at 2-week intervals. Blood was taken 4 days after the last immunization; lymphocytes were isolated and RNA extracted. A nanobody library was constructed using a golden gate strategy. ORF2 specific nanobodies were selected after 3 rounds of panning on transformed TG1 E. coli, using the phage display technology. Then, 96 colonies were randomly selected and the expression of HA-His-C-tagged nanobodies was induced with 1 mM IPTG. Recombinant VHH-HA-His proteins were tested for their capacity to recognize the ORF2 HEV-3 protein using an indirect ELISA. Positive clones were sequenced and nanobodies were selected according to the hypervariable complementary-determining region 3 (CDR3) sequence. Scaling up of the selected nanobodies was done after transformation of WK6 E. coli, the protein was extracted out of the periplasm and purified by affinity chromatography. Finally, the VHH were cloned and modified.

Results: A nanobody library of 1.8x109 individual colonies was obtained and a 100% of the tested clones contained a VHH fragment. A phage library of 1x1012 phages/ml was generated after infection of the TG1 E. coli with the M13K07 helper phage. After 3 rounds of panning, 96 clones were randomly selected and successfully extracted from the periplasm to identify specific binders by ELISA. 86 individual positive colonies were identified, and 16 clones were sent for sequencing after a pre-selection by restriction enzyme analysis. Six different HEV-3 ORF2 specific nanobodies were selected and modified with different tags (polystyrene binding peptide, biotin binding site, and proteins with enzymatic activity). These VHHs with and without tags were successfully expressed and purified by affinity chromatography. Conclusion:

To our knowledge, this is the first report of the selection and production of nanobodies specific for the ORF2 protein of HEV-3. We are currently working on the design of a low-cost immunoassay, with different formats such as sandwich ELISA and competitive ELISA, to detect anti-HEV antibodies or the biomarker ORF2 in human or pig plasma/sera. It is important to mention that these nanoantibodies could also be used for research and passive therapy to prevent HEV infection in pigs to avoid zoonotic transmission.

Lipids

LI-01

The pair ceramide-1-phosphate/ceramide kinase regulates intracellular calcium concentration during sperm exocytosis

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Before fertilization, spermatozoa must undergo calcium-regulated acrosome exocytosis in response to physiological stimuli such as progesterone and zona pellucida. Our laboratory has elucidated the signaling cascades accomplished by different sphingolipids during human sperm exocytosis. Recently, we established that ceramide increases intracellular calcium by activating different channels and stimulating sperm exocytosis. However, whether ceramide induces exocytosis on its own, activation of the CerK/C1P pathway, or both is still an unsolved issue. Here, by using functional assays, we demonstrate that C1P addition induces exocytosis in intact, capacitated human sperm. Real-time imaging in single-cell and calcium measurements in sperm population show that C1P needs extracellular calcium to induce intracellular calcium increase. The sphingolipid triggers cation-influx through Catsper, VOC, and SOC channels. However, requires calcium efflux from internal stores through IP3R and RyR to achieve sperm

secretion. For the first time, we report the presence of the ceramide kinase (CerK) in spermatozoa, the enzyme that catalyzes C1P synthesis. Furthermore, CerK exhibited calcium-stimulated enzymatic activity during sperm secretion. Exocytosis assays using a CerK inhibitor demonstrate that ceramide induces sperm exocytosis, partly due to C1P synthesis. Importantly, progesterone requires C1P to trigger the acrosome exocytosis. This is the first report, implicating the bioactive sphingolipid C1P in the physiological pathway of the sperm acrosome reaction.

LI-02

The biosynthesis of sphingolipids with very-long-chain PUFA is enhanced by testosterone in spermatogenic cells

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Ceramide (Cer), sphingomyelin (SM), glucosylCer (GlcCer), and complex glycosphingolipid species with very-long-chain PUFA (VLCPUFA), in nonhydroxy (n-V) and 2-hydroxy (h-V) forms, characterize the spermatogenic cell lipidome in rodents. The h-V/n-V ratio increases with differentiation from pachytene spermatocytes (PtS) to round spermatids (RS) and further stages. We first established that PtS and RS in culture are able to synthesize de novo Cer, SM, and GlcCer autonomously, and then studied the influence on such synthesis of testosterone, alone and after adding the medium conditioned by Sertoli cells (SCM). Using [^{3}H]16:0 as precursor, the formation of [^{3}H]Cer and [^{3}H]SM species with VLCPUFA was quite active, especially n-V SM. The label was mostly in the sphingoid base, 16:0, and a part was in [^{3}H]VLCPUFA. *De novo* synthesis inhibition affected distinctly the [^{3}H]SM/[^{3}H]Cer ratios in PtS and RS. The genes CerS3, SMS1, SMS2, and GCS diverged in expression with differentiation. Testosterone stimulated the de novo biosynthesis of n-V [^{3}H]SM in PtS and increased the [^{3}H]Cer/[^{3}H]SM labeling ratio in RS. Supplementation of testosterone-containing medium with the SCM robustly stimulated these reactions. Testosterone also stimulated the expression of two fatty acid elongases and fatty acid 2-hydroxylase. We speculate that germ cells must have a form of receptor to testosterone. The SCM effect is consistent with one of the known secretory products from Sertoli cells, most likely the androgen-binding protein, facilitating the availability of the hormone to germ cells, but we cannot rule out other molecules or even structures (e.g. exosomes) that might be present in the SCM. Supported by SGCyT UNS-PGI-UNS [24/B272 to GMO], FONCyT, [PICT2017-2535, PICT2020-02056 to GMO].

LI-03

Fusion of micron-size vesicles: interplay between the mitochondrial Mfn2 protein and lipids

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Membrane fusion is crucial for the coordination of mitochondrial dynamics. An imbalanced mitochondrial dynamic leads to the formation of fragmented mitochondria and a decrease in intracellular ATP levels, contributing to the development of important diseases, including neurodegenerative, cardiac or cancer conditions. The fusion process is energetically unfavorable, thereby requiring specialized proteins. In mammals, Mitofusins (Mfn) 1 and 2 are responsible for mitochondrial outer-membrane (OMM) fusion. They belong to the dynamin superfamily of multi-domain GTPases. Recent structural studies suggest that, upon GTP hydrolysis, Mfns dimerize to promote the approaching and fusion of OMM. However, the OMM fusion seems to require multiple regulatory factors that control the dynamics and kinetics of mitochondrial fusion throught the formation of heterotypic Mfn1-Mfn2 dimers. In this study, we purified and functionally reconstituted the full-length mouse Mfn2 in large and giant unilamellar vesicles (LUVs and GUVs, respectively). Vesicles were prepared with POPC alone or with 30% of plasmalogen-PC or DOPE. Unlike GDP, after incubation with GTP, vesicles underwent fusion. Fast video microscopy imaged the Mfn2-dependent membrane fusion pathway which involves the formation and expansion of a membrane diaphragm and the opening of a fusion pore. The incorporation of DOPE (30% mol) in the lipid composition did not alter the fusion sequence but enhanced the fusion kinetics significantly, as revealed by a lipid-mixing assay. Our observations show that Mfn2 alone can promote the fusion of micron-sized vesicles, without the presence of other proteins in the membrane. In addition, the lipid environment is an important factor in the modulation of Mfn2-dependent membrane fusion, a process that seems to require topological lipid intermediates with negative curvature.

LI-04

Cytosolic phospholipase A2 modulates triacylglycerides accumulation in renal cells under osmotic stress through arachidonic acid-PPARg axis

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Hyperosmolarity is a key controversial signal for renal cells. Under physiological conditions, it induces renal cell differentiation and maturation of the urine concentrating system. However, abrupt changes in environmental osmolarity may also induce cell stress that can lead to cell death. To adapt and survive in such adverse conditions, renal cells implement different osmoprotective mechanisms that include both the upregulation of cyclooxygenase-2 (COX-2) expression and prostaglandins (PGs) synthesis from arachidonic acid (AA), and a coordinated increase in phospholipids (GP) and triacylglycerides (TG) biosynthesis. In this work we evaluated whether hyperosmolarity modulates AA metabolism in MDCK cells under osmotic stress and the role of the phospholipase A2 (PLA2)-AA-PPARγ axis in TG synthesis activation.

MDCK cells were subjected to hyperosmolarity (298-512 mOsm/kg H2O) for different periods of time (0, 12, 24 and 48 h) and treated in the presence or not of different PLA2 subtypes, COX and PPARγ inhibitors or a PPARγ agonist. RT-qPCR and WB studies showed that hyperosmolarity increased cPLA2 expression at 24 and 48 h in a time-dependent manner. Moreover, cells treated with hyperosmolar media showed changes in cPLA2 intracellular distribution. Inhibition of cPLA2 but not iPLA2 nor sPLA2 prevented hyperosmolarity-induced TG synthesis and lipid droplets accumulation.

It is worth to point out that prostaglandin synthesis inhibition with Indomethacin (Indo) not only failed to prevent hyperosmolarity-induced TG synthesis and accumulation but also exacerbated it. Similar results were obtained when cells were treated with PPARγ agonist Rosiglitazone (Rosi) under hyperosmotic conditions.

In addition to this, hyperosmolarity increased free intracellular AA levels which was even higher when AA conversion into prostaglandin was blocked with Indo. Furthermore, inhibition of PPARγ with GW-9662 not only prevented the effects of Indo and Rosi on TG synthesis but also reduced TG synthesis stimulated by hyperosmolarity.

Our results suggest that hyperosmolarity induces AA release from GP by cPLA2 activity and that AA stimulates TG synthesis and LD formation through PPARγ activation. Therefore, this work highlights the role of cPLA2 as an osmoprotective gene.

LI-05

Resveratrol effect on renal osmoprotection: Modulation of COX-2 expression

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Resveratrol (RSV) is a polyphenol naturally present in several plants. Nowadays it is sold as an over-the-counter dietary supplement due to its antioxidant, anti-inflammatory and antitumoral effects. Paradoxically, it was documented that RSV may also present pro-oxidizing and pro-proliferative effects. Likewise, RSV effects on renal tissue are still controversial, as some injury models described beneficial effects, while others observed nephrotoxicity. Many molecular targets were described to explain RSV effects, one of them is ciclooxigenase 2 (COX2). Most of the articles exploring RSV effect on COX2 used cancer or inflammation models and found that it inhibited COX2 expression or activity. In the kidney, this protein plays an important role in osmoprotection of tubular cells subjected to physiological hyperosmolarity. We demonstrated that renal epithelial cell line MDCK undergoes an adaptive process during the first 24h of hyperosmolarity, in which the transcription of the osmoprotective gene COX2 is activated, among others. After 48h, cells are already adapted and begin to differentiate, acquiring a polarized morphology. Considering that many articles found that RSV can inhibit COX2, in this work we evaluate RSV effect on adaptation and differentiation mechanisms, focusing particularly on COX2 role. To do this, MDCK cells were pretreated with different concentrations of RSV (1-25 μM) and cultured in hyperosmolar media (~512 mOsm/kg H2O) for 24 and 48h. Cells were harvested to obtain cell number and viability. Cell cycle, immunofluorescence (IF), western blot and RT-PCR analysis were performed. We found that RSV decreased cell number in a concentration-dependent manner at 24 and 48h. Cell cycle analysis revealed that RSV increased S-phase and Sub-G0 population. Additionally, treated cells did not reach typical epithelium morphology. COX2 activity was not increased by RSV, but mRNA and protein levels were surprisingly upregulated, and IF revealed protein accumulation in cytoplasmic granules. To investigate the pathways leading to this increase, we evaluated TonEBP, NF-κB and MAPKs pathways, which are activated by hyperosmolarity; and SIRT1 implication, a target of RSV. TonEBP target genes mRNA did not show significant changes under RSV treatment, while NF-κB target gene mRNA showed an

increase similar to that of *COX2* mRNA. NF-κB IF revealed an increase in its nuclear localization. Regarding MAPKs, the only treatment that blocked COX2 protein expression was ERK1/2 selective inhibitor. SIRT1 specific inhibition did not change COX2 expression. These results show that RSV pretreatment caused harmful effects in hyperosmolar cultures of MDCK cells, affecting cell number and cell cycle and impeding cells to reach differentiated phenotype; in contrast with our hypothesis, RSV did not decrease COX2 expression but significantly upregulated the protein which was not active. Herein we report a possible nephrotoxic effect of Rsv.

LI-06

FPR2/ALX signaling and their lipid mediator pathways: pleiotropic roles in neurotoxicity.

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Neurotoxicity generated by several environmental factors has been widely associated with Parkinson's disease (PD). Human prolonged exposure to the pesticide Maneb (MB) has been reported as a triggering insult for dopaminergic neurodegeneration and the onset of PD. Even though this effect has been well documented in numerous epidemiological and research studies, little is yet known about the mechanisms underlying MB neurotoxicity in neuron-glia crosstalk. Based on our previous reports about the involvement of cyclooxygenases (COX) and lipoxygenases (LOX) in the neuronal response to MB toxicity (SAIB2021), our aim was to elucidate the role of these lipid mediators' pathways in neuron-glia communication. To study the possible alterations in neuron-glia crosstalk caused by MB exposure, dopaminergic N27 cells were exposed to astrocyte (C6 cell line) secretome and vice versa. Astrocytes' secretome showed to be neuroprotective against MB, whereas neurons secreted glial proliferative factors after pesticide exposure. Neither COX-2 nor CYP450 pharmacological inhibition were able to revert the effect of secretomes on their respective acceptor cells. In contrast, the inhibition of LOX-15, enzyme responsible for the generation of anti-inflammatory lipid mediators, abolished the glial proliferative effect of neuronal secretome during MB toxicity. In addition, the neuroprotective effect of astrocyte-derived secretome was blocked.

Next, we evaluated the role of FPR2/ALX receptor, whose main ligands are lipid mediators associated with resolution. The antagonist of FPR2/ALX, Quin-C7, blocked the effect of the astrocytic secretome on

neuronal survival upon MB challenge. In agreement, FPR2/ALX activation by a specific agonist enhanced the neuroprotective effect of the astrocytic secretome.

To determine the role of FPR2/ALX downstream signaling, cells were incubated with PI3K and ERK1/2 pharmacological inhibitors, After MB exposure, neuronal and astrocytic viability was nondependent of ERK1/2 activation. On the contrary, the blockage of PI3K showed to increase pesticide-induced cell death. Moreover, ERK1/2 phosphorylation was diminished by MB in both cell types. Interestingly, we found that the astrocyte proliferation caused by the secretome derived from MB-exposed neurons was mediated by ERK1/2 activation. Our results suggest that FPR2/ALX signaling and their lipid ligands are involved in the neuronal-glial crosstalk during MB exposure. These findings pay the way for interventions aimed at enhancing the resolution response during pesticide-induced neurotoxicity.

Signal Transduction

ST-01

2-arachydonoyl glycerol (2-AG) drives TRPV-dependent sensory signals that increase the intracellular cholesterol trafficking in Caenorhabditis elegans

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Cholesterol is an essential lipid constituent of eukaryotic cell membranes. Furthermore, its derivated metabolites have important biological roles as signalling molecules. Because of its relevance,

impairment in cholesterol metabolism has been related to several pathologies such as diabetes, cancer, among others. Caenorhabditis elegans is an useful model organism to study pathologies which have impaired cholesterol metabolism for the reason that this worm has homologous of 40% of genes that are associated with human diseases. Besides, C. elegans is auxotrophic for sterols and requires exogenous addition of them to survive. Cholesterol depletion leads to an early developmental arrest due to its essential role as precursor of signalling molecules. Thus, tight regulation of cholesterol storage and distribution within the organism is critical. We have recently demonstrated that the endocannabinoid 2-arachidonoylglycerol (2-AG) plays a key role in C. elegans modulating sterol mobilization (Galles et al, Sci Rep. 2018; 6398). However, the mechanism by which 2-AG controls cholesterol trafficking in C. elegans is not known. Here we show that 2-AG controls cholesterol homeostasis trough pathways that are independent of C. elegans neuropeptide receptors mediating endocannabinoid-dependent regulation of regenerative axon navigation and aversive behavior. We found that the calcium-activated regulator of neural dense-core vesicle exocytosis (DCVs) UNC-31 is essential for 2-AG-mediated stimulation of cholesterol mobilization. This result, combined with mutant analysis, suggested that 2-AG-dependent cholesterol traffic requires signaling of insulin peptides through the DAF-2 insulin receptor. In addition, mutations in the ocr-2 and osm-9 genes coding for transient receptors potential type V (TRPV) ion channels, dramatically reduces the effect of 2-AG in cholesterol mobilization. These findings indicate that 2-AG act as endogenous modulators of TRPV signal transduction to control intracellular sterol traffic through modulation of DCVs secretion.

ST-02

Xrn1 regulates cAMP-PKA specificity during thermotolerance

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Organisms are constantly challenged by changing environments. Complex signalling circuits sense these changes. Signal transduction pathways play a major role in controlling enzyme cascades that ultimately promote transcriptome and proteome remodelling in multi-stress and stress-specific manners. Several physiological mechanisms help organisms to prepare for recurring stressors. In the “acquired stress resistance” or "adaptive response" cells exposed to a mild stress dose survive a subsequent lethal dose

of the same or a different stress condition. One of the most studied signalling pathways is the conserved cAMP-PKA pathway. PKA from S. cerevisiae is composed of two catalytic (Tpk1, Tpk2 or Tpk3) and two regulatory subunits (Bcy1). Although each Tpk isoform controls different biological processes, little is known about the principles that govern PKA signalling specificity. One of the mechanisms involved in signalling specificity is regulation of PKA subunits expression. We have previously demonstrated that all PKA subunits share a negative expression regulation mechanism mediated by PKA activity. However, Tpk1 is the only subunit transcriptionally upregulated during heat shock. To further understand the molecular basis of cAMP-PKA specificity, we evaluated the mechanisms involved in PKA subunits expression during thermal stress adaptation. To this aim, we exposed cells to a scheme of two consecutive heat shocks: 37ºC for 30 minutes and 45ºC for 10 minutes (thermotolerance). We observed that only TPK1 promoter activity and mRNA levels increase during thermotolerance. Gene expression in eukaryotes does not always follow a linear progression from transcription to translation and mRNA degradation. There is an important crosstalk between transcription and mRNA decay machineries which functions from the nucleus to the cytoplasm and vice versa. The transcription-degradation crosstalk is grounded on the co-transcriptional imprinting of mRNAs with general factors such as RNA Pol II subunits (Rpb4/7) and mRNA decay factors (Xrn1 or Ccr4-NOT). Thus, we evaluated the role of Xrn1 in PKA activity and TPK1 expression. PKA activity was assessed by analysing different physiological readouts of the cAMP-PKA pathway such as heat resistance and glycogen accumulation. The xrn1Δ strain is more resistant to heat shock and shows a higher glycogen accumulation than the WT strain, consistent with a lower PKA activity. These results indicate that Xrn1 could play a role in the regulation of PKA activity. Moreover, TPK1 mRNA half-life increases in a xrn1Δ strain. TPK1 promoter activity and mRNA levels are upregulated upon thermotolerance in a Xrn1-dependent manner. Accordingly, Xrn1 is recruited to the TPK1 promoter upon stress. Although during thermotolerance TPK1 mRNA levels are strikingly upregulated in a xrn1Δ strain, Tpk1 protein levels severely decrease. These results indicate that Xrn1 regulates TPK1 expression at different levels during thermotolerance, affecting transcription, mRNA decay and translational efficiency. However, neither thermotolerance nor Xrn1 have an effect on the regulation of the other PKA subunits. Our results demonstrate that TPK1 expression is specifically regulated during thermotolerance and that Xrn1 has an important role in this process. Thus, we disclosed a tricky mechanism of the expression regulation of PKA subunits that impacts on cAMP-PKA pathway specificity.

ST-03

Quantitative proteomics reveals possible mechanisms of regulation of adipogenic differentiation in 3T3-L1 cells

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The murine preadipocyte 3T3-L1 cell line is a widely used model for the study of adipogenic differentiation. During its differentiation, a complex network of signaling processes takes place, including many unknown interactions. Currently, a growing number of studies reveal the participation of the 14-3-3 family proteins in adipogenesis, and these proteins have been recognized as a possible point of convergence of this intricate process. 14-3-3 proteins bind to phospho-serine or phospho-threonine residues in target proteins, affecting their activity, stability, subcellular localization or molecular interactions. Previous results in our laboratory revealed higher levels of mRNA and protein of the 14-3-3γ isoform during adipogenesis. We also reported that 14-3-3γ silencing produced a significant increase in lipid droplet accumulation in 3T3-L1 cells, compared to the wild-type, both in untreated or adipogenic induced cells. Therefore, the aim of this work was to examine the proteome and study how 14-3-3γ participates and regulates other proteins during the adipogenic differentiation process in 3T3-L1 cells. To do this, we infected cells with lentiviruses containing shRNA for the 14-3-3γ paralog; the silencing of this protein was confirmed by Western blot. Initially, cells were cultured with high-glucose DMEM and supplemented with 10% fetal bovine serum. Then, adipogenesis was induced by adding an adipogenic differentiation medium (ADM) containing insulin, dexamethasone, 3-isobutyl-1-methylxanthine, and rosiglitazone. Four biological independent protein samples of wild-type and 14-3-3γ-silenced cells were analyzed by mass spectrometry at day four of differentiation. Over 2750 individual proteins were identified in each cell lysate. 14-3-3γ silenced samples expressed a significant increase in adipogenesis-relevant proteins, such as C/EBPβ and GLUT1, and proteins related to the oxidative stress. Interestingly, the expression of the enzyme PARP3 was decreased compared to the wild-type ($p < 0.01$). Subsequent bioinformatic analysis indicated that PARP3 has two binding sites for 14-3-3γ. PARP3 is an enzyme primarily involved in many cellular processes, such as DNA repair and programmed cell death. Recently it was discovered to be involved in the inhibition of C/EBPβ activity and consequently, cellular adipogenesis. Our results showed that 14-3-3γ could be an inhibitor of the adipogenesis process. This work suggests a possible regulatory mechanism, completely unknown until now, which involves the binding of 14-3-3γ to PARP3 and the indirect modulation of C/EBPβ activity.

ST-04

Analysis of cytosolic calcium burst of Saccharomyces cerevisiae during the response to the sexual pheromone

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Saccharomyces cerevisiae constitutes an ideal system in which to study cell signaling mechanisms generating results that can often be extrapolated to other eukaryotes (Botstein, Chervitz, and Cherry 1997; Engelberg, Perlman, and Levitzki 2014; Khurana et al. 2015). S. cerevisiae haploid cells of are of particular interest since they respond to the sexual pheromone secreted by cells of the opposite sexual type, generating a sequence of events that leads to cell cycle arrest and subsequent morphological changes, such as cell polarization, interaction and fusion with a mating partner (Dohlman and Slessareva 2006). Previous studies have shown that calcium incorporation during pheromone response was necessary to coordinate genes involved in signal transduction and cell survival (Aguilar, Engel, and Walter 2007; Iida, Yagawa, and Anraku 1990; Muller et al. 2003).

We have previously adapted the fluorescent calcium sensor GCaMP6f in yeast. Through in vivo fluorescence microscopy experiments followed by single cell segmentation and fluorescence quantification, we showed that the pheromone does not generate, as previously reported, a single continuous increase in cytosolic calcium levels, but transient increases in bursts of short duration (Carbó-Tarkowski et al., 2017). Likewise, the presence of the pheromone seems to translate into an increase in the frequency of appearance of these calcium bursts, suggesting that the information transmitted by calcium is encoded in the temporal distribution of these bursts. Here, analyzing strains that are deficient in each known calcium flux pathway we show that the calcium response not only depends on transport from the extracellular medium, but it can also depend on intracellular calcium flow pathways. Moreover, we discovered that Yvc1 also plays an important role in this response suggesting the existence of a calcium-induced calcium release system in this organism. Modeling of cytosolic and vacuolar calcium dynamics enabled us to assign specific features to the different calcium pathways which explained part of our experimental results.

EN-01

COMPARATIVE STRUCTURAL, BIOCHEMICAL AND KINETIC STUDIES BETWEEN THERMOSTABLE AND COLD-ADAPTED GLUCOAMYLASES

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Glucoamylases (GAs, EC 3.2.1.3, glucan 1,4-α-glucosidase) are hydrolytic enzymes also known as amyloglucosidases. GAs belong to the GH15 and GH97 families of glycoside hydrolases (www.cazy.org). These enzymes are exo-amylases that hydrolyze α-1,4 glycosidic bonds by the successive removal of glucose residues from the non-reducing end of starch and related substrates, releasing β-D-glucose. In previous studies, we identified and characterized two novel GAs: *Te*GA, from *Thermoanaerobacter ethanolicus*, a thermophilic anaerobic bacterium and *Sd*GA, from *Saccharophagus degradans*, a marine bacterium which degrades different complex polysaccharides at high rate. *Te*GA is a thermophilic enzyme while *Sd*GA is a cold-adapted enzyme. Structurally, these proteins are composed by an N-terminal GH15_N domain linked to a C-terminal catalytic domain (CD), found in the GH15 family of glycosyl hydrolases. In this study we compared the global structure, catalytic residues, catalytic clefts and the flexibility of SdGA, TeGA and other thermophilic, cold-adapted and psicrophilic enzymes. We identified some characteristics of cold-adapted GAs that would explain the adaptation of these enzymes to low temperature.

EN-02

Biochemical and structural characterization of two phosphoenolpyruvate carboxykinases fromthe green alga Chlamydomonas reinhardtii

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Phosphoenolpyruvate carboxykinase (PEPCK) is an enzyme distributed in all groups of organisms and catalyzes the reversible reaction of decarboxylation and phosphorylation of OAA to generate PEP and CO2. The partitioning of carbon skeletons between PEP and OAA by the action of PEPCK is potentially important in the inter-conversion of sugars, organic acids, amino acids, aromatic compounds and lipids, suggesting that PEPCK must be strictly regulated. Since neither PEPCK function nor kinetic or regulation properties have been described in green microalgae, we decided to study the physiological and biochemical role of the PEPCK from the model green algae *Chlamydomonas reinhardtii* (*Chlre*PEPCK).

In this work we identified two possible PEPCK isoforms in *C. reinhardtii* crude extracts, both originated from a unique gene and were named *Chlre*PEPCK1 and *Chlre*PEPCK2.We cloned and purified the two enzymes and studied their kinetics, oligomerization forms and response to different metabolites. We assayed the carboxylating and decarboxylating activity of *Chlre*PEPCK1 and *Chlre*PEPCK2 to find that the two isoforms are capable of catalyzing both reactions *in vitro* but they have different decarboxylating parameters, being *Chlre*PEPCK1 more active than *Chlre*PEPCK2. The native molecular masses of the *Chlre*PEPCKs were determined by gel filtration chromatography and we observed that *ChlreP*EPCK1 has an hexameric form while *Chlre*PEPCK2 is mainly monomeric, implying that *Chlre*PEPCK2 lacks the needed residues for the monomer-monomer interaction. Last, we studied the influence of different metabolites in the activity of both isoforms. *Chlre*PEPCK1 activity is affected by the presence of citrate, L-Phe and malate, while *Chlre*PEPCK2 is regulated by citrate, L-Phe and L-Gln. All in all, it can be concluded that both *Chlre*PEPCKs are active *in vitro* and have different kinetics parameters, oligomeric structures and they also respond differently to the presence of amino acids and Krebs cycle intermediates. The obtained results allow us to gain insight into the green algae biochemical regulation of PEPCK and may reflect an in vivo intricate regulation involving allosteric regulation accompanied by oligomeric state changes.

EN-03

Cell-free glycobiology: enzymatic modification of α-glucans with glucosamine moieties

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Cell-free glycobiology is the application of synthetic strategies to obtain glycans in vitro combining purified enzymes, substrates and optimal conditions. This modern field allows the direct control of the system composition and avoids the limitations of the biological membranes or cell viability requirements, favoring real-time monitoring and process automatization. The study of enzymes related to carbohydrate metabolism and their promiscuity is a source to improve the availability of biocatalytic tools for synthetic glycobiology. In order to modify α-glucans, we analyzed an ensemble of bacterial enzymes capable of channelling glucosamine (GlcN) subunits to glycogen molecules. This arrangement is composed by three enzymes: an ADP-glucose pyrophosphorylase (GlgC, EC 2.7.7.27) that forms ADP-Glc from glucose-1P (Glc-1P) and ATP; a GT4-type maltosyl-1P synthase (GlgM, EC 2.4.1.142) that uses Glc-1P and ADP-Glc to form maltose-1P; and a maltosyl transferase (GlgE, EC 2.4.16.99) elongating glycogen molecules in two glycosidic moieties using maltose-1P. We previously found that the GlgM from *Rhodococcus jostii* (*Rjo*GlgM) is capable of use GlcN-1P as alternative substrate, reporting an enzymatic activity of 70 U/mg. This reaction would produce a putative GlcN containing hetero-disaccharide-1P (Glc-α-1,4-GlcN-1P). We then carried out a coupled enzymatic assay adding GlgE from *Streptomyces coelicolor* (*Sco*GlgE) to the reaction mixture. Thereby, the GlcN hetero-disaccharide, synthetized in situ by *Rjo*GlgM, could be used by *Sco*GlgE to elongate glycogen, releasing a phosphate molecule that can be measured. Surprisingly, the *Sco*GlgE activities with either the canonical (maltose-1P) or the alternative (Glc-α-1,4-GlcN-1P) substrates where in the same order of magnitude (~5 U/mg). Additionally, we reported that GlgC from *Geobacillus stearothermophilus* (*Gst*GlgCD) catalyzes GlcN-1P utilization alternatively to Glc-1P with the highest catalytic activity reported among several studied enzymes (10-12 U/mg). Thus, another system was studied composed by *Gst*GlgCD and *Rjo*GlgM enzymes using ATP and GlcN-1P. We measured the formation of a homo-amino-disaccharide-1P molecule by quantifying ADP release, suggesting the synthesis of GlcN-α-1,4-GlcN-1P. Finally, we assayed the ensemble of the three enzymes using as substrates GlcN-1P, ATP and glycogen as final acceptor. Our preliminary results indicate that glycogen molecules are modified by the addition of terminal GlcN moieties. We also studied the enzymatic system with Glc-1P and different final acceptors (maltose, maltotriose and starch). In all cases, similar activities were obtained to the analyzed aglycons. In a whole view, results indicate that the system presents substrate promiscuity for GlcN-1P and different α-glucans. The work provides insights into the design of new biotechnological tools to produce glucans (oligo- and poly-saccharides) with added α-1,4-amino sugars, being molecules not described in nature so far.

BT-01

BIOSENSING PERFORMANCE OF THREE DIFFERENT WHOLE CELL BIOSENSOR BASED ON LEAD-RESPONSIVE TRANSCRIPTION FACTORS

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This study utilizes synthetic biology principles to develop plasmid-based whole-cell bacterial biosensors for detection of lead. Lead is one of ten chemicals that the World Health Organization (WHO) has identified as a major public health concern (it is recommended to be lower than 10 μg/L (ppb) in drinking water). The lead biosensors evaluated are based on the natural metal detoxification mechanism of the *Cupriavidus metallidurans* (previously *Ralstonia Metallidurans*) CH34 strain. The genetic element of the lead biosensor constructs consists of i) PbrR1 or ii) pbrR2 of *C. Metallidurans* and of iii) PbrR of *K. Pneumoniae* combined with PbrT of *C. Metallidurans* genes sequences. All PbrR genes encode the lead-specific binding proteins (regulatory proteins), that specifically bind to their respective divergent promoter regions. Depending on the presence or absence of lead, they regulate the expression of a reporter gene. The PbrT gene encodes the Pb(II)-uptake protein. Results obtained with i) and ii) biosensors presented good sensitivity at low levels but showed high variability between assays and high basal expression of the reporter protein. The results obtained with iii) showed that our biosensor can detect lead in drinking water at levels as low as 10 ppb and could be used in a low-cost, portable and easy to use device to test home's water.

NS-01

Assembly of mitochondrial complexes in the brain of a rat model of Alzheimer

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Mitochondrial respiratory complexes (CI, CII, CIII and CIV) are associated in supercomplexes (SCx) (SCx1: I+III2+IIn; SCx2: I+III2+IV1, SCx3: I+III2+IV2, SCx4: I+III2+IV3 y SCx5: I2+III2). Although its role in mitochondrial respiration is still controversial, the study of SCx (dis)assembly is relevant to understand the causes of brain bioenergetic dysfunction reported in Alzheimer's disease (AD). In this work we evaluated the organization of SCx in an animal model of AD-like brain amyloidosis, the McGill-R-Thy1-APP transgenic (Tg) and controls (CNT) rats. Mitochondria from the hippocampus of 9-12-month-old animals (n = 3-6/group) were isolated and the organization and abundance of SCx were analyzed by electrophoretic runs in native gels (BN-PAGE) stained with Coomassie Brilliant Blue G-250. The functionality of CI was assessed by in-gel activity. Densitometric analysis of the bands showed that Tg and CNT rats do not assemble SCx2 and SCx3, unlike that was reported in mice. By contrast, both genotypes (in cortex and hippocampus) assemble SC1; SC4 (respirosome) and SC5; show the same amount of SC1 and the same respirosome (SC4) and SC5 activities. CNT and Tg show a significant decrease in the abundance of SC4 and SC5 in cortex and there is a significant decrease in the activity of SC1 in the cortex and hippocampus of Tg vs. CNT. Our results support the plastic model of organization of mitochondrial complexes in rat brain. We discard the hypothesis that the assembly of SCx is interrupted by the accumulation of amyloid at the early stages of the AD pathology and suggest that the decrease in SC1 activity (I+III2+IIn) could be the cause of the brain bioenergetic failure reported in this model.

NS-02

Addressing the molecular signatures of selective vulnerability to Parkinson's disease using cell-type specific α-syn expression and single-nucleus RNA-seq

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Parkinson's disease (PD) is a neurodegenerative disorder that spreads out across specific regions in the adult brain. One of the hallmarks of PD is the presence of α-synuclein (α-syn) aggregates, which has led to the hypothesis that the disease follows a pathogenic α-syn propagation path. However, aggregates are observed only in restricted cell types and don't always correlate with cell degeneration. In order to characterize the early stages of the neurodegeneration process and to dissect the mechanism underlying neuronal vulnerability to α-syn aggregation, we decided to search for common molecular signatures among several sensitive neuronal subpopulations, and their counterparts in resistant regions. To specifically assess α-syn triggered signatures, we overexpressed a human mutated version of the protein (hα-synA53T), a system that causes neurodegeneration in vulnerable cells. Taking advantage of Cre transgenic mice and Cre-inducible AAV, we selectively targeted hα-synA53T to specific neurons in precise brain regions of adult mice. We combined this with gene expression analysis by single-nucleus RNA-seq of selected cell populations. To set up our experimental approach, we focused on one sensitive region, the noradrenergic locus coeruleus (LC), which is less characterized than the more widely studied substantia nigra. We have co-inyected in the LC of NET::Cre adult mice the AAV-hα-synA53T (or a control AAV in the opposite hemisphere) with a vector that constitutively expresses GFP fused to the nuclear lamina domain (GFP-KASH). We found that two months after the injection neurons are still alive but show signs of degeneration, representing a suitable time-point for analysis of cellular response to the pathology. Therefore, we used that time-point to dissect the LC, extract nuclei and conduct single-nuclei RNA-seq of both neuronal and non-neuronal cell populations. We sequenced 18000 single nuclei in total, pre-processed them and analyzed their expression profiles using Seurat R package. Our preliminary results identified two cell clusters both enriched in GFP-KASH and hα-synA53T expression, suggesting the presence of at least two possible noradrenergic cell populations. Also, both clusters showed different signatures of gene expression responses to hα-synA53T overexpression, when compared to control treatment, assessed by differential expression analysis and Gene Ontology terms enrichment. This could

indicate the presence of different molecular mechanisms underlying pathogenic hα-syn degeneration in the different subpopulations of the LC. While still at our first steps, we believe that this project can help to elucidate the mechanisms that underlie PD progression and give rise to interesting candidate genes or pathways, whose targeting could halt-down neurodegeneration.

POSTERS

Cell Biology

CB-15

Chemotherapeutic drugs induce the activation of proteins associated with tumorigenesis and drug resistance in low aggressive tumor cells.

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Acyl CoA synthetase 4 (ACSL4) is an enzyme which regulates steroidogenesis in normal physiological conditions. However, in pathological scenarios, an increase in ACSL4 expression is associated with the promotion of a highly aggressive cell phenotype in breast, prostate, colon and liver cancer. ATP-binding cassette (ABC) transporters are transmembrane proteins which use energy through ATP hydrolysis to translocate low-weight molecules. In normal physiological conditions, these transporters are in charge

of maintaining homeostasis in different tissues. In contrast, their participation in pathological events is associated with drug resistance through chemotherapeutic drug efflux, among other events. Chemotherapeutic drugs inhibit the uncontrolled growth and proliferation of cancer cells, while cell resistance to drugs, either existing before treatment or generated after therapy, is responsible for most relapses in cancer, one of the major causes of death from the disease. We have shown that ACSL4 takes part in the resistance to chemotherapeutic agents by regulating the expression of ABC transporters; thus, the objective of this work was to study the effect of chemotherapeutic drugs on ACSL4, ABCG2 and ABCC4. The experimental model consisted in challenging H295R adrenal and MCF-7 breast cancer cell lines, characterized by low aggressive phenotypes and low expression of ACSL4, ABCG2 and ABCC4 proteins, with non-lethal doses of doxorubicin (20 nM) and cisplatin (200 nM). We evaluated cell functionality using viability (MTT) and proliferation (BrdU) assays, and compound exclusion (efflux) using fluorescent doxorubicin and Hoechst 33342. ACSL4, ABCG2 and ABCC4 were evaluated by western blot (WB). Treatment with doxorubicin and cisplatin improved H295R and MCF-7 cell viability (MTT-$p<0.001$) and increased the expression of ACSL4 (WB-$p <0.001$). Treatment with an ACSL4 inhibitor reduced the proliferation of H295R and MCF-7 cells (BrdU-$p <0.001$). Chemotherapeutic treatment also increased doxorubicin exclusion (efflux-$p <0.001$), an effect reversed by ABCG2 inhibitor Ko143 but not by ABCC4 inhibitor ceefourin1 in H295R cells. Interestingly, drug treatments increased the expression of ABCG2 (WB-$p <0.001$) and ABCC4 (WB-$p <0.05$), but also improved the exclusion of Hoechst 33342 (efflux-$p <0.01$). This effect was reversed by Ko143. These results are in line with previous work by our group showing that ABCC4 is not involved in doxorubicin efflux. Thus, ACSL4 and ABCG2 could be therapeutic targets at the initial stages of chemotherapeutic treatment to prevent the activation of pathways associated with increased tumor aggressiveness and drug resistance.

CB-16

INCREASED CONTENT OF TUBULIN TYROSIN LIGASE IS ESSENTIAL TO MAINTAINING NORMAL ARTERIAL PRESSURE: PREVENTIVE EFFECT OF TYROSINE AND NO3-TYROSINE ON HYPERTENSION DEVELOPMENT

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We previously showed that detyrosinated tubulin (Glu-Tub) is increased in the membranes of hypertensive erythrocytes. In addition, we reported reduced content and activity of the enzyme tubulin tyrosine ligase (TTL) in erythrocytes from hypertensive subjects with respect to normotensive subjects. Now, we show that the content and enzymatic activity of erythrocyte TTL remain constant in SHR rats in their pre-hypertensive stage until the pathology develops, while normotensive rats increase both the activity and the amount of TTL throughout their lives. This is consistent with an increase in Glu-Tub in SHR membranes with developed hypertension compared to normotensive rats. We also show that the decrease in Glu-Tub in the erythrocyte membrane in SHR rats, treated with tyrosine or 3-NO3-tyrosine as TTL substrates, partially reverses the low deformability of their erythrocytes and high blood pressure caused by the high content of Glu-Tub in the membrane. The results suggest that alterations in the tubulin tyrosination/detyrosination cycle are involved in biochemical and rheological changes in the blood of hypertensive subjects and, consequently, in the development of hypertension.

CB-17

CHARACTERIZATION OF THE sRNA CARGO IN BIOLOGICAL FLUIDS AND EVALUATION OF THEIR POTENTIAL ROLE ON EPITHELIA

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The discovery of RNAs in biological fluids has opened new questions about the function of these molecules besides their traditional role as intermediates in protein synthesis. Among them, small RNAs (sRNA) are particularly interesting since they are highly stable in these fluids. sRNAs circulate through the body both free and inside vesicles and it is postulated that they could participate in intercellular communication processes. Various studies have characterized the profile of these molecules in biofluids finding significant differences in their composition. A bioinformatic analysis carried out in our laboratory comparing sRNA-seq data from different human fluids (20 in total, including serum, plasma, seminal plasma, sputum and synovial fluid, among others) shows that the most abundant sRNAs in extracellular

compartments are fragments derived from RNApol I and III transcripts (yRNA, tRNA and rRNA). These sequences account for 60% or more of the total reads depending on the analyzed fluid, unlike the small percentage that can be assigned as miRNAs, and correspond to specific regions of these molecules. To address their potential role in extracellular fluids, we focus on the top 10 most abundant sRNAs found in serum. Using synthetic RNA oligonucleotides we tested their effect on cell viability in different cell lines (HEK293, HaCat, THP1, Caco-2 and Vero) showing that these molecules do not affect cell proliferation in a wide range of concentrations. These abundant extracellular sRNA are more stable than average RNAs and most of them are detected even 24hs after incubation in cell culture medium at 37 °C. Also, we evaluated whether these sRNA could play a role in maintaining the epithelial layer's structure, integrity and functionality by actin cytoskeleton staining, migration studies and immunoassays. Our results are consistent with a mild effect of two of the tested sRNA in preserving barrier function, providing encouraging preliminary data regarding previously unknown functions of the extracellular sRNA cargo.

CB-18

STUDY OF THE BIOLOGICAL ACTIVITY OF PEG-COATED MAGNETIC NANOPARTICLES IN BREAST CANCER CELLS

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Breast cancer is a malignant tumor that may invade surrounding tissue and spreads to distant areas of the body. Quercetin is a phytoestrogen with anticancer activity; however, it has limited bioavailability and adverse effects on healthy tissues. In order to generate a non-toxic carrier to optimize the delivery of drugs for the treatment of breast cancer, in this work we firstly studied the biological activity of iron oxide magnetic nanoparticles coated with polyethylene glycol (MNPs) in MCF-7 cells. The MNPs were synthesized by the hydrothermal method and characterized with a hydrodynamic diameter of 204 nm

and a zeta potential of -22.1 mV. Different concentrations of MNPs (1-150 μg/ml) were employed for the biological assays. The effect of MNPs on the cell morphology and the proliferation was analyzed using the Crystal Violet colorimetric technique, whereas cell viability was evaluated by the neutral red uptake assay. The results showed that the presence of MNPs do not decrease these parameters at the concentrations tested during 48 h. Moreover, incubation of the cells for longer periods of time (144 h) with the lower concentrations of MNPs did not provoke significant changes in the proliferation. In addition, to study the internalization and the accumulation of MNPs in MCF-7 cells, the iron content of the MNPs was evaluated by the Prussian blue assay. The representative images obtained under optical microscope and the quantification of the iron revealed that cellular uptake of the MNPs occurred after 48 h of incubation in a concentration dependent manner. The targeting of MNPs in the cell culture was assessed in the absence and presence of an external magnetic field of 0.3 tesla for 48 h. The results indicated that the direct exposure of the cell cultures incubated with 100 μg/ml of MNPs to a static magnetic field provoked a high intracellular accumulation of MNPs in the cells growing near the magnetic field without being incorporated by the surrounding cells. Altogether, these studies suggest that the nanoformulation employed would be suitable as a biosafe carrier of quercetin and other drugs, to optimize the treatment of breast cancer, by inducing the drug delivery in the target site, avoiding side effects and lowering the required doses.

CB-19

Anticancer potential of 4-hydroxy-3-(3-methyl-2-butenyl)-acetophenone (4-HMBA) on melanoma cell cultures

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According to IARC statistics, Argentina is the fifth country in South America with high cancer incidence (IARC, 2020) rising two places from 2018. Since a common feature of all tumor types is uncontrolled cell proliferation, it is crucial to develop new prevention strategies and efficient treatments. In this regard, there is a growing interest in developing chemotherapeutic or chemopreventive drugs from traditionally used medicinal herbs. Senecio graveolens (Compositae) is one of the 300 Senecio species which occur

in Argentina. It grows in the north west of the country, where it is known by the popular name of 'chachacoma'. The ethnobotanic resources are used regularly for their medicinal properties to counteract mountain sickness, and also as a digestive and cough suppressant. The main component of the essential oil found in chachacoma's leaves is 4-hydroxy-3-(3-methyl-2-butenyl)-acetophenone (4-HMBA) belonging to the alkylphenyl ketone group, a family of compounds whose therapeutic effects are used to treat inflammatory diseases, kidney disorders and organ dysfunction, among others. Senecio graveolens´s essential oil was found to selectively affect the proliferation of breast cancer tumor cells in vitro. And the main compound 4-HMBA shows antifungal activity against Candida albicans.

The aim of this work was to explore in vitro the cytotoxic and differential effects of 4-HMBA on proliferation and viability on B16F0 melanoma cells and on the pre-adipocytic, non-tumorigenic 3T3L1 cell line.

For cell viability assays, 5 × 103 B16F0 and 3T3L1 cells/well were seeded in 96 well multiwell plates incubated with RPMI medium and treated for 72h with 10 µl/ml of 4-HMBA fractions/RPMI medium in vehicle Ethanol (F1: 0.10 µM; F2: 0.25 µM; F3: 0.50 µM; F4: 0.75 µM; F5: 1.00 µM; F6: 1.50 µM; F7: 2.00 µM). For controls, 10 µl of the vehicle was added per ml of RPMI medium. Then, cell viability was measured by MTT assay. At 72 h of culture F3, F4 and F5 reduced B16F0 cell viability (49±3% SD, 34±7% SD, 29±11% SD respectively vs control). None of the three fractions affected 3T3L1 viability.

For cell proliferation assays 3 x 104 B16F0 and 3T3L1 cells/well were seeded in p6 well and treated for 72h with 10 µl/ml 4-HMBA fractions/RPMI of F3 and F5. Then the number of cells per field was quantified in 10 random fields/well at 0, 24, 48 and 72h of treatment. At 24h, F3 reduced B16F0 cell proliferation (44±11% SD vs control). Meanwhile, F3 reduced 3T3L1 cell proliferation only at 72h (34±15 SD).

In conclusion, 4-HMBA shows in vitro a cytotoxic and differential effect that reduces viability and proliferation of B16F0 tumor cells without affecting 3T3L1 non tumor cells.

CB-20

PHYSICAL INTERACTION BETWEEN DUX4 AND HORMONE NUCLEAR RECEPTORS

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DUX4 is a double homeobox transcription factor required during early embryonic development in placental mammals. We have demonstrated that abnormal expression of DUX4 in human muscle cells is toxic, pro-apoptotic and is associated with facioscapulohumeral muscular dystrophy (FSHD), a prevalent human inherited neuromuscular disease. In addition, we recently demonstrated that DUX4 is a corepressor of the nuclear receptors (NRs) of progesterone (PR) and glucocorticoids (GR). In this work, we used co-immunoprecipitation (Co-IP) assays to investigate the potential physical interaction between DUX4 and these NRs. Co-IP studies were performed using protein extracts from: i) HEK293 cells co-transfected with plasmids expressing tagged versions of DUX4 and GR, and ii) T47D cells, which endogenously express PR, transfected with a plasmid expressing DUX4-V5. In these studies, DUX4-V5 was consistently able to bind and pull-down the FLAG-tagged GR. A weaker interaction, however, was observed between DUX4-V5 and the PR. Our results suggest that the corepressive effect of DUX4 on the GR, but not PR, could be related to a direct protein-protein interaction. In silico analysis of the C-ter of DUX4 showed the presence of NR-box like motifs, which are present in coactivators of NRs. As it has been shown for corepressors of NRs, either deletions or amino acid substitutions at NR1 and NR2 do not affect the corepressor activity of DUX4 on the PR and GR. Predictions of the DUX4 C-ter secondary structure indicate that this region has a triple α-helical domain, similar to the repressor domain (CoRNR) present in the well characterized corepressors NCoR and SMRT. Moreover, a short amino acid sequence (AQPLL388-392), at the DUX4 C-ter, matches the core ϕXXϕϕ proposed as a consensus sequence for CoRNR domain. Additional studies are required to demonstrate whether the corepressor activity of DUX4 on NRs requires the AQPLL388–392 motif and/or the predicted α-helical domains at its C-ter.

CB-21

Anatomical and functional changes of the human erythrocyte cytoskeleton are mediated by tubulin in response to increased calcium levels

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Erythrocytes affected by age and diseases such as hypertension, diabetes, exhibit abnormally high intracellular Ca2+ ion levels and appear to have altered cytoskeletal properties. In previous research, we observed that tubulin can be found in three fractions within erythrocytes, as membrane-bound, as a soluble fraction, or as part of a structure that can be sedimented by centrifugation. This tubulin may be forming part of the cytoskeleton. We have observed that its differential distribution within these fractions can alter several hemorheological properties, such as the deformability of erythrocytes, the present work studied how this distribution is in turn affected by the increase in Ca2+ levels, another key player in the regulation of the stability of the cytoskeleton of erythrocytes. The results showed that when Ca2+ levels increase in the cell, either due to the addition of the ionophore A23187, due to specific inhibition of plasma membrane Ca2+-ATPase (PMCA), or due to arterial hypertension, tubulin moves to the membrane, erythrocyte deformability decreases, and phosphatidylserine exposure increases. Furthermore, the increase in Ca2+ was associated with an inverse correlation in the distribution of tubulin and spectrin, another important protein of the cytoskeleton. Based on these findings, we propose the existence of a mechanism of action through which higher concentrations of Ca2+ in erythrocytes trigger the migration of tubulin to the membrane, a phenomenon that results in rheological and molecular alterations of the membrane itself, as well as of the integrity of the cytoskeleton.

CB-22

Prevention of tubulin/aldose reductase association delays the development ofpathological complications in diabetic rats

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In recent studies, we found that compounds derived from phenolic acids (CAFs) prevent the formation of the tubulin/aldose reductase complex and, consequently, may decrease the occurrence or delay the development of secondary pathologies associated with aldose reductase activation in diabetes mellitus. To verify this hypothesis, we determined the effect of CAFs on Na,K-ATPase tubulin-dependent activity in

COS cells, ex vivo cataract formation in rat lenses and finally, to evaluate the antidiabetic effect of CAFs, diabetes mellitus was induced in Wistar rats, they were treated with different CAFs and six parameters were determinants: i-cataract formation, ii- erythrocyte deformability, iii-nephropathy, iv-blood pressure, v-phosphatidylserine exposure in erythrocytes and vi- blood coagulation time. After confirming that CAFs are able to prevent the association between aldose reductase and tubulin, we found that treatment of diabetic rats with these compounds decreased membrane-associated acetylated tubulin, increased NKA activity, and thus reversed the development of six AR-activated complications of diabetes mellitus determined in this work. Based on these results, the existence of a new physiological mechanism is proposed, in which tubulin is a key regulator of aldose reductase activity. This mechanism can explain the incorrect functioning of aldose reductase and Na,K-ATPase, two key enzymes in the pathogenesis of diabetes mellitus. Moreover, we found that such alterations can be prevented by CAFs, which are able to dissociate tubulin/aldose reductase complexes.

CB-23

Characterization of “in vitro” models of Parkinson's disease: a rescue effect of phosphatidylcholine

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Parkinson´s disease is the second most prevalent neurodegenerative disease in the world. It is caused by death of dopaminergic neurons in the “substantia nigra”, and characterized by the aggregation of the α-Synuclein protein in cytoplasmic inclusions called Lewy bodies, mitochondrial dysfunction and generation of reactive oxygen species. The present study aimed to establish "in vitro" models of Parkinson's disease. For this, SHSY5Y neuroblastoma cells were transfected with plasmids designed to overexpress the wild type α-Synuclein or the mutant A53T (mutation of adenine by threonine in amino acid 53 that increases protein aggregation), or cells were treated with 6-hydroxydopamine, a drug that induces mitochondrial deficits and stimulates several pro-apoptosis molecular factors. To validate the models, survival was analyzed and cellular death was identified and quantified by biochemical and morphological methods. We have previously demonstrated that liposomes of phosphatidylcholine exert a neuroprotective effect under inflammatory conditions by increasing neuronal plasticity and

differentiation. Thus, we evaluated the effect of this phospholipid in the established models of Parkinson's disease to further characterize its neuroprotective effect.

CB-24

PROTEOMIC ANALYSIS OF NEURAL STEM CELLS DERIVED EXTRACELULAR VESICLES

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Neural stem cells (NSC) have the capacity to regenerate the nervous tissue and represent a promising approach for the treatment of several neurodegenerative disorders. In addition, an alternative beneficial effect of stem cells is exerted by paracrine mediators, like extracellular vesicles (EVs). Extracellular vesicles are nanovesicles that mediate local and systemic cell-to-cell communication by transporting functional molecules such as proteins into target cells, thereby affecting the behavior of receptor cells. Despite their similar functions, extracellular vesicles from different origins present heterogeneous characteristics and components. In this study, extracellular vesicles secreted by NSCs (NSC-EVs) were isolated by ultracentrifugation and size exclusion chromatography. Results showed that extracellular vesicles, which have an average diameter expected for this vesicle, exhibit a cup-shaped morphology and express exosomal markers. By proteomics and using a label-free method, about 200 proteins were identified. Bioinformatic analysis revealed that exosomes carry a high number of proteins involved in important cellular processes. We also compare NSC-EVs proteome with proteins found in mouse NSC and EVs derived from mesenchymal stem cells. Gene Ontology analysis was performed and proteins were classified according to cellular component, biological process and molecular function. The identified proteins might serve as potential therapeutic targets for some neurodegenerative diseases.

CB-25

ANDROGEN RECEPTOR NEGATIVELY REGULATES ACSL4 TRANSCRIPTION IN BREAST CANCER CELLS

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Acyl-CoA synthetase 4 (ACSL4) is an enzyme involved in arachidonic acid metabolism. ACSL4 overexpression is known to play a key role in the development of aggressive prostate and breast cancer. In prostate cancer, it has been shown that inhibition of the androgen receptor (AR) leads to overexpression of ACSL4, suggesting that this enzyme may have an important role in these hormone-resistant tumors. Actually, AR signaling is emerging as an important factor in the pathogenesis of breast cancer. Several studies imply that AR may be a new marker and a potential therapeutic target among AR-positive breast cancer patients. The objective of this work is to study whether androgenic regulation is involved in ACSL4 transcription in breast cancer cells.

We previously demonstrated that the hormone receptor-positive MCF-7 breast cancer cell line expresses low levels of ACSL4 whereas the quadruple negative MDA-MB-231 cell line contains high expression of this enzyme. In this context, we verified that high levels of AR mRNA are present in MCF-7 cells and absent in the highly aggressive cell line MDA-MB-231. In a bioinformatic analysis performed with Genomatix software on a 1.8 kb fragment of the human ACSL4 promoter, we identified a consensus site for the AR with a score close to 1. This finding was also confirmed with the Alggen Promo tool. AR overexpression in MDA-MB-231 cell line results in a reduction of ACSL4 promoter activity measured by NanoGlo luciferase reporter gene assay. Moreover, we also observed a decrease in mRNA expression of ACSL4 in MDA-MB-231 overexpressing AR. Breast cancer cells treated with 10 nM dihydrotestosterone (DHT) in steroid-free medium showed a reduction of promoter transcriptional activity and of ACSL4 mRNA levels, both in MCF-7 and in MDA-MB-231 cells transiently transfected with human AR. No effect of DHT was observed in control MDA-MB-231. Furthermore, as previously described in a prostate cancer model, in MCF-7 and MDA-MB-231 cells with ectopic AR expression, transcriptional activity of ACSL4 promoter was induced by 10 μM of the AR antagonist bicalutamide (Casodex). In summary, these results demonstrate that androgens could regulate ACSL4 expression in breast cancer cells. Therefore, in the future it would be important to evaluate the effectiveness of the combined use of therapies that target the AR together with ACSL4 inhibitors in breast cancer.

CB-26

HCT116 cells as experimental model to study nuclear fucosylation

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Glycosylation has been found to be involved in tumor malignancy, developmental processes, and immune system, which has led to new insights into many human diseases. In cases of colon cancer, many reports have suggested that changes in glycosylation are involved in carcinogenesis and metastasis. Particularly, aberrant glycosylation is associated with the acquisition of all characteristic features of tumor cells. Fucosylation represents the transfer of a fucose residue (from GDP-fucose) to oligosaccharide chains. Most GDP-fucose is synthesized by the de novo pathway, in which GDP-mannose is transformed into GDP-fucose through three steps catalyzed by GDP-mannose-4,6-dehydratase (GMDS), and FX, an epimerase-reductase enzyme. The salvage pathway synthesizes GDP-fucose from free fucose derived from extracellular or lysosomal sources. The deficiency of GMDS has been reported for HCT116 cell line, caused by a deletion of 400 bp, which corresponds to three exons encoding functionally critical domains for enzyme activity. In the present work, we evaluate HCT116 cells as an in vivo model for the study of nuclear fucosylation. Since GDP-fucose is deficient in HCT116 cells, this cell line shows a marked decrease in the fucosylation level, which we characterize by western-blot and microscopy analysis. In addition, we were able to recover the fucosylation phenotype by taking advantage of the salvage pathway, supplementing the culture medium of the cell line with free fucose. This caused an increase in the fucosylation levels not only in the whole cell but also in purified nuclei. These results place the HCT116 cell line as a good model for fucosylation studies at the cellular and nuclear level, where we are able to “turn on and off” the fucosylation pathway as needed just by supplementing with free fucose.

CB-27

Histones with carbohydrate-binding ability

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Histones are nuclear proteins with the ability of binding to DNA molecules. Its main function is associated with the packaging of genetic material by the formation of nucleosomes regulating gene expression. They can be classified into five families: H1, H2A, H2B, H3 and H4. Previous evidence demonstrated the presence of different types of glycans in the cell nucleus and the ability of histones to interact with glycans. Here we investigate whether histones can specifically bind to glycosidic structures. Initially, histones purified from cow thymus were successfully biotinylated for follow-up in subsequent assays. An agarose column conjugated with ε-aminocaproylgalactosamine was used in affinity chromatography to evaluate its ability to retain them. Purified cow histones were incubated with the chromatographic support and retained histones were recovered by elution with lactose. The eluated proteins reacted with streptavidin indicating that some biotinylated histone interacted with the ligand on the column and was consequently eluted with lactose. Polyacrylamide gels stained with Coomassie Brilliant Blue allowed the identification of bands with molecular weights similar to histones. Furthermore, direct interaction assays of histone recovered from affinity chromatography with glycoproteins showed a clear interaction preference for Glucosamine-Bovine Serum Albumin (GlcN-BSA). Histone-glycan interaction assays in the presence of carbohydrates showed inhibitory effect (lactose) while other sugar (GlcN) increased the binding of histones with GlcN-BSA. Similar results were observed with recombinant human histone H2A, showing an important interaction with GlcN-BSA and differential behavior in presence of carbohydrates: GlcN enhances binding and lactose shows an inhibitory effect. Taken together, these results indicate that histones have some capacity to recognize glycans, showing similar behavior to a lectin. Further studies regarding the ability of histones to interact with glycans are in development by our research group.

CB-28

BIOINFORMATICS ANALYSIS OF POTENTIAL BIOLOGICAL EFFECTS OF HERBICIDES ON HUMAN HEALTH

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There is great controversy concerning the potential toxicological effects of several herbicides commonly used in agriculture. A growing body of scientific evidence suggests a negative impact on human health. The objective of this work is to evaluate -in a comparative manner- the potential toxicological effects on human health of four herbicides commonly used in Argentina: glyphosate, atrazine, 2,4-D, and paraquat. A bioinformatic analysis was performed to identify which proteins could be affected by their interaction with these herbicides, identifying in turn those biological processes and diseases that could be altered as a result of the interactions between these herbicides and proteins. To determine which proteins could be affected in their regulation by the use of the mentioned herbicides in humans, the STITCH platform was used. This platform integrates databases, text bases and prediction methods, generating interaction networks between a given chemical compound and those proteins involved in different biological processes. The STRING platform was used in turn to obtain a second shell of protein interactors. For both platforms, only those interactions from curated data and/or experimental data ("Database" and "Experiments", respectively) were selected. Only those interactions with a confidence score of medium or higher (≥0.4 points) were considered and a maximum of 500 interactors was set. To identify those biological processes mostly affected as a consequence of the interaction between each of the herbicides under study and those proteins identified in the interaction networks, overrepresented terms associated with the "Biological Process" category of Gene Ontology were extracted from the protein list from the interaction networks by performing an enrichment analysis. Moreover, an enrichment analysis of overrepresented terms in the protein list for those diseases annotated in the DisGeNET database was performed. Firstly, interaction networks for each one of the herbicides under study revealed the presence of proteins involved in two signaling pathways linked to cell proliferation, cell death, stress response and cell survival: the PI3K/Akt pathway and the Unfolded Protein Response (UPR). Our results also showed that all herbicides interact with proteins involved in key biological processes such as proliferation, cell growth, cytoskeletal organization, and axogenesis, among others. For all the herbicides under analysis, we could observe an enrichment in terms associated with cancer such as carcinoma, sarcoma, lymphoma, and immune tumors, and in terms associated with neuronal pathologies such as the Alzheimer's family of diseases. These results are consistent with past reports on the toxicological effects of these herbicides on human health and their impact on key biological processes. Furthermore, our results indicate that the regulation of both the PI3K/Akt pathway and the UPR could be disrupted by exposure to these herbicides.

CB-29

TUMORAL PD-L1 ORCHESTRATES MACROPHAGE IMMUNOSUPPRESSION DURING BREAST CANCER PROGRESSION

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One of the main immunosuppressive mechanisms by which cancer avoids eradication by the immune system is the expression of PD-L1, the ligand for T-cell inhibitory receptor PD-1. PD-1 activation by PD-L1 leads to CD4+/CD8+ lymphocyte exhaustion, which is at the focal point of today's cancer immune therapies. However, despite PD-1 is also expressed by immune populations of the myeloid lineage, it is not clear how PD-L1 is triggering immune evasion mechanisms mediated by myeloid immune cells. To interrogate these interactions, we generated a PD-L1 KO model in the EO771 TNBC-like cell line using CRISPR/Cas9 gene editing, allowing us to profile the immune system compartments associated to the tumor microenvironment (TME) during tumoral growth in the syngeneic line C57BL/6J.

As expected, we found that tumoral PD-L1 is required for tumor growth in independent clones. Using flow cytometry, we discovered that tumoral PD-L1 is required for monocytic-myeloid derived suppressor cells (M-MDSCs) and macrophage differentiation to pro-tumoral profiles in vivo. Co-culturing bone marrow-derived macrophages (BMDMs) and tumoral cells, we found that Macrophage M2 differentiation is directly regulated by tumoral PD-L1 in vitro. Interestingly, preliminary data show the same effect exposing BMDMs to tumor cell conditioned medium, suggesting that cell-to-cell contact is not necessary for M2 macrophage polarization.

Using flow cytometry in early vs. late-stage tumors, we found that the increase in macrophage M2 polarization during tumor growth correlates with higher PD-1 expression levels in this immune cell

population. Finally, in vitro co-cultures experiments allowed us to observe that tumoral PD-L1 does not regulate changes in macrophage PD-1 expression.

All together, these data suggest that tumor-intrinsic PD-L1 plays a key role in TNBC progression by shaping the TME, triggering immunosuppression mechanisms in the myeloid landscape beyond its canonical role. By interrogating these non-canonical mechanisms, we could gain insights into novel mechanisms of resistance to PD-L1/PD-1 immune therapies.

CB-30

Arylalkylamine N-acetyltransferase (AANAT): "Nuclear regulation by blue light and its potential role in the neuron viability of vertebrate retina".

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Arylalkylamine N-acetyltransferase (AANAT), is the key regulatory enzyme of melatonin (Mel) synthesis within the vertebrate retina and pineal gland, the rhythmic synthesis of melatonin is controlled by the enzyme Arylalkylamine N-acetyltransferase (AANAT) which converts serotonin to N-acetyl serotonin (NAS). In the retina, MEL is mainly synthesized by the photoreceptor cells (PRCs), with high levels at night and lower levels during the day as well in the pineal gland. In a previous report, we found that AANAT activity in RGCs displayed circadian rhythmicity with highest levels during the subjective day in constant dark (DD), light (LL), or in the light phase of a LD cycle on the contrary, AANAT in PRCs exhibited the typical nocturnal peak in DD and LD, but no detectable oscillation was observed under LL whereas a brief nocturnal light pulse significantly decreased AANAT activity in these cells, but had no inhibitory effect on RGCs at any time. Strikingly AA-NAT activity in RGCs of chickens undergoes a completely different regulation by light than in PRCs. The AANAT activity is controlled by a cAMP dependent phosphorylation, this post-translational modification in AANAT (pAANAT) activates the enzyme to form a protein complex with 14-3-3 proteins, leading to an increase in its activity and protecting the enzyme against the proteasome degradation. Previous results, in primary cultures of retinal cells from chicken embryos, showed that the exposure to blue light (BL) at low intensity for 15 min to 1h induces a change in the AANAT subcellular localization from the cytoplasm to nuclei, and the levels of AANAT remains higher in dark after

the BL exposure as well the pAANAT levels. Here we studied the effect of BL exposure at low intensity in primary cultures of retinal neurons cells from chicken embryos, and the effect of the silencing of AANAT using specific Sh-RNA, by electroporation, in the cellular viability by MTT assay and flow cytometry using Calcein red- AM and the reactive species of oxygen (ROS) levels usuing 2′,7′-Dichlorofluorescein diacetate (DHCDA). Our results showed that the Sh-AANAT decreases the AANAT-like protein and its transcripts levels in cultures compared with the control. In addition, the silencing of AANAT also affected the cellular viability, however the BL exposure did not affect the cellular viability in the different experimental conditions. Moreover, the ROS levels showed an increase in the cultures treated with the Sh-AANAT compared with the control. Taken together, these results suggest an important role of AANAT in the retina, playing a protective role controlling the balance of ROS, and this function could be associated with the antioxidant products NAS and Mel. Further experiments will be necessary to investigate the nuclear role of AANAT by BL in the local production of NAS and Mel as a new mechanism of protection in retinal neuron cells.

CB-31

MYO1C KNOCKDOWN AND PHARMACOLOGY INHIBITION DISTURB CHLAMYDIA TRACHOMATIS DEVELOPMENT AND BACTERIAL EXIT BY EXTRUSION IN AN ACTIN-DEPENDENT MANNER.

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Chlamydia trachomatis is an obligate intracellular Gram-negative bacterium and the most frequent bacterial agent of sexually transmitted infections. This bacterium modifies the actin cytoskeleton to ensure its entry, development, and exit. It has been reported that an actin belt surrounds chlamydial inclusion during bacterial growth. Anti-chlamydial immune response and conventional antibiotics are not enough to complete bacterial clearance and avoid female infertility associated with this infection. The development of vaccines and pharmacologic targets seems like a possible solution to *C. trachomatis* increasing prevalence. The myosin protein superfamily plays a central role in the modulation of the actin

cytoskeleton. However, the involvement of myosin in chlamydial infection has not been explored deeply. We previously described the participation of Myo1C in actin cytoskeleton stability. In this context, we hypothesize that Myo1C could be involved in actin cytoskeleton modulation during *C. trachomatis* infection. Myo1C is a single-headed class I myosin strongly present in dynamic regions of the plasma membrane, where it modulates the actin network. Working with our model of *C. trachomatis* infection in Hela cells, we determined, by confocal microscopy, that endogenous and over-express Myo1C was recruited to the chlamydial inclusion. The knockdown of Myo1C impaired the *C. trachomatis* invasion and development, causing destabilization of the actin belt that surrounds the inclusion. Moreover, this phenotype was rescued in HeLa cells overexpressing mouse GFP-Myo1C transfecting with siRNA- human Myo1C. Likewise, the over-expression of the negative mutant Myo1C ΔABL, which does not bind to actin, induces a significantly higher percentage of incomplete or absent actin belt surrounding the inclusion. Besides, it’s known that an actin belt is necessary for *C. trachomatis* to exit by extrusion. So, our objective was to determine if Myo1C is involved in this process. We purified extrusions by differential centrifugation at 48 hours of infection. We infected a monolayer of Hela with extrusion obtained from cells that overexpressed GFP, GFP-Myo1Cwt, or GFP-Myo1CΔABL cells to quantify chlamydial extrusion levels per condition. We determined a significant decrease in the extrusions recovered after over-expression of GFP-Myo1CΔABL. In line with these results, we detected significantly lower extrusion levels after Myo1C depletion. Likewise, extrusions recovered at 48 h pi from cells incubated for 6 hours with pentachloropseudilin (PClP), a Myo1-specific inhibitor, were significantly lower than the control condition. Altogether these results indicate that MYO1C would stabilize the surrounding actin ring and that the knockdown and pharmacological Myo1C inhibition disrupts *C. trachomatis* development and exit by extrusion.

CB-32

The expression level of the cyclin kinase inhibitor, p21, in cells transiting s-phase modulates the rate of dna fork speed to prevent genomic instability

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Cell cycle arrest outside S phase requires the expression of high levels of p21, a cyclin kinase inhibitor. In opposition, during S-phase, cyclin kinases activity is high due to low levels of p21. Therefore, basal levels of p21 were considered to have no role in S-phase until recently when two manuscripts published by us and another laboratory showed that such low levels of p21 in S-phase modulate the speed of nascent DNA synthesis. Both reports concluded that such an effect of p21 on DNA replication depends on its interaction with PCNA (Proliferating Cell Nuclear Antigen), an auxiliary factor that recruits DNA polymerases to DNA. Intriguingly, however, the two manuscripts indicated opposite effects of p21 depletion on the speed of nascent DNA synthesis (decrease vs increase). Here we show that the extent of p21 downregulation explains such apparently contradictory results. Full p21 downregulation achieved by p21 knockout or efficient siRNA-mediated knockdown decrease fork speed in a manner that depends on the enhanced participation of the low processivity DNA polymerase, Pol Kappa, in DNA replication. In contrast, partial p21 downregulation by siRNA-mediated knockdown increases fork speed by enabling the participation in DNA replication of PrimPol, a DNA polymerase with primase activity that promotes DNA replication restart. Remarkably, both the excess contribution of Pol Kappa or PrimPol to DNA replication events when p21 is fully or partially downregulated trigger the augmentation of several genomic instability markers. Furthermore, our work points to the sources of genomic instability when p21 is fully or partially down regulated being different, and they depend on Pol Kappa and PrimPol activity, respectively. We also identified that transcriptional modulation of p21 as a consequence of loss of p53 on established p53-deficient cell lines triggers genomic instability dependent on Pol Kappa or PrimPol if the basal p21 level is non-existent or low, respectively. Such results reveal an unexpected and quite complex tumor suppressor role of p21 as a guardian of the quality of newly synthesized DNA that prevents spontaneous mutation events.

CB-33

Improving the quality of cell killing by Chk1 inhibitors

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Due to their fast proliferation, tumor cells replicate their genome more frequently than normal cells. Such a difference is exploited by many of the currently approved chemotherapy regimens that cause replication stress-mediated cancer cell killing. The main drawback of this strategy is the accumulation of chromosome instability (CIN) in the fraction of cancer cells that survive the treatment. The accumulation of CIN is a potential cause of treatment failure as there are no current tools to prevent it, and it can introduce mutations that promote the acquisition of resistance to the treatment. One novel approach under clinical investigation is using checkpoint kinase 1 inhibitors, Chk1i. As with other approved therapies, Chk1i causes replication stress, CIN and cell death (CD) in cancer cells. Here, we present data showing that the inhibition of PLK1, a master regulator of mitosis progression, prevents CIN without affecting the CD caused by Chk1i. We have previously shown that, whereas Chk1i-triggered CD is associated with acute replication stress in S phase, CIN accumulates in cells that finalize S phase and fail to duplicate under-replicated DNA (UR-DNA) in M phase fully. These results demonstrate that CD and CIN after Chk1i are dissectible at the molecular level, suggesting that M-phase triggered CIN could be reduced without impairing S-phase associated CD. While more work is required to understand the molecular basis of the counteracting effect of PLK1 inhibition on CIN induction, these results provide a proof of concept for the design of novel anti-cancer strategies aimed at inducing a CIN-free CD of cancer cells.

CB-34

ALPHA 2-MACROGLOBULIN, A NATURAL LRP1 LIGAND, STIMULATES MITOPHAGY IN ERYTHROLEUKEMIC CELLS

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Erythropoiesis is a highly regulated process by which red blood cells are generated from hematopoietic stem and progenitor cells (HSPCs) located in the bone marrow. During this process, the erythroid precursors undergo a series of physiological changes necessary for the maturation of the erythrocyte. These changes involve membrane remodeling, cell volume decrease, specific protein synthesis such as hemoglobin, membranous organelles clearance by autophagy and enucleation. Autophagy is a

degradation and recycling process by which cytosolic macromolecules and even entire organelles are transported to lysosomes. During mitophagy (a specialized autophagic mechanism), mitochondria become trapped in double-membrane vesicles (autophagosomes) and are subsequently degraded and removed by autophagosome-lysosome fusion. This process is related with red blood cells differentiation and maturation, as well as certain mechanisms involved in chemotherapy treatment resistance of particular types of leukemia. On the other hand, low-density lipoprotein receptor-related protein 1 (LRP1) is a transmembrane endocytic receptor involved in many cellular processes, such as cell migration, apoptosis, autophagy and the metabolism of molecules such as alpha-2-macroglobulin (α2m). Previously, we have shown that hemin, a physiological inducer of erythropoiesis, is capable of inducing autophagy in the erythroid cell line K562 through its binding to LRP1. We want to determine the specific role of the LRP1 receptor, as a mitophagy modulator, during human terminal erythropoiesis using different ligands (hemin, LDL and α2m). Interestingly, our recent results indicate that α2m, which is a natural ligand of LRP1 and is not an inducer of erythropoiesis (like hemin), is also capable of autophagy stimulation in erythroleukemic cells. Furthermore, as we have identified the presence of mitochondria inside autophagosomes of α2m incubated K562 cells, we propose that α2m is responsible for the mitophagy induction. These findings lead us to consider that LRP1 would be a key receptor in the erythropoietic process, participating in mitophagy stimulation during the erythroid maturation process. This study could provide valuable knowledge for understanding the physiology of the erythroid maturation process, as well as for possible treatment developments against some pathologies associated with this process, such as leukemia.

CB-35

Activated alpha 2-macroglobulin and aggregated LDL regulate differential LRP1 activation and receptor accumulation at early endosomes

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Low-density lipoprotein (LDL) receptor-related protein-1 (LRP1) is an endocytic and signaling receptor expressed in several cell types. This receptor binds more than 40 different ligands, included alpha 2-macroglobulin-proteinase complex (activated a2M or a2M*) and aggregated low-density lipoprotein

(agLDL). Previously, we showed that a2M* induces the LRP1 endocytic recycling to plasma membrane, whereas agLDL leads this receptor to locate in degradation compartments. These data suggest that LRP1 follows different intracellular routes depending on the ligand interacting. Here we evaluate the effect of a2M* and agLDL on LRP1 expression, endocytosis and intracellular signaling activation in HeLa cells. By Western blot we found that a2M* induces a significant increase for LRP1 protein expression from 8 h of stimulation, whereas agLDL does not produce any change after 24 h of stimulus. Both ligands unmodified the sortilin protein expression, a sorting protein fundamental for LRP1 traffic. By confocal microscopy we showed that a2M* and agLDL promote LRP1 endocytosis with significant accumulation in EEA1+-early endosomes (EEA1+-EE) for 30 min of continuous incubation. Through image analysis we established that a2M* produces EEA+-EE vesicles with increased surface values compared to agLDL-stimulated and non-stimulated cells. Finally, a2M*, but not agLDL, activates Akt pathway. These data suggest that ligands produce different processes of membrane fusion at early endosomes during LRP1 endocytosis and regulate the receptor activation, which in part may explain non-redundant functions of a2M* and agLDL ligands.

CB-36

The Ursodeoxycholic acid targets PERK and ameliorates neurite atrophy in a cellular model of GM2-gangliosidosis.

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Progressive neurodegeneration in Tay-sachs and Sandhoff diseases are characterized by the accumulation of GM2 ganglioside in the brain, which is known as GM2 gangliosidosis. This triggers Endoplasmic Reticulum (ER) stress hence activating the transduction signaling cascade UPR (Unfolded Protein Response). We have previously proved PERK sensor contributes either to regain ER homeostasis or to neuronal apoptosis, in acute or chronic phase of stress correspondingly. Although extensive work is being performed to develop treatments for these disorders, still there is no current approved therapy. In cell and animal models of other ER stress related diseases, chemical chaperones are known to be able

to alleviate ER stress. We particularly tested ursodeoxycholic acid (UDCA), since it's able to move across the blood brain barrier and it has low toxicity which makes it an interesting therapeutic tool. In this study, we showed that pre and post treatment UDCA significantly diminishes neuritic atrophy in primary neuron culture by performing microtubule-associated protein 2 (MAP2) immunocytochemistry. We evaluated whether in this protective effect participates PERK. Indeed, in our cellular model, we demonstrated that UDCA, significantly increases PERK autophosphorylation and decreases the upregulation of its pro-apoptotic factor CHOP Afterwards, potential UDCA action mechanism were studied in vitro by performing kinase and crosslinking assays, using different recombinant protein PERK variations, either in solution or in rebuilt liposomes. Data from these assays allows us to propose a direct interaction between UDCA and the cytosolic PERK domain which stabilized PERK dimerization enhancing the auto- phosphorylation. All these, suggests that UDCA modulates PERK changing the susceptibility to undergo neurite atrophy in a GM2 gangliosidosis cellular model.

CB-37

APEX-seq a new tool to investigate membraneless organelles such as Smaug MLOs

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There are a variety of membraneless organelles within cells, formed from a liquid-liquid phase separation where several molecules and transcripts end up condensing in these cell bodies. However, the study of its composition with techniques such as biochemical fractionation can be complex. Here, we apply a proximity labeling technique called APEX-seq, to study the composition of cytoplasmic membraneless organelles called Smaug-bodies. This powerful technique uses an ascorbate peroxidase (APEX), which oxidizes biotin substrates into highly reactive radicals capable of interacting with both proteins and RNAs within a radius of approximately 25 nanometers. We fused APEX to Smaug1 and Smaug2 proteins that are components of the Smaug bodies, and we were able to verify the performance of this technique by

immunofluorescence. This technique will allow us to study the identity of the RNAs enriched inside the Smaug bodies and improve the understanding of these organelles.

CB-38

Cytosolic Ca2+ increase generated by the translocon modulates PERK activation

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The endoplasmic reticulum (ER) is a dynamic organelle that plays a critical role in a variety of processes, which can be performed only if the Ca2+ concentration in the lumen is optimal. When the load of newly synthesized proteins exceeds the folding capacity in the organelle, the ER enters into stress condition. To restore the homeostasis, it´s activated a signaling transduction pathway termed Unfolded Protein Response (UPR). An immediate response is the attenuation of protein synthesis due to the activation of (PKR)-like-ER kinase (PERK) a ER-transmembrane protein that is generally inactive due to its association with the chaperone BiP. During ER stress, BiP is titrated by the unfolded protein, leading to PERK activation and phosphorylation of eIF2α, which attenuates protein synthesis. We demonstrated that calcineurin A/B (CN), directly associates with PERK, increasing its auto-phosphorylation and significantly enhancing inhibition of protein translation. Besides, the β isoform of subunit A of CN, in astrocytes has an important PERK-dependent cytoprotective effect. Although Ca2+ is a versatile signal that can regulate many different cellular functions, little is known about its role in restoring homeostasis, once UPR is activated. In this respect, we recently described an active Ca2+ release initiated by microdomains in stressed human astrocytes and in a HEK cell line with knockout of IP3R isoforms (HEK-TKO). Pharmacological and molecular studies indicate that these local events are generated by the translocon, a protein complex (Sec61α, β, γ), where Sec61α, extends on the ER lipid bilayer and forms the pore. Here we evaluated the effect of Ca2+ originated by translocon on PERK activation. Astrocytes expressing ER-anchored GCamP6-Cytb5 were pre-incubated with BAPTA-AM, which chelate the Ca2+ events evoked by the stressor Tunicamycin (Tm). Also, we quantified the number of particles of PERK clustering by immunocytochemistry and the eIF2α-P expression level by western blot. PERK activation was partially

reverted in BAPTA-AM-loaded and stressed astrocytes. We demonstrated that BiP limits Tm-induced Ca2+ release. In order to investigate the effect of Ca2+ originated by translocon on PERK phosphorylation, we overexpressed BiP in HEK-TKO cells and performed phospho-PERK immunolabeled. We found that PERK clustering significantly decreases in overexpressing BiP cells with respect to the stressed control cells. We have shown that, under acute Tm treatment, in HEK-TKO cells, PERK forms a macromolecular complex with the translocon pore (Sec61α) and CN by performing a blue native PAGE followed by 2nd dimensional SDS-PAGE gels. By pharmacological agents that modify the activity of the translocon, and an antibody anti-PERK that recognizes (phosphorylated and non-phosphorylated forms). We observed a dynamic arrangement of PERK complexes participating in three large assemblies. The P-PERK/PERK ratio in each macromolecular complex appears to change according to the condition tested. Overall, these data strongly suggest that PERK is activated by Ca2+ increase generated by the translocon and that this signal is promoted by the macromolecular arrangement between Sec61α, CN and PERK during the acute phase of UPR.

CB-39

Hyaluronan and chemical derivatives affect monocyte/macrophage interaction with mesenchymal stem cells under co-culture conditions

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Monocytes/macrophages (Mo/MØ) are key regulators of tissue repair and regeneration, and their proper activation is necessary for tissue homeostasis. Along with Mo/MØ, mesenchymal stem cells (MSCs) have gained importance in regenerative contexts. Hyaluronan (HA), one of the main components of the extracellular matrix, interacts with MSCs, being able to regulate their functional profile. Numerous antecedents demonstrate a proactive interaction between MSCs and Mo/MØ, however, the role of this

interaction in a regenerative context is unknown. Since the activation of Mo/MØ is an event that leads to changes in the redox balance and the development of the inflammatory process, we evaluated the production of oxidative stress and inflammatory factors by these cells under stimulation of the MSCs secretome and in direct co-culture. To do this, MSCs were treated with HA (MSCs HA) and with its sulfated chemical derivatives (MSCs sHA1 and MSCs sHA3) at a concentration of 100 µg/ml for 48 h. Next, Mo/MØ from peripheral blood, enriched by Ficoll/Percoll gradient, were cultured with treated and untreated MSCs (MSCs BC) in a 1:10 ratio (MSCs:Mo/MØ) for 72 h or stimulated with the secretome derived from these cells. The total antioxidant capacity (TAC) was determined by the ABTS•+ cation inhibition assay, the induction of the antioxidant enzyme catalase (CAT) by the Aebi method, and the levels of nitrite, a water-soluble and stable nitric oxide metabolite, were measured spectrophotometrically by Griess reaction. Furthermore, we analyzed the expression level of TSG-6 mRNA under these conditions by RTqPCR. The data obtained was analyzed using the GraphPad Prism software. We show that the antioxidant status of Mo/MØ did not change after secretome stimulation of MSCs treated with HA and sulfated derivatives compared to the basal condition. However, the antioxidant status of Mo/MØ was regulated in the co-culture system. We found a higher TAC ($p<0.01$) in Mo/MØ co-cultured with MSCs sHA3, in agreement with a higher specific activity of CAT ($p<0.05$) and a higher production of nitrite in this condition compared to co-culture under basal conditions (Mo/MØ-MSCS BC). The opposite effect was observed for Mo/MØ that were cultured with MSCs HA and MSCs sHA1. Furthermore, TSG-6 production was increased in Mo/MØ cultured with MSCs sHA1 in agreement with the reduction in antioxidant activity in this condition. Our results indicate that in a Mo/MØ and MSCs sHA3 co-culture system, the antioxidant system is up-regulated, contrary to what occurs in a Mo/MØ and MSCs HA and sHA1 co-culture, and that this effect depends on cell-cell interaction. They further suggest that TSG-6 lowers oxidative stress under co-culture conditions. In conclusion, HA and its derivatives affect the interaction of Mo/MØ with MSCs by altering the activity of the antioxidant system and the expression of TSG-6 in Mo/MØ, suggesting that it is an inducer of inflammatory signals that could affect tissue regeneration.

CB-40

ID4 an oncogene in TNBC with properties to repress luminal genes

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Background: Inhibitor of differentiation protein 4 (ID4) is a dominant negative regulator of the basic helix-loop-helix (bHLH) family of transcription factors. In different tumor types ID proteins are associated with loss of differentiation, stemness, and neoangiogenesis. In breast cancer, ID4 is highly expressed in the triple-negative breast cancer (TNBC) subtype and high ID4 expression is associated with stem-like phenotype and worst prognosis amongst other BC subtypes. It has been proposed that ID4 regulates the expression of genes that participate in luminal differentiation such as estrogen receptor (ER), FOXC1, and NOTCH1. Since ID4 downregulates the expression of genes involved in luminal specialization and since ID4 is overexpressed and meets an oncogenic function in TN tumors, we hypothesize that silencing ID4 in TN tumors will unlock the expression of genes required for luminal differentiation and will revert the aggressive behavior of this tumor type. Methods: In vitro: MDA-MB231 TN cell line ID4 silencing by siRNA or by shRNA, gene and protein expression analysis by RT-qPCR and western blot. In vivo: MDA-MB231 cells lines (silenced and control) were inoculated in the fourth mammary gland of NSG mice. In-silico analyses involved the evaluation of differentially expressed genes according to ID4 expression on human breast tumors of public datasets. Results: We first evaluated differential gene expression patterns among breast cancer samples with high vs low ID4 expression. To do so we performed *in silico* differential gene expression analysis from the TCGA database from 680 samples. Our analysis revealed a total of 922 differentially expressed genes (DEGs) (290 down-regulated and 632 up-regulated) ($logFC > 1.5$ and $logFC < -1.5$, $p\text{-value} < 0.05$). Amongst downregulated genes we found that ER, GATA3, and FOXA1 were the ones with the highest fold change values and highest significance. To determine the biological processes in which the downregulated genes are involved, we did pathway analysis which revealed that the most significant downregulated genes ($logFC < -2.9$) belong to the regulation of intracellular estrogen receptor signaling pathway (GO:0033146) ($p < 0.001$). These results suggest that when ID4 expression is high it may have a role in suppression of luminal differentiation genes. To further test our hypothesis, we carried out in vitro experiments with the objective to silence ID4 and evaluate changes in the expression of luminal genes and in aggressive phenotypic features. Our results revealed that ID4 silencing induced an increase in the expression of two luminal genes tested: ER and GATA3 ($p < 0.05$). In vitro migration assay determined that ID4 silenced cells migrated less ($p < 0{,}01$) and in vivo experiments revealed that tumor size was significantly smaller in the ID4 silenced group compared to control tumors ($p < 0.01$). Conclusions: Our findings reveal that ID4 may unlock the expression of luminal genes and revert some aggressive characteristics of TN tumors.

CB-41

Role of the transcription factor CREB3L1 in the regulation of lipids homeostasis

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The transcription factor CREB3L1 regulates the expression of proteins involved in the secretory pathway (transport factors) as well as the expression of tissue-specific genes that require the secretory pathway to reach their final localization. We have previously shown that, in thyroid cells, CREB3L1 acts as a downstream effector of the Thyroid Stimulating Hormone (TSH) inducing the expression of proteins of the secretory pathway along with an expansion of the Golgi volume. We hypothesize that CREB3L1 also modulates the lipids homeostasis allowing thyroid cells to adapt to TSH stimulation. In this regards, lipidomic analysis of the rat thyroid follicular cell line PCCL3 shows that CREB3L1 knockdown (by siCREB3L1) induces higher expression levels of certain phosphatidylcholines (PC) and LysoPC species. Furthermore, both the CREB3L1 overexpression (by using CREB3L1CA) or TSH stimulation increase the same lipids species such as triacylglycerols (TAG) and lysobisphosphatidic acid (LBPA), as well as the metabolic processes associated with these lipids. In contrast, the lipids that increase in siCREB3L1 treated cells bear some degree of similarity to those that increase in TSH-deprived cells. This is comparable to what occurs in the absence of TSH (where CREB3L1 levels are low). These results highlight the role of CREB3L1 in the regulation of lipids homeostasis in thyroid cells.

CB-42

Increment of Complexin-2 in insulin resistance-like states. Possible involvement in deficient GLUT4-translocation

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Introduction: Insulin-induced glucose uptake in muscle and adipose tissue is mediated by the glucose transporter GLUT4. In the resting state, GLUT4 is stored in intracellular compartments (recycling compartment and vesicles GSVs -GLUT4 store vesicles- and IRVs -insulin-responsive vesicles-). The insulin increase promotes the trafficking and fusion of GSV/IRV with the plasma membrane (MP), therefore increasing the levels of GLUT4 on the cell surface (GLUT4 exocytosis). As with many exocytic processes, in GLUT4 exocytosis the SNAREs and accessory proteins play a major role in regulating GSV/IRV fusion with MP. Our group has recently shown, for the first time, the existence of complexin2 both in muscle tissue and in myoblastic cells, which, upon insulin stimulus, migrates towards MP. We also show that the lack and excess of complexin2 decrease GLUT4 exocytosis, these observations being consistent with the role of complexin2 in the stabilization of the trans-SNARE complex. On the other hand, there is evidence that the sustained insulin stimulus, (given by a high yeast diet), induces an increase in complexin levels, compromising motor neurotransmission in *Drosophila*. Objective: To evaluate the expression level of complexin2 in myoblastic cells maintained under conditions of high insulin concentration for prolonged periods (insulin resistance conditions). Materials and Methods: L6 myoblast cells were incubated with 100nM insulin for 2, 4, and 7 days. The level of protein expression and mRNA were evaluated by Western blot and RT-PCR, respectively. Results: So far, the results show a gradual increase in both the protein and the mRNA of complexin2. Conclusions: The increase in mRNA and protein would indicate an increase in the transcription and translation of complexin2 by insulin action. Based on the mechanism of action of complexin on the SNARE complex in other working models, and based on our results in L6 cells, we believe that the increase in complexin2 observed by high concentrations of insulin could slow down or decrease GLUT4 exocytosis, contributing, at least partially with to the decrease of GLUT4 over the cell surface observed in states of insulin resistance.

Biosynthesis of N-acetyllactosamine glycans in human cell nucleus

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O-glycosylation is a protein post-translational modification in human cells that is critical for cell physiology and involved in several pathologies. In particular, O-GlcNAc glycosylation is found in nuclear proteins with regulatory functions in the cell nucleus and showing altered expression in different cancer types. Moreover, there is no evidence about the elongation of this glycan in nuclear proteins. In the present work we study the catalytic activity of β galactosyltransferase in the cell nucleus, necessary for the elongation of O-GlcNAc glycan in N-acetyllactosamine. We detected the presence of β-4 Galactosyltransferase 1 in the nucleus of human cells such as CaCo2, HeLa and A549 by confocal microscopy including z-axis cutting. This enzyme catalyzes the covalent union between galactose (using UDP-galactose as donor) and O-GlcNAc terminals (acceptor) via β 14 bond to form nuclear O-acetyllactosamine. It is important to mention that UDP-Gal is permeable to the nuclear membrane, so it is biologically available in the cell nucleus. We studied the nuclear β-galactosyltransferase activity through in vitro assays for purified nucleoplasm of different line cells (HeLa and CaCo2). This activity was measured on different glycoprotein acceptors such as GlcN-BSA and ovalbumin, and detected by using biotinylated Erythrina cristagalli lectin (ECL) that is able to bond to terminal lactosamine residues. In these studies we found an important β -galactosyltransferase activity in cell nucleoplasm. We also find constitutive presence of lactosamine residues in nuclear proteins by immunofluorescence and western blot, using ECL as a probe. Finally, we measured lactosamine residues in nuclear proteins such as Lamin B1 and RNA pol2 by ELISA sandwich assays. All together show that the machinery needed for O-GlcNAc elongation is located in the human cell nucleus. We are working on additional evidences of this important biochemical discovery.

CB-44

RAB24, A NOVEL REGULATOR OF TFEB ACTIVITY

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Autophagy is a highly-conserved degradation/recycling pathway that allows cells to recycle unwanted or damaged material to preserve cellular homeostasis. This highly dynamic process requires the formation of double-membrane vesicles, termed autophagosomes, which engulf and deliver the cargo to lysosomes, to digest the incorporated material. RAB proteins are a family of small GTPases that regulate intracellular vesicle trafficking events, including autophagy. RAB24 is one of the small GTPases which has been involved in vesicle trafficking events and autophagosome maturation.The serine/threonine protein kinase mTORC1, represents a major suppressor of autophagy. When nutrients and growth factors are abundant, mTORC1 is translocated from the cytoplasm to the lysosomal surface, promoting cell growth and suppressing autophagic activity. Activated mTORC1, suppresses lysosome catabolism by inhibiting the nuclear translocation of the transcription factor EB (TFEB). TFEB acts as a master transcriptional regulator of lysosome biogenesis and autophagy, regulating several genes involved in autophagosomal biogenesis, such as Beclin1 and Atg5. Beclin1 is a component of the class III phosphatidylinositol 3 kinase complex (PIK3C3), that generates PtdIns3P (phosphatidylinositol 3-phosphate), and this compound is subsequently bound by effectors like WIPI (WDrepeat-interacting phosphoinositide proteins) and DFCP1 (double FYVE domain containing protein 1), that are necessary for the biogenesis of the autophagosome. Atg5, a part of E3 ubiquitin ligase-like complex (Atg12-Atg5/Atg16L1), is considered to be an essential molecule for the induction of autophagy. Our group have previously demonstrated that Rab24 participates in both the endosomal degradation process and autophagy, but its precise role in this latter pathway remains elusive. For this reason we have studied, in different cellular models, the effects of Rab24 in mTORC1 activity, TFEB localization and ATG5/Beclin1 levels. To do this we use several techniques, such as protein transfection, RNAi knockdown, immunofluorescence and Western blot assays. Taken together, our results point to a novel function of RAB24 in autophagosomal biogenesis and provide relevant insights about the role of Rab24 in autophagy.

CB-45

Distinctive nuclear methylation patterning according to the degree of mitochondrial DNA heteroplasmy. Opportunity for treatment of mitochondrial diseases?

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Introduction: Mitochondrial disorders (MD) are caused by mutations in nuclear or mitochondrial DNA (mtDNA). mtDNA disease-causing mutations co-exist with wild-type molecules and the percentage of mutated mtDNA, called heteroplasmy, defines the emergence of disease when higher than a threshold. So, reducing the mutant to wild-type proportion or "heteroplasmy shift" is a valuable strategy for treatment. There is evidence that different degrees of mitochondrial stress modify the nuclear epigenome in a specific manner. Particularly, nuclear DNA methylation patterning is proposed as an adaptive response to intense chronic mitochondrial stress. Considering that high-heteroplasmy is associated with intense mitochondrial dysfunction, we hypothesized that these highly stressed cells have this epigenetic mechanism turned on. If disrupted, this could favor the survival of low-heteroplasmy and wild-type cells, and so become a strategy for heteroplasmy shift. Methods: We characterized the nuclear DNA methylation of cybrid cells with the m.13513G>A disease-causing mutation at different heteroplasmy levels using Infinium 850k Methylation EPIC array®. Gene enrichment analysis of the differentially methylated regions (DMR) was performed with ENRICHR®. We measured proliferation rates using the BN00566 fluorescence kit and mtDNA heteroplasmy with qPCR.

Results: The nuclear DNA methylation pattern of the m.13513G>A cybrids distinctively separated the samples according to their mutation load. Enrichment analysis of the DMR (wild-type versus mutated and high versus low heteroplasmy) showed significant hypermethylation of apoptotic and tissue differentiation pathways and hypomethylation of proliferation-related routes. We measured proliferation rates of wild-type, low (~10% heteroplasmy) and high (~70%) heteroplasmy cybrids. Basal proliferation rates were increased in the high heteroplasmy cells (1.7 times high versus low heteroplasmy; 1.2 times high heteroplasmy versus wild-type cells). When treating these cells with general DNA methylation disruptors (5-Azacytidine and Decitabine), proliferation was distinctively decelerated in the high heteroplasmy cells versus low heteroplasmy cells: 37% versus 0% for 5-azacytidine; 50% versus 15% for decitabine conditions. Treatment with decitabine of the high heteroplasmy cells decreased the m.13513G>A heteroplasmy (~20% reduction, $p<0.01$).

Conclusions: There is a distinctive nuclear DNA methylation pattern associated with the level of heteroplasmy in cybrid cells. This epigenetic programming seems to induce an adaptive pro-survival response that is proportional to the heteroplasmy level. When this pattern is disrupted, high heteroplasmy cells lose their proliferative advantage and heteroplasmy levels are reduced. Being that many epigenetic modulators are FDA-approved drugs, this could be an opportunity for drug repurposing and treatment for MDs.

CB-46

Is GLUT1 a new PURA target?

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Introduction: Genetic alterations of PURA or Pur-alpha have been detected as a cause of human disease not too long ago with the advent in the use of next-generation sequencing technologies in the clinic. PURA is expressed in the brain, muscle, heart, and blood. It is known to be essential for normal brain development. It binds to purine-rich nucleotide sequences and acts as a transcriptional regulator, enhancing or blocking gene transcription. Also, it can transport mRNAs to facilitate specific site protein translation. In 2018, we described for the first time the presence of hypoglycorrhachia in a patient with PURA deficiency. Until then, hypoglycorrhachia was considered a pathognomonic sign of GLUT1 deficiency. Glucose transporter GLUT1 or SLC2A1 is essential for the correct entry of glucose into the Central Nervous System through the blood-brain barrier. When defective, this generates a neurological phenotype with seizures, hypotonia, acquired microcephaly and dystonia. Therefore, PURA deficiency and GLUT1 deficiency phenotypes overlap. In our previous work, we verified a lower expression of GLUT1 in the patient's blood. We then hypothesize that PURA regulates GLUT1's expression, translation and/or function. Methods: In silico analysis of PURA and GLUT1 expression was performed using xenabrowser.net, TCGA TARGET GTEx database. Cell culture model: Hela cells. PURA knock down cells were obtained using shRNA through lentiviral transfection. PURA over-expression was carried out using a mRNA PURA plasmid through polyethylenimine transfection. mRNA quantification was performed using RTqPCR. PURA and GLUT1 proteins were quantified using Western Blot. PURA and GLUT1 proteins were visualized using indirect immunofluorescence through confocal microscopy. Results: In silico: the analysis of the expression of PURA and GLUT1 in normal nervous tissue resulted in a positive correlation between the expression of both genes. Pearson's rho $r = 0.4959$ ($p = 7.328e-72$). In vitro: When knocking down or overexpressing PURA we did not see differences in the mRNA level of GLUT1. Nevertheless, when we explored GLUT1 protein levels, PURA knock-down slightly reduced its expression, whereas PURA overexpression slightly increased it, although not significantly ($p=0.1$). Anyhow, when comparing GLUT1 protein levels between PURA knock-

downs and PURA overexpressed cells, they were significantly higher in the PURA overexpression condition ($p<0.05$). When looking at the localization of PURA and GLUT1 inside the cell, their interaction became evident in cells that overexpressed PURA and were stressed with a mitochondrial inhibitor (Rotenone, which increases GLUT1). In this condition, PURA and GLUT1 colocalization appeared. Conclusions: GLUT1 appears to be a target for PURA regulation. This regulation seems to be mainly important in stress conditions where glycolysis flux is exacerbated and GLUT1's function is pivotal for the cell's metabolism.

CB-47

Dictyostelium discoideum as a new model for rare disease Friedreich ataxia.

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Friedreich ataxia is a neurodegenerative disease caused by mutations of frataxin. Frataxin is a protein that participates in the mitochondrial desulfurase supercomplex. In this work we propose to model this disease using *D. discoideum*, in order to better understand its pathophysiology and to be able to develop and evaluate therapeutic strategies. Using the CRISPR-Cas9 genetic editing technology, we have obtained clones with mutations in the frataxin gene that present different cellular alterations. We have selected two clones for further analysis. One clone presents a premature stop codon that would produce a truncated most likely non-functional protein (clone 8) and another clone carries a small insertion of 2 residues that should produce a less severe effect. We present a preliminary characterization of both clones. We have been able to describe a variety of partially overlapping phenotypes, such as decreased growth, slowing of multicellular development, greater sensitivity to oxidative stress and decreased activity of aconitase and succinate dehydrogenase. These mitochondrial enzymes depend on iron-sulfur centers and their disfunction have been reported as a hallmark of the disease. We have been able to express

frataxin variant (DdFXNG122V) that mimics a mutation found in patients and to evaluate its ability to rescue the phenotypes in our CRISPR clones. Interestingly some phenotypes are fully rescued while others are only partially restored. This constitutes a powerful tool to deepen our understanding of the pathological consequences of different mutations. This new FA model we hope will help in the search for new therapeutic compounds and strategies for Friedreich Ataxia.

CB-48

Zika virus proteins regulate the expression of the secretory protein Synaptotagmin-9

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Despite the fact that the flavivirus life cycle has been extensively studied, there are still many open questions to answer. One of them consists in the mechanism for the release of the new virions, as well as the secretion pathways for certain viral proteins, mainly the Non Structural protein 1 (NS1). Previous reports have suggested that these processes may involve vesicular transport, in which vesicles containing new virions or secretable isoforms of the viral protein NS1 are transported from the Endoplasmic Reticulum and Golgi apparatus to the cell membrane. However, the cellular proteins involved in these processes are still missed. In this context, we decided to study the role of a cellular protein barely studied until now, called Synaptotagmin-9 (SYT9). Previous studies determined that SYT9 plays a role in the intracellular trafficking of protein-laden vesicles in different tissues. Therefore, we first evaluated the effect of the secretable NS1 viral protein over the expression of SYT9. Our results showed that the presence of this viral protein significantly increases the expression of SYT9 using in vitro transfection models. In addition, we demonstrated a marked subcellular redistribution of SYT9 and an intense colocalization pattern of both proteins by immunofluorescence assays. Interestingly, the changes of SYT9 expression in the presence of NS1 were also observed in ZIKV infected cells. Subsequently, we wanted to assess a potential contribution of SYT9 in virus assembly and release. For this, we initiated the analysis of the impact of one of the

structural ZIKV proteins, the envelope (Env) protein, on SYT9 expression. We could show that Env protein induces an increase in SYT9 abundance together with changes in its subcellular localization with an intense co-localization with Env. Again, these observations were also found in ZIKV infected cells. Furthermore, we evaluated the expression of SYT9 in the presence of a viral protein not related to secretion processes such as the Non Structural protein 4A (NS4A). The presence of NS4A did not induce clear changes in either the levels or localization of SYT9, suggesting that our previous findings were not an artifact due to the expression of any viral protein. Altogether our results suggest that SYT9 plays a role during ZIKV infection, probably being involved in the transport of vesicles loaded with viral proteins and/or new virions produced in infected cells. Although much more studies are necessary, this work shows for the first time the association of a member of the synaptotagmin family protein with the life cycle of flaviviruses, laying the bases for further research to identify potential new therapeutic targets to control viral dissemination in the infected host.

CB-49

Interaction analysis of Hsc70 and CSPβ proteins during the acrosome reaction in human sperm

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The fertilization of the ovule by the sperm gives rise to a new individual. In this process, the sperm executes a regulated exocytosis called acrosome reaction (AR), which is mediated by evolutionarily conserved proteins. Molecular chaperones participate in protein complex association. Cysteine String Protein (CSP) and Heat shock cognate 70 kDa (Hsc70) are chaperones that contribute to exocytosis interacting with the SNARE complex. In a previous work of our lab, we showed that recombinant non-palmitoylated CSPβ inhibited the AR triggered by calcium. But, the mechanism of this interaction has yet to be fully explained in human sperm, so we have focused on these chaperones' interplay. On the other hand, it has been reported that a loop in the CSP protein, called HPD loop, is crucial for the interaction and functionality of the Hsc70-CSP complex. This work aimed at studying the interaction between Hsc70 and CSPβ, both in vitro and in silico, and proposes a mechanism to explain the AR inhibition by the recombinant CSPβ. Using

dot blot and functional assays, we demonstrated that a CSP mutant (D45A) cannot bind Hsc70 and does not affect the AR stimulated with calcium. Based on already reported complexes in other species, we have performed a model for the Hsc70-CSPβ complex and further molecular dynamics simulations to understand critical determinants for the interaction between both proteins at an atomistic level. Our results suggest a possible mechanism to explain the inhibition of AR in which the recombinant non-palmitoylated CSPβ sequesters endogenous Hsc70, restricting its function. These results contribute to the importance of adequately forming this complex and offer insight into the implication of impairing proper acrosome reaction during fertilization. A discussion on implications in exocytosis regulation and further open questions will be presented.

CB-50

ZIKA VIRUS LIKE PARTICLES CHARACTERIZATION AS A USEFUL STRATEGY TO STUDY THE VIRAL MATURATION PROCESS.

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Zika virus (ZIKV) belongs to the Flaviviridae family and spreads mainly by the Aedes mosquitoes. ZIKV can also be transmitted sexually and by body fluids contact. ZIKV was originally detected in rhesus monkeys in Uganda in 1947, but was really put under the scope after its last outbreak in North and South America (2015-2017). ZIKV can cause an autoimmune disorder in adults that affects the nervous system (Guillain-Barré syndrome); and a severe brain damage in newborns from infected women (Microcephaly). Unfortunately, no vaccines or effective treatments to prevent ZIKV-related diseases are available, highlighting the urgent necessity of compelling research in this field.

ZIKV enters the host cell by specific receptor mediated endocytosis: once inside the cell, the virion fuses with lysosomes and releases its genome into the cytoplasm. Afterwards, a viral polyprotein is synthesized by translation of the viral genome and, after proper processing throughout the secretory pathway, a mature virion is generated and released from the cells. A key step in this maturation process, involves the cleavage of the viral protein prM by the host cell endopeptidase Furin to produce the small M protein (involved in ZIKV-host cell recognition) and the fragment pr. Viral Like Particles (VLPs) are non-infective

particles lacking the viral genome but having key viral proteins anchored to their membranes. VLPs have been widely used as a model to study different aspects of Flavivirus biology. Dr. Jose Galarza (CEO, TechnoVax, Inc, USA) gently transferred us two constructs, that when co-expressed generate ZIKV VLPs: i) ZO2, to express the ZIKA capsid, prM, and the E envelope protein (CprME), and ii) ZO3, to express the viral protease NS3 and its cofactor NS2B (NS2B/NS3P). In the present work, we set up the use of ZIKV VLPs generated by co-transfecting HeLa cells with the ZO2 and ZO3 constructs. We used a time-lapse of 24, 48 and 72 h post-transfection (h p.t.) and by using an anti prM antibody, we detected its expression levels by Western Blot and intracellular distribution by Immunofluorescence. We found that prM localizes at ER, cis, medial and trans-Golgi compartments, as we assayed its co-localization with different components of the secretory pathway (calnexin, GBF1, BIG1, BIG2, and Furin) and they all showed good colocalization indexes (between 0.6-0.8 Pearson coefficient). Thus, suggesting that the nascent VLPs mimic the same ZIKV pathway. Finally, we tested ZO2 and ZO3 co-transfected HeLa cells (72 h p.t.) by electron microscopy. We identified "VLP-like structures" with an average diameter size of 60 nm and a morphology that correlates with the data published before. Summarizing, here we show a useful strategy that can be developed in a regular class I biosafety cabinet, to eventually study the ZIKV maturation process. Our findings set the scene for the further testing of novel antiviral drugs, contributing to stop the diseases that ZIKV infection causes.

CB-51

Myc-regulated miRNAs modulate p53 expression and impact animal survival under nutrient deprivation

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MicroRNAs (miRNAs), are endogenous small non-coding RNAs that mediate post-transcriptional regulation of gene expression. miRNAs exert diverse functions including the regulation of cell growth, proliferation and apoptosis, and the deregulation of many of them has been associated with cancer. In the last decade, individual miRNAs have been associated with developmental and tissue-specific

functions, however the transcription factors that regulate miRNA expression remain uncharacterized. Here we show that the transcription factor Myc regulates miRNA expression and processing in *Drosophila*. Chromatin immunoprecipitation (ChIP) assays revealed that dMyc binds to DNA binding sites located at the loci of 56% of *Drosophila* miRNA coding genes and reduction of dMyc levels resulted in widespread downregulation of miRNAs gene expression. By using in vivo miRNA activity sensors we demonstrate that dMyc promotes miRNA-mediated silencing in different tissues, including the wing primordium and fat body. We also show that dMyc-dependent expression of miR-305 in the fat body modulates Dmp53 levels according to nutrient availability, which has a profound impact on the ability of the organism to respond to nutrient stress. dMyc depletion in the fat body resulted in extended survival to nutrient deprivation, which was reverted by coexpression of either miR-305 or a dominant negative version of Dmp53. Our study reveals a previously unrecognized function of dMyc as an important regulator of miRNA expression and processing, and suggests that Myc-dependent expression of specific miRNAs may have important tissue-specific functions.

CB-52

Looking for cell fusogens that work in yeast

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The fusion of the biological membranes is not spontaneous, but specific proteins, called fusogens, catalyze this process. In contrast to enveloped virus fusion and intracellular fusion, the fusogens that catalyze cell fusion are hardly known. In this sense, different works show that the HAP2/GCS1 protein is involved in the fusion of gametes of organisms as diverse as *Arabidopsis thaliana*, *Chlamydomonas reinhardtii, Tetrahymena thermophila*, *Plasmodium falciparum* and *Dictyostelium discoideum* (Speijer et al. 2015, doi: 10.1073/pnas.1501725112). In collaboration with others, our group showed that HAP2 is a bona fide fusogen. Phylogenetic and structural studies demonstrated that HAP2 is homologous to somatic cell

fusogens (FFs) and class II viral fusogens (Valansi et al. 2017, doi: 10.1083/jcb.201610093). This superfamily of fusion proteins was named FUSEXINS: fusion proteins essential for sexual reproduction and exoplasmic merger of plasma membranes. On the other hand, archaeal proteins that are homologs of fusexins have recently been identified (Moi et al. 2022, doi: 10.1038/s41467-022-31564-1). Fusexins are ancestral and are present in most eukaryotic lineages. However, they have not been detected in fungal and vertebrate genomes yet. The aim of this study is to identify and characterize candidate genes to participate in the gamete fusion reaction in humans and other species of interest. In this work, the mating of yeast *Saccharomyces cerevisiae* is the gamete fusion model used for the identification and characterization of cellular fusogens. Here we use the cell fusion phenotype of *prm1* mutants to evaluate whether different heterologous fusogens (e.g., AtHAP2, VSVG, FASTs) can complement this defect. Functional heterologous fusogens will be employed to carry out complementation genetic screenings searching for mutants that are incapable of forming diploids and that are only complemented by the heterologous fusogen.

CB-53

Golgi Phosphoprotein 3 mediates the association between β3GalT-IV and ST3Gal-II glycosyltransferases.

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Glycosphingolipids (GSL) are sialylated glycolipids located on the surface of eukaryotic cells. There they modulate the activities of membrane proteins, regulating several physiological processes. The expression levels of GSLs are specific to the cell type and changes in the glycosylation of these glycolipids are a hallmark of tumors that contributes to their development and progression. Recently it has been proposed that the Golgi oncoprotein GOLPH3 could regulate the synthesis of GSL by modulating the retention of glycolipid´s glycosiltransferases in Golgi. A feature that might contribute to GOLPH3 oncogenic trait. In the present work we analyzed the effect of GOLPH3 downregulation in the metabolism of complex sialylglycolipids. We observed by high performance thin-layer chromatography, flow cytometry and immunofluorescence that GOLPH3 knockdown (KD) in a glioblastoma cell line, produces a

downregulation of GD1a ganglioside and an increase of GM1 ganglioside. Similar results were also obtained in a breast cancer cell line, suggesting that GOLPH3 may have a general role in glycolipid metabolism in multiple tumor types. By immunofluorescence we noticed that GOLPH3 depletion did not affect the subcellular localization of β3GalT-IV-HA-YFP (GM1 synthase) and ST3Gal-II-mCherry (GD1a synthase). Also, low levels of GOLPH3 did not alter the molecular weight of N-glycosylated ST3Gal-II, meaning that GOLPH3 levels are not involved in N-glycosylation of ST3GalI-II. Hence, the down regulation of GD1a in GOLPH3 KD cells may noy be attributable to a lack of posttranslational modifications of ST3Gal-II. Previously, it has been shown that the formation of complexes between certain glycosyltransferases, determines the channeling of substrates, thus the product of one enzyme is preferentially used by the second one and not a competing glycosyltransferase. This prompted us to ask whether there is a physical association between β3GalT-IV and ST3Gal-II and if it is modulated by GOLPH3 levels. By immunoprecipitation and FRET assays on control and GOLPH3 KD cells we show for the first time that β3GalT-IV and ST3Gal-II form a complex that depends on GOLPH3 expression. This association would be responsible for enhancing the synthesis of GD1a from GM1. Our results suggest a novel mode of regulation of glycolipids synthesis, where GOLPH3 plays a crucial role.

CB-54

Studying the role of 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 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. We are interested in understanding eisosomes´ role in cell lifespan using *S. cerevisiae* as a model. Replicative lifespan (RLS) is the number of daughter cells a mother yeast cell can produce before

senescing, traditionally measured by manually dissecting mother cells from daughter cells. Using this method we found that knockout strains for the *PIL1* gene (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. Microfluidic systems have been developed to automate the dissection process, accelerating RLS determination. We were able to measure RLS of wt and eisosome´s mutants yeast strains analyzing microscope bright field images of mother cells trapped in microfluidic devices during 90 h. A decrease in the concentration of glucose or certain amino acids in the culture medium extends RLS in *S. cerevisiae*. In order to understand the mechanism underlying *pil1* mutant extended lifespan we studied if the absence of eisosomal organization leads to a nutrient imbalance state and/or alters nutrient signaling extending RLS. We found that extension of longevity in *pil1* mutant is not given by a difference in glucose consumption. Eisosomes are storage compartments of many amino acids transporters including arginine and tryptophan permeases Can1 and Tat2. Deletion of *CAN1* and *TAT2* genes decreases amino acid cytosolic contents and correlates with an extension in RLS. While the importance of proper domain association for protein functionality has been demonstrated for Can1, the role of eisosomes in Tat2 functionality and/or availability was unknown. Measuring ^{3}H-Trp import in vivo we found that *PIL1* deletion does not generate a decrease in Trp incorporation. Therefore, a deficiency in Tat2 activity does not seem to be underling the RLS extension mechanism in study. General Amino acid Control pathway activity was determined performing reporter gene assays in eisosomal mutants. Together with cytosolic amino acid quantifications by NMR these experiments will enable us to determine whether an amino acids imbalance state is underlying eisosome disassembly-dependent RLS extension. Our work on microfluidic devices in conjunction with mutants´s physiology analysis will be key in order to under eisosomes´ role in aging, likely contributing to further describing the complex aging process and nanoscale PM domains function.

CB-55

Endometriosis affects the mouse oocyte quality by altering the molecular machinery involved in cortical reaction

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Endometriosis is a chronic gynecological disease characterized by the presence of endometrial-like tissue outside the uterine cavity, for example, in the pelvic cavity. Among the symptoms generated by the disease, infertility is one of the most prevalent and oocyte quality has been identified as the most important factor. Nevertheless, the factors that define this poor quality are unknown. Previous studies carried out in our laboratory showed that oocytes obtained from female mice with experimentally induced endometriosis (endo oocytes) presented some parameters related to oocyte quality altered, indicating that endometriosis might affect the oocyte competence to be fertilized. It was observed that matured metaphase II (MII) endo oocytes were not able to secrete the cortical granules content during parthenogenetic activation when compared with control oocytes. This secretory event -also known as cortical reaction- involves the fusion of cortical granules with the oocyte plasma membrane, and it is physiologically activated by the sperm during fertilization avoiding the fusion of new sperm (polyspermy). Therefore, this work aimed to determine possible factors that may affect the cortical granules exocytosis (CGE) in endo oocytes. Considering that CGE is regulated by the actin cytoskeleton and specific exocytic protein machinery -that we have previously characterized-, we hypothesized that the expression of F-actin and two key proteins for CGE -alphaSNAP and NSF- were altered in endo oocytes. First, an autologous endometriosis model was induced in female CF-1 mice (8 weeks of age). After 4 weeks, endo, sham, and control females were hormonally stimulated to obtain matured MII oocytes. Cells from three groups were fixed and permeabilized to perform indirect immunofluorescence assays. Fluorescent phalloidin was used to identify F-actin and specific primary antibodies were used to detect alphaSNAP and NSF. The analysis of fluorescence intensity by confocal microscopy showed that cortical F-actin was thickened in endo oocytes when compared with sham and control oocytes, while there was no difference between these last groups. On the other hand, when the fluorescence intensity corresponding to alphaSNAP and NSF were studied, we observed that both protein expressions were reduced in endo oocytes compared to sham and control oocytes. These results confirm our hypothesis and suggest that the molecular machinery involved in cortical granule exocytosis is altered in endo oocytes affecting their quality and competence to be fertilized.

CB-56

Identification of the palmitoyltransferase DHHC4 interactome using proximity-dependent biotinylation by BioID2

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S-acylation or palmitoylation is a reversible post-translational lipid modification of proteins by which a long chain fatty acid is covalently bound to cysteine residues. Palmitoylation is involved in processes of great biological importance and its dysregulation has been linked to several diseases. A family of enzymes named Palmitoyltransferases (PATs) catalyzes this modification. There are 23 members of this family (DHHC1-23) in the human genome. There are more palmitoylated substrates than PATs, and very few enzyme-substrate pairs have been confirmed, thus the specificity of PATs is not well understood. Here we focus on DHHC4, a PAT for which substrates remain largely unknown. Identifying protein-protein associations is crucial to studying protein, and BioID (proximity-dependent biotin identification) is a novel and powerful tool to uncover them. This system relies on fusing a protein of interest with a promiscuous biotin ligase mutant, which will add Biotin to the amine groups of neighboring proteins. Biotinylated proteins can then be isolated by conventional biotin capture methods and identified using liquid chromatography-mass spectrometry (LC-MS). One of the greatest benefits of BioID2 is that it is not limited to strong, stable binders, and therefore ideal to uncover enzyme-substrate pairs. Here we explore the endogenous substrates/interactors of DHHC4, using BioID in cultured human cervical carcinoma-derived HeLa cells. We electroporated HeLa with either DHHC4-BioID2-HA or BioID2-HA as a control. The correct subcellular localization of our constructs was assessed by indirect immunofluorescence microscopy. Differences in protein biotinylation between the samples were confirmed by immunoblotting. The data obtained in LC-MS were analyzed in the Proteome Discoverer V2.4.1.15 and Mascot software, using the database corresponding to *Homo sapiens* (Human). The search identified 273 proteins that had cysteines in their sequence and may represent new possible substrates of DHHC4. 137 of the 273 have predicted palmitoylation sites in the SwissPalm database. 7 of these proteins are confirmed palmitoylation substrates, but the PAT/s responsible are unknown, these include Thioredoxin-related transmembrane protein 1 (TMX1), Parkinson disease protein 7 (PARK7), Platelet-activating factor acetylhydrolase IB subunit α1 (PA1B3), Ras-related protein Rap-1b (RAP1B) and Rab-7a (RAB7A), Rho-related GTP-binding protein RhoB (RHOB) and Protein transport Sec61 subunit β (SC61B). We also found proteins with assigned PATs, like CI Man-6-P receptor (MPRI), Guanine nucleotide-binding protein G subunit α-2 (GNAI2), and Transferrin receptor protein 1 (TFR1). We believe that this approach is complementary and might be more sensitive and less prone to false positives than current techniques

for the high-throughput identification of palmitoylated proteins. We suggest that the method will be particularly useful for assigning palmitoylation substrates to each PAT.

CB-57

Palmitoylation, stability and function of the CYSTM family of proteins in yeast.

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CYSTM proteins are a superfamily of proteins widely distributed among Eukaryotes. These proteins are small, ranging from 60 to 120 amino acids and characterized by the presence of a conserved motif at the C-terminal region, which is rich in cysteine residues and has been annotated as a transmembrane domain (TMD). In the yeast *Saccharomyces cerevisiae*, the family has no known functions and comprises the genes YBR016W, YDL012C, YDR034W-B, YDR210W and possibly MNC1 (YBR056W-A). Some plant homologues of these proteins have been linked to resistance to pathogens and also to abiotic stresses, but no mechanistic explanations for these phenotypes have been proposed.

Protein S-acylation, commonly known as palmitoylation, is a highly prevalent, post-translational modification (PTM) that consists of the addition of long-chain lipids onto cysteine residues. This PTM is mediated by a family of Palmitoyltransferases (PATs) and plays multiple roles in important biological processes. We have suggested that CYSTM proteins do not possess a TMD as postulated, but are bound to membranes by palmitoylation of the cysteines in the CYSTM domain. Here, present additional evidence for this claim, which includes extensive mutagenesis of the CYSTM domain, followed by fluorescence microscopy, subcellular fractionation and biochemical determination of their palmitoylation status. These experiments allow us to postulate a model for how CYSTM domains interact with membranes. Expression of Ybr016w in strains lacking each of the seven PATs indicated that this protein is completely

degraded, in a proteasome-dependent manner, in the absence of the PAT Akr1, and to about half of its steady-state levels, in the absence of Erf2. We generated a series of constructs in which the CYSTM domain of YBR016w was either deleted, replaced by the transmembrane domain of Sso1 or mutated so that all cysteines were replaced by Alanines. Analyses of these constructs in wt, akr1Δ and erf2Δ strains indicated that the stability of Ybr016w is affected by the lack of Erf2-mediated palmitoylation. Interestingly, the complete degradation of YBR016W observed in akr1Δ strains is independent of the CYSTM domain. Finally, we began to explore the possible functions of this family of proteins. Growth tests in different media showed that the lack of these genes makes strains more resistant to the presence of 6 mM Zn2+ and to a lesser extent to the presence of 8 mM Mn2+. Other bivalent cations such as Cd2+ or Cu2+ did not elicit growth differences with respect to the wt.

Finally, we are carrying out proximity labelling experiments (Turbo-ID) to uncover interacting partners of the CYSTM family of proteins by LC followed by mass spectrometry. We expect that these experiments will lead to a better understanding of this protein family.

CB-58

Senescence activation modified the internalization but not the intracellular traffic of *S. aureus*

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In cells, a mechanism to avoid damage dissemination in an organism is senescence activation. This process is defined as a state where proliferating cells lose their replicating capacity. This mechanism is activated by different stimuli, such as oxidative stress, telomere shortening, DNA damage, mitochondrial damage and also is induced by oncogenes activity. Characteristics of senescent cells include irreversible growth arrest, increased cell size, expression of cyclin-dependent kinase inhibitor (CDKI), formation of senescence-associated heterochromatin foci and senescence-associated secretory phenotype (SASP).

These changes attempt to prevent damaged cells from proliferating and causing expansive damage. It takes place during different physiological and pathological processes such as tissue remodeling or injury, cancer, and aging. Phagocytosis is a process where pathogens are taken and eliminated by the cells. Previously, we have observed that senescence activation in cells infected with *S. aureus* produces a lower recovery of bacteria. This effect may be due to two mechanisms, a lower internalization or an increase in bacterial degradation inside the cell. To distinguish between these two effects, we studied the intracellular traffic of *S.aureus* by confocal fluorescence microscopy. We analyzed the colocalization between the bacteria and different markers of the endophagocytic pathway, observing no differences comparing the infection in control and senescent cells. Subsequently, we performed an analysis by colony-forming units (CFU) of bacteria recovery using different inhibitors of endophagosomal maturation. To do this, we infected control and senescent cells in the presence and absence of several inhibitors. Then, at different time points, we lysed the cells and counted bacteria by CFU. We did not observe differences between control and senescent cells. These results demonstrate that the trafficking and degradation of *S. aureus* shows no differences in senescent cells. We could conclude that a lower recovery of bacteria in senescent cells is due to a lower internalization of the same.

CB-59

Differential uptake of apoptotic material during *Pseudomonas aeruginosa* infection

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Macrophages are cells specialized in the destruction of pathogens through phagocytosis, but they also play a role in the elimination of dead cells and cellular debris through efferocytosis. Since infection sites are usually full of pathogens and dead cells, macrophages encounter both stimuli simultaneously. However, depending on the perceived stimuli, macrophages are thought to acquire either a pathogen-killing or an efferocytic/healing phenotype, previously considered to be mutually exclusive. Besides, we have previously found that the bacterial pathogen *Pseudomonas aeruginosa* adheres to apoptotic cells. Therefore, we aimed to study whether pathogens and apoptotic cells can be internalized and processed jointly by macrophages and the modulation of efferocytosis during inflammation. We exposed primary

bone marrow-derived macrophages (BMDM) to *P. aeruginosa*, apoptotic cells, and *P. aeruginosa*–laden apoptotic cells, to study the response of macrophages under different stimuli. To achieve this, we measured phagocytic and efferocytic efficiencies by confocal microscopy image analysis, and changes in cytokine expression levels by RT-PCR. We found that BMDMs are very efficient in engulfing both *P. aeruginosa* and apoptotic cells, with a high bactericidal capacity not affected by the concomitant presence of apoptotic material. Furthermore, after phagocytizing and processing *P. aeruginosa*, through IL-6 production, macrophages increase their efferocytic capacity. Thus, our results show that the inflammatory response generated by bacterial processing enhances the ability of these macrophages to control inflammation. We further described that, when macrophages are exposed to apoptotic cells, they engulf mainly intact cells that subsequently fragment within the phagocyte. In contrast, apoptotic cells loaded with bacteria are internalized into multiple fragments, preceding the formation of multiple efferosomes. We also found that apoptotic material and bacteria localize into separate LAMP-1 positive vesicles. Since macrophages produce an immune response only after phagocytosis, we hypothesize that a differential processing or trafficking of cargo must occur. Regulation of vesicle formation, trafficking, and maturation, involves the Rab family of small GTPases. Therefore, we plan to study the role of Rab GTPases in the sorting and trafficking of this differential cargo and the biological implications this may have.

CB-60

Staphylococcus aureus infection activates the autophagic pathway.

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Autophagy is a degradative cellular mechanism activated in response to different stress conditions. This process is induced as a degradative pathway when cells are subjected to nutrient limitation, high temperatures, oxidative stress or accumulation of damaged organelles. The sequestered material is degraded, and the generated molecules are recycled and translocated back to the cytoplasm to be reused by the cell. Some pathogenic microorganisms (bacteria, viruses, and parasites) are removed by autophagy, but others are benefited by this pathway. *Staphylococcus aureus* is a Gram-positive bacterium responsible for serious infectious processes. This pathogen modifies the autophagic pathway

to invade and replicate into host cells. *S. aureus* localizes into autophagosomes and inhibits maturation and fusion with lysosomes. The *S. aureus*-containing phagosome is clearly marked by the autophagic protein LC3 and transit via this pathway is beneficial for pathogen survival, replication, escape, and further dissemination. We have previously demonstrated that α-hemolysin (Hla) is a *S. aureus* secreted factor required for the activation of the autophagic pathway. This activation is independent of both PI3Kinase activity and Beclin 1 but it was regulated by calcium.

In the present work, we have used CHO cells (CHO-K1), wild type (WT) or overexpressing GFP-LC3 (CHO GFP-LC3) infected with three different *S. aureus* strains: WT, a mutant deficient for Hla [Hla (-)] and a mutant deficient for Hla but complemented with an Hla plasmid [Hla (+)]. Cells were incubated in complete medium or under starvation. In order to activate the autophagic pathway, cells were infected with the *S. aureus* bacterial strains or treated with a bacterial-free culture supernatant. The conversion of LC3-I to LC3-II was analyzed by Western Blot and the recruitment of LC3 to autophagic vacuoles was analyzed by confocal microscopy. Our results indicate that *S. aureus* infection activates autophagy similar to the starvation condition. However, incubation with bacterial free supernatants does not seem to stimulate this pathway.

We have recently demonstrated that the kinase PKCα has a role in the regulation of autophagy induced by *S. aureus* infection. The use of PKC activators inhibits the autophagy response and the recruitment of LC3 to autophagic vacuoles. A positive modulation of PKC causes a marked inhibition of bacterial replication, decreasing their survival. It has been shown that LC3 could be directly phosphorylated on Thr6 and Thr29 by PKC. CHO-K1 cells were transfected with GFP-LC3 WT and GFP-LC3 T6A/T29A, a non-phosphorylable mutant. Cells were incubated with starvation medium or infected with *S. aureus* Hla (+), and autophagy activation was assessed as indicated above. Our results indicate that the phosphorylation of LC3 on T6 and T29 are not critical for the autophagic response induced either by starvation or by *S. aureus* infection. Other possible phosphorylation sites in LC3 are currently under study.

CB-61

Exosome secretion and Rab27a like as modulators of *Chlamydia trachomatis* infection

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Chlamydia trachomatis (Ct) is an obligate intracellular Gram-negative bacterium and the most frequent bacterial agent of sexually transmitted infections. During its intracellular life cycle, the bacteria interacts strongly with several intracellular pathways including multivesicular bodies (MVB) transport. In our laboratory it was described that part of this interaction is mediated by Rab39a and that the bacteria use this mechanism to acquire host lipids that are necessary for their development. Exosomes are extracellular vesicles that are released from the cells for the fusion between MVB and the plasma membrane. It has been reported that exosome release contributes to intracellular physiology by controlling homeostasis of nucleic acids, proteins, and lipids. Because of the robust interaction between Ct inclusion and MVB, and the intracellular homeostasis that could demand the Ct lifestyle, we proposed that MVB and exosome biology could be relevant for Ct development. We harvest by differential centrifugation exosomes present in the supernatant of infected and non-infected Hela cells. By western blot, we studied the levels of exosome-specific markers in exosome fraction (100k fraction) for an infected and non-infected equal number of cells. Interestingly, we detected substantially higher levels of exosome markers in the 100k fraction from infected in comparison with control non-infected condition. It is known that Rab27a regulates exosome secretion controlling docking of MVB to the plasma membrane. In this context, we decided to work with Rab27a knockdown Hela cells (KD27a) and describe Ct development. We detected significantly lower levels of Ct infection in KD27a condition in comparison with control (Scrb) at 24 hours post-infection by confocal microscopy. Besides, we observed a significantly lower percentage of infected progeny in the KD27a condition in comparison to Scrb condition at 72 hours post-infection. After that, we worked with overexpression of Rab27a-GFP in Hela cells, and overexpression of GFP was used as control. We observed a significantly higher percentage of infected cells in Rab27a overexpression condition. Besides, Ct inclusion showed a significantly higher size in Rab27 overexpression context and finally, the infected progeny was higher in this condition too. This data suggests that exosome secretion would be a necessary process for Ct physiological development in Hela cells.

CB-62

TRANSMEMBRANE DOMAIN OF GLUCOSIDASE I IS INVOLVED IN ITS FUNCTION IN THE FISSION YEAST ENDOPLASMIC RETICULUM

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Protein N-glycosylation is a highly conserved process and one of the most relevant post-translational modifications in eukaryotes. It begins with oligosaccharyltransferase (OST), a complex present in the membrane of the endoplasmic reticulum (ER), transferring the pre-assembled oligosaccharide Glc3Man9GlcNAc2 (G3M9) to asparagine residues of proteins that are being translocated into the ER. ER-glucosidase I (GI), trims immediately the outermost glucose of G3M9 converting it to G2M9 and allowing glucosidase II (GII), to convert it to G1M9, which is in turn recognized by the Endoplasmic Reticulum Quality Control (ERQC) mechanism that facilitates folding and of secretory and membrane proteins and marks terminally misfolded polypeptides for degradation. Defects associated with GI, produce type IIb "Congenital disorders of glycosylation" (CDG-IIb). This phenotypically manifests with multisystemic failures that appear at different ages in human patients. CDG-IIb has been associated with an unactive GI that would be responsible for not trimming G3M9 into G2M9. Our previous findings demonstrated that the accumulation of G3M9 due to the lack of GI in *Schizosaccharomyces pombe* is extremely toxic for the cell, resulting in a sick phenotype of GI yeast mutants. This adverse phenotype could be partially -but not fully- rescued by knocking out an alpha-1,2-glucosyltransferase (alg10p) responsible for adding the outermost residue of glucose during glycan biosynthesis, the same that is a substrate for GI. Interestingly, mutations found in patients show that most are not within the proposed catalytic pair residues of GI. Both results could imply that not only the catalytic function of the enzyme is critical for the correct development of the cell, but that other domain/s of GI could also be important. While GII is a soluble resident protein of the ER, GI is a membrane bound protein that contains three domains: a globular catalytic domain inside the ER, a transmembrane domain, and a short cytosolic tail. In this work, we evaluated the phenotypic effect of the genetic complementation of a catalytic-domain-only GI (cGI) of ΔGI fission yeast mutants. GI variant's proper expression and localization was confirmed by fluorescence microscopy and western blot. We assessed viability and growth rate by spot assay in solid and liquid media, respectively. Also, phenotypic cell differences were observed by transmission microscopy, and cells lengths were measured and compared by a one-way ANOVA. Our findings show that adding back cGI to ΔGI mutants does not rescue the sick phenotype of fission yeasts lacking full length GI, supporting the idea that GI has an additional unknown role yet to be discovered and that membrane localization might be involved in it.

CB-63

Taxonomic analysis of white gypsum-halite precipitations from Laguna Verde

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-AFILIACION??

The Argentine Puna represents a unique environment, characterized by high UV radiation, low oxygen pressure and extreme temperature fluctuations. There we find saline water deposits such as Laguna Verde (Salar de Antofalla, 3300 m.a.s.l.) surrounded by large extensions of white gypsum-halite precipitating crusts. These crusts harbor microbial communities distributed in layers (microbial mats) defined by physicochemical requirements, light and oxygen. These mats colonize both solid and sedimentary surfaces. Metagenomic, physiological and geochemical studies reveal a series of strategies that allow these communities to survive in hypersaline wetlands by performing photosynthesis and serving as CO_2 sinks. The goal of this job was to determine the biodiversity associated with these large evaporitics biofilms surrounding the lake.

It was observed that the microorganisms are organized in two layers in the salt crust, an upper yellow layer, and a lower green layer. Samples were taken using a cut off sterile 10-ml syringe, were fixed immediately in RNAlater and transported to the laboratory on ice. Total genomic DNA was isolated from each of the layers using the FastDNA ™ SPIN Kit for Soil (MP Biomedical) and it was sequenced with Illumina HiSeq. Raw data obtained, was uploaded to the European Bioinformatic Archive (ENA). Once uploaded, it was analyzed using the MGnify Annotation Pipeline developed by Dr. Rob Finn's Team at EMBL-EBI. This pipeline performed the taxonomic annotation of the raw reads. The taxonomic annotation obtained, showed that the orange layer is dominated by oxygenic photoautotrophic bacteria (Cyanobacteria, ca. 65%) followed by anoxygenic photoautotrophs (Proteobacteria, ca. 10%) from the classes Alphaproteobacteria and Gammaproteobacteria and heterotrophic bacteria (Bacteorideтes, ca. 10%). The green layer is dominated by the phyla Proteobacteria, ca. 50% followed by Bacteroidetes, ca. 30% and Cyanobacteria, ca. 20%. In both layers, the phyla Actinobacteria and Euryarchaeota represent at least 1% of the relative abundance.

CB-64

The role of CYP46 at inflammatory context: 24(S)hydroxycholesterol as predisposing agent for Alzheimer’s disease

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CYP46 carries out the hydroxylation of cholesterol to 24(S)HOC, which is the main mechanism of cholesterol elimination from the brain. CYP46 has been mainly reported in neuronal populations, however, in cases of brain damage such as traumatic brain injury or Alzheimer’s disease CYP46 increases its expression in astrocytes. However, the role that CYP46 would play in astrocytes in pathological conditions is unknown.

We found that CYP46 levels are greatly increased in reactive astrocytes treated with lipopolysaccharide (LPS) and IL-6. Accordingly, IL-6 was able to increase APP synthesis in primary astrocytes. Providing a link between CYP46 and APP, our results show that 24(S)HOC-treated primary cortical astrocytes showed a marked increase in APP levels compared to control cells. In addition, when we inhibit the activity of CYP46 with Voriconazole in an inflammatory context induced by IL-6, astrocytes lose the ability to produce APP, compared to control cultures Our data indicate that 24(S)HOC appears to exert its role through epigenetic mechanisms. Also, we observed that this oxysterol acts differentially in the different cell types. In astrocytic cultures, 24(S)-HOC downregulates the H3k9Ac epigenetic mark, whereas in cortical neurons it upregulates it.

We propose that under a proinflammatrory context, as for example a microbial infection in the brain, 24(S)HOC would mediate the production of APP and Ab in astrocytes to face the aggression but on the other side it would predispose to Alzheimer's disease.

CB-65

Macrophage TNFα activity drives Chlamydia trachomatis triggered activation through an autocrine mechanism.

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Chlamydia trachomatis (CT) represents a major burden in public health systems worldwide as it is the most frequently sexually-transmitted bacterium and as it may cause severe sequelae through the chronification of the disease. The dangerous complications of CT infection are supported by immunopathological responses that fail to eliminate the pathogen while promoting the inflammation and scarring of sensitive tissues, a process that frequently ends up in female infertility. We set up a model of ex-vivo infection of murine uteri to evaluate the inflammatory response upon infection and upon the use of novel anti-chlamydial agents. We selected four chemically and functionally different inhibitors of AKT at different stages of clinical trials based on our previous reports confirming their antibacterial activity. By WB and IHC we corroborated that the use of the four inhibitors reduced the levels of TNFα and IL1β triggered by CT infection of the tissues. These two cytokines are mostly produced by tissular macrophages in this ex-vivo culture model. For this reason, and with the aim of evaluating a potential antiinflammatory activity of AKT inhibition, we continued using a model of CT infection of the RAW246.7 cell line. By WB, we confirmed that recombinant TNFα activated the AKT/IκBα pathway in infected macrophages. Incubation of infected and TNFα-stimulated macrophages with AKT inhibitors reduced the levels of phosphorylated IkBα. To continue with the evaluation of the signaling cascade, we assessed by RT-qPCR the expression of genes regulated by the AKT/IκBα/NFκB pathway (IL1β, TNFα, IL10, IL6). In this same line, we evaluated by FC the M1/M2 activation profile of macrophages that were infected and then incubated with AKT inhibitors or after blockade of the TNFα autocrine signaling. We discovered that TNFα autocrine signaling was essential for the activation of the macrophages while the inhibition of the downstream effector AKT results in the skewed activation toward an anti-inflammatory M2 profile. Finally, we corroborated, through the use of a model of ex-vivo infection of human explants, the anti-inflammatory activity of AKT inhibition. This novel insight into the origin of the inflammatory response triggered by macrophages may have impactful implications in future research trying to understand the immunopathology that underlies chlamydial disease.

Plants

PL-18

Characterization of PI3K complexes in *P. patens*: towards unraveling differential roles in development

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Phosphatidylinositol-3-phosphate (PtdIns3P or PI3P), which represents a small fraction of the phosphoinositides pool, is a key regulator of membrane dynamics and transport processes. Its synthesis is catalyzed by the enzyme Phosphatidilinositol-3-kinase (PI3K). In plants and yeasts, unlike mammals, these processes depend only on one type of PI3K: VPS34. This enzyme forms complexes which share three proteins (VPS34, VPS15 and ATG6) and differ in a fourth: ATG14 in complex I (PI3K-CI), and VPS38 in complex II (PI3K-CII). PI3K-CI participates in autophagosome formation, whilst PI3K-CII is involved in different endosomal trafficking events depending on the species. Although these complexes are widely characterized in yeasts and mammals, studies in plants have just started to arise and there are still several questions to be assessed. In this work, we characterized the different subunits of PI3K complexes I and II in the moss *Physcomitrium patens.* First, we analyzed their gene expression pattern and modelled their protein structure. Differential gene expression was observed when protonemata were grown in the two commonly used media. In addition, the analysis showed up-regulation of almost every gene during the dark hours of a normal day/night photoperiod and in extended darkness. Protein structure analysis revealed that PpVPS34, PpVPS15a and PpVPS15b conserve all domains and major features previously described in human and yeast. Structures of PpATG6a-b are mostly conserved, but slight changes in the amino acid composition of the Aromatic Fingers, membrane contact sites, were detected. On the other hand, PpATG14 lacks the BATs domain essential for membrane binding in humans; although, as in Arabidopsis, the C-ter region contains bulky hydrophobic residues. PpVPS38 also presented differences regarding known structures, lacking the C2 domain and the C-ter region known to be important for post translational modifications in humans. Second, we characterized the expression pattern of VPS34 in colonies and protonemata using a PpVPS34::GFP-PpVPS34 reporter line. We also generated stable knock-down mutants for VPS34 that show accelerated senescence and less protonemal expansion under optimal growth conditions. However, these are pleiotropic phenotypes caused by the great variety of processes affected by the decrease in PI3P levels. To solve this dilemma, we are currently working on mutant lines of the differential subunits, ATG14 and VPS38. These knock-out genotypes will allow us to

identify effects caused by the inhibition of autophagy (PI3K-CI) or endosomal trafficking (PI3K-CII). With these studies, we aim to contribute to knowledge about the role of PI3P in plants, a field that has recently emerged. Moreover, using a bryophyte as a model system allows us to dive into the evolutionary aspects of this area.

PL-19

Proteomic modulation of *Vitis vinifera* cv. Malbec in response to solar UV-B in a high-altitude vineyard of Mendoza – Argentina

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Grapevine cultivation at high altitudes offers cooler, more suitable environmental conditions for premium quality winemaking in the context of climate change. Apart from lower mean temperatures and higher thermal amplitudes, grapes experience a significantly larger intensity of UV-B radiation due to lower air turbidity. UV-B serves both as a developmental signal and an oxidative-stress-inducing element depending on its intensity and the cultivar's ability to cope with it. Grapes usually respond to high UV-B by generating UV blocking anthocyanins and a myriad of antioxidant compounds and enzymes to prevent damage. This is often desirable for wine production, as it enhances organoleptic properties and aging capacity. In this work we show how Malbec grapes modulate their proteome and secondary metabolism in response to the solar UV-B found at a high-altitude vineyard (1350 m a.s.l.) in Mendoza, Argentina. Functional categories and STRING clusters constructed with differentially expressed proteins display a strong regulation of primary and secondary metabolism, redox balance and antioxidant system. Additionally, proteins related to ABA biosynthesis and defense response were upregulated by UV-B, suggesting an enhanced capacity to deal with general stress. In accordance, low molecular weight polyphenols with antioxidant and phytoalexin properties, such as procyanidins, flavonols and stilbenes were found in much higher concentrations with high UV-B in pre-veraison. While total and di-hydroxylated anthocyanins were more abundant with higher UV-B at harvest. This is the first report of grape proteomic modulation in response to solar UV-B, and it bears special importance for grape cultivation for high-quality winemaking in present and future climate scenarios.

PL-20

Metabolite profiling and cytotoxic activity of Andean potatoes: polyamines and glycoalkaloids as potential anticancer agents in human neuroblastoma cells in vitro

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Andean potatoes (*Solanum tuberosum L. ssp. andigena*) are a good source of dietary antioxidant polyphenols. We have previously demonstrated that polyphenol extracts from Andean potato tubers exerted a dose-dependent cytotoxic effect in human neuroblastoma SH-SY5Y cells, being skin extracts more potent than flesh ones. In order to gain insight into the bioactivities of potato phenolics, we investigated the composition and the in vitro cytotoxic activity of total extracts and fractions of skin and flesh tubers of three Andean potato cultivars (Santa María, Waicha, and Moradita). Potato total extracts were subjected to liquid-liquid fractionation using ethyl acetate solvent in organic and aqueous fractions. We analyzed both fractions by HPLC-DAD, HPLC-ESI-MS/MS and HPLC-HRMS to confirm the annotations. Results corroborated the expected composition of each fraction. Organic fractions were rich in hydroxycinnamic acids (principally chlorogenic acid isomers), whereas aqueous fractions contained mainly polyamines conjugated with phenolic acids, glycoalkaloids, and flavonoids. Organic fractions were not cytotoxic against SH-SY5Y cells, and indeed, some increased cellular metabolism compared to controls. Aqueous fractions were cytotoxic and even more potent than their respective total extracts. Treatment with a combination of both fractions showed a similar cytotoxic response to the corresponding extract. According to correlation studies, it is tempting to speculate that polyamines and glycoalkaloids are crucial in inducing cell death. Our findings indicate that the activity of Andean potato extracts is a combination of various compounds and contributes to the revalorization of potato as a functional food.

PL-21

Expression analysis of Protease inhibitors in potato plants exposed to different environmental conditions.

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Analysis of the potato genome (SPUD DB) allowed us to identify 142 protease inhibitors (PIs); 42 PI genes are clustered in tandem in chr.3, position 43 Mbp (3.43): 14 encode Kunitz-type PIs (KTIs), 4 encode trypsin type PIs (TPI) and 1 encodes a cystein PI (CPI), and at position 48 MBp (3.48), 10 genes encode KTIs, 8 encode PIN-II and 5 encode serine type PI (PIN-I). On the other hand, 11 genes encoding 5 KTIs, 3 cystatin B, and 3 PIN-I are grouped in Chr. 6, and 12 PIN-I genes are in chr. 9. This multigene family regulates protein turn-over preventing catabolism of essential proteins during metabolic processes and plays a role in the defense against heterologous proteases from pathogens and pests. PIs have been proposed as an alternative to chemical pesticides for the control of herbivorous insects, and as abiotic stress-protective factors. Degenerate primers were designed against five PIs (Soltu.DM.03G018480, -G018520, -G018580, -G018620, -G018650) of 3.43, seven PIs (Soltu.DM.03G023490, -G023500, -G23510, -G23520, -G23530, -G23540, and -G024110) of 3.48, and seven PIs of Chr. 9 (Soltu.DM.09G025840, -G025850 -G025860 -G025880, -G025900, -G025910, and -G025930) that share at least 83.46, 55,2 and 77,53 % of nucleotide identity. According to RNAseq data these three gene clusters are induced upon drought, salt, mannitol and N2 fertilization, and are downregulated during tuber dormancy release. To confirm the RNAseq data, RT-qPCR assays were performed using RNAs from in-vitro plants cultured under control conditions or a) with different NH4+ and NO3- concentrations, b) exposed during 1 week to continuous darkness, and c) to 150 mM NaCl during 24 and 96 h. In addition, RNA was extracted from greenhouse plants exposed to drought conditions. To analyze, the role of PIs during source-sink processes, RNA was obtained from dormant and sprouting tubers, from etiolated and green sprouts, and from young, fully developed and senescent leaves. In the future we aim to characterize one gene of each cluster in overexpressing or knockdown plants to elucidate their potential as biotechnological tools.

PL-22

S-nitrosation modulates auxin signaling during thermomorphogenesis in Arabidopsis

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Plasticity is the ability of plants to modulate their growth and development in response to environmental fluctuations. A mild increase in environmental temperature stimulates the growth of Arabidopsis seedlings by promoting the biosynthesis of the plant hormone auxin. Additionally, an increase in ambient temperature also induces an accumulation of HSP90 protein that enhances the stability of the auxin co-receptor TIR1, required for adequate auxin signaling activation. This work aimed to determine if nitric oxide (NO) through S-nitrosation of TIR1 is part of an additional layer of regulation involved in temperature-induced hypocotyl elongation in Arabidopsis. Twenty-nine degrees stimulated hypocotyl length; however, the treatment with the NO scavenger cPTIO inhibited temperature-induced hypocotyl elongation. Additionally, seedlings triggered an increase in NO level (measured by NO-sensitive DAF-FM DA probe) after 5 h at 29 °C, and nia1 nia2 mutant seedlings showed impaired hypocotyl elongation at 29 °C, suggesting that NO production through Nitrate Reductase enzyme is required for thermomorphogenesis. Finally, plants overexpressing a non-nitrosable tir1 mutant protein (tir1 C140A) failed to elongate their hypocotyl at 29 °C suggesting that NO, at least in part, through S-nitrosation of TIR1 protein participates in the modulation of auxin signaling in response to temperature changing environment.

PL-23

Non thermal plasma improve of wheat seed conditioning

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The present work is based on the search and development of sustainable technological tools that guarantee the physical, physiological and sanitary quality of the seeds. The continued growth of agriculture demands greater challenges to maintain productivity for the seed and production sectors. In recent years, interest in physical treatments has been renewed, based on the risk of excessive use of chemicals. Non-thermal plasmas (NTPs) represent an effective, durable and green footprint solution. These plasmas are based on the generation of oxidizing agents from electrically ionized gases; this production varies according to the gases and plasma configurations used. Our group has spent more than seven years developing innovative devices that generate NTPs and investigating their potential use in agriculture. In this sense, the general objective of this work was to investigate the NTPs as a conditioning element of seed quality and the growth of plants originating post-treatment, effects observed in previous studies by our group and independent groups. For this purpose, a selected NTP configuration (dielectric barrier: ternophase, gas: nitrogen) was used to guarantee a microbicidal power. Two different NTP exposure times (N2: 2 min. and N3: 3 min.) and GDM wheat seeds of the Algarrobo variety with good quality (GP>80) were used. Seed quality and biometric parameters, antioxidant enzymes and field growth (1.47mx8m field plots and 4 repetitions) were evaluated. In treated seeds, a tendency to increase GP was recorded (N2: 8.3%, N3: 9.6%). Additionally, an increase in the germination index (N2: 4.7%, N3: 5.1%) and germination speed (N2: 21%, N3: 40%) was observed. A decrease in the surface roughness of the seeds was determined by 2.6 and 2.8 times respectively using AFM analysis, and additionally an increase in water consumption (N2: 28%, N3: 36%) was observed. In studies on plants originating post-treatment, an increase in growth parameters height (N2: 40%, N3: 51%) and AF (N2: 5.4%, N3: 8%) was recorded. The antioxidant activity did not present alterations in the protein and lipid profile, but a high tendency of the oxidoreductase enzymes was observed. In field trials, an increase in yield (N2: 9.7%, N3: 9%) and grain weight (N2: 9%, N3: 8.6%) was achieved. In conclusion, we have found an NTP configuration capable to stimulate the intrinsic capacities of the seed, in order to improve the implantation and emergence of seedlings and finally enhancing crop yield.

PL-24

Bioinformatic analysis of LysM domains from plant receptor kinases.

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The LysM domain is a widespread protein module involved in binding peptidoglycan (PGN), chitin (CO), and lipo-chitooligosaccharides (LCOs), most likely recognizing the N-acetylglucosamine moiety. It has a length of 44 to 65 amino acids and it can be found, either in a single, or multiple copies per protein. They have been found in bacterial lysins, PG hydrolases, peptidases, chitinases, and they can act as antigens bind to albumin, elastin or immunoglobulin. In plants, LysM domains are present in the LysM Receptor-Like Kinase (RLK) and LysM Receptor-Like Protein (RLP) families, involved in the interaction with bacteria and fungi, both pathogenic and symbiotic. RLKs and RLPs contain three extracellular LysM domains with highly conserved C pairs separated by one AA (CXC) involved in the formation of disulphide bonds that pack the three LysMs together. Browsing the pfam database we found that some RLK/Ps were annotated with a single, or only two LysM domains, including the nod factor perception (NFP) protein required for nodulation in legume plants. In this work, we generated improved HMM profiles for LysM domain detection based on the structural alignment of manually curated LysM domain structures from PDB and Alphafold. We also defined an HMM profile from the structural alignment of triple LysM bundles from plant RLK/Ps, and of their LysM1, LysM2 and LysM3 domains. Using these profiles we attempted the phylogenetic classification of RLK/Ps by their affinity for PNG, CO and LCOs. Our results show some clusters associated exclusively with proteins that bind LCO or CO which could be useful in the prediction of RLK/Ps ligand specificity. However, since only a limited number of the analyzed RLK/Ps have been previously characterized at the ligand level, to improve our findings a more extensive molecular characterization of plant RLK/Ps is needed.

PL-25

THE ATPNP-R1 RECEPTOR INTERACTS WITH PLANT (ATPNP-A) AND BACTERIAL (XACPNP) TYPE NATRIURETIC PEPTIDES.

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Natriuretic Peptide (NP) systems have been identified in many vertebrates and are associated with osmoregulatory homeostasis. In higher plants, PNPs are heterologous of animal NPs and elicit a number of responses that are essential in solute and solvent homeostasis and responses to biotrophic pathogens. The *Arabidopsis thaliana* PNP, AtPNP-A, interacts with a leucine-rich repeat protein denominated AtPNP-R1. Through this interaction, it is able to trigger different responses in plants, to adapt to changes in the environment and regulate internal homeostasis.

Xanthomonas citri subsp. citri, the biotrophic bacterial pathogen responsible for citrus canker, also has a PNP-like protein named XacPNP. Our previous results support the hypothesis that XacPNP mimics AtPNP-A in eliciting physiological responses in plants, such as stomatal opening, increases in net water flux and sustains photosynthesis during the early stage of pathogenic infection. We aligned the tertiary structures to AtPNP-A y XacPNP, obtained *in silico*, and we determined that these are similar even though their amino acid sequences have a percentage of identity of 49%. It is important to mention that amino acids are conserved within the active domain. This, together with previous results, is compatible with the hypothesis of horizontal gene transfer.

We were able to determine quantitatively that the receptor AtPNP-R1 is associated with the plasma membrane in plant epidermal cells, and it moves along the membrane and also, intracellularly. For this, we expressed the construct AtPNP-R1-Venus, we labeled the plasma membrane with FM4-64 label and we performed colocalization analyses and plasmolysis assays. Also, we analyzed the interactions between constructs AtPNP-Turquoise – AtPNP-R1-Venus and XacPNP-Turquoise – AtPNP-R1-Venus *in planta* using colocalization and FRET assays.

PL-26

Phylogenetic analysis of early symbiotic genes from bean, lentil and chickpea

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The projected increase in world population in next decades demands an increase in food supply with minimal damage to the environment. In this scenario, Legumes represent a friendly crop alternative to

face climate change and soil deterioration. Legumes have the ability to establish a symbiotic relationship with soil bacteria known as rhizobia, which leads to nitrogen fixation within specific root organs named nodules. Biological N2 fixation contributes legumes to grow in poor soils conditions, so this is a natural process with environmental and agro-economic significance. In particular, common bean is one of the most popular legumes used for direct human food in occidental regions such as Latin America and Africa whereas chickpea is important for Asia and India. Nodule formation by rhizobia is a complex multistep process that is initiated by mutual signaling between both partners. Lipochitooligosaccharides produced by rhizobia are perceived as nodulation factors by plant receptors (Nodulation factor receptors NFR), which trigger the activation of several genes of the early response that leads to nodule formation. Genes of this nodulation pathway have been identified mainly in the model legumes *Medicago truncatula* and *Lotus japonicus*. By taking advantage of the availability of sequences of symbiotic genes in public databases , and also from the associated laboratories, orthologous genes of nfr (Nodulation Factor Receptor), nin (Nodulation Inception), ccamk (kinase Calcium Calmodulin), and cyclops were examined in common bean, lentil, chickpea and other legumes, and compared among them by maximum likelihood phylogenetic analysis. Our results indicate that most of the studied symbiotic proteins follow the species phylogeny. However, for certain proteins a deviation from the reference phylogeny was observed (e.g., the cluster *G. max*, *V. radiata* and *C. cajan* for the NIN protein, or the difference observed in lentils for the Nfr1 protein). Moreover, certain genes exhibit a significant intraspecies variability, which we expect to characterize in greater depth. Further studies will help elucidate whether those variants would be associated with the protein function.

PL-27

Overexpression of a typical aspartic protease (At1g11910) confers to *Arabidopsis thaliana* resistance to *Botrytis cinerea* infection.

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Aspartic proteases (APs) are a family of proteolytic enzymes widely distributed among living organisms. Plant APs are involved in various biological processes as in the plant defense mechanism to abiotic and biotic stresses. In our laboratory we identified, purified, characterized, and cloned two typical APs from

Solanum tuberosum (StAP1 and StAP3). These typical APs are bifunctional enzymes, with antimicrobial and proteolytic activities, and their expression is directly correlated with the resistance of potato plants to *P. infestans* infection. We detected three StAP1 homologous genes in *A. thaliana.* These genes are At1g11910 (80% id) (named as APA1), At1g62290 (82% id) and At4g04460 (83% id). According to data described in the Arabidopsis eFP browser APA1 gene is induced in leaves after *Botrytis cinerea* infection. However, there are no data that correlate the induction of this gene with the increase or decrease of the defense response of *A. thaliana* to *B. cinerea* infection. The current study aimed to evaluate this correlation and the potential role of APA1 in the mechanism of the response of *A. thaliana* plants to *B. cinerea* infection. Obtained results show that the foliar area affected by *B. cinerea* infection was larger in mutant plants (apa1) than the observed in wild type (WT) and OE- AP1 plants. This result indicates that the APA1 gene is involved in the defense response mechanism of *A. thaliana* plants to *B. cinerea* infection and, that overexpression of the APA1 gene increases the resistance of plants to *B. cinerea.* Data obtained from real-time PCR assays show that overexpression of APA1 induces the expression of genes that regulate the jasmonic acid signaling pathway, like PDF1.2, in response to necrotrophic pathogen infection. In apa1 plants expression levels of the PDF1.2 gene were lower than the PDF1.2 expression levels determined in WT and OE- APA1 plants, at all times analyzed. On the other hand, and according to data described for other antimicrobial peptides and the plant-specific insert (PSI) overexpressed in *A. thaliana,* OE- APA1 plants showed increased PR-2 expression levels upon *B. cinerea* infection compared to apa1 and WT plants. The results obtained show that this typical AP (APA1) is involved in the defense mechanism of *A. thaliana* and that its overexpression confers significant enhancement in the defense response against *B. cinerea* infection. We propose the development of crops with increased levels of APA1 as a strategy to improve the resistant to pathogens and thereby minimize the use of agrochemicals.

PL-28

Seed priming with Spm improves germination and seedling growth of wheat under nitrogen deficiency

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Nitrogen (N) is an essential macronutrient that plays a vital role in plant growth and development. Excess application to crops to increase yields raises production costs and causes environmental damage. N

availability is crucial for seedling establishment, so at this stage, N deficiency could be detrimental for vegetative growth and crop yield. Among modern and eco-friendly approaches to improve seed germination and early growth, seed priming has become a promising strategy. Polyamines putrescine (Put), spermidine (Spd) and Spermine (Spm) are essential molecules for plant growth, involved in stress tolerance, and recognized to be effective priming agents against several abiotic and biotic stresses. We have examined Spm as seed priming agent in wheat, to overcome N deficiency at early growth stages. Seeds were treated with H2O (control, C) or 0,1 mM Spm (S) for 3h and then placed in plastic trays for germination for 48h. Seedlings were transferred to hydroponic system in a growth chamber, in a complete (N+) or deficient (N-) N medium. After 8d in culture, no differences in shoot or root length were detected among treatments in N+ medium. N limitation produced shoot growth inhibition and increased root length in both cases, but SN- roots resulted 16% longer than CN- roots. Moreover, Spm caused the earlier appearance of the 3rd leaf. Total N in CN- and SN- roots decreased about 50% compared to CN+ and SN+, whereas in leaves, the decay was 48% in CN- and 41% in SN- compared to the respective N+ medium. Thereby, SN- leaves had 14% higher total N than CN- leaves. Shoot N/root N ratio was 5% higher in CN- than in CN+ and 25% higher in SN- than in SN+. Under N limitation, nitrate reductase (NR) activity was greatly reduced compared to N+ medium. NR activity resulted 71% and 53% higher in SN- 1st and 2nd leaves compared to the activity in CN- 1st and 2nd leaves, respectively. Glutamine synthetase (GS) activity was similar in CN+ and SN+, and it resulted 31% and 36% higher in the 1st and 2nd leaves, respectively, in SN-seedlings compared to SN+. In the 1st leaf, GS activity was 30% higher in SN- than in CN-. Total protein content in leaves was 28% and 19% lower in CN- and SN- compared to CN+ and SN+, respectively. In roots, protein content decreased about 20% both in CN- and SN- compared to CN+ and SN+. Nitrate (NO3-) content was remarkably lower in plants grown under N deficiency compared to those in N sufficiency; it was significantly higher in SN+ leaves compared to CN+ leaves while no differences were observed in roots. No remarkably changes were observed in ammonium (NH4+) content in leaves. However, in roots, it was 31% higher in SN- than in CN-. The decline in total amino acid content was less pronounced in SN- than in CN- compared to SN+ and CN+, respectively. These results showed that seed priming with Spm have a positive impact in N metabolism in wheat seedlings and could improve seed germination and early seedling growth under N deficiency.

PL-29

Identification of new transcription factors involved in tomato defense against *Pseudomonas syringae* pv. tomato (Pst)

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During their life cycle, plants are exposed to a wide variety of pathogens and to defend themselves they have evolved a two-layered immune system. The first barrier is called PTI (pattern-triggered immunity) and is activated upon the recognition of MAMPs (microbe-associated molecular patterns) by plants' PRRs (pattern-recognition receptors) located on the cell's surface. In early stages of the infection, tomato detects mainly two flagellin MAMPs with receptors flagellin sensing 2 (FLS2) and flagellin sensing 3 (FLS3) which recognise flg22 and flgII-28 respectively, leading to PTI activation. Virulent pathogens like Pst can deliver effector proteins into the cytoplasm to promote susceptibility. However some plants have evolved a second layer of immunity called ETI (effector-triggered immunity) which requires resistance proteins (R) that can recognise these effectors and activate a fast and localized response that culminates in a hypersensitive response (HR) where infected cells die to contain pathogen infection. Tomato Rio Grande Pto-R plants detect two Pst effectors (AvrPto and AvrPtoB) with a two protein complex Pto/Prf which lead to ETI activation. PTI and ETI activation include large transcriptional changes. Current studies mainly focus on a few transcription factors (TF) families such as bHLH, MYB, WRKY, AP2/ERF and NAC. We wonder if there are other TFs outside these families that have not yet been implicated in plant immunity, regulating key processes in plant defense against bacteria. In previous studies we identified around 300 genes that encode for TFs whose expression is increased due to the treatment with MAMPs and Pst mutants. With this information we propose to identify and characterize new TFs implicated in the regulation of tomato defense against Pst, with special emphasis in those belonging to families not previously linked to the defense response. We selected five TFs (ERF30, Ethylene responsive factor 30; HSF, Heat shock factor; LBS, LOB domain protein; DBP, DNA binding Phosphatase; OFP, Ovate family protein) and silenced their orthologous genes in *Nicotiana benthamiana* (Nb) using VIGS (virus-induced gene silencing) technique. To assess their implication in ETI, silenced plants were infiltrated with a combination of A. tumefaciens GV2260, one which possesses pBTEX::pto vector, and another one with pBTEX::avrPto, that strongly induce macroscopic HR. Plants silenced for ERF30 and HSF showed a delay in HR development compared to control plants. Next, Nb plants expressing 35S:Pto transgene were silenced with our candidate constructs and infiltrated with P. s. pv. tabaci strains carrying AvrPto or an empty vector as control. Plants silenced with ERF30 and HSF showed increased disease symptoms compared to control plants. Together, these experiments suggest that TFs ERF30 and HSF may play a role on plant defense response signaling pathways against Pst infection.

PL-30

THE OVEREXPRESSION OF GZF1 GENERATES BRANCHED PHENOTYPES IN *ARABIDOPSIS THALIANA*

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The grass family is distinguished from many species by their inflorescence architecture, whose final form is the result of a balance between the ability of the axillary meristems to form branches or terminate in spikelets. GRASS ZINC FINGER 1 (GZF1) is a Cys2-His2-type zinc finger transcription factor that seems to play a central role at this stage of maize inflorescence development. By comparison of GZF1 amino acid sequences from model and non-model grass species, we observed a partial conservation of the sequences towards the C-terminal and a variability in the number of EAR motifs. To answer if the divergence of protein sequences is correlated with a functional divergence of GZF1, we decided to generate plants of *Arabidopsis thaliana* that overexpressed different GZF1 sequences. In particular, we decided to work with GZF1 of three grass species: *Zea mays*, *Setaria viridis* and *Cenchrus echinatus*. Transgenic plants showed phenotypes composed of many orders of branching and, in some cases, the branches were fasciated and sterile. In addition, transgenic plants developed abnormal flowers and open carpels with free ovules. According to the results, we think that GZF1 may be involved in the regulation of apical and axillary meristem fate affecting the original plant architecture.

PL-31

Study of the UTR regions in the regulation of SHORTROOT

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In the field of plant biology, the mechanisms that gave rise to Kranz anatomy and, with it, the emergence of C4 photosynthesis, is still one unsolved mystery. We seek to study the molecular mechanisms responsible for the diversification of leaf anatomies of plants. In particular, the aim of this work is to

increase the knowledge about the role of the SHORTROOT gene (SHR) that is known to be influencing the maize foliar anatomy and development of the Kranz bundle sheath cells. We know that SHR has multiple copies in grasses, however, little is known about the function of these copies during leaf development. Based on sequence comparison we identified highly conserved motifs along the 5'UTR and this motivated us to explore whether such regions affect the levels of SHR expression. To achieve this, we cloned the 5'UTR sequence of SHR between the constitutive 35S CaMV promoter and the GUS reporter gene and used this to agroinfiltrate tobacco leaves. We observed that the detection of GUS protein on samples varied in correlation with differences observed in the 5'UTR of SHR copies. These results suggest that 5'UTR of SHR have gene regulation elements that control the expression pattern of each copy. In consequence, the study of the 5'UTR region may bring us closer to understanding the SHR mode of action in leaf development and differentiation of Kranz anatomy.

PL-32

Transcriptome analysis of fruit development and maturity date by integrating mRNAs and long non-coding RNAs data in *Prunus persica*

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The Chilean fruit export industry, which includes many species of fruit (such as peaches and nectarines), is the largest in the Southern Hemisphere. Consumers of fresh fruit demand high-quality products, which is achieved through the combination of several fruit quality traits. In *Prunus persica*, the maturity date (MD) is a crucial fruit trait for a market focused on maintaining fresh fruit and extending fruit shelf-life. MD is a consequence of the fruit developmental process, and as a result, many physiological parameters

associated with maturity are also defined in the early developmental stages. Currently, some candidate genes reported for this trait are ethylene biosynthesis, jasmonate biosynthesis, and cell wall modification-related genes. However, the roles of these genes in the molecular regulation of MD in stone fruits are still unknown. Previously, we used a combination of RNA-seq and DNA-seq from contrasting individuals for MD at the harvest stage. We identified genes related to JA biosynthesis and cell wall metabolism. In this sense, using transcriptome data (mRNA and lncRNA) would allow us to understand the MD better. Taking this background into account, the goal was to identify the transcriptomic control involved in the expression of genes related to jasmonate biosynthesis and cell wall modifications associated with maturity date (MD) during fruit development in *Prunus persica*. We have identified differentially expressed lncRNAs and mRNA during fruit development (6 stages) using contrasting varieties (Early Juan; SJ and Super August; SA) for MD by RNA-seq. We have identified 6480 differentially expressed genes in at least one stage between SJ and SA, some of them associated with JA biosynthesis and cell wall metabolism. Of the 956 lncRNAs identified in the transcriptome, 829 lncRNAs were differentially expressed in at least one comparative. On the other hand, network analysis found transcription factors at different developmental stages associated with fruit development. Results suggest that these transcription factors are expressed in both varieties. However, their expression is conditioned to different temporalities during fruit development, explaining the contrasting phenotype for MD between EJ and SA. These transcription factors are mainly related to the biosynthesis of hormones such as abscisic acid, ethylene, auxins and jasmonic acid, and cell wall remodeling genes. Finally, we have integrated mRNAs and lncRNAs using MixOmics Software and have correlated the candidate genes with lncRNAs. This study could be the beginning of understanding the MD-related genetic control and generation of the basis of biomarker development and the following application in peach breeding programs.

PL-33

The splicing factor RS31 regulates seed germination and flowering time in *Arabidopsis thaliana.*

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Environmental signals such as light and temperature shape the plant transcriptome by impacting on each possible level of gene expression. Alternative splicing (AS) significantly modulates the transcriptome, and to some extent the proteome, during development and in response to environmental cues. The regulation of AS depends on the expression level and post-translational modification of serine/arginine-rich (SR) proteins and other splicing factors. We have previously shown that when Arabidopsis seeds receive a pulse of Red light (Rp), the AS pattern of 226 genes associated with mRNA processing, RNA splicing, and mRNA metabolic processes, including the splicing factor RS31, are drastically changed. Interestingly, these AS events are regulated by R and Far-Red light, although not directly associated with the propensity of the germination response, suggesting that AS is a source of gene expression variability, and that it is a necessary mechanism for plants to adapt to a changing environment. Here, we show that the overexpression of the RS31 coding isoform (namely mRNA1) promotes germination even under suboptimal light conditions. Moreover, the mRNA1 isoform abolishes light-induced alternative splicing changes of some genes (i.e., U2AF65A, DRM1) when compared to wild type seeds. These data suggest that RS31 functions during light-induced seed germination in Arabidopsis and that the striking impact on germination of the AS outcomes could be a relevant mechanism underlying seed physiology. We also show that mRNA1 overexpression lines have a delayed flowering time and, accordingly, FLOWERING LOCUS C (FLC) and other flowering genes (i.e., FCA) have altered expression levels in these plants when compared to the wild type, suggesting that RS31 is involved in the regulation of flowering. Hence, affecting splicing, potentially through FLC, may also alter key physiological traits.

PL-34

Subcellular localization and putative functions of alternative splicing isoforms in *Arabidopsis thaliana*

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Alternative splicing (AS) produces multiple transcripts (variants or isoforms) from a single gene through the variable and regulated usage of different splicing sites, significantly modulating the transcriptome, and to some extent the proteome, during development and in response to environmental cues. In addition to generating different isoforms that can be translated into different proteins, this process can also give rise to variants without coding capacity. Some of these are degraded, allowing delicate regulation of the amount of protein generated. However, others are substantially stable. Interestingly, non-coding transcripts can control chromatin status, modulate the abundance of other RNAs (miRNAs, lncRNAs, mRNAs, etc.), and even translation. By using publicly available RNAseq datasets linked to RNA translation, non-sense mediated mRNA decay and subcellular localization, we found that certain non-coding isoforms of a set of genes of interest are not degraded, despite the presence of NMD associated sequences, as they accumulate in the nucleus. To validate these data, the subcellular localization of the different isoforms of RS31 and other genes of interest (SR30, U2AF65) was evaluated. Consistently, certain isoforms that retain intronic sequences are kept in the nucleus. We hypothesize that these non-coding transcripts could be fulfilling a nuclear regulatory function, acting as lncRNA generated by alternative splicing of coding genes. Therefore, transgenic plants that overexpress each particular isoform were generated, and their phenotype was compared with that of wildtype plants. Next, we will determine if the non-coding isoforms regulate the activity of the protein coded by the same gene.

PL-35

Role of ancestral retrograde signals in the regulation of *Arabidopsis thaliana* alternative splicing

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Plant development and physiology depends on light. This environmental cue produces a massive change on gene expression, regulating it at different scales such as alternative splicing. This mechanism might be regulated in nucleus by chloroplast (retrograde signal). On the one hand, it has been demonstrated that TOR kinase is involved in light-induced retrograde signaling pathway in roots. On the other hand, it is very well-known that chloroplasts are derived from free-living photosynthetic bacteria. In all bacteria, stringent control, which is activated by (p)ppGpp (alarmone) in response to nutritional deficit, regulates gene expression. As bacteria, chloroplasts in land plants can synthesize the alarmone in response to stress. Alarmone levels are regulated by RelA-Spot homologue enzymes (RSHs) that are encoded in the nuclear genome and their expression is regulated by TOR kinase. Another plastid response is the formation of stromules (stroma-filled tubules). These tubules might exchange signals and metabolites among plastids and other cellular compartments. We aim to determine whether these signaling mechanisms are shared between bacteria and chloroplasts, as a control pathway able to modulate nuclear gene expression. Specifically, we will study how dark/light-induced alternative splicing regulation is affected by alarmone and stromules in *Arabidopsis thaliana.* We will establish reporter transgenic lines to correlate alarmone levels, stromules abundance and changes in alternative splicing patterns. We will discuss the preliminary data and try to establish how light and TOR kinase signals regulate alarmone levels, stromules formation to modify alternative splicing.

PL-36

ROLE OF ARGONAUTE PROTEINS IN THE RESPONSE OF *ARABIDOPSIS THALIANA* PLANTS TO UV-B RADIATION

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Plants use sunlight to direct and regulate essential processes. Among the components of solar radiation is UV-B (280-315 nm), which at high intensities generates harmful effects on plants. Because of this, plants have developed multiple mechanisms of tolerance and adaptation to UV-B radiation. Some of the effects

caused by high doses of UV-B radiation in plants include damage to DNA (induces the formation of CPDs), lipids and proteins. At the cellular level, UV-B can affect cell cycle progression, inhibition of cell expansion, or both effects. Two common plant phenotypes after UV-B exposure are inhibition of leaf growth and primary root elongation. ARGONAUTE proteins (AGOs) are key players in all known small RNA-directed pathways. The genome of Arabidopsis contains ten AGO genes, designated AGO1 to AGO10, belonging to three functional groups: RNA slicers, small RNA binders, and chromatin modifiers. AGOs are the main components in RISC complex, (RNA-induced silencing complex) which associate with small RNAs. In plants, they regulate different processes such as development, response to stress, structure maintenance and genome integrity, among others. It has been previously reported that AGO2 can promote the recruitment and/or retention of RAD51 at DNA double-strand breaks (DSBs) to facilitate homologous recombination repair. It is also reported that there are direct interactions between DNA repair and the dynamics of DNA methylation, which involves proteins AGO4 and AGO6. Therefore, the objective of this work was to study the role of different AGO proteins in DNA repair mechanisms that are activated after the damage produced by UV-B radiation in plants. In particular, we focused on the role of AGO1, AGO4 and AGO6 in these processes. Thus, we analyzed the effect of UV-B radiation on the primary roots of Arabidopsis plants. Our results show that AGO4 and AGO6 expression levels decrease in the roots after a UV-B treatment using lines that express the mcherry reporter. However, using Arabidopsis mutants in these proteins (*ago4-5*, *ago6-2* and *ago4-5xago6-2*), we found that depletion of these proteins does not generate a differential response in the elongation of the primary roots or a change in the meristematic zone (analyzed by confocal microscopy) as a result of UV-B treatment. On the contrary, we showed that *ago1* mutants were less affected by a UV-B treatment than WT plants, while transgenic lines that retain AGO1 in the nucleus were more sensitive to the treatments. Together, AGO1, AGO4 and AGO6 participate in different responses to UV-B radiation in primary roots of *Arabidopsis thaliana* plants.

PL-37

Trichoderma spp.: a sustainable alternative to protect *Arabidopsis thaliana* against biotrophic and necrotrophic phytopathogens

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Trichoderma spp. is a filamentous fungus that inhabits the rhizosphere and beneficially interacts with plants. This interaction induces a plant systemic defense response against a high phytopathogen diversity. This response is activated from the colonization of roots by Trichoderma spp. In this study, the ability of Trichoderma spp. to protect *Arabidopsis thaliana* against *Pseudomonas syringae*, a biotrophic pathogen, and *Botrytis cinerea*, a necrotrophic pathogen, was evaluated. *P. syringae* is associated with salicylic acid (SA)-dependent defense responses and induces plant cell death at the site of infection. *B. cinerea* is associated with jasmonic acid (JA)-dependent defense responses that promote cell death prior to or during colonization and acquire nutrients from dead cells. In order to analyze the differential expression of defense-related genes after pathogen attack, five *A. thaliana* genes were selected: *PR1*, *PAL1* and *SID2* involved in the SA pathway; *VSP2* involved in the JA pathway and *GLY4* that modulates the crosstalk between the SA and JA signaling pathways. First, roots from two weeks old *A. thaliana* plants (n=14) were inoculated with a suspension of 106 conidia/ml of *Trichoderma atroviride*, *Trichoderma harzianum*, *Trichoderma virens* or *Trichoderma reesei*. Control plants were inoculated with distilled water. After 10 days, 7 plants were infected by spraying with a *P. syringae* suspension (DO=2) and 7 plants were sprayed with distilled water (control plants). The same procedure was applied for *B. cinerea*. Leaf samples from all treatments were taken at 1 hour post infection (hpi) and 3 days post infection (dpi) and were used for *P. syringae* colony-forming unit (CFU) determination. No significant differences in CFU (ANOVA; $p>0.005$) were observed between treatments at 1 hpi. In contrast, statistical significant differences were observed between control and inoculated Trichoderma plants, being *T. atroviride* the species with the highest pathogen protection ($p<0.001$). Then, real time-PCR was performed to compare priming response of *A. thaliana* plants inoculated or not with *T. atroviride*. No statistical differences (t-test; $p>0.05$) were observed between treatments at 1 hpi. *PR1*, *GLY4* and *SID2* showed a significantly higher expression ($p<0.001$) at 3 dpi in *T. atroviride* inoculated and *P. syringae* and *B. cinerea* infected plants than in control plants. These results suggest that *T. atroviride* would activate the SA and JA pathways and induce a systemic response in *A. thaliana*, protecting plants against phytopathogens with different lifestyles.

PL-38

ROLE OF GRP1A IN TOMATO FRUITS: SUSCEPTIBILITY TO FUNGI PATHOGENS AND RESPONSE TO DIFFERENT POST-HARVEST CONDITIONS

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Tomato is one of the main food crops worldwide. Its climacteric fruit has a short period of time during which its organoleptic properties are optimal for marketing and consumption. Currently, the commercialization period of these fruits is from the mature green stage at temperatures between 12-15ºC. Although lower temperatures delay ripening, allowing marketing, they induce cold damage leading to a deterioration in the fruit quality when they are purchased by the consumer. Glycine-rich RNA-binding proteins (GRPs) are involved in the regulation of gene expression at the post-transcriptional level. In previous research by the working group, transgenic tomato plants cv Microtom that express the GRP1a protein from the mature green stage (*PG-preGRP1a* plants) have been obtained. The fruits of these plants have been characterized as having a higher protein content, compared to fruits of wild type (WT) plants, when these were stored for 7 days at 4ºC. In this work, progress was made in the knowledge of the response of WT and transgenic tomato fruits (*PG-preGRP1a* plants) under prolonged cold postharvest storage and their susceptibility to pathogen-induced attack at different temperatures. A postharvest treatment that evidenced cold damage in tomato fruits was set up. The fruits were harvested at the yellowish mature green stage (D0) and stored at 4ºC (damage-inducing temperature) and 15ºC (control temperature) for 21 days (21D4 and 21D15, respectively). Storage of tomato fruits at 4ºC for 21 days, although it delays ripening, induces cold damage, which was greater in WT fruits compared to transgenic fruits. This damage is exacerbated by storing the fruits at room temperature 1 day after treatment (21D4+1 and 21D15+1). Fruit samples were collected under the different treatments and prior to them (D0) for evaluation. Proteome analysis of the different genotype fruits and conditions help to explain the phenotypes observed in WT and *PG-preGRP1a* fruits. For example, different levels of proteins involved in the cell wall remodeling and proteins found to interact with GRP1, as it is the case of ribosomal proteins L10, were found in the different samples. The response to infection of tomato fruits with *Botrytis cinerea* (BC) and *Fusarium oxysporum* (FO) at different postharvest temperatures (4ºC and 15ºC) was also studied. The results obtained indicated a greater susceptibility to these pathogens in fruits WT with respect to *PG-preGRP1a* fruits. This observed characteristic is consistent with proteins differentially expressed in genotypes and treatments (proteomic data), among which, cell wall restructuring proteins and pathogen attack response proteins stand out. The differential response to pathogen attack adds to the observed differential response to cold stress, and it represents an aspect to consider in cold injury index evaluations.

PL-39

ARABIDOPSIS THALIANA MUTANTS IN POLYAMINE CATABOLISM EXHIBIT DIFFERENTIAL RESPONSES TO CADMIUM STRESS

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Polyamines (PAs), namely putrescine (Put), spermidine (Spd) and spermine (Spm), are low molecular weight nitrogenous compounds ubiquitous in all living organisms. In plants, they are involved in multiple functions, such as cell division and differentiation, seed germination, flowering, senescence and stress responses. Polyamine homeostasis is rather complex, with many processes regulating free polyamine levels (synthesis, transport, conjugation and catabolism, among others). According to their substrate specificity, two kinds of enzymes are involved in PAs catabolism. One is a copper-dependent diamine oxidase (DAO, EC 1.4.3.6, also named as CuAO) and the other is a flavin adenine dinucleotide (FAD)-dependent polyamine oxidase (PAO, EC 1.5.3.11). Regarding abiotic stresses, cadmium (Cd) pollution is an important issue because of the widespread utilization of phosphorous fertilizers and industrial sewage. This non-essential metal hampers plant growth and development and causes a redox imbalance, leading to oxidative stress. In this work, we assessed the response of wild type (WT) and mutants of *Arabidopsis thaliana* deficient in polyamine catabolism (pao5, cuaoζ and cuaoα2) exposed to Cd stress. Arabidopsis seeds were germinated in soil in a growth chamber under controlled conditions. At day 21, plants were transferred from soil to a hydroponics medium (Hoagland solution ½) and 3 days later, they were incubated in 10 µM Cd for 4 days. Cd effect was different depending on the plant genotype, being the wild type and pao5 the most affected. These genotypes showed shorter root length, lower root and shoot dry weights and a slightly smaller rosette size. Besides, signs of foliar immaturity were observed (rounded leaf shape and longer petioles). Chlorophyll levels were not differentially altered under Cd stress, but a significantly higher content was found in *cuao* mutants in the absence of the metal. In this condition, the same genotypes also showed a larger rosette diameter and root length. Anthocyanin content was higher in the three mutants in presence of Cd compared to wild type genotype, more markedly in pao5. Fluorometric detection of ROS in roots tips revealed that Cd generated a higher accumulation of O_2.- but lower H_2O_2 content in the four genotypes. The mutants showed more H_2O_2 and O_2.- production than the wild type, both in absence or presence of the metal. In summary, we could observe that Arabidopsis WT or mutant genotypes were differentially affected by Cd treatment, suggesting that alterations in polyamine catabolism can modulate Cd stress response in Arabidopsis, partially through changes in the homeostasis of H_2O_2, O_2.- and phenolics. Other stress parameters are currently being evaluated to shed light on the mechanisms involved.

PL-40

Nitric oxide homeostasis is required for primary nitrate response in Arabidopsis

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Nitric oxide (NO) is a polyfunctional gasotransmitter that regulates several physiological processes in plants. NO participates in the response to many biotic and abiotic stresses as well as developmental processes, primarily due to its known antioxidant capacity, being a key player in the cell redox balance. The main enzymatic pathway for NO production in plants is via Nitrate reductase (NIA) activity. Besides NIA assimilates nitrate (NO3-) by reducing it to nitrite (NO2-), according to an unknown regulatory mechanism that probably involves NO3- / NO2- balance, it can reduce NO2- to produce NO. Besides being the most important source of inorganic nitrogen (N) for plants, NO3- is also a signal molecule that has implications for the global physiology of plants. The addition of NO3- to N starved Arabidopsis seedling triggers the primary NO3- response (PNR) affecting the expression of sentinel NO3- genes like NIA1, nitrite reductase (NiR) and NO3- transporters (NTR1.1, NRT2.1). Recently, the participation of reactive oxygen species (ROS) during PNR was demonstrated, elucidating an antioxidant regulatory mechanism. In this work, using a pharmacological and genetic approach, we evaluated the participation of NO in the PNR in Arabidopsis. The sequestration of NO by the specific NO scavenger (cPTIO) drastically blocked the PNR-associated gene response. Similar results were obtained using the Arabidopsis mutant line *noa1*, with low NO content, where the expression of most of the NO3- sentinel genes was not modified by NO3- addition. Moreover, results showed that the expression of Phytoglobin 1 (Phytoglb1), an enzyme involved in NO homeostasis, is upregulated during PNR. Furthermore, Arabidopsis mutant lines lacking the main genes involved in PNR, *nrt1.1* and *nlp7*, failed to induce Phytoglb1 expression. The latter allows us to speculate that the expression of Phytoglb1 during PNR is under the control of the NRT1.1-NLP7 signaling cascade. Finally, our results suggest a fine redox balance that not only involves ROS but also NO is required as a key process during NO3- sensing and response in Arabidopsis.

PL-41

Integrated metabolic profile of *Prunus persica* leaves exhibiting peach leaf curl disease

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Prunus persica (L.) is a species of great economic importance due to its tasty fruit. Several conditions affect its production; among them, the biotrophic fungus *Taphrina deformans* causes the peach leaf curl disease. This worldwide-distributed disease generates losses ranging between 2.5 and 3 million dollars per year. The characteristic symptoms in leaves include pink pigmentation, thickening and leaf curling.

In this work, healthy leaves (G0) and leaves exhibiting an advanced degree of the disease were collected from naturally infected trees in the orchard. Using sick leaves, symptomatic areas (GC) were carefully dissected from the asymptomatic regions (GN) and both fractions were used in the analysis. In order to understand the metabolic changes triggered by the fungus in infected leaves, lipidomic, metabolomic and a proteomic analysis, complemented with other biochemical determinations, were carried out. Comparison between healthy leaves and symptomatic or asymptomatic tissue were made.

Lipidomics conducted by UPLC-MS showed a disturbance of the lipid composition in sick tissue. Whereas the relative levels of digalactosyl-diacylglicerols did not significantly change in GN or GC with respect to G0, monogalactosyl-diacylglicerols levels were generally induced in GN samples. Plastidic sulfoquinovosyl-diacylglycerols (34:3, 36:3 and 36:6) decreased in GC with respect to GN. Furthermore, most of the triacylglycerols (TAG) relative levels were modified in infected tissue with respect to healthy one. TAGs with low number of unsaturations tended to increase in symptomatic tissue while the levels of TAGs with a high number of unsaturations decreased in symptomatic tissue with respect to healthy

leaves. Metabolomics profiling conducted by GC-MS together with other biochemical measurements and the study of the proteome by LC-MS-MS, revealed a decrease in photosynthesis, carbon fixation and synthesis of photoassimilates as accounted by decreases in sucrose, sorbitol, starch and other sugars and alcohol sugars in symptomatic tissue with respect to healthy tissue. Concomitantly, it was observed an induction of enzymes involved in the glycolytic, fermentative and Krebs cycle pathways, which provide energy from carbohydrates. On the other hand, while most of the amino acids decreased in symptomatic tissue with respect to healthy leaves, asparagine was largely increased. Principal component analysis showed that asymptomatic areas are closer to healthy tissue than to the symptomatic areas. Collectively, data obtained reveal an alteration in the chloroplast functioning of leaves exhibiting "peach leaf curl disease" and give cues of the changes in the metabolism that occur in the diseased tissue. In addition, information obtained expands our knowledge of the interaction between *P. persica* and *T. deformans*.

PL-42

MMR SYSTEM IN THE CROSSTALK BETWEEN DNA DAMAGE RESPONSE AND IMMUNE RESPONSE IN *ARABIDOPSIS THALIANA*

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As sessile organisms, plants are continuously exposed to a variety of adverse environmental factors. These factors, both biotic and abiotic, can cause damage to several biomolecules, such as DNA. Fortunately, all living organisms including plants have multiple mechanisms for detecting and repairing DNA damage in order to maintain the integrity of the genome. One of them is the DNA mismatch repair (MMR) system. MMR proteins are implicated in sensing and correcting DNA-replication associated errors and other nucleotide lesions induced by different stresses. Biotic stress and immune response in plants have been studied in depth and so have the responses to DNA damage. However, little information is available on the signaling crosstalk that occurs between DNA damage and biotic stress. The aim of this work was to study the role of MMR proteins during the immune response of *Arabidopsis thaliana* plants. Our previous data indicate that plants lacking the MutS homolog 6 (MSH6) were less susceptible to the bacterial pathogen *Pseudomonas syringae* pv. tomato strain DC3000 (Pst DC3000) than WT plants,

probably due to the reduced stomatal opening observed in *msh6* mutant plants. Here, we extended our research and observed bacteria-induced host DNA damage, but also found that *msh6* mutant plants have increased levels of DNA double-strand breaks (DSBs) than WT plants under control conditions. Taking into account these observations, we measured the transcript levels of genes involved in both DNA DSB damage responses (*RAD51D* and *SOG1*) and immune responses (*NPR1* and *ICS*) and found that *msh6* mutant plants have higher levels of these transcripts compared with WT plants. Since reactive oxygen species (ROS) are produced as a defense mechanism against pathogens and that these are known to damage DNA and regulate stomatal opening, we also investigated the response of *msh6* mutant plants to hydrogen peroxide (H_2O_2) treatment and found that they were more affected than WT plants by this treatment. Moreover, we observed higher levels of H_2O_2 accumulation in msh6 mutant leaves compared with WT after staining with 3,3'-diaminobenzidine (DAB). Taken together, these results suggest that MSH6 could play a role in DSB response and in ROS homeostasis regulation in *A. thaliana* and highlight the high level of functional redundancy among DNA repair pathways. Future experiments will allow us to test this hypothesis.

PL-43

Cytochrome *c* regulates hormone-mediated hypocotyl elongation in Arabidopsis.

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Energy production through oxidative phosphorylation is one of the main processes that take place inside mitochondria. Oxidative phosphorylation is performed by the sequential operation of complexes (I–V) located in the inner mitochondrial membrane. In addition to these complexes, Cytochrome *c* (CYT*c*) catalyzes the transfer of electrons from complex III to complex IV. Phenotypic analysis of mutant and overexpression lines suggested that CYT*c* is a positive regulator of hypocotyl elongation. Changes in hypocotyl growth mainly result from changes in longitudinal cell elongation. Accordingly, *cytc* mutants show a decrease in hypocotyl cell length, relative to the wild type. We show that the defect in *cytc* mutants

is rescued by exogenous application of brassinosteroid (BR) and auxin (AUX). In addition, *cytc* mutants exhibit altered sensitivity to a BR biosynthesis inhibitor and the polar auxin transport inhibitor NPA, suggesting that AUX and BR homeostasis are affected. The gain-of-function mutation bzr1-1D suppresses the elongation defect of *cytc* mutants indicating that BZR1 acts downstream of CYT*c* in promoting plant growth. Previous studies have shown that BZR1 directly interacts with PIF4, and this complex controls cell elongation and hypocotyl growth. Our results suggest that CYT*c* is capable of regulating the elongation of Arabidopsis hypocotyls acting via the BZR1–PIF4 complex.

PL-44

Characterization of a *Chlamydomonas reinhardtii snrk2.1* mutant: response to salt and oxidative stress

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INBIOTEC

In plants, the SnRK (Snf1-Related protein Kinase) gene family plays a central role in energy sensing and stress-adaptive responses. Recent studies have shown that orthologs of the plant SnRK protein exist in the model green algae *Chlamydomonas reinhardtii* but their functions are still unknown. In this study, we characterize a Chlamydomonas snrk2.1 mutant to start unraveling its role under abiotic stress. With this goal, wild-type (wt) and mutant cells were cultivated in complete TAP media or under salt or oxidative stress conditions, and growth parameters (OD, dry weight), chlorophyll, and starch/lipid content were determined at different time points. We observed that under mixotrophic growth conditions, the *snrk2.1* mutant strain reached a higher cell density (OD750), dry weight, chlorophyll, lipids, and starch contents than the wt strain. The *snrk2.1* mutant cells tend to form characteristic clusters of 4 cells, which is mostly uncommon in the wt strain. However, the *snrk2.1* mutant cells were markedly affected by stressing conditions, showed a 40% reduction in cell density (OD750) upon salt and oxidative stress. On the other hand, the wt strain only decreased its cell density by 20% under salt conditions, in comparison with non-stressed cultures. Furthermore, we performed the same analysis with the well-characterized nnr1 and psr1 mutants, which are null mutants of key proteins for N and P starvation responses, respectively. In conclusion, these results point to SnRK2.1 as a regulator of the stress responses in Chlamydomonas and as a potential target for biomass production and feedstocks for bioenergy and /or other purposes.

PL-45

Gains and losses of foreign mitochondrial chromosomes in a holoparasitic plant

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In plants, horizontal gene transfer (i.e. the movement of DNA between non-mating organisms) has been widely reported in mitochondrial genomes (mtDNAs). It is particularly frequent in parasitic plants due to the intimate connections they establish with their host plants. *Lophophytum mirabile* (Balanophoraceae) is a root holoparasite that lacks chlorophyll and depends completely on their mimosoid (Fabaceae) hosts. About ~70% of the mtDNA of an individual of L. mirabile sampled in the Calilegua National Park, province of Jujuy (LmCal), consists of foreign transferred tracts from mimosoid hosts. This mtDNA (821,906 bp in length) assembles into 64 circular chromosomes, of which 24 harbor protein, rRNA, and tRNA coding genes while the other 40 are completely devoid of known coding regions. The evolutionary dynamics and perpetuation of the mitochondrial chromosomes in L. mirabile are unknown. The aim of this work is to compare the mtDNAs from different populations of *L. mirabile* to examine the presence/absence of mitochondrial chromosomes and their phylogenetic origin. For this purpose, total DNA was extracted from flowers of two individuals of *L. mirabile* growing in Santa Clara, province of Jujuy (LmSC) and in Bolivia (LmBol), respectively, and sequenced with DNBseq technology. De novo assembly of LmSC mtDNA was performed using SPAdes v.3.15.2 and finished with CONSED v.29 resulting in a mtDNA of 66 circular chromosomes and 811,073 bp in length. All chromosomes carrying genes in LmCal (24) are also present in LmSC with an identity of 99.22-99.99%. In contrast, there is extensive variation in the presence/absence of non-coding chromosomes among the three *L. mirabile* populations. Of the 40 noncoding chromosomes of LmCal, six are absent and five are partially present (30-60%) in the LmSC mtDNA. Similarly, of the 42 noncoding chromosomes of LmSC, six are absent and six are partially present in LmCal. Furthermore, a draft assembly of the LmBol mtDNA revealed that four of the 12 chromosomes variably absent or present in the mtDNAs of LmCal and LmSC are present in LmBol. Interestingly, more than 70% of the noncoding chromosomes that are not shared among populations of L. mirabile are mostly foreign.

Therefore, these results suggest that noncoding chromosomes evolve under genetic drift and their presence is sporadic in the different populations of L. mirabile as a result of gains and losses of foreign DNA.

PL-46

Analysis of the effect of sorbitol incubation on the stability of biochemical properties of peach slices exposed to hot-air dehydration

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Peach fruit is characterized by its fleshy pulp and delicate aroma. It provides vitamins, fiber and antioxidant compounds, among others. Since it is perishable, conservation strategies are needed to expand peach consumption. Traditionally, dehydration with hot air is used to preserve fruit. Osmotic dehydration (OD) before conventional drying is a pre-treatment applied to fresh fruit in order to prevent browning or sugar caramelization during prolonged hot air exposure. The aim of this work was the study of the effect of sorbitol treatment before drying on the nutraceutical and nutritional properties of three peach commercial cultivars. Biochemical properties of OD+D were compared with those of D using slices of Elegant Lady (EL), Dixiland (DX) and Flordaking (FD) peaches. Fruit harvested at commercial maturity were washed, disinfected, cut in slices and was used as fresh material (F). A fraction of F was dried in the oven (D) at 58 °C to reach a 10% water content (WC). Another fraction was incubated at 40°C in a solution of sorbitol (47°Brix, OD) during 3 hours of stirring. A water loss of 32% was achieved in the three cultivars. Next, a set of OD slices were dried at 58°C during 4 h (OD+D) until 10% WC. The following parameters were evaluated in F, OD, OD+D and D slices: total phenolics, flavonoids, carotenoids, ascorbic acid, tannins, total protein, glucose, sucrose, sorbitol and antioxidant activity. As expected, in the three cultivars, OD and OD+D slices exhibited an increase in sorbitol –in a dry weight (DW) basis- with respect to F due to sorbitol uptake from the solution. In consequence, dry mass weight of slices incubated in sorbitol was increased between 1.62 and 1.75 times. Thus, this correction factor was applied for analysis and results were

expressed in dry weight (DW) basis since samples greatly differ in water content. In all cultivars tested, antioxidant activity measured by DPPH and ABTS methods, total proteins, phenolic compounds and tannins, were greater in OD+D than in D slices. Flavonoids were greater in OD+D than in F in FD; while exhibited the same levels in OD+D and D in DX and EL slices. In DX, carotenoids and ascorbic acid were greater in OD+D than in D. In FD carotenoids were also higher in OD+D than D slices. Regarding sugars, sucrose levels were smaller in OD+D than D in the three cultivars. While glucose was lower in OD+D than in D in DX and EL, it exhibited the same levels in both types of dehydrated fruit. With respect to the color of the pulp, even though OD+D and D slices were less luminous than F- irrespectively of the genotype-browning was prevented with the OD treatment. Collectively, our results show that OD pretreatment with sorbitol is effective to ameliorate the decay in nutraceutical and nutritional components that usually occurs in D fruit. In addition, the decrease in glucose and sucrose at the expense of the increase in sorbitol makes this treatment suitable for diets restricted in sugars.

PL-47

Energy and growth: cross-talk between Cytochrome *c* and the SnRK1-dependent growth and stress regulatory pathway

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In eukaryotic organisms, respiration takes place in mitochondria and involves the transport of electrons through a series of protein complexes that oxidize reduced coenzymes and reduce oxygen. Cytochrome *c* (CYT*c*) is a small heme protein that participates in electron transport coupled with ATP synthesis. Mutants in CYT*c*-1, one of the Arabidopsis CYT*c*-encoding genes, have shorter roots, smaller rosettes and delayed growth. Under the hypothesis that an alteration in CYT*c* levels modifies the activity of growth regulatory pathways related with energy availability, we studied a possible connection between CYT*c* and the SNF1 (sucrose non-fermenting 1)-related protein kinases 1 (SnRK1) pathway, involved in adjusting cellular metabolism during starvation and stress conditions. Transcriptomic analysis of CYT*c* mutants

showed that CYT*c* and SnRK1 affect the expression of a common set of genes. Then, we analyzed whether the decrease or overexpression of CYT*c* alters the SnRK1 pathway. We determined that changes in CYT*c* levels affect the phosphorylation state of SnRK1, which is related to its activation. In agreement, transcript levels of starvation marker genes regulated by SnRK1 were affected in *cytc* mutants. To further explore the connection between CYT*c* and the SnRK1 pathway, we made genetic crosses between CYTc and SnRK1 overexpressing and mutant lines. Our results suggest that CYT*c* levels affect the activity of the SnRK1 pathway, probably through changes in mitochondrial energy production, to adjust cellular metabolism and growth to changes in respiration.

PL-48

Evaluation of three alfalfa cultivars under waterlogging and drought based on agronomic and molecular approaches

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Alfalfa (*Medicago sativa* L.) is the most important forage worldwide. It is considered the queen of forages for being a natural source of proteins, vitamins and minerals and due to its high palatability. Argentina is one of the largest producer of alfalfa in the world with a production of between 8,000 and 22,000 kg of dry mass/ha/year and an average cultivated area of 4.5 million hectares. Due to the expansion of extensive crops, such as soybean, alfalfa production has moved to marginal areas with poor soils and located in regions subject to cycles of drought and flooding. The selection of the most suitable cultivar according to the growing conditions is key for the correct management of the crops by rural producers to increase yield. In this work, we analysed at agronomic, biochemical and molecular level the response of three alfalfa cultivars under hydric stress. We selected the cultivars MONARCA SP INTA (GRI 8), NK MATRERA (GRI 8) and NK VAQUERA (GRI 9) because they are widely used in our country. They were subjected to three treatments: 14 days of waterlogging, 14 days of drought and normal irrigation. Agronomic measures were calculated, such as fresh and dry weight of roots and leaves, survival rate, and the leaf greenness index was determined. Moreover, using RT-qPCR we quantified the expression of three genes implicated in the synthesis of the hormones abscisic acid, jasmonic acid and ethylene. Our analysis revealed a close

correlation among genes involved in hormone synthesis, alfalfa cultivars and hydric stress. In this sense, these results could be used in the genetic improvement of alfalfa plants.

PL-49

VACUOLELESS GAMETOPHYTES is differentially regulated according to several abiotic stress conditions in *Arabidopsis thaliana*

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In our laboratory we performed the first functional characterization of a protein with DC1 domains, which we called VACUOLELESS GAMETOPHYTES (VLG). We demonstrated that VLG is required for the central vacuole formation during male and female gametophyte development. We also proved that VLG physically interacts with proteins related to endomembrane dynamics and vesicular fusion. In this work we present results that show that VLG also participates in stress responses in *Arabidopsis thaliana.* The study of promoter:GUS reporter lines allowed us to evaluate the activity of the VLG promoter in different organs subjected to various abiotic stresses. VLG promoter regulation occurs most notably on leaves, although some regulation can be seen in roots during particular stress conditions such as salt and cold treatments. The expression of VLG, corroborated by q-PCR, increases upon abiotic stress conditions such as 4ºC cold, NaCl treatments and also during senescence; and decreases in response to oxidants and UV-B light. VLG-silenced plant lines expressing a specific miRNA under the 35S promoter were generated and analyzed. VLG-silenced plants exposed to high fluence UV-B radiation display lower ROS accumulation as evidenced by DAB staining. In addition, silencing of VLG causes an atypical accumulation of anthocyanins in leaves and shoots during senescence, providing an overall higher antioxidant capacity. Altogether, considering these results and the previously suggested role of VLG as a scaffold protein, we suggest that VLG plays an active role in the processes of response to abiotic stresses, likely interacting with other regulatory proteins and coordinating the activity or formation of protein complexes. In particular, VLG could be involved in the regulation of the accumulation of antioxidant compounds as anthocyanins during the response to UV-B light. In sum, these results suggest that VLG is

involved in the abiotic stress response, particularly leading to a higher capacity to deal with oxidative stress when silenced.

PL-50

COMPARATIVE RESPONSES OF MORPHOPHYSIOLOGY AND NITROGEN METABOLISM IN THE INTERACTION BETWEEN PEANUT CULTIVARS WITH CONTRASTING DROUGHT TOLERANCE AND *BRADYRHIZOBIUM SP*

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Legumes are environmentally important due its ability to fix atmospheric nitrogen into organic compounds through biological nitrogen fixation (BNF). They grow in temperate and warm climates, being susceptible to unpredictable episodes of water deficit. Drought stress constraints legume vegetative growth stages such as germination, growth, dry matter partitioning as well as reproductive stages such as tiller production, organ development, seed filing and seed quality. Therefore, this study aimed to evaluate morphophysiology, nitrogen metabolism and yield in the interaction between peanut cultivars with contrasting drought tolerance and the strain able to infect peanut plants *Bradyrhizobium sp.* For that, seeds of two peanut cultivars, Granoleico (sensitive) and EC-98 (tolerant), were inoculated with *Bradyrhizobium sp.* SEMIA6144 and 30 days after sowing, plants were separated into the experimental groups: control, drought stress (withholding irrigation until the apparition of wilting symptoms), and drought stress and subsequent rehydration (rewatering of plants subjected to drought stress). Root and nodule morphology were analyzed by light microscopy (histological preparations); N-compound contents (i.e. aminoacids, ureides and nitrates) were quantified spectrophotometrically; N and C contents in shoots were analyzed by inductively coupled mass spectrometry (ICP-MS) and, finally, crop yield was evaluated in two seasons (2016/17 and 2017/18). The results obtained on root anatomy and histology, although revealed differential responses between cultivars were not associated with tolerance traits in the tolerant cultivar (EC-98). However, nodules of EC-98 showed invariable diameter and

infection zone upon the exposition to drought stress and rehydration compared to nodules of Granoleico plants exposed at the same treatments. The balance of C- and N-contents and N-compounds in EC-98, i.e., an invariable content of C in shoots and of nitrates and ureides in roots and nodules of stressed and rehydrated plants, an increase in total amino acids only in nodules of stressed plants and small reduction in shoot N-content (compared with the sensitive cultivar), were associated to an efficient BNF. This cultivar may be able to transport the N products from nodules to shoots in order to prevent the N-feedback limitation of BNF. These traits would contribute to greater yields in the peanut-*Bradyrhizobium sp*. interaction as observed in the dry season 2017/2018. Thus, the EC-98-Bradyrhizobium sp. interaction exhibits tolerance traits derived from the N-balance and nodule metabolism (as revealed by histology and physiology) which would contribute to yield during challenging seasons with drought stress episodes affecting the crops.

PL-51

Characterization of DPa and DPb transcription regulators in *Arabidopsis thaliana*: participation in DNA damage responses

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Because of their sessile condition, plants are continuously exposed to solar radiation. One component of the solar spectrum is UV-B radiation (280-315 nm). At high intensities, it can damage lipids, proteins and most important, DNA. As a consequence, exposure to UV-B can inhibit plant growth and elongation of primary roots. If damaged DNA cannot be adequately repaired, cells enter the death cell program. The G1/S transition is an important checkpoint of the eukaryotic cell division cycle, in which the cells decide to continue or to stop dividing. One of the most important regulators is the Retinoblastoma (Rb) protein, which can negatively regulate E2F transcriptional factors. In *Arabidopsis thaliana*, there are six E2F proteins (E2Fa – E2Ff), which control the expression of G1/S transition genes. E2Fa-c are called "typical E2Fs" and they need to heterodimerize with two DP interaction proteins (DPa and DPb) to regulate transcription of target genes. Different studies in our and other labs showed that the effect of UV-B radiation on E2F mutants (*e2fa*, *b* and *c*) exert different responses. These results demonstrate that E2F TFs play crucial and in some cases antagonist roles in several pathways related to cell division, DNA repair

and differentiation. However, little is known about the specific roles of the DP interacting proteins in these responses. In this work, we have phenotypically characterized *DPa* and *DPb* overexpressing (OE) and mutant lines. We also studied the participation of those proteins in the regulation of cell proliferation and plant growth in response to UV-B exposure. *DPa* and *DPb* overexpressing lines showed shorter primary roots than WT seedlings under control conditions. We used confocal microscopy to analyze the primary root meristematic zones from these plants. *DPaOE* and *DPbOE* have shorter meristematic zones with fewer cells than those from WT primary roots. On the contrary, when primary roots from dpa and dpb mutants were studied, they looked similar to those from WT seedlings, showing meristematic zones with similar size as those from WT seedlings. Previously, inhibition of primary root elongation after UV-B irradiation in WT Arabidopsis seedlings was correlated to a decrease in the length and in the number of cells of the meristematic zone. After UV-B exposure, *DPaOE* and *DPbOE* primary roots were also shorter than those from WT seedlings; however, the effect of the treatment was similar in the transgenic and the WT plants. When the effect of a single UV-B exposure in *dpa* and *dpb* mutants was analyzed, the results showed that *dpb* mutants showed a similar inhibition of primary root elongation as WT roots, but after the treatment, *dpa* roots were longer than those from WT seedlings, showing less sensitivity to UV-B. This result was confirmed by the analysis of the primary root meristems, showing those from dpa seedlings larger sizes with more cells. Finally, we analyzed how programmed cell death is activated after UV-B exposure in the different lines. Interestingly, while all plants showed dead cells in the primary root meristems after UV-B exposure, both *DPaOE* and *dpb* mutants had a lower number of dead cells after UV-B treatment than WT, *DPbOE* and *dpa* mutants. Despite this, all plants accumulated similar levels of cyclobutane pyrimidine dimers after UV-B, suggesting that none of these proteins directly participate in DNA repair. Together, our results suggest that DPa and DPb are both involved in the UV-B response in roots, having different functions.

PL-52

Root apex proteome response to cadmium and copper stress during the early maize seedling growth

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Metal progressive increase in the environment because of the sustained anthropogenic pollution threatens cultivable regions. To cope with this adverse condition, plants adjust their metabolism and reprogram their growth. We focus our work on studying the effects of copper (Cu) and cadmium (Cd) in the root of *Zea mays* L. (maize) seedlings. Herein, proteomic analysis of the root growth zone (first 5 mm of the apex) is presented. Uniformly germinated seeds were transferred to a hydroponic culture containing 250 mL of diluted (1/10) Hoagland's nutrient solution without (C) or with 50 µM $CdCl_2$ and $CuCl_2$; 30 seedlings per tray. Plants were grown in a controlled climate room at 24 ± 2 ºC with 50% relative humidity, and trials were carried out in darkness to mimic soil conditions during germination and post-germinative growth. Protein extracts were obtained from the root apex. Three independent experiments were performed. Protein digestion and mass spectrometry analysis were conducted at the Proteomics Core Facility CEQUIBIEM (UBA-CONICET). Data were processed using Proteome Discoverer software. Protein identities were assigned taking the maize Uniprot protein database as the reference (UP000007305). Perseus software was used to perform comparisons among samples, area values, and statistical tests. A total of 2271 different proteins were detected. Pearson correlation coefficients were calculated for all aliquot pairs using the normalized abundance after a log2 transformation. Correlation analysis indicates high assay reproducibility. Principal component analysis revealed that C, Cu, and Cd clustered separately. Samples shared up to 90% of identified proteins. Proteins whose abundance varied significantly ($p<0.05$) and exhibited a difference higher than 2-fold or less than 0.5-fold in metal-treated seedlings compared to the C were considered differentially accumulated proteins (DAPs). Under metal stress, 114 and 288 proteins were up represented, 80 and 241 were down represented for Cd and Cu respectively. Metals shared 76 and 35 DAPs (for the increased and decreased ones respectively). Enrichment analysis based on GO categories and functional interactions between DAPs were conducted using the STRING database (string-db.org/). For Cd and Cu overlapped DAPs, most of the identified proteins for the biological process category were involved in Metabolic process (63), Organic substance metabolic process (43), Oxidation-reduction process (28), Catabolic process (22) and Response to stress (21); for the molecular function category, were Catalytic activity (60), Ion binding (35), Metal ion binding (25), Oxidoreductase activity (24), and Hydrolase activity, hydrolyzing o-glycosyl compounds (10). And for the cellular component category, Cellular anatomical entity (70), Cytoplasm (44), and Extracellular region (21) were the most represented groups. Taken together, these proteins might have important roles in maize defense mechanisms against metal stress.

PL-53

Metabolic Engineering of chlorophyll metabolist in tomato

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Production and characterization of plants overexpressing genes involved in chlorophyll metabolism is the starting point to address fundamental question about this pathway and could be useful for the development of plants resistant to diverse environments.. In this work, the impact of overexpression of the genes that encode the phytol kinase (VTE5) and phytol kinase kinase (VTE6) enzymes, committed steps of the chlorophyll degradation pathway, on photosynthetic activity and on the accumulation of tocochromanols , was assessed in tomato transgenic plants. Variations in chlorophylls contents, photosynthetic parameters and germination rate were observed in the overexpressing lines. However, no differences in tocochromanols levels were recorded under control conditions. Crosses between single transgenic lines are now being analyzed.

PL-54

Wheat seeds priming with spermine or 1,3-diamine propane was effective in supporting early seedlings growth by the adjustment of polyamine metabolism after Cd exposure

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Anthropogenic contamination affects crop production. Among the environmental pollutants, cadmium increment in the soil is affecting agricultural areas. Within the areas with increased risk are those for intending wheat (*Triticum aestivum* L.), one of the most important cereals in the world. Post-storage seed reinvigoration is one of the beneficial strategies proposed to improve early plant growth. On-farm seed priming is one of the many invigoration techniques increasingly used. It involves soaking the seeds in water or inorganic/organic compounds solution before sowing. Here, we used spermine (Spm) or 1,3 diamine propane (DAP) for seed priming to boost seedling growth under normal or Cd-stressed conditions. The study is focused on root and shoot polyamine (PA) homeostasis of two wheat varieties, Saeta (S) and Turandot (T). Seeds were placed in glass flasks containing 30 mL of distilled water or 25 μM Spm or DAP, 30 seeds per flask. After incubation for 8 h in darkness, at 120 rpm and 24 ± 2 °C, seeds were germinated on filter paper moistened with distilled water, in trays protected from desiccation for 24 h. Germinated seeds were transferred to trays containing Florcom SV® substrate previously moistened to field capacity with water or 100 μM CdCl2. Plants were cultivated in a controlled climate room; substrate humidity was maintained by watering with water or Cd solution.On day 7, root and shoot length were measured, and extracts for metabolite determination and PA-metabolism gene expression analysis were prepared. Cadaverine, putrescine, spermidine, spermine, agmatine, homospermidine (HomoSpd), and norspermidine (NorSpd) total and free-form contents were determined. Bioinformatic analysis was performed to find out genes associated with PA metabolism in the wheat genome. Primers were designed for diamine oxidase (DAO), polyamine oxidase (PAO), S-adenosylmethionine decarboxylase (SAMDC) and ornithine decarboxylase (ODC) genes. Seed priming reverted Cd-induced root growth inhibition. Root and leaf PA homeostasis was differently affected by Cd, as well as by seed priming. The metal increased gene expression of DAO and PAO and decreased that of SAMDC. The increase in H2O2 due to PA catabolism could be accounting for Cd-induced cell redox imbalance. Priming did not reverse the effects of Cd on gene expression but modified ODC and SAMDC transcript accumulation. This could suggest that priming regulates PAs homeostasis by controlling the entry of ornithine into the PA biosynthesis pathway and further biosynthesis of Spd and Spm. Results showed that seed priming was effective in sustaining early root growth during Cd exposure by regulating the interconversion between PA and their free/conjugated forms. Interestingly, this is the first report of NorSpd and HomoSpd presence in wheat.

PL-55

Lysine acetylation of NADP-malic enzyme of *Setaria viridis*

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Lysine acetylation is a post translational modification that serves as an important metabolic modulator in many organisms. In our investigation, we evaluate the hypothesis that acetylated lysines could have a prominent role in the modulation of the function of the photosynthetic C4 cycle in plants. Plants performing C4 photosynthesis have a higher productivity per crop area related to an optimized use of water and nutrients. This occurs because C4 plants partition photosynthetic reactions between two cell types. They initially fix the carbon to C4 acids within the mesophyll cells (MC) and then transport these compounds to bundle sheath cells (BSC), where they are decarboxylated. This carbon shuttle results in a more efficient incorporation of CO_2 into the Calvin cycle (CC) because it allows CO_2 concentration to increase in the RuBisCO environment, which increases carbon fixation. In previous analysis, we have investigated metabolic characteristics underlying the C4 cycle in *Setaria viridis*, a model plant closely related to several important forage and bioenergy grasses. We observed variations in Acetyl-CoA producing pathways between BSC and MC. Since Acetyl-CoA is a well-known acetyl-donor, in this work we searched the proteome data for lysine acetylated peptides. We found that NADP-dependent malic enzyme, a key enzyme in the C4 pathway, in BSC presents an N-acetylation-type modification in lysine 582. Furthermore, the immunoprecipitated NADP-ME from *S. viridis* leaf extract was recognised by anti-acetylated lysine antibodies. These results indicate that NADP-ME would be acetylated *in vivo*, which could impact on the catalytic characteristics thus functioning as a C4 photosynthesis modulator. To prove this hypothesis further experiments are needed, which are now under progress.

PL-56

Role of small RNAs in the systemic response against pathogens in Arabidopsis

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When plants are exposed to pathogens, they induce both local and systemic defenses to evade them. After pathogen recognition in aerial tissues by extra and or intracellular receptors, the activation of MAPKs-dependent signaling, accumulation of ROS, and accumulation of Salicylic Acid (SA) induce local resistance. Moreover, the spreading of mobile signals such as SA and Pipecolic Acid (PIP), among others, trigger the systemic response called systemic acquired resistance (SAR). SAR is characterized by the establishment of a primed condition, which prepares the plant for a faster and/or more robust activation of defenses in further infections. In both local and systemic tissue, the stress-responsive genes are subject to a fine-tuning of their transcription and stability of their mRNAs for proper defense activation. In this process, miRNAs trigger post-transcriptional gene silencing of many defense-master genes. The miRNA precursors are transcribed by RNAP-II. At the co-transcriptional level, Dicer-Like1 degrades precursors to generate mature miRNAs. Its recruitment to chromatin is allowed by HASTY. Then, mature miRNAs are loaded in AGO1, exported to the cytoplasm, and directed to their target molecule. Interestingly, both HST and AGO1 also modulate the systemic mobilization of miRNAs. We are interested in knowing how the biogenesis/activity of miRNAs controls the systemic responses and priming in Arabidopsis. To this end, we evaluated the ability to induce systemic defenses in mutants affected in different components of the miRNA biogenesis pathway. Either wild type or mutants were treated with Mock, PIP, SA, or *Pseudomonas syringe* pv tomato (Pst) AvrRpt2 to induce systemic responses. In contrast to ago1 mutant plants, hst were more resistant to Psm than wild type. Interestingly, both hst and ago1 were unable to induce systemic responses after PIP treatment as wild type plants did. Interestingly, both mutants respond to SA and Pst-AvrRpt2 treatment for systemic defense induction. By the analysis of mRNA-seq of wild-type plants with SAR or treated with systemic inductors as well as sRNA-seq of hst and ago1 and RT-qPCR, we found four miRNA candidates to modulate systemic resistance and/or priming. Our current studies are directed to understand how HST and AGO1 modulate PIP- but not SA-systemic signaling, focusing on a potential general modulation of miRNA systemic mobilization and/or the activity of specific miRNAs in the triggering of this response.

PL-57

PLANT GROWTH AND RESISTANCE PROMOTED BY STREPTOMYCES SPP. IN THE MODEL ORGANISM *ARABIDOPSIS THALIANA*

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Rhizobacteria, so called due to their association with roots in the rhizosphere, play a very important role in plant growth promotion and protection by controlling or inhibiting pathogens. Actinomycetes is one of the major components of rhizosphere microbial populations. As part of this group, Streptomyces spp. are Gram-positive filamentous prokaryotes, ubiquitous in soils. Many rhizobacteria have a beneficial effect on plant growth and development, showing at least two of the three following criteria: competitively root colonization, stimulation of growth and reduction of disease incidence. In recent years, the use of Streptomyces has become an attractive tool for crop disease management, being considered as a component for sustainable agriculture. Previously, we have isolated two Streptomyces spp. from the soybean-rhizosphere that showed protection and promoted growth of soybean plants. Here, this study aimed (i) to analyze isolates Streptomyces spp. as growth promoter for *Arabidopsis thaliana* wild type (Col-0 ecotype) plants and (ii) to study protection of Streptomyces spp. against the attack of the pathogen *Pseudomonas syringae* in the model organism *Arabidopsis thaliana* wild type (Col-0 ecotype). Our results demonstrate that treatment with either Streptomyces sp. promoted plant growth and development, and also increased seed production per plant, showing higher yield under greenhouse conditions. Moreover, the results obtained demonstrate that, in *A. thaliana*, plants treated with each of these two Streptomyces species showed increased resistance against *Pseudomonas syringae* infection. Altogether, these results provide evidence that Streptomyces spp. from soybean rhizosphere could be considered as possible biocontrol agents against phytopathogenic microorganisms and also stimulating growth and development in other plants.

PL-58

Lack of cytochrome c affects early stages of plant growth and development

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Cytochrome *c* (CYT*c*) is a soluble heme protein responsible for the transport of electrons from reduced coenzymes to molecular oxygen in the electron transport chain located in mitochondria. These are organelles capable of sensing and signaling the cellular energy state, coordinating development through interaction with different growth regulatory pathways. Mutants in CYT*c* genes show altered shoot and root growth, with smaller rosettes and delayed flowering. Here, we show that these alterations begin in the first steps of plant development, particularly due to leaf primordium and primary root decreased growth rate. In addition, lack of CYT*c* impacts in primary root structure, with a reduced meristematic and elongation zone and shorter mature elongated cells. The root architecture is also modified, with a lower amount of lateral root primordia and higher lateral root density. Finally, CYT*c* seems to be involved in the maintenance of a correct auxin response, since altered expression of auxin reporters was observed in *cytc* mutants in both shoot and root tissues. This changes are correlated with leaf primordium growth arrest and higher lateral root densities, suggesting that changes in CYT*c* levels may affect auxin distribution.

PL-59

Identification and expression analysis of *Brachypodium distachyon* B3 superfamily genes involved in flowering

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The plant-specific B3 superfamily encompasses five major families of genes containing the B3 DNA binding domain. Some of them are well characterized such as the auxin response factor family (ARF), but little is known about the Reproductive Meristem (REM) subfamily. To date, the only REM gene for which a function has been determined is VRN1, a key factor in vernalization response. Other REM members have been proposed as flowering regulators in *Arabidopsis thaliana* and rice, for example, AtREM16 and OsREM20. *Brachypodium distachyon* is the model plant for Pooideae subfamily, which includes important crops such as wheat, barley, and oats. In these cereals, the tight regulation of flowering is key to breeding the most important agricultural trait: grain yield. For this reason, we decided to study *B. distachyon* to characterize novel genes involved in flowering, potentially useful to breeding in cereal crops. Here, we aimed to identify REM family transcription factors involved in flowering development. For this purpose, we classified phylogenetically the *B. distachyon* REM family and then selected some members to analyze their expression profile during flowering. Three developmental stages, stem elongation, booting, and spikelet emergence were chosen for this analysis. Furthermore, the expression of *B. distachyon* homologs of known flowering time genes FT, SOC1, LFY and VRN1 was determined in the same samples. One of the B3 genes, BdREM29 remained undetectable during the stem elongation stage but was expressed both in booting and spikelet emergence. Additionally, BdREM32 was detectable in the three stages, showing a similar pattern to FT and SOC1 homologs, whose expression levels increase when spikelet emergence occurs. The results suggest the role in flowering of these genes in *B. distachyon*, and further functional characterization analyses will be carried out due to their potential use as breeding tools in Pooideae.

PL-60

MORE TIES THAN BREAKUPS AMONG THE BEH FAMILY OF TRANSCRIPTION FACTORS IN STREPTOPHYTES

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The piecemeal assembly and cooption of pre-existing modules into novel signalling pathways is a recurrent feature of land plants. BEHs (BES1-Homologues) proteins are a small gene family of transcription factors that are master regulators of brassinosteroid (BRs) signalling. BRs are polyhydroxylated steroidal compounds with wide-ranging developmental effects in angiosperms; this signal transduction pathway is one of the best characterized in plants. BR perception by membrane-associated receptors relieves the phosphorylation and subcellular localization checkpoint that GSK3 kinases exert on BEHs, which as a consequence accumulate in the nucleus to regulate the expression of a significant number of genes. Most of our knowledge on this pathway has been gathered from studies on *Arabidopsis thaliana* and a few other angiosperms, and the full set of BR signalling components only appears in the seed-plant clade. However, BEH transcription factors are present in Klebsormidiales and Phragmoplastophyta, raising the question on the role these proteins play in unicellular and multicellular streptophytes, and the reasons for their recruitment into the BR pathway. BRs have been associated with cell division and expansion, but mounting evidence also links them to stem cell homeostasis and differentiation in meristems. In fact, we've found that imbalances in the abundance of MpBES1, the closest homolog to angiosperm BR-transducing BEHs in the bryophyte *Marchantia polymorpha,* cause phenotypic alterations that point to a crucial role of this protein in the control of cell division and differentiation. Overexpression of MpBES1 in Arabidopsis, in turn, phenocopies gain-of-function mutations in endogenous BEHs. These results suggest that BEHs perform a similar function in liverworts and in angiosperms, but are subject to different regulatory cues. Through a broad phylogenomic analysis of BEH homologs in all major streptophyte lineages, we disclose diagnostic characters and evolutionary trends in this gene family. In embryophytes, BEH genes have undergone few duplication events, particularly at the origin of euphyllophytes, seed plants and core eudicots, that contributed to the expansion of the family and probably its functional diversification. However, motif-conservation analysis also reveals that new or combinatorial variants seldom occur in this pretty conservative family, but rather that sequence adjustments in predicted subdomains are the rule. Features that set apart Zygnematalean and land-plant sequences, moreover, suggest that BEH proteins in the latter were wired early on to a phosphorylation-dependent modulatory regime. Insights into the evolution of BEHs may not only shed new light on the basic processes driven by BRs, but also reveal relationships between streptophyte lineages and regulatory constraints upon the conquest of land by plants

PL-61

Differential malate catabolism influences contrasting oil and protein content in soybean seeds

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Over time, breeders have striven to obtain soybean varieties with higher yields, that is, higher grain weight at harvest per unit land area. However, this has led to a decrease in grain quality, referring to the concentration of protein it contains. This attribute is very important when using soybeans in the food industry. Also, there is evidence of negative correlation between the amount of protein and oil in the soybean grain. Therefore, understanding of the metabolic routes that the grain uses for the biosynthesis of these reserve compounds is of great agronomic and biotechnological interest. In order to study molecular determinants associated with the accumulation of each component of the grain, we selected soybean lines with a similar genetic background but whose protein/oil ratio is contrasting: line 91, with a high protein concentration; and line 39 with a high oil content. Differences were observed in the composition of polar metabolites in the grains of both genotypes, mainly in the levels of the organic acids, malate and citrate. To study the enzymes involved in the metabolization of these compounds, we prepared protein extracts of grains harvested at different times from the beginning of filling (R5 stage) to desiccation (R8). Then, we carried out spectrophotometric determinations of NAD and NADP-dependent malic enzyme activity (ME), malate dehydrogenase (MDH), and citrate synthase (CS). Our results indicate that the level of NAD-ME is different between both lines under study, being higher in the high protein line 91, which is consistent with the higher content of malate in this line. Thus, the regulation of the levels of this enzyme would function as a possible strategy to regulate the content of proteins and oils in the soybean grain.

PL-62

Deciphering FITNESS gene regulatory network. A focus on JA-signaling pathways

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We have previously demonstrated that the transcription factor (TF) FITNESS which is highly repressed by oxidative stress acts as a node regulating signal transduction pathways in response to both abiotic and biotic stresses modulating SA-related processes. To further characterize the gene regulatory network of FITNESS in Arabidopsis we used estradiol (EST) – inducible overexpressing (*FITNESS-IOE*) plants to perform a targeted transcript profile of 42 genes including 31 non-TF- and 11 TF-encoding genes which were either significantly altered in fitness mutants or which are involved in specific hormone or stress signaling pathways. Among them, four genes (*LOX2*, *ANAC072*, *JAZ3*, and *JAZ10*) were highly induced, and eight were significantly repressed (*ICS1*, *COI1*, *WAK1*, *PCC1*, *ACD6*, *EDS1*, *ORA59*, *ERF1*) 4 h after the induction of *FITNESS* expression Noteworthy, many of these putative target genes are involved in JA synthesis or signaling. For example, *LOX2* (*LIPOXYGENASE2*) encodes a key enzyme in the octadecanoid pathway leading to JA biosynthesis, COI1 is an essential component of the bioactive JA perception apparatus and its strongly downregulation in FITNESS-IOE lines indicates that JA perception might be affected. Additionally, transcripts of JAZ3 and JAZ10 proteins, which act as repressors of JA signaling, were highly upregulated after FITNESS induction. The aforementioned results suggest that FITNESS modulates JA-related responses. To test this hypothesis, we first measured root growth inhibition in the presence of Methyl Jasmonate (MeJA). Arabidopsis fitness mutants showed the highest growth inhibition to MeJA when compared to control conditions. Additionally, given that the expression of the genes encoding the transcription factors ORA59 and ERF1 as well as the marker genes *PDF1.2* and *VPS2* were also affected in FITNESS-IOE plants, we tested the behavior of plants with altered levels of *FITNESS* against the infection of the necrotrophic pathogen *Botrytis cinerea*. Our results showed that fitness mutants exhibit enhanced disease resistance to *B. cinerea* when compared to WT plants. Summarizing, our work demonstrates that changes induced in the transcriptional network in fitness mutants stimulates SA- and JA- mediated defense responses.

PL-63

Elucidating the mechanisms of chilling injury resistance in peach through proteome analysis

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Peach is a perishable fruit that deteriorates quickly once harvested at room temperature. Storage at 0ºC is used to prevent fruit decay and extend shelf life. However, this practice produces physiological disorders called “chilling injury” (CI), leading to loss in quality. The aim of this study was to analyze and compare the proteome of a cultivar resistant to CI (Elegant Lady) subjected to different post-harvest conditions: in harvested fruits (H), the stage where it reached physiological maturity; in fruits kept in a chamber at 20◦C for 5 days (organoleptic characteristics suitable for consumption), called RS (room temperature-storage); in fruits stored at 0◦C for 21 days (CS21, cold storage for 21 days); and in fruits stored at 0◦C for 21 days followed by 5 days at 20◦C (CS21+RS). Proteins were extracted from mesocarp tissue and run on polyacrylamide gels before sending them to Proteomics Core Facility CEQUIBIEM for Mass Spectrometry analysis. Proteins containing a fold-change ≥2 and p ≤0.05 were considered for the analysis. In the comparison H vs RS, several proteins involved in cell wall degradation such as pectinesterase and endo-polygalacturonase were increased, according to the loss of firmness observed. Also, a laccasse was detected, which plays a role in the biosynthesis of lignin, and a xyloglucan endotransglucosylase, involved in cell wall biogenesis, was found decreased. In addition, an increase in the enzymes involved in the ascorbate-glutathione cycle, ascorbate peroxidase and glutathione reductase, was detected, which could be involved in maintaining the redox balance during ripening and senescence. In the comparison H vs CS21, an increase of the expression of a chitinase, a pathogenesis-related protein, and two thaumatin proteins were observed, which could have a role in the protection of cell wall structure against chilling injury and pathogen attack. Finally, in the comparison RS vs CS21+RS, various enzymes implicated in the carbohydrate metabolism, such as phosphoglycerate mutase, glucose-6-phosphate isomerase and citrate synthase, decreased their levels, suggesting a decline in glycolysis and Krebs pathways in the ripening after a prolonged cold treatment. Also, it was observed an increase and decrease of proteins involved in the metabolism of proteins that could suggest an active turnover in these samples. Overall, our results show that in H vs RS, the reconfiguration of the proteome was as expected for a ripening process accumulating predominantly wall degradation enzymes. While in the comparison RS vs CS21+RS, an inhibition of pathways that degrade carbohydrates was observed. Our previous results in this variety showed a large increase in sucrose, glucose, fructose and raffinose in the comparison H vs CS21. These sugars that act as osmoprotectants plus thaumatins that protect the cell wall, producing a decrease in wooliness symptom (lack of juice), might be the most probable cause of resistance to CI in EL.

PL-64

CHARACTERIZATION OF AN UDP-GLYCOSYLTRANSFERASE INVOLVED IN FLAVONOID GLYCOSIDES BIOSYNTHESIS IN MAIZE

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Flavonoids are specialized metabolites widely distributed in plants with diverse biological functions. In addition, these compounds have benefits for human health. Flavonoids are accumulated in the vacuoles as O-glycosylated derivatives; however, several species including cereals synthesize flavonoid C-glycosides. The aim of this work is to characterize a maize UDP-glycosyltransferase (ZmUGT2), which may be involved in the biosynthesis of flavonoid glycosides. First, by phylogenetic analysis of plant UGTs that accept flavonoid as substrates, we determined that ZmUGT2 clustered with UGTs that add sugars on 5-hydroxyl group, suggesting that this enzyme may have this specificity. We then cloned the coding sequence and expressed the protein E. coli to perform in vivo and in vitro enzymatic activity assays. The analysis of the products formed by HPLC showed glycosylation of flavonols, flavanones and flavones when tested as substrates. In order to investigate its function in planta, we generated *Arabidopsis thaliana* transgenic plants expressing *ZmUGT2* (*p35S:ZmUGT2*). Under anthocyanin-inducing conditions, the transgenic plants showed higher levels of these compounds than wild type plants. We also studied the effect of UV-B radiation over the elongation of the primary root in transgenic and wild type plants. Although both genotypes showed a decrease in primary root elongation after UV-B treatment (along four days) compared the plants under control conditions (no UV-B), the transgenic plants showed less inhibition of the primary root elongation in comparison with wild type plants. We are purifying ZmUGT2 recombinant protein to determine its kinetic parameters for the different flavonoids as substrates. We also plan to analyze by LC-MS how the flavonoid glycoside accumulation patterns are altered in *ZmUGT2* transgenic roots with respect to WT plants and also to deepen on the studies about UV-B radiation tolerance in these transgenic plants.

PL-65

Characterization of mitochondrial citrate synthase isoforms from soybean seeds

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Soybean [*Glycine max* (L.) Merr] is known as one of the most relevant crops in our country because of the several usages such as raw material for export, oil production and cattle feeding. Citrate synthase enzyme (CS), which plays an important role in the glyoxylate cycle into the peroxisome and in the Krebs cycle into the mitochondria, catalyzes the condensation of oxaloacetate and acetyl-CoA. Several reports indicated that the overexpression of CS in roots increased the aluminum stress tolerance by organic acid internal accumulation and exudation. According to our previous evidence, CS also would facilitate the export of carbon derived from amino and other organic acids from soybean seed mitochondria to the plastids, site where fatty acid synthesis occurs. Thus, CS would play an important role in the generation of precursors for the biosynthesis of storage compounds during seed filling. As a promising target enzyme for crop improvement, this work studies the two mitochondrial CS isoforms found in soybean which differ from each other solely in three amino acidic residues. First, the enzymes were expressed in *E. coli* BL21 DE3 transformed with pET28a(+) carrying each synthetic gene and purified using Ni-NTA affinity columns. Each protein was detected at high levels in the soluble fraction of bacterial extracts and its molecular mass and quality after purification was as expected. We determined the optimum pH, substrate affinities, and the effect of several metabolites on enzyme activity using Ellman's reagent coupled assay. Despite the high sequence similarity, our results indicated subtle differences that could indicate a differential contribution of both CS isoforms to the carbon flux during soybean seed maturation.

PL-66

CRISPR/CAS9 mediated gene-editing of HD Zip Class I transcription factors enhances salt tolerance in rice

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Rice (*Oryza sativa* L.) is one of the most important crops worldwide. However, both biotic and abiotic stress conditions cause enormous economic losses. According to the Food and Agriculture Organization of the United Nations (FAO), the demand for agricultural products will be around 70% by 2050, so the annual production of cereals must be increased accordingly to feed the world population. We study members of the HD-Zip type I family of transcription factors in plants. The overexpression of these proteins in Arabidopsis increased sensitivity to Abscisic acid (ABA), decreased water deficit tolerance, and increased soil salinity. Conversely, insertional mutants with a significant decrease in the expression of these proteins exhibited less sensitivity to ABA treatment and increased tolerance to water and salt stress. To obtain plants with adaptive advantages and of agronomic interest, we generated rice varieties with altered expression of two members of the family of HD-Zip I rice proteins, OsHDZIPI.2 and OsHDZIPI.4, using CRISPR/Cas9 edition technology. We obtained ten edited lines in OsHDZIPI.2 and fifteen edited lines in OsHDZIPI.4. As a selection criterion for the best-edited plants, we performed a germination test using different concentrations of NaCl ranging from 0 to 300 mM. We selected lines showing more than 50% germination in the highest NaCl concentration. We analyzed these edited lines by sequencing, obtaining two homozygous lines for OsHDZIPI.2 and 2 for OsHDZIPI.4. We determined that proteins expressed by the edited plants are aberrant and lost the DNA-binding and dimerization domains characteristic of HD-Zip transcription factors. Seven-day-old seedlings of edited lines for OsHDZIPI.2 and OsHDZIPI.4 showed higher tolerance to 100 mM and 200 mM NaCl relative to Kitaake (WT) plants. Likewise, edited plants exhibited increased tolerance to 50 mM NaCl than WT plants 30 days after sowing. In a field trial under normal growth conditions, the edited plants did not exhibit yield penalty, being the productivity parameters comparable to WT plants. This result must be confirmed and reproduced to incorporate grown under saline soil conditions. We also performed a drought assay following the rehydration and observed that edited lines recovered the healthy phenotype while WT plants died after the stress. In conclusion, both gene-edited rice plants in HD-Zip I transcription factors showed better tolerance to salt conditions than the WT. Gene expression and ABA-related metabolic pathways are being analyzed to characterize the molecular mechanism in this stress-tolerant phenotype observed on the edited plants.

PL-67

Relationship between TCP proteins and protection against photo-oxidative damage during de-etiolation in *Arabidopsis thaliana*

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The transition to light is a challenge for dark-germinated seedlings, since light can be harmful to the plant during the greening or de-etiolation process. When the plant grows in dark conditions, the chlorophyll is not synthesized but its precursor, protochlorophyllide, is. The illumination of an etiolated plant directly induces the conversion of the precursor into chlorophyllide, an immediate precursor of chlorophyll, but it also generates a situation of stress and an increase of reactive oxygen species (ROS) due to the illumination of free protochlorophyllide. This can produce photooxidative damage, which affects the accumulation of chlorophyll, photosynthesis and, eventually, the growth or survival of the plant. To avoid photodamage, a number of key regulatory factors are required for the control of chlorophyll biosynthesis during this transition. In this work, we observed that plants with loss-of-function in members of the TEOSINTE BRANCHED 1, CYCLOIDEA, and PROLIFERATING CELL FACTORS (TCP) family of plant transcription factors show increased photodamage and ROS accumulation during de-etiolation. This is correlated with higher levels of protochlorophyllide and increased expression of genes that encode proteins involved in chlorophyll biosynthesis in etiolated plants. The effect of mutation of the TCPs was counteracted by treatment with gibberellin (GA) or overexpression of the transcription factor PHYTOCHROME INTERACTING FACTOR 4 (PIF4), a repressor of the expression of chlorophyll biosynthesis genes, suggesting that the TCPs affect GA metabolism or responses upstream of PIF4. Related to this, mutants in the TCPs show reduced expression levels of genes that encode GA biosynthesis enzymes. Altogether, our results indicate that TCP transcription factors fine-tune the biosynthesis of chlorophyll precursors in etiolated plants and during de-etiolation, affecting GA metabolism and the activity of PIFs.

PL-68

Trehalose 6-phosphate modulates the levels of SWEET13 transcripts in bundle sheath cells from *Setaria viridis* leaves

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Trehalose 6-phosphate (Tre6P) is a signal metabolite that coordinates plant carbon metabolism with growth and development. The Tre6P-sucrose nexus model postulates that Tre6P is a signal and a negative feedback regulator of sucrose levels. Our current understanding of Tre6P metabolism and signalling in plants is based almost entirely on studies performed with *Arabidopsis thaliana*, an eudicot performing C3 photosynthesis. To better understand the role of Tre6P in C4 species, we used Setaria viridis as a model species. In this work, we analysed the expression pattern of the genes involved in Tre6P metabolism, their intercellular distribution in leaves and the role of Tre6P in the regulation of sucrose export. Setaria has 21 putative genes coding for Tre6P-related enzymes: 10 Tre6P synthases (TPS), 10 Tre6P phosphatases (TPP) and 1 trehalase (TRE). To analyze their expression pattern, we performed real-time qPCR in the aerial part of five-days old seedlings, whole leaves (pool from leaves 5 and 6), the fourth internode, the flag leaf and inflorescences. The SvTPSI.1 transcript (encoding the SvTPSI.1 protein, putatively involved in Tre6P synthesis) was mainly found in whole leaves and the fourth internode. SvTPSII transcripts (encoding Class II TPS proteins, putatively involved in Tre6P perception and/or signaling) were preferentially accumulated in whole leaves and seedlings. SvTPP transcripts were detected in all the analyzed tissues, with higher levels in samples from whole leaves and the fourth internode. Analysis of samples enriched in mesophyll cells (MC) and bundle sheath cells (BSC) showed that the SvTPSI.1, SvTPPB1.1 and SvTPPB1.2 transcripts were mainly found in BSC. Finally, we incubated isolated BSC strands with different metabolites, including Tre6P, Glc6P, Suc and trehalose, to test their effect on the levels of transcripts preferentially accumulated in BSC. We found that incubation of BSC with Tre6P and Suc increased the levels of SvSWEET13a and SvSWEET13b. Our results strongly suggest that Tre6P metabolism occurs in BSC, where it would regulate sucrose export to the apoplast by modulating the expression and/or stability of transcripts encoding SWEET13 transporters.

Endogenous nitric oxide influences acclimation responses to phosphorus restriction in soybean plants

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The bioavailability of phosphate (P) in soils is usually low, where its mobility is also restricted. To sustain P levels, plants have developed mechanisms to improve P acquisition from the soil solution, and P reutilization from internal sources. Nitric oxide (NO), a gaseous free radical molecule, is a critical component in plant acclimation responses to abiotic stress conditions, such as mineral nutrient disbalances. In this work, we evaluated changes in endogenous NO levels following P deprivation in soybean plants, and its influence in some typical acclimation responses. Seedlings (*Glycine max* cv. Williams 82) were grown for 7 days under controlled conditions (28ºC/22ºC day-night, 400 µmol m-2 s-1 of PAR, and 16-h photoperiod) in modified Hoagland nutrient solution (500 µM H3PO4, +P). Then, plants were separated into three groups receiving the following treatments: P deficiency (nutrient solution without H3PO4 addition, -P), P deficiency added with 100 µM sodium tungstate (an inhibitor of NR activity), and full nutrient solution (+P) as a control. Typical acclimation responses were evaluated in plants up to 96h of P restriction, where P concentration significantly decreased in roots and unifoliate leaves (64 % as compared to control plants), but neither growth nor photosynthesis was hardly affected. NO levels were evaluated employing the DAF-FM-DA probe and fluorescence microscopy. In this sense, an increase in NO levels in -P leaves was observed, and the treatment with sodium tungstate reduced this increase in -P plants. Acid phosphatase (AP) activity, RNase activity, and organic acids (malic and citric acids) content were evaluated at 96h of treatment. RNase and AP activities were significantly increased in -P roots (55% and 80% respectively) as compared to control, and the same trend was observed in leaves. Citric acid and malic acid levels, evaluated by HPLC, also increased in -P roots. The treatment of plants with sodium tungstate reduced the magnitude of these acclimation responses to control levels. In addition, changes in hormone levels (such as ABA and GA) were induced under P restriction. Overall, the data here presented might indicate the involvement of NO in signaling pathways related to acclimation responses during the first hours of P deprivation and the involvement of NR as NO source.

A rapid, non-destructive and highly efficient *A. rhizogenes*–mediated hairy root transformation protocol based on colour selection.

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Agrobacterium-mediated genetic transformation is a widely used and efficient technique for gene functional research in crop breeding and plant biology. On the other hand, reporters have been widely used to visualize gene expression, protein localization and other cellular activities, but the commonly used reporters require special equipment, expensive chemicals, or invasive treatments. Here, we pursue to develop a rapid, non-destructive and highly efficient method to analyze gene expression, based on a carrot (*Daucus carota* L.) Hairy Root (HR) system, induced by *Rhizobium rhizogenes* (*A. rhizogenes*). In this work we test a RUBY reporter that converts tyrosine to vividly red betalain, which is visible to the naked eyes, without the need of special equipment or chemical treatments. Factors influencing the transformation rate like Agrobacterium strain and co-cultivation medium (CC) were evaluated. At the present, tree Agrobacterium strains were tested, A4 (pRiA4), K599 (pRi2659) and Arqua1 (pRiA4b), all harbouring the plasmid pHDE expressing RUBY under the control of CaMV 35S promotor. At the same time, to evaluate the effect of CC medium on transformation, we used 1/2 B5, MS, 1/2 MS, and B5 salts in CC liquid. Assays were performed on tap roots of commercially available orange carrot. The first development of carrot tissue was observed 10 days after inoculation. In most discs we observed, an enlargement of vascular cambium region and development of neoplastic tissue. Adventitious roots were initiated about 5 days later, at a region along vascular cambium. In this procedure, the selection was performed based on the colour production. Thus, A4 and Arqua1 strains always stimulated higher adventitious root production on carrot discs than K599, although the transformation frequency was higher with A4 strain. Oh the other hand, tap roots inoculated and co-cultivated with *R. rhizogenes* in MS medium exhibited the highest positive transformation frequency. Presence of the transgene and their expression levels were confirmed by PCR and qPCR respectively. Thus, qPCR analysis showed a higher expression of the reporter compared with actin endogenous gene. In this work, we showed that RUBY is an effective selection marker for transformation events in *A. rhizogenes*-mediated hairy root transformation protocol. Moreover, we describe an efficient *A. rhizogenes*-mediated hairy root transformation system that takes just 17 days to complete the whole workflow and enables the rapid characterization of gene function without employing further equipment nor chemicals.

PL-71

Optimization of the vegetative micropropagation of Cannabis sativa from axillary buds

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Cannabis (*Cannabis sativa* L.) is one of the oldest plant sources of oils, resins, and textile fibers. Moreover, this plant is a natural reservoir of countless compounds with various medicinal properties. Currently, more than 150 phytocannabinoids have been characterized. The two major pharmacologically active components are delta-9-tetrahidrocannabinol (Δ9-THC) and cannabidiol (CBD). In Argentina, the law 27669 on Medicinal Cannabis and Industrial Hemp was recently approved, which provides a regulatory framework for public and private investment in the entire cannabis production chain. Some estimations suggest that cannabis could become another export commodity, just like grains or beef. Therefore, it is essential to advance in the improvement of propagation techniques that will allow maximizing cannabis production and guarantee the generation of new plants faster and more efficiently, keeping the desired genetics. In this regard, micropropagation makes possible to obtain many genetically identical and healthy plants in a short period of time. Furthermore, this technique is a necessary complement for the conservation of germplasm in vitro or in the form of cryopreserved synthetic seeds. However, there are found several and persistent bottlenecks to achieve a fast, reliable, and efficient production system in the regeneration and growth stages of plants cultivated in vitro. In the present work, the conditions for the micropropagation of different C. sativa specimens of chemotypes II (THC~CBD) and III (THC<<CBD) were optimized using axillary buds as explants. Two different cytokinins, benzyl amino purine (BAP) and thidiazuron (TZD), were tested at different concentration levels (0.5, 1, and 2.5 μM). In each case, the explants growth was evaluated by length, number of shoots, and total plant area. A dose cytokinin dependence was observed under these parameters. Our results show that the incubation with 2.5 μM TDZ was the most favorable condition. For the in vitro rooting stage, different concentrations of indole butyric acid (IBA) were evaluated, observing the presence of roots at 5 μM IBA. After the rustication, two extraction methods were used for cannabinoids from dried and ground flowers using ethanol. Next, CBD and Δ9-THC content was analyzed between flowers obtained from mother and micro-propagated plants, and

among the different chemotypes, using GC/MS. The results suggest that this propagation technique is suitable for the mass production of each chemotype studied without significantly affecting the CBD/THC contents. Regarding the methods used to extract cannabinoids, one of them gave a higher yield in the amount of CBD and THC.

PL-72

Mechanical treatment induces a basal tolerance against necrotrophic pathogens in tomato plants

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Tomato (*Solanum lycopersicum* L.) is one of the most important horticultural crops due to its high consumption, nutritional value, and extensive production area worldwide. Strategies to increase its production through the modification of growth parameters or by increasing disease resistance are objectives pursued by many research and breeder groups. *Fusarium oxysporum* f. sp. Lycopersici (Fol) is a necrotrophic fungus responsible for the tomato vascular wilt disease by colonizing the xylem vessels of roots and stems. Previous results of our group showed that a mechanical treatment on Arabidopsis stems increased the xylem area and enhanced seed yield. A commercial tomato variety also showed an increment in the stem diameter after 48 h of treatment. We then analyzed the impact of mechanical treatment on different cultivars, which allowed us to study the mechanisms deployed at the physiological and molecular levels using available mutants. We observed that the Money Maker (MM), M82, and Ailsa Craig (AC) varieties responded to the mechanical treatment, each requiring a different weight and application time. AC-tomato seedlings of 4-5 cm in length responded after 48 h of treatment with a 3 g-mechanical dispositive. We observed an increment of 35 % in the stem diameter and 60 % in the aerial biomass in the mechanical-induced plants. In addition, we quantified 1,6 more lateral roots compared to the control and an increment of 20 % in the root area in treated AC tomatoes, which could indicate the induction of an adaptive advantage for growing better under different soil conditions. We performed a

detailed analysis of the morpho-anatomical and molecular changes at the vascular level at 3 h, 6 h, 12 h, and 24 h post-mechanical challenge. The histological analyses of the stem cross-sections of treated AC-tomato plants showed an increment in the number of vascular bundles and high stele area 6 h and 12 h post-treatment, respectively. In addition, cross-sections near the root collar showed an increment in the number of vascular bundles 6 h post-treatment. Furthermore, AC-tomato plants induced by mechanical treatment were more tolerant to Fol-infection than non-treated tomatoes since they were less affected, remained greener, and showed fewer signs of wilting after the Fol infection. Concerning the physiological and molecular mechanisms elicited by the mechanical treatment, no differences in ROS levels were observed in the challenged plants between 3 to 24 h post-treatment. A global transcriptional analysis is underway. We focus on the expression profile of genes differentially expressed in Fol-resistant varieties in connection with those increased after mechanical treatment. Gene expression patterns, modifications in the regulation of hormonal pathways, and biochemical and metabolic processes must be analyzed to evaluate the global impact of mechanical treatment in conferring the ability to overcome Fol infection to tomato plants.

PL-73

The ultraviolet- B response of *Arabidopsis thaliana* hypocotyls is regulated by nitric oxide via the UVR8 signaling pathway

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The photomorphogenic response is triggered by sensing light and involves adaptative changes that prepare the plant to optimize photosynthesis under environmental changes. Ultraviolet- B (UV-B) radiation has dual effects on these organisms: while high doses induce stress, low doses act as a regulatory signal. The UV-B receptor UVR8 is a master regulator of several of these responses. It was reported that both UVR8 and the gasotransmitter Nitric oxide (NO) are involved in the hypocotyl shortening. This work aimed to find if the NO participates in the UVR8-regulated photomorphogenesis. Results obtained showed that UV-B induced the accumulation of NO in A. thaliana wild type (wt)

hypocotyls, but not in the lines lacking UVR8 (*uvr8-1*). The use of the NO donor S-nitrosoglutathione (GSNO), restored the inhibition of hypocotyl elongation caused by UV-B irradiation in uvr8-1 mutants. In this sense, the AtNOA1 mutant line with reduced levels of NO, partially inhibit hypocotyl elongation when grown with UV-B. In accordance, RT- qPCR analysis showed that GSNO treatment overlaps the effect of UV-B irradiation, downregulating cell elongation-related (PRE5, PRE1, HBI1) and gibberellic acid-related genes (GA3oxi1, GA2oxi1). Furthermore, the irradiation of GSNO-pretreated seedlings failed to induce the expression of cyclobutane pyrimidine dimers (CPDs) photolyase (PHR1) which correlated with increased levels of CPDs, the major type of UV-B induced DNA damage. Moreover, the accumulation of anthocyanins and chlorophyll induced by GSNO in wt hypocotyls was impaired in uvr8-1. Altogether, these results suggest a key role of NO in the complex regulatory network of UV-B-induced photomorphogenesis.

PL-74

Physcomitrella patens Reticulata, PpRet, an unknown function protein from the inner membrane of the chloroplast

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Drought tolerance was an ancient adaptation that must be considered to have played an essential role in colonization of the terrestrial environment. The most primitive of extant land plants, the bryophytes may provide resources as to how this adaptation was achieved. *Physcomitrella patens,* the model bryophyte, is highly tolerant to dehydration, salinity and other abiotic stress factors, and thus also a model for stress tolerance. In previous work, we describe the functional characterization of an abscisic acid up-regulated gene (PpCOR413im) from P.patens, encoding a WCOR413 protein. These protein types were first described in wheat in relation to low temperature stress, but their precise function is still unknown. In silico analysis indicate the presence of several transmembrane domains (TrM) in their deduced amino acid sequence, suggesting a subcellular localization in plasma or chloroplast thylakoid membranes. To assess the function of PpCOR413im in abiotic stress responses, we generate gene target disruption mutants of this gene, and phenotypic characterization showed a severe reduction in plant growth under high light conditions. In this work, we study the only candidate protein that has been suggested to interact with

PpCOR413im, a protein called RETICULATA (PpRet), of unknown function, that targets to the inner membrane of the chloroplast. Although the function of PpRet remains unknown, knockout mutants of this gene in *Arabidopsis thaliana* have alterations in lipid and amino acid metabolites and are affected in developmental processes.

PL-75

Identification of strains isolated from symptoms of bacteriosis in maize leaves

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Maize is one of the most important cereals used in human and animal diets as well as industrial alcohol, fuel, and bioethanol. In Argentina, maize is the primary grain grown and the province of Córdoba is the main producer providing about 33% of the national production. Bacteriosis is a significant disease in crop production; little is known about the bacteria that produce this disease in maize. A recent increase in the incidence of bacteriosis in maize is possibly due to the massive adoption of direct seeding and the absence of resistant genotypes. This work aimed to isolate and identify bacterial pathogens from foliar symptoms in maize during the 2019 summer season. A phenotypic evaluation of symptoms was carried out in the field. Yellow and brownish necrotic lesions were observed. Infected leaves were collected, and samples were taken from the lesions, macerated in physiological solution, serially diluted, and grown in Petri dishes containing LB with fungicide. Plates were incubated at 30 °C for 24 h. Colonies with different morphological characteristics of size, shape, texture, and color were selected. The isolates were identified using MALDI-TOF–MS, and sequencing of the 16S rRNA gene. The 16S rDNA sequences of isolates were analyzed and compared with sequences retrieved from the GenBank database using the Basic Local Alignment Search Tool (BLAST). A comparative phylogenetic analysis with phytopathogenic strains was carried out using MEGAX software. Pathogenicity tests were carried out with candidate strains. Four

different genera were successfully identified by both MALDI-TOF and 16S rDNA sequencing: Enterobacter, Pseudomonas, Pantoea, and Curtobacterium. In addition, the MALDI-TOF MS analysis demonstrated a higher resolution power at the species level than 16S rDNA sequencing in several cases, allowing the identification of *Enterobacter pyrinus* with high scores. Members of the genus Pantoea were predominant in the studied samples. Isolates were grouped in the phylogenetic analysis with other plant-associated strains of the same species. The pathogenicity tests showed that most of the evaluated strains brought about bacteriosis symptoms in maize. A bibliographic search showed that the genus *Enterobacter pyrinus* has been previously reported as a maize pathogen. The isolation and identification of pathogenic bacteria from maize provides a starting point for identifying diseases in crops and future strategies to mitigate them.

PL-76

Circular economy strategy to obtain chitin/chitosan as a plant growth promoter from black soldier fly

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Chitosan is a deacetylated derivative of chitin that is the most abundant polysaccharide present in the fungal cell wall, insect, and crustacean exoskeletons from which it can be extracted. Mostly chitosans, at an industrial level, have been obtained from the shells of crustaceans due to their easy extraction, versatility, and high yields. However, currently, and because it fits in the policy of circular economy initiatives to obtain food and feed from bioconverter insects such as black soldier fly, *Hermetia illucens* are being studied. Our main goal was to evaluate the feasibility of black soldier fly grown on potato skins discarded by industrial foods as source of chitin/chitosan. Chitin was extracted from the adults and pupal exuviae of *H. illucens* by applying chemical methods, and converted into chitosan by homogeneous chemical deacetylation. Then, a fraction was subjected to a bleaching procedure using 5% H_2O_2 for 2 h at 70 °C, under stirring. Fourier-transformed infrared spectroscopy confirmed the identity of both the bleached (ChB) and unbleached (Ch) chitosan produced from *H. illucens*. The antioxidant properties of

ChB were evaluated by the scavenging of 1,1-diphenyl-2-picrylhrazyl radical (DPPH) radical. On the other hand, we evaluated their biological properties as promoters of plant growth. Both Ch and ChB activate the auxin signaling pathway in DR5:GUS auxin reporter tomato plants. Moreover, when lettuce seedlings were supplemented with 10 μg/ml Ch and grown under nutrient-deficient medium an increase of approximately 75% and 40% in fresh and dry biomass, respectively were quantified. Total chlorophyll also increased by around 200% in Ch/ChC supplemented plants. Therefore, *H. illucens* can represent a new and sustainable alternative of chitosan-based compounds for the horticulture field.

PL-77

Assessing the role of branched- chain amino acids metabolism in soybean responses to drought stress

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Water deficit is one of the major stress factors that affect soybean productivity and quality. Plants employ a variety of response mechanisms to cope with adverse environmental conditions. One of the most general responses to abiotic stress is metabolic reprogramming, including the accumulation of amino acids such as proline and branched chain amino acids (BCAA). We have analyzed BCAA levels in soybean genotypes exhibiting contrasting phenotypes to drought stress. We found that all soybean lines accumulate high levels of BCAAs under drought conditions. Moreover, drought tolerant slow wilting lines accumulate higher levels of BCAAs in response to water deficit than fast wilting genotypes, which supports the role of this type of responses in drought tolerance. Changes in BCAAs levels appear to be associated with the expression of branched-chain amino acid transferases (BCAT) that participate in their synthesis and degradation. BCAT activity is correlated with their subcellular localization, being the chloroplastic enzymes involved in BCAAs synthesis, and mitochondrial BCAT involved in BCAAs degradation. In this study, we aim to functionally characterize a drought induced soybean gene (GmDIAAT-Glycine max Drought Induced Amino Acid Transferase), which shares sequence similarity to BCATs. To determine the subcellular localization of the enzyme, transgenic Arabidopsis thaliana lines overexpressing GmDIAAT-GFP have been generated and visualized by confocal microscopy. To assess the role of GmDIAAT in BCAAs abiotic stress tolerance, we generated transgenic *Arabidopsis thaliana* lines

overexpressing GmDIAAT and analyzed the phenotype of these lines under different stress conditions (water deficit, high salinity and osmotic stress). Finally, BCAA content will be monitored in GmDIAAT overexpressing lines to assess the role of this gene in BCAAs metabolism. Together, these results will contribute to understand the role of BCAAs in abiotic stress tolerance in soybean and to design strategies to increase drought tolerance through the modulation of BCAA metabolism in soybean.

PL-78

Marine macroalga as source of bioactive compounds for horticultural crops

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The rising population and global demand for food have led traditional agriculture to excessive use of agrochemicals for increasing crop productivity. This intensive agricultural production has eventually resulted in a seriously negative impact on the environment and human health. In this sense, seaweed extracts are currently regarded as an important plant biostimulants category with beneficial effects to improve crop health and stress resilience. *Undaria pinnatifida* is an abundant but also, invasive macroalgae in Argentinean coast. The aim of this work was to analyze the effectiveness of ethanolic algae extracts as sources of bioactive compounds in two horticulture plant species, tomato and lettuce. For this purpose, differential extractions with 75% (v/v) ethanol from total biomass (ET) and sporophylls (ES) were carried out and polyphenols, soluble proteins, free proline and antioxidant activity were quantified. The mean concentration of total phenolic compounds (expressed as mg of gallic acid equivalent in 100 g of dry weight) was higher in the ES (96,3) than in ET (68,9). Similarly, ES has 25% more antioxidant activity than ET. Regarding soluble protein and free proline content, no differences were found between both extracts. Then, we examined the dose-dependent effects of ES (0.001%, 0.01%, and 0.1% v/v) on tomato (*Solanum Lycopersicum*) seed germination. Treatment of seeds with 0.001% (v/v) ES promoted a statistically significant increase of 20% on germination efficiency and fresh biomass in 10-day-old seedlings compared with control. In order to evaluate the effect of ES on plants grown under stress environmental condition, lettuce (*Lactuca sativa)* seedlings were subjected to nutritional stress. For that,

seedlings were cultivated hydroponically in nutrient-deficient media (0.25x ATS), supplemented or not with ES (0.01%, and 0.1% v/v). Lettuce plants treated with 0.01% (v/v) ES showed an increase of approximately 160% and 170% in fresh and dry biomass, respectively. ES-supplemented plants also showed an increase of around 200% in total chlorophyll content with respect to control. Therefore, our evidence indicates the high technological potential of macroalgal biomass and allows us to deep into the bioprospection study as a source of bioactive compounds with productive beneficial in plants.

PL-79

Evaluation of a biotechnological tool for the control of *Pectobacterium carotovorum*, causative agent of potato soft rot

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Iron incorporation is problematic for terrestrial organisms, and many infectious agents have developed mechanisms to hack host ferroproteins. The most abundant iron-containing plant protein is ferredoxin (Fd), which delivers redox equivalents to numerous essential oxido-reductive pathways, including photosynthesis. One of the phytopathogens that have taken advantage of this abundance to their benefit is *Pectobacterium carotovorum* (Pcc), a necrotrophic bacterium that causes soft rot in numerous crops of agro-economic importance, such as potatoes and onions. Several observations showed that this mechanism of iron acquisition via Fd could be key to the success of Pcc infection, suggesting that plants with decreased Fd levels could be resistant to soft rot. Unfortunately, Fd is an essential gene, and its deficiency has catastrophic phenotypic consequences for the plant. In this context, many cyanobacteria and algae contain an iron-free protein called flavodoxin (Fld), which can functionally replace Fd, and whose expression is induced in situations of iron starvation. Although the Fld-encoding gene is absent from the plant genome, the introduction of a cyanobacterial Fld directed to chloroplasts increased tolerance to environmental stresses including iron limitation. Therefore, we tested the virulence of Pcc in transgenic plants with decreased levels of Fd and expressing Fld. We worked on leaves of two plant species in which Fd levels were decreased by different methods: Arabidopsis knockout mutants in the gene encoding the major leaf ferredoxin (Fd2); and tobacco plants using transient virus-induced gene

silencing (VIGS). We evaluated the disease progression by monitoring the onset of symptoms and their spread, estimation of bacterial populations and electrolyte leakage in leaf tissue. Our results show that Fd deficiency would limit the availability of iron during Pcc infection and affect the development of the disease.

PL-80

Phenotypic changes in *Solanum lycopersicum* due to expression of NADP-malic enzyme in phloem companion cells

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Plants are frequently subjected to numerous adverse environmental conditions that can affect the normal growth and development of plants. Evidence indicates that NADP-malic enzyme (NADP-ME), key enzyme regulating malic acid metabolism, is induced by diverse biotic and abiotic stresses. It has been shown that it is involved in various processes, including housekeeping roles such as the maintenance of cellular pH, generation of reductive power, regulation of guard cells, regulation of osmotic pressure for stomatal movement and fruit ripening. The plastidic non-photosynthetic NADP-ME from *Zea mays* has been integrated into the *Solanum lycopersicum* genome under the control of *SUC2* promoter from Arabidopsis, which drives the expression to the phloem companion cells of all green tissues. In this work, two tomato transgenic homozygous lines (*SUC174* and *SUC176*) obtained from one transformation event were characterized phenotypically and compared with WT plants. The expression of NADP-ME in the root, stem, leaves and flowers was verified by quantitative real-time PCR in transgenic plants while it was undetectable in WT plants. Five plants of each line were evaluated in relation to flowering time, plant height, internode length, number and length of leaves, number of leaflets in leaf, length and width of terminal leaflet, weight and diameter of fruit, number of seeds per fruit and weight of seeds. Mean values were compared through Student's t test at a significance level of $p < 0.05$. The analyses indicated that WT plants had opened the first flower before *SUC* lines (47.80 ± 1.16 vs 56.87 ± 1.27 days). At this stage transgenic plants were larger (13.35±0.29 vs 10.56±0.85 cm), with longer internode lengths (1.523±0.085 vs 1.130±0.091 cm) and more average leaflets by leaf (4.27±0.09 vs 3.83±0.10). The first ten mature fruits of every plant were harvested and analyzed. Transgenic tomatoes showed superior values for weight

(3.61±0.13 g) and diameter (19.71±0.28) than WT tomatoes (2.81 ± 0.18 g and 18.16 ± 0.47 cm, respectively) but the weight of the transgenic seeds decreased slightly (2.27±0.07 vs 2.99±0.21 mg). These preliminary results indicated detectable changes under stress-free conditions in tomato lines that express NADP-ME in phloem companion cells, being the first step to advance in the study of changes that occur in this fundamental tissue responsible of photosynthates transport, shedding light on new strategies to deal with global climate change and the growing demand for food.

PL-84

The role of mitochondrial H_2S in stomatal closure induced by pathogens

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Stomata are microscopic structures in the epidermis of most land plants, formed by a central pore delimited by pairs of highly specialized cells, the guard cells. Stomatal pores have the key physiological function of exchange gasses between the plant and its environment. Guard cells are able to sense external and internal stimuli and integrate them into a complex signalling network that triggers changes in the cellular volume to control pore size, leading to stomatal aperture or closure.

Some bacterial and fungal pathogens use stomatal pores as a natural entrance to infect plants. As a consequence, plants evolved to recognize conserved motifs in pathogens surface, the pathogen-associated molecular patterns (PAMPs), and trigger different signalling pathways in order to induce stomatal closure, as a first mechanical barrier to prevent infection. The gasotransmitter hydrogen sulfide (H_2S), a highly reactive molecule, participates in the modulation of different physiological processes including stomatal closure. Although H_2S can be produced in different subcellular compartments, the role of mitochondrial H_2S in plants remains little explored. In this work, we present results obtained in our lab showing that mutants of the mitochondrial enzymatic source, β-cyanoalanine synthase (*cas-c1*) has an impairment on stomatal closure induction and a reduced apoplastic ROS production with respect to wild type plants when treated with the bacterial PAMP flagellin (flg22). Moreover, employing genetically-encoded sensors, we observed that guard cells from cas-c1 have altered cytosolic H2O2 levels and

glutathione (GSH) oxidative status at basal conditions and in response to flg22. In summary, the results presented in the poster show that the mitochondrial H_2S source CAS-C1 is involved in pathogen-induced stomatal closure.

PL-85

The interplay between sugar and ROS: sugar nature and its concentrations as determinants of the redox state in *Arabidopsis thaliana*

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Sugars, in addition to being energy resources in plants, are important signaling molecules that regulate development and responses to environmental stresses. Sucrose (Suc) is the main sugar for systemic transport and a specific signaling pathway for this disaccharide has been described. Furthermore, glucose (Glc) has been shown to have sensing and signaling pathways that could be hexokinase dependent or independent. Noticeably, high sugar concentrations, resembling some stress conditions, could act as reactive oxygen species (ROS) scavengers or ROS producers. In this regard, we studied different types and concentrations of sugars on ROS homeostasis and growth in the model plant *Arabidopsis thaliana.* We performed the sugar treatments under dark conditions to avoid light effects. The results showed that sugars differentially affected the ROS state and antioxidant system at high concentrations (3 and 5%). Although seedlings treated with both Glc and Suc showed high levels of superoxide ion, they presented opposite responses mainly at the reduction power measured through FRAP assay and cell damage estimated by malondialdehyde content (MDA). The treatments with exogenous Suc at 5% showed a higher content of MDA and a lower reducing power than those with Glc at the same concentration. Regarding the enzymes of the antioxidant system, we observed increases in catalase (CAT) activity at high concentrations of both sugars. The total activity of the superoxide dismutase (SOD) enzyme increased slightly in seedlings treated with Glc and Suc at 5% compared to the control without sugar. However, the distinct SOD isoforms (CuZn, Mn and Fe) exhibited differential

behavior. In particular, we observed a significant increase in activity of the isoforms Fe-SOD and CuZn-SOD in seedlings grown in Suc 3-5% and Glc 5%, respectively. These results demonstrate that there is an interplay between sugar nature and concentration with the generation of different types of ROS, their levels and subcellular localization. Associated with this, root growth was negatively affected at Glc 5% while seedlings treated with the same concentration of Suc continued growing. Taken together, Suc maintains ROS homeostasis and promotes growth, even when present at high concentrations, whereas elevated levels of Glc seem to produce oxidative damage despite inducing antioxidant systems. More research is needed to unveil the roles of ROS in the molecular mechanism behind the sugar responses.

PL-86

Compartmentalized expression of maize non photosynthetic NADP-ME resulted in improved plant productivity in normal and drought stress conditions

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NADP-malic enzyme (NADP-ME) has been suggested to play important roles in diverse stress responses in plants. Nicotiana tabacum transgenic plants expressing maize non photosynthetic NADP-ME (*npNADP-ME*) under the *KAT1* promoter resulted in enhanced water use efficiency, earlier flowering and shorter life cycle under normal conditions. Also, these lines exhibit reduced stomatal aperture, increased sugar export from leaves and increased net CO_2 fixation rate compared to WT possibly due to *npNADP-ME* activity in guard and companion cells driven by the *KAT1* promoter. To better understand the phenotype of these plants we studied them under normal and drought stress growing conditions. Under normal conditions, quantitative real-time PCR was performed, showing that *npNADP-ME* was expressed at stem and leaves of transgenic lines; but it was undetectable in roots and seeds, while it was not observed in

any tissue of WT plants. In order to investigate the performance of these plants under drought conditions, plants were grown for 60 days and then irrigation was stopped for 30 or 45 days. Finally, plants were irrigated until the end of the cycle and total dry stems, leaves and fruits were collected. Three plants of each line were evaluated for each treatment. WT plants were not able to recover after the 45 days of drought. In all the other conditions, the harvest index, calculated as the ratio between seed yield and dry biomass, was higher in transgenic plants (at least 2 fold), with an average value of 0.41 in control conditions and 0.43 after 30 days of drought versus 0.15 and 0.20 values for WT. Average number of seeds per fruit and number of seeds per plants were also greater in the transgenic lines, either in control conditions (61224.12 and 2514.54 vs 25800.73 and 1245.84) or after 30 days of drought 42579.34 and 2146.38 vs 24612.13 and 1174.46). These lines produce a larger number of smaller seeds. 1000-seed weight decreased compared to WT in both treatments (0.05 and 0.06 vs 0.08 and 0.08) but there is no significant difference among the genotypes in the proportion of protein or glucose content. Considering all, the overall effect in yield is an increase between 1.4 to 1.8-fold in the grams of seed per plant of the transgenic lines in comparison to the WT. Leaf relative water content (RWC) was also followed. In control conditions, RWC was similar in transgenic and WT plants. During drought stress, the RWC declined in the same proportion for all the genotypes (34-41%). Xylem vessels diameter in control conditions were characterized using light microscopy evidencing differences between genotypes. The results demonstrated that modification of the levels of NADP-ME in the guard and vascular companion cells of tobacco is a promising approach for the improvement of plant productivity, especially in drought conditions.

PL-87

Different types of sugars alter the growth and development of *Physcomitrium patens* in a unique way while affecting the autophagic process

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Autophagy is an important cellular process involved in the recycling of diverse molecular structures. In plants, it plays a key role in modulating the response to diverse stresses, as it provides both pro-life and pro-death functions. The metabolic state of the cell, especially the amount of carbon and nitrogen, is known to affect and be affected by autophagy. It has previously been established that both carbon starvation and excess can activate the autophagic process. Our group uses the model bryophyte *Physcomitrium patens* to study these processes. In this organism, the loss of autophagic function mutant atg5 shows accelerated senescence and impaired growth. In this work we characterized the phenotypes of wild-type and atg5 in the presence of different sugars (glucose, sucrose, fructose and mannitol) in increasing concentrations, to discern possible different signaling and/or energetic responses. It was observed that as the concentration of sugar increases (except for the osmotic control mannitol) the senescent phenotype is reverted but the growth of the adult gametophores is inhibited. Each sugar produced a different phenotype, suggesting that the modulation of autophagy is affected by or affects the cellular concentration of each kind of metabolite. We have also quantified the soluble sugar and starch content of wild-type and atg5 protonema during a normal day-night cycle and established that the mutants have lower starch, but higher free sugar levels compared to the wild-type. We measured autophagic activity during the night (during which autophagy is normally activated) with or without the addition of these sugars, by observing GFP-labeled autophagic particles and measuring autophagic flux. We found a different pattern of autophagy activation or inhibition depending on the presence of each sugar and its concentration and depending on the tissue type (chloronemata or caulonemata). This response appears to be metabolic, not osmotic, as mannitol (which is not metabolized) produces a completely different pattern compared to the metabolized sugars. We have concluded that the growth and development of *P. patens* are affected in a unique way with the addition of each distinct kind of sugar, suggesting that each molecule plays a unique role in influencing the autophagic process and its involvement with the modulation of energetic and/or signaling pathways.

PL-88

HYPONASTIC LEAVES 1 IS REQUIRED FOR PROPER ESTABLISHMENT OF AUXIN GRADIENT IN APICAL HOOKS OF ARABIDOPSIS

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Seedlings germinating under the soil surface have evolved to an exquisite developmental program termed skotomorphogenesis. In darkness, seedlings rapidly elongate the hypocotyl toward the surface in a desperate search of light, while protecting the apical meristem against mechanical damage by forming a hook between the hypocotyl and the two closed cotyledons. A proper skotomorphogenic growth must be achieved until seedlings reach the light to ensure survival as

they depend on limited seed reserves. In our previous work we uncovered that microRNA biogenesis is necessary for proper skotomorphogenesis in Arabidopsis. By studying mutants in the core components of miRNA microprocessor, DICER LIKE 1 (DCL1), HYPONASTIC LEAVES 1 (HYL1) and SERRATE (SE), we surprisingly found a microprocessor-independent function of HYL1 as a repressor of hook development. Our findings have led to hypothesized a possible connection between HYL1 action in skotomorphogenesis and the well-established and crucial function of the phytohormones auxins in hook development. In this work we dissect different aspects of auxin biology in HYL1 mutants (auxin sensitivity, transcriptional responses, biosynthesis and transport) and found that HYL1 is needed to establish the auxin gradient in apical hooks. Our research led us to propose that HYL1 might integrate light/dark and auxin signals to control skotomorphogenic growth in Arabidopsis.

PL-89

Multi-holistic approach to the comprehension of the epigenetic basis determining tomato crop productivity and increased temperature response of tomato local varieties

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To date, more than 70% of the mass production of tomato cultivation in Argentina, is explained by the use of imported hybrids. The use of wild species and "landraces", locally available, would provide desirable

characteristics related to responses to abiotic conditions, such as changes in environmental temperature and nutritional quality, to commercial cultivars. Given their sessile nature and their need to adapt to fluctuating environmental conditions, plants respond to these changes in environmental temperature by regulating gene expression at different levels; epigenetic, transcriptomic, epi-transcriptomic and proteomic, resulting in metabolic responses. In our laboratory, we described how the expression of a key gene in vitamin E biosynthesis is controlled by the methylation status of a SINE-type retrotransposon. In this work we explore the natural biodiversity of metabolic responses to high temperatures aiming to elucidate the (epi)genetic nature of such responses in tomato. Thus, phenotypic traits and environmental data were recorded and analyzed: temperature, humidity and radiation, time to flowering, set of fruits in general, total fresh fruit (g), metabolite profiles of primary soluble compounds, lipids in particular isoprenoids (mainly tocopherols and carotenoids) from source leaves and mature fruit of tomato "landraces". Those genotypes showing significant effects of temperature on metabolism, will subsequently analyzed for their transcriptional methylome profiles in order to elucidate mechanism(s) and gene(s) mediating temperature responses.

PL-90

SCL28 PROMOTES ENDOREPLICATION AND CELL EXPANSION IN ARABIDOPSIS BY ACTIVATING A GROUP OF SIAMESE-RELATED CYCLIN-DEPENDENT KINASE INHIBITORS.

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Plant organ growth initiates with proliferating cells generated by the mitotic cell cycle (MCC). Then, cells enter an expansion and differentiation program. During this later stage, plant cells often switch to an alternative cell cycle named endoreplication (ER), in which cells replicate their DNA without progressing

through mitosis nor cytokinesis, generating somatic polyploidy. The transition from MCC to ER involves regulatory mechanisms that specifically inhibit mitotic cyclin/cyclin-dependent kinase (CDKs) complexes causing cells to oscillate between S and G1 without engaging into mitosis. Previously, we´ve shown that the *Arabidopsis thaliana* SCL28 transcription factor promotes mitotic G2/M progression. To extend this characterization, we performed a transcriptome analysis of *scl28-3,* a mutant in this gene. Among the differentially expressed genes we found genes related to cell elongation and differentiation, including cell wall and cytoskeleton assembling related genes. Also, we found downregulated 6 members of *SIAMESE/SIAMESE-RELATED* (*SIM/SMR*) family in the mutant. These proteins have been reported to trigger the transition from the MCC to ER by its association with specific CDKs. In this work we characterize the role of SCL28 in both roots and leaves development. We found that this transcription factor is involved in the switch from the proliferation to the cell expansion phases, promoting ER and regulating cell wall and cytoskeleton assembly genes.

PL-91

Chloroplast localization of citrus psorosis virus movement protein is essential to exert its viral movement activity

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Plant viral infections are responsible of huge crop losses and given their adaptability against defenses strategies, their eradication seems impossible. Understanding virus infection cycle and the interaction with host cellular machinery becomes essential to design protection strategies to ensure productivity and quality of vegetable products. *Citrus psorosis* virus (CPsV), the type member of Ophioviruses is the causal agent of Citrus psorosis, a worldwide distributed disease that affects all citrus species and is responsible for the death of 5% of productive plants per year. Early during infection CPsV causes chlorotic elongated spots in young leaves and progresses with the accumulation of gum in the vascular system, affecting plant yield and eventually causing bark scaling and plant dead. Natural resistance against CPsV

has not been reported. CPsV is a three-partite negative sense RNA virus. RNA2 encodes a protein of 54 kDa (54K) that contains an aspartic protease domain and can self-cleave producing a 20 kDa protein at the C-terminal part that maintains protease activity, and a 34 kDa protein (movement protein, MP) at the N-terminal part. MP localizes almost exclusively in chloroplasts (Chl) and plasmodesmata (PD) and alters PD structures, increasing size exclusion limit (SEL) and forming tubules that facilitate cell-to-cell virus movement. Chloroplasts play a central role in photosynthesis and are involved in the synthesis of plant defense and signaling hormones, which highlight the importance of this organelle in pathogen interaction and immune response coordination. Most plant viruses cause chlorosis early during infections; this is produced by alterations at chloroplasts and the consequent affection of photosynthetic rate. Transmission electron microscopy observations in herbaceous hosts infected with CPsV show modifications in both the structure and size as well as in cell chloroplasts arrangement compared to non infected plants. In silico analysis of the MP from CPsV and other ophioviruses identify a conserved chloroplast transit peptide (cTP). Together with MP and 54K localization, all this results suggest that CPsV targets the organelle as part of their infection cycle. To test the hypothesis, we designed 54K and MP mutants at the cTP as well as chimeras swapping cTPs with known chloroplast located proteins, such us *Nicotiana benthamiana* Rubisco small subunit (RbsS). These mutants were used to evaluate the role of chloroplast targeting in MP subcellular distribution, PD SEL regulation, virus cell-to-cell movement and chloroplast-mediated immune responses. Our results show that deleting native cTP from MP delays dramatically virus movement. Using a strong cTP (RbsS) which directs MP exclusively to chloroplast have a similar effect, which indicates that MP needs a dual localization to function properly. Further work will allow as to mechanistically describe the specific role of MP at chloroplast.

PL-92

Soybean plants exhibit drought stress memory involving transcription factors belonging to the homeodomain-leucine zipper I family

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Plants are exposed to dynamic and stressful conditions affecting their fitness and seed production. To cope with harmful environments, they have evolved different molecular mechanisms. Interestingly, plants can alter the subsequent response to repetitive detrimental episodes, positively impacting their performance. Such adaptive response is known as "stress memory". Transcription factors (TFs) play crucial regulatory roles in the adaptation responses to environmental variations with consequences in growth and differentiation. Among plant TFs, the homeodomain-leucine zipper (HD-Zip) proteins are unique and reported as involved in abiotic stress responses. For example, soybean plants expressing the sunflower gene HaHB4 exhibited drought tolerance and increased yield in 27 field assays. Soybean is one of the most economically important crops, losing about 40% of potential yield by water deficit worldwide. While plant adaptation to drought has been extensively studied, the physiological changes and molecular factors involved in the response of soybeans to consecutive stresses are unknown. Preliminary observations of soybeans pre-exposed to a mild drought indicated that they were better prepared to respond to water deficit than those stressed for the first time. We wondered if HD-Zip I TFs were involved in such a positive response. To investigate this, we firstly analyzed phylogenetic trees and found that several HD-Zip I proteins were duplicated in the soybean genome. However, no functional information is available about these TFs so far. To understand the role of HD-Zip I TFs during drought, we analyzed the transcript levels of these genes in 7-day-old Williams 84 (WT) and HB4 (transgenics bearing the *HaHB4* gene) soybean plants upon dehydration stress. We observed that *GmHB12-1* expression was induced, whereas *GmHB12-2* levels were upregulated to a lesser extent, indicating that both putative paralogs were expressed. A similar behavior was found for *GmHB52-1* and *GmHB52-2*, whereas other family members did not respond to drought in this developmental stage. In addition, we found that these genes were induced in HB4 plants under normal growth conditions, suggesting that the transgenic are constitutively prepared to deal with this stress. Notably, we uncovered a differential expression of *GmHB12* and *GmHB52* when 14-day-old soybean plants were pre-exposed to water deficit; such analysis indicated these genes are response memory genes. Unlike seedlings, expression of both copies of *GmHB7*, *GmHB12,* and *GmHB52* was induced to similar levels when plants were subjected to single dehydration stress at the V4 stage, suggesting a differential regulation for these genes throughout the plant life cycle. Together, our results suggest that HD-Zip I proteins are involved in drought responses and stress memory in soybean. We are currently studying how stress memory affects soybean plants during different phenological stages and the role of HD-Zip I proteins in this process.

PL-93

Connections between OXR proteins and the Cytokinin pathway in Arabidopsis

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Our group aims to identify new proteins involved in defense mechanisms against oxidative stress in *Arabidopsis thaliana.* Plants over-expressing AtOXR2 (oeOXR2) exhibited interesting phenotype characteristics like a larger size, higher biomass and increased resistance to hemibiotrophic pathogens infection compared to WT plants. These characteristics could result from higher Cytokinins (CKs) content or differences in sensitivity and signaling pathways. Using a GFP-reporter, we observed that oeOXR2 plants showed increased CK levels while the T-DNA knockout mutant (*oxr2*) presented a decreased accumulation of CKs compared with de WT (Col-0) plants. In agreement, the root development of oeOXR2 plants coincides with those of plants with higher CK levels. Therefore, we focused on this plant organ to analyze in depth the role of OXR proteins in the metabolism and CKs signalling pathway. oeOXR2 plants were less sensitive to the addition of the exogenous BAP (6-Benzylaminopurine) since they showed a significant increase in primary root length (17 mm vs 14 mm) and a larger rosette area (10 mm2 vs five mm2) compared to 7-day WT plants grown in the presence of at 50 nM BAP. In contrast, under the same conditions, the *oxr2* mutant develops shorter roots (12 mm) and significantly reduced rosette area (2.5 mm2). Furthermore, we obtained plants with increased expression of AtOXR2 in the background of *ahp1/2/3/4* quadruple and *arr3/4/5/6/8/9* sextuplet mutants and made crosses between oeOXR2 and *ahk4* mutants and over-expressing plants in the CK oxidases (oeCKX). Higher expression of AtOXR2 failed to reverse the root phenotype of the *ahp* and *arr* mutants both in control and at 25 nM BAP levels. However, it partially reverses the root phenotype of oeCKX plants. We propose that the accumulation of the hormone and the stimulated response to it could be the response to a partial inhibition of the catalytic activity of CKXs enzymes in the redox-modified environment of oeOXR2 plants.

PL-94

Up and down, the effect of the OsOXR-Q12 protein on development and salt stress response in rice seedlings

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The OXR (OXidation Resistance) protein family was characterized by the presence of the TLDc domain in the C-terminal portion of the protein. Members of the OXR/TLDc family were firstly identified in mammals for their protective effect against oxidative stress. In *Arabidopsis thaliana,* we identified six members whose expression profile is differentially regulated on different plant tissues and during situations that generate oxidative stress. Arabidopsis plants overexpressing AtOXR2 show higher biomass and seed production and are more tolerant to biotic and abiotic stresses. In addition, we observed that Arabidopsis lines overexpressing the chimeric AtOXR-Q12 protein are plants with increased root and aerial development, photosynthetic efficiency, and seed production. In order to characterize the role of TLDc proteins in monocots, we obtained rice plants (*Oryza sativa* var. Kitaake) with high ectopic expression of this AtOXR-Q12 chimeric protein (OsOXR-Q12). The biometric parameters of 6-day-old seedlings grown on 0.5x liquid MS medium were measured. OsOXR-Q12 lines showed 150% more height in the aerial part, while the root was 160% longer than WT. Thus, OsOXR-Q12 plants produced 50% more aerial and 20% more root biomass. In addition, histological analysis of transversal root sections of 30-day-old plants grown under these conditions was performed. As we expected, due to the root diameter quantified, the OsOXR-Q12 lines showed an increase of about 50% in the number of parenchyma cells. Rice photosynthetic parameters suggest enhanced vegetative and radicle development in OsOXR-Q12 plants under normal growth conditions. We then evaluated the behaviour of these plants under salt stress imposed by the addition of 90 mM NaCl during the first six growing days. OsOXR-Q12 seedlings showed beneficial characteristics in parameters such as height and total biomass, increased by about 50% and 100%, respectively. Since the expression of the AtOXR-Q12 protein in Arabidopsis shows increased resistance to oxidative stress, we are evaluating the levels of ROS and phenolic compounds, as well as the activity of these antioxidant enzymes and stress pathway marker genes. All the results will allow us to characterize the mechanisms by which OsOXR-Q12 plants show beneficial characteristics and to find candidates for generating plants of interest and usefulness from the Agrobiotechnological view.

DIFERENTIAL ROLE OF NITRIC OXIDE AND ABSCÍSIC ACID ON DRY BIOMAS PARTITIONING AND SUGAR DISTRIBUTION IN DIFFERENT ORGANS OF TOMATO PLANTS (*S. lycopersicum* cv. Micro-Tom) UNDER DROUGHT STRESS.

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The purpose of this work was to evaluate the regulatory effects of nitric oxide (NO) and abscisic acid (ABA) on sugars and organic acids distribution in different organs of tomato plants (*Solanum lycopersicum* cv. Micro-Tom) subjected to water stress. For this, since flowering to harvest period, Micro-Tom plants were exposed to the following treatments: water stress (WS, irrigation treatment, 50% field capacity), 100 μM SNP (NO donor) and 100 μM ABA. Leaves, roots and fruits were liofilized and used to quantify sugars by Gas Chromatography-Mass Spectrometry (GC-MS). Both ABA and NO increased sucrose content in leaves, but differentially modified monosaccharide (glucose and fructose) levels in roots and fruits. The interaction between irrigation and chemical treatments (SNP and ABA) was significant for dry biomass in roots, stem, leaves and fruits. Under irrigation, both ABA and NO decreased dry biomass in stems and fruits, while in root and leaves did it only by ABA. Under drought, both ABA and NO decreased dry biomass in leaves and roots, while in fruits did it only by ABA. These results suggest that ABA and NO can act differentially in carbon allocation during water stress.

PL-96

Role of Arabidopsis MutSβ in sensing and deploying a saline stress-mediated DNA damage response

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In order to maintain the proper integrity of their genetic material, plants have evolved both conserved and idiosyncratic mechanisms to cope with genotoxic stress. One of such mechanisms is the mismatch repair system (MMR) which recognizes and corrects different types of post-replicative errors introduced in the DNA. In addition, the MMR components MutSα (MSH2/MSH6) and MutSγ (MSH2/MSH7) were shown to inhibit genetic recombination and cell cycle progression in the presence of moderate to severe biotic- and abiotic stress-derived DNA damage. However, less information is available about the participation of plant MutSβ (MSH2/MSH3) in the DNA damage response pathway upon exposure to environmental stress. In this work, we characterized the responses of three *msh3* T-DNA-derived insertional mutant lines to acute salt stress in comparison to their parental wild-type (WT). We chose to work with high salinity, since it is one of the major environmental constraints that affect plant growth and productivity, and it is reported to perturb genome stability by inducing DNA double strand breaks (DSBs). Indeed, formation of DSBs in salt-treated seedlings was confirmed by a neutral comet assay, where differential levels of DNA fragmentation could be observed between *msh3* mutants and the WT line. We also performed seed germination assays under saline stress, where we observed an up to 40% increase in the germination rates of all *msh3* mutant lines when compared to WT at 150 mM NaCl. This suggests an impaired cell cycle control in the *MSH3* defective lines in response to DNA damage. This concurs with our in silico coexpression network analysis, where we observed a coregulation of *MSH3* expression with several cell cycle-related genes. Moreover, enrichment analysis of promoter cis-regulatory motifs revealed an overepresentation of E2Fa/b binding motifs in the promoter regions of densely connected coregulated modules, among which MSH3 could be found. This was confirmed by RT-qPCR analysis, which showed a significant increase in relative *MSH3* transcripts in both *E2Fa*- and *E2Fb*-overexpressing lines. Experiments are underway to demonstrate whether this interaction is direct by both EMSA and ChIP assays, and to assess whether *msh3* mutants display alterations in somatic recombination rates under saline stress. Our preliminary data suggests a novel link between the mismatch recognition protein MSH3 and abiotic stress-mediated DNA damage perception and response.

PL-97

Assessment of the methylation index in *Arabidopsis thaliana*

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Methylation of DNA and histones has profound effects on the epigenetic regulation of plants. In addition, methylation of polysaccharides, such as pectin and hemicelluloses, is essential for assembling the plant cell wall. S-adenosylmethionine (SAM) is the methyl donor for the methylation of nucleic acids, proteins, polysaccharides, lipids, lignin and is also involved in the synthesis of ethylene and polyamines; thus, SAM plays a critical role in plants. One of the products of the methylation reactions is S-adenosylhomocysteine (SAH); which acts as an inhibitor of methylation reactions; thus, methylation reactions are regulated by the concentration of the methyl donor (SAM) and the concentration of the inhibitor (SAH). Hence the SAM/SAH ratio has become known as the methylation index. However, little is known about how this index is regulated in plants. Arabidopsis thaliana is a model plant that became a valuable tool for genetic analysis. Arabidopsis has a low mass, a fully developed plant weighs less than 1 g, and different organs weigh a few milligrams; therefore, highly sensitive assays are required to work with a low amount of material. In order to develop a quantitative, highly sensitive assay to assess the methylation index in Arabidopsis, we develop a UPLC-MS assay to measure SAM and SAH that allows us to analyze individual organs from a single plant. Differences in SAM content were detected among different organs; however, the methylation index remains relatively constant. Despite this, some changes were observed between day and night. Finally, a valuable tool to assess the methylation index has been developed, which should help us to identify genetic and environmental cues that affect methylation in plants.

PL-98

Application of CRISPR/Cas9 in soybean quality improvement

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Soybean is a major crop providing protein-rich food and edible oil for human and animal nutrition. Soybean seed contains approximately 40% protein which is provided by the main grain storage proteins, glycinin (11S globulin) and β-conglycinin (7S globulin). 7S β-conglycinin is particularly poor in sulfur-containing amino acids such as methionine and cysteine, and thus, the higher the 11S:7S ratio, the better the nutritional quality. In this work we used CRISPR-Cas9 to improve two different characteristics of soybean grains: seed size and protein quality. For increasing seed size, we selected the "BIG SEEDS" (BS) genes as a target for gene editing. These genes code for transcription factors of the TIFY family, which interact with transcription corepressors. Silencing of the two soybean BS genes has been reported to increase the size of plant organs the seeds and to increase the content of 16 amino acids. On the other hand, to increase the ratio 11S:7S of seed storage proteins, we selected several target genes encoding for low sulfur amino acids containing proteins from α, α´, and β subunits of 7S globulin. To generate CRISPR-induced knockout mutants in the target genes, we designed three sgRNA for each gene of the α, α´, and β subunits of 7S globulin and the BS1 and BS2 genes. Soybean somatic embryos were transformed by biolistic with gene constructs expressing Cas9 and the different gRNAs. Embryos resistant to the selection agent were selected and potential edited events were genotyped by PCR, HRM, and sequencing. To date, we have identified 3 BS-edited lines containing a large deletion in the target site of the gRNAs and three edited lines in 7S globulin genes, containing small deletions in the target sites. The seeds of these lines will be evaluated for protein and amino acid content and profile, as well as for their agronomic characterisceccatics.

PL-99

ACCUMULATION OF A MISFOLDED PROTEIN IN CHLOROPLASTS TRIGGERS AN ORGANELLE-SPECIFIC RESPONSE

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A quality control system of heat shock proteins, chaperones, and proteases ensures that proteins entering the chloroplast are properly folded. Yet, the system may be overloaded in stressful situations and can get

overwhelmed by unfolded proteins. In this case, a genetic program called the unfolded protein response (UPR) is unleashed and consists of increased levels of the components of the quality control network. In a laboratory setting, the UPR can be triggered by chemicals, but it is suspected that the molecular signature of the chemically induced UPR is different from other more physiological stresses. In this work, we used aggregation-prone versions of ferredoxin NADP+ reductase (Taq3-FNR and ΔC-FNR) that show a marked reduction in solubility. Genetic constructs containing the *Taq3-FNR*, *ΔC-FNR*, or wild-type *FNR* coding sequences were used to transform *Nicotiana benthamiana* and *Arabidopsis thaliana*. We confirmed that Taq3-FNR protein accumulates in chloroplasts at a lower concentration than WT-FNR, suggesting increased turnover. The levels of the chloroplastic ClpB3 disaggregase were used as a marker for the UPR. Leaves overexpressing *Taq3-FNR* and *ΔC-FNR* showed a significant accumulation of ClpB3. We then analyzed alterations in the leaf proteome by proteomics using a Q-Exactive HF mass spectrometer. Data were analyzed by MaxQuant/Perseus and Proteome Discoverer. We then performed network and GO enrichment analysis using String and Cytoscape. We found that, in the presence of Taq3 and ΔC-FNR, the plant cell responds by activating the expression of molecular chaperones (such as ClpB3), proteases (FtzH), and proteins involved in retrograde signaling to the nucleus (GUN5). Our results suggest that Taq3-FNR and ΔC-FNR generate a specific response in chloroplasts, due to their presence in the stroma. Also, since most proteins with elevated levels during the UPR are nuclear-encoded, the response should involve chloroplasts-to-nucleus communication, a common feature in other UPRs

PL-100

Involvement of hydrogen sulfide (H2S) in stomatal immunity

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Stomata are pores in the aerial part of the plants surrounded by a pair of cells, guard cells. The regulation of the pore size is crucial since it allows gas exchange with the environment and the loss of water by evapotranspiration. The regulation of stomatal aperture is key to maintain carbon and water homeostasis and also for plant defense since pathogens can enter the plant through the stomatal pore.

Hydrogen sulfide (H2S) is a gaseous signaling molecule. In plants, the main enzymatic source is L-cysteine desulfhydrase 1 (DES1) which degrades L-cysteine to H2S, pyruvate and ammonia in the cytosol. The involvement of H2S has been reported both in response to stress and in development processes in plants. Today it is considered that H2S mechanism of action is given mainly by protein persulfidation, a redox posttranslational modification (PTM) in protein cysteine residues. This modification can modify the activity or localization of the target protein. In our lab, we have demonstrated that exogenous application of H2S induces stomatal closure in several plant species involving the participation of other signaling molecules such as nitric oxide (NO) and reactive oxygen species (ROS). Moreover, in Arabidopsis thaliana endogenous H2S/DES1 participates in pathways that lead to stomatal closure as abscisic acid (ABA) and the bacterial elicitor flagelin (flg22). Our principal aim is to unveil the signaling pathway and the mechanism of action of endogenously produced H2S that lead to stomatal closure in response to pathogen perception. Stomatal closure assays, with different pathogen elicitors in isolated epidermal peels of wild type and des1 mutant Arabidopsis leaves, show that des1 does not respond to flg22 but does respond to elf18 and chitin suggesting a specific role of DES1 in flg22 signaling. Moreover, we used the ratiometric Orp1-GFP2 biosensor and observed that flg22-induced H2O2 increase is blocked in presence of H2S scavenger, hypotaurine (HT). We further explored the cytosolic H2O2 dynamics in response to H2S in rbohD mutant and observed a response that was similar to that of wild type suggesting that H2O2 induction by H2S is RBOHD-independent. We also analyze apoplastic ROS burst in response to flg22 in leaf discs by luminol-based assay. The result demonstrates that DES1 participates in this response since des1 mutant induces significantly less ROS than wild type. Finally, we are performing a Dimedone-switch assay on Arabidopsis epidermal peels treated with flg22 in order to identify persulfidation targets. Preliminary results show several targets associated to the immune response, suggesting this PTM is an active mechanism in H2S–dependent stomatal closure in response to flg22. Further investigations are needed in order to know the H2S targets and the role of persulfidation during stomatal immunity.

Microbiology

MI-16

EXPRESSION, PURIFICATION AND BIOACTIVITY OF A PERMEABLE VERSION OF THE CATALYTIC SUBUNIT OF BOTULINUM TOXIN B

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Exocytosis is the process by which cells release the contents of secretory vesicles to the extracellular milieu. This is accomplished through the fusion of the membranes that sorroundsurround the vesicles with the plasma membrane, a process catalyzed by the SNARE proteins. Tetanus and botulinum (BoNT) toxins are potent inhibitors of secretory vesicle release due to their highly specific, proteolytic cleavage of the neuronal isoforms of SNARE proteins. Thus, neurotoxins are powerful tools to study the molecular mechanisms that drive exocytosis. Native toxins are the most potent of all toxic substances. To eliminate the risk of working with holotoxins, we and others use the light chains - which are innocuous because they lack the ability to bind neurons - of these enzymes heterologously expressed in *Escherichia coli*. Recombinant light chains can be used in permeabilized cells and in cell-free systems. Here, we describe a novel version of botulinum toxin B (His6-TAT-BoNT/B) that is delivered to live cells by protein transduction because it is fused to the TAT-permeable peptide from HIV. We optimized the expression in *E.coli*, purification by affinity chromatography and storage conditions of His6-TAT-BoNT/B. The neuronal isoform 2 of the SNARE protein synaptobrevin is the target of BoNT/B. This protein is required for human sperm exocytosis (the acrosome reaction, AR). When introduced into permeabilized human sperm, His6-TAT-BoNT/B prevented the AR induced by calcium with the same potency as the non-permeant version of the toxin (used as positive control), which suggested that the novel toxin was catalytically active. Furthermore, His6-TAT-BoNT/B transduced through the membranes of rat brain synaptosomes and cleaved its target synaptobrevin 2, whereas non-permeant BoNT/B did not. The AR is an ideal model to investigate protein transduction because sperm lack endocytosis machinery. Co-incubation of non-permeabilized human sperm with His6-TAT-BoNT/B prevented the AR induced by the calcium ionophore A23187 in a dose response fashion, as expected, non-permeant BoNT/B did not. These findings indicate that His6-TAT-BoNT/B transduced through sperm's plasma membrane and cleaved endogenous synaptobrevin 2.

MI-17

Non-Saccharomyces wine yeast microsatellite loci encode polymorphic proteins

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Yeast microsatellite loci consist of short tandem-repeated DNA sequences of variable length useful for strain differentiation, population genetics, and evolutionary biology. Coding microsatellite sequences have been recognized in the yeasts *Saccharomyces cerevisiae* and *Candida albicans*. These particular loci are dynamic components of yeast genomes and play a relevant role in gene evolution. We have previously shown that indels and non-tandem DNA repeat variations are frequent among microsatellite loci of the wine yeasts *Starmerella bacillaris*, *Hanseniaspora uvarum*, *Saccharomyces cerevisiae*, *Saccharomyces uvarum*, *Torulaspora delbrueckii*, *Brettanomyces bruxellensis*, and *Meyerozyma guilliermondii*. Our results indicated that, besides a variable number of their tandem-repeated motifs (TRM), allelic variants for some microsatellite loci of these yeast species were also dependent on SNPs and/or indels flanking their TRMs. In this work, we explored the possibility that wine yeast microsatellite sequences are also located within protein coding regions. *In silico* analyses confirmed that some microsatellite loci from H. uvarum, *S. cerevisiae*, *T. delbrueckii*, *B. bruxellensis*, and *M. guilliermondii* localize into protein-coding sequences, many of them corresponding to proteins having regulatory roles. Many of these coding microsatellite sequences involve trinucleotide repeats, which result in allelic variants that do not disturb the corresponding open reading frames. Coding TRMs show a remarkable bias in nucleotide composition, most of them encoding small hydrophilic amino acid residues (i.e., E, Q, D, and N). Additional sequence analyses of allelic variants revealed that many of the SNPs in regions flanking the TRM result in silent mutations that do not affect the corresponding protein sequences. Sequence variations in coding microsatellite loci may reveal specific adaptive metabolic profiles of wine yeast strains.

MI-18

Antimicrobial activity of zinc, copper and silver nanoparticles against fungal pathogens

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The main economic losses for the lemon fruit production in Tucumán are due to postharvest fungal diseases. In order to control them, chemical fungicides are used, carrying a high risk to environmental health and increasing the appearance of resistant isolates. For this reason, the need to investigate and develop alternative methods for the control of resistant pathogens becomes evident. A promising option is represented by metal ion nanoparticles, with recognized antimicrobial action. In the present work nanoparticles of zinc oxide, silver or copper oxide (ZnO-Np, Ag-Np, CuO-Np) were synthesized and evaluated to control local isolates of fungicide resistant phytopathogens. The nanoparticles were obtained by chemical or green synthesis and were characterized in respect to their morphology and size. The antifungal activity was tested in vitro using conidial suspensions adjusted to 10^6 UFC/ml of *Penicillium digitatum*, *Penicillium italicum*, sensitive or resistant to fungicides. The obtained results show that ZnO-Np did not significantly inhibit the development of pathogens up to a concentration of 40 mg/ml. The CuO-NP were effective against *P. digitatum* with a MFC (minimum fungicidal concentration) of 50 mg/ml, while for *P. Italicum italicum* the MFC was 100 mg/ml. The Ag-Np showed the highest antifungal activity with a MFC 10 µg/ml for all pathogens. At lower concentration, 1µg/ml Ag-Np was able to inhibit conidial germination. AditionallyAdditionally, as a first step to characterize the mechanism of action, it was observed that Ag-Np increase in the conidial membrane permeability for all the pathogens tested by fluorescent probe SYTOX Green. The resistant isolates exhibited similar behaviors to their wild type. Ours results represent the first findings in the evaluation of metallic nanoparticles as alternative antifungals for the control of relevant phytopathoges that affect the lemon production in our region

New strategies alternatives to synthetic fungicides to control postharvest fungal pathogens

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Tucuman is the main national citrus and blueberries producer, but both activities must face important economic losses due to postharvest fungal diseases. To control these diseases, chemical fungicides are applied and, although they are very efficient, have associated serious problems for the consumers and environment health, as well as the increase of fungicide resistant isolates. For this reason, there is a permanent search for new alternative control strategies, both evaluating new compounds and edible coatings, that which provide support and durability to postharvest treatments. In this work we evaluate the antifungal action of silver nanoparticles (NP-Ag) obtained by green synthesis from cultures of *Streptomyces* sp. M7 and a new edible coating, Levan, a natural fructan with 2,6-β bonds produced by the *Bacillus* sp. Mcn4. For both compounds, we determined the effect over conidial germination and micellar growth inhibition of citrus pathogens such as *Penicillium digitatum* and *P. Italicumitalicum*, sensitive and resistant to fungicides and *Geotrichum citr-aurantii* and blueberries pathogens such as *Botritys cinerea* and *Alternaria*. sp. The results showed that Np-Ag 2mg/ml of were able to inhibit mycelial growth for all pathogens. Furthermore, conidial germination was affected using Np-Ag 0.5 mg/ml, showing conidial and hyphae deformations. On the other hand, Levan 5% had no antimicrobial action in vitro against the pathogens. To validate their activity as edible coatings, we evaluated green mold incidence in lemons artificially inoculated with *P. digitatum* and treated with levan alone or as a vehicle for the administration of potassium sorbate (SK), a GRAS salt with recognized antifungal action. The results showed that the application of 1% levan and 6% SK reduced the diseases incidence by 80%. Here we demonstrate that the application of silver nanoparticles or levan, as natural edible coatings, would be a promising compound to development new alternative strategies applied in postharvest to control fungal diseases.

Screening of antimicrobial peptides with potential to treat mycobacterial infections

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Tuberculosis, caused by *Mycobacterium tuberculosis* (Mtb), is one of the first causes of death worldwide and other mycobacterial infections are increasing globally. *Mycobacterium abscessus* (Mab) causes pulmonary disease and is particularly deadly among cystic fibrosis and other immunocompromised patients. The failure of mycobacterial treatments is due, in part, to development and spread of drug-resistant and multidrug-resistant strains, for which the available antibiotics are poorly or not at all efficient, in addition to produce severe side effects in patients. Despite of the exhaustive research in the academic and private sector, only a few compounds have been proved to be efficient against mycobacteria and safe for humans, highlighting the need for expanding the horizons to less conventional or less explored compounds. Such is the case of antimicrobial peptides (AMPs), a diverse class of short peptides, usually between 10 and 60 amino acids long and a net positive charge, produced as a first defense line by a variety of organisms, ranging from procaryotesprokaryotes to humans. Here we focus on the screening of AMPs with antimycobacterial activity produced by soil bacteria. We developed a modified version of the traditional supernatant-agar method for selecting those bacteria capable of inhibiting mycobacterial growth. We screened over a hundred isolates from three diverse bacterial collections previously stablishedestablished in our lab. A total of 12 and 5 strains displayed promising growth-inhibition activity against the non-pathogenic and fast-growing bacterium *M. smegmatis* (Msm, a model mycobacterial organism) and Mab, respectively. The bacteria displaying inhibition against mycobacteria belong to the genus genera *Terribacillus*, *Staphylococcus*, *Curtobacterium*, *Microbacterium*, *Pseudomonas*, *Paenibacillus*, *Serratia*, and *Pantoea*, most of which have not been reported to produce antimycobacterial compounds. Those isolates that exhibited inhibition against Msm or Mab are currently being assayed against Mtb. Overall, our results show several promising isolates secreting toxic compounds for mycobacteria and point out the soil as an important reservoir of bacteria producing natural compounds with potential to treat mycobacterial infections. Future studies are needed to establish the mechanistic of growth-inhibition activity.

A Brucella secreted type IV effector with a linear N-WASP binding motif promotes niche formation

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Bacterial pathogens are extremely versatile microorganisms that have evolved various virulence strategies to subvert host cell functions and establish successful infections. Due to the different life styles these pathogens have, the mechanisms they use as well as the cellular pathways they exploit, are very diverse. Several virulent bacteria have the ability to manipulate the host cell actin cytoskeleton as part of their pathogenic strategy. These pathogens subvert host cell actin polymerization machinery for several purposes, including motility within the cells, cell-to-cell spread, and to prevent phagocytic uptake by professional phagocytes. Unlike intracellular pathogens, pathogenic *Escherichia coli* (which includes both enterohemorrhagic and enteropathogenic *E. coli*) subvert actin polymerization from an extracellular localization to facilitate adherence. Actin is a major component of the cytoskeleton of eukaryotic cells and is critical for cell shape and motility, as well as many other cellular functions. The wide variety and versatile mechanisms that bacterial pathogens have evolved to modulate actin activity are incredibly rich and highlight the central role that this family of proteins plays in the host cell-pathogen interaction. In the present work, we describe the identification of a novel Brucella type IV secreted effector with a Short linear motif (SLiM) predicted to mimic the C-helix of the GTPase binding domain (GBD) present in WASP. With the goal of identifying new virulence factors, we performed an *in-silico* search on the genome of *Brucella abortus* searching for potential SLiMs present in predicted proteins with disordered regions. Using this approach, we identified a gene coding for a predicted protein with no known function or homology to other proteins. We showed that this protein is secreted in a type IV secretion dependent manner, that a null mutation affects replication of *B. abortus* in host-cells and that overexpression facilitates intracellular replication. We also demonstrated that this novel effector binds WASP and propose that it functions promoting the formation of the replicative niches.

Functional characterization of Dtr genes from a novel family of MOBQ mobilizable plasmids from *Acinetobacter* spp.

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Acinetobacter baumanii is an opportunistic pathogen that has become relevant due to its capacity of overcome extremely unfavorable environmental conditions and its tendency of developing antimicrobial multiresistance. The spreading of such capacities among *Acinetobacter* and other nosocomial genera is a consequence of multiple events of horizontal gene transfer. Plasmids are small self-replicating extra-chromosomal bacterial DNA molecules. Such structures serve as vehicles for propagation of genes capable of bringing adaptive advantages to its recipient cell. Plasmids interchange by conjugation is considered one of the main mechanisms of horizontal gene transfer among bacteria.

Given its transference capacity, plasmids can be classified as conjugative, if containing the entire conjugative machinery, or mobilizable, which possess the DNA transfer and replication (Dtr) genes but lacks the Mating pair formation (Mpf) elements. Mpf genes code for a Type iV IV secretion system that serves as a platform for bacterial interaction and transference of a single strand of plasmidic DNA to the recipient individual. For a mobilizable plasmid to be transferred is necessary the presence of a second DNA molecule carrying the Mpf, called helper.

Characterizing a plasmid collection of *Acinetobacter* spp. from nosocomial isolations, our group has described a novel subfamily within MOBQ plasmids. Dtr genomic structure of such plasmids resembles pTF1 plasmid of *Thiobacillus ferrooxidans*, a IncQ replicon, composed by an accessory protein (MobS) and a relaxase (MobL). Plasmids from IncQ incompatibility group are broadly distributed and involved in dispersion of antibiotic resistance. Given the importance of plasmids in the spreading of antimicrobial resistance genes among clinically relevant bacteria and the development of multirresistant strains, the aim of the present study is to functionally characterize the novel subfamily of MOBQ plasmids of *Acinetobacter* spp. from nosocomial isolation. In previous work we analyzed the conjugative transfer capacity of two plasmids from this MOBQ family: pIH6 and pIH7. Quantitative assessment of conjugative transference revealed a differential behavior of Dtrs from plasmid pIH6 regarding pIH7 when using IncP, IncN, IncW and IncX as helper plasmids. In addition, bioinformatical analysis of relaxasas revealed that, while the N-terminus of MobL gene product is highly conserved among plasmids, the C-terminal end of such proteins are extremely variable.

In order to study if such differential conjugative capacity is a particular feature only of these two plasmids, we analyzed a third member of this novel MOBQ family: pIH16. Plasmids containing the mobL and mobS

genes from pIH16 resembles the conjugative behavior of pIH6, supporting the idea that the N-terminus of relaxasas wouldn't be solely involved in the regulation of conjugative plasmid transfer. We propose a novel regulatory function of the C-terminal end of relaxasas from the MOBQ family, being such domain involved in the recognition of the coupling protein, the Mpf element involved in the recruitment of the plasmid to the transference machinery.

The study of plasmid conjugative transfer regulation and the molecular mechanisms involved in such process will allow us to develop strategies that let us control the gene flow among clinically relevant bacteria, one of the main processes responsible for the antibiotic resistance epidemic existent in nosocomial environments.

MI-23

Use of NaCl 5M for the extraction of *Lentilactobacillus kefiri* S-layer proteins: characterization of the extracts and immunomodulatory activity on murine macrophages

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Self-assembled S-layer proteins (SLP) are non-covalently bound to the cell wall of several prokaryotic species, including potentially probiotic *L. kefiri* (Lk) strains. Recently, we have demonstrated that different SLP-Lk are able to enhance macrophage activation *in vitro*, and particularly, SLP-Lk8348 showed adjuvant capacity *in vivo*. Thus, regarding the potentiality of these proteins for their animal or human use, we aimed to replace LiCl by NaCl as a safer and cheaper extraction agent to obtain SLP from Lk strains of interest. To achieve this, cultures of Lk8348 and Lk83111 were grown in MRS broth at 37ºC for 48 hs, and then the SLP were isolated using 5M NaCl for 10 or 30 minutes at room temperature under stirring. Usual extraction using 5M LiCl for 10 minutes was carried out as control. SLP extracts were characterized by SDS-PAGE and concentration of total proteins was determined by Bradford´s method. Effect on murine macrophage cell line RAW264.7 activation was determined evaluating both IL-6 secretion and CD86 surface expression as

markers. Regarding the amount of protein isolated after incubation for 10 minutes, 5M NaCl showed lower efficiency of extraction than 5M LiCl for both SLP-Lk8348 and SLP-Lk83111. The SDS-PAGE revealed no apparent differences between 5M LiCl and 5M NaCl extracts obtained after 10 minutes of incubation. However, the incubation for 30 minutes with 5M NaCl showed evidence of protein degradation in both SLP-Lk8348 and SLP-Lk83111 extracts. Regarding the immunomodulatory properties, both NaCl and LiCl extracts of SLP-Lk83111 were able to induce secretion of IL-6 ($P<0.05$) as well as surface expression of CD86 ($P<0.05$) in murine macrophages. On the other hand, it is noteworthy that SLP-Lk8348 isolated with NaCl induced activation of RAW264.7 cells ($P<0.05$), while no cellular response was observed after stimulation of SLP-LK8348 LiCl extract. These findings might be attributed to differences in the composition of the NaCl vs LiCl extracts, or even to conformational changes of the SLP-Lk8348 isolated with NaCl respect to LiCl-isolated SLP-Lk8348. Our results showed that 5M NaCl could be used as extraction agent for SLP-Lk, however, further studies are needed to get deeper into characterization of the extracts.

MI-24

Inhibitory activity of *Bacillus* sp. strains and their metabolites against *Penicillium Penicillium digitatum* and *P. italicum*, causal agents of green and blue mold on lemons

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Argentine citrus production must face significant economic losses caused by postharvest diseases, mainly green mold (*P. digitatum*) and blue mold (*P. italicum*). Synthetic fungicides are used to control these fungal pathogens but, their widespread use has led to appearance of resistant fungal isolates. Therefore, there is an urgent need to develop safe alternatives strategies to control citrus postharvest diseases. Biopreservation, the control of one organism by another, has received much attention in the last years. *Bacillus* sp. are a promising candidates to be used as fungal antagonists, since they have been reported to have antimicrobial properties. Thus, the aim of this study was to evaluate the potential antifungal activity of FZB42 (*B. velezensis*), the local isolate CA1 (*B. velezensis*) and the commercial strain of *B. subtilis* QST713 against *P. digitatum* (PD) and *P. italicum* (PI), and to determine the appropriate media

culture to produce their antifungal metabolites. The inhibitory activities on the growth of PD and PI of the *Bacillus* sp strains were assayed by the dual culture technique method on APG medium. All strains exhibited a strong micelial growth inhibition of both fungi. To evaluated the media influence in the production of antifungal metabolites, FZB42, CA1 and B QST713 were grown in LB, M9 or PG medium at 30ºC. After 12h, cells were removed by centrifugation, and cell-free supernatants (CFS) were obtained by filtration and separated in 1x or concentrated to 10X. The antifungal activity of each CFS was evaluated in a 96-well polystyrene microtiter plate containing the fungal conidial suspensions adjust to 10^5 UFC/ml and the different CFS 1X (50:50). Microplates were incubated during 5d at 22ºC and the conidia germination evaluation were performed by observation using an inverted light microscope. Results showed that, after 1d of incubation, CFS-PG of all *Bacillus* sp. strains inhibited conidia germination of both fungi being CFS-PG of FZB42 the most active, also showing that the conidia structure was altered by deformation and vacuolization. In the CFS-LB and CFS-M9 of all strains the conidia germination was retard, since the growth was observed after 5d of incubation. Finally, to detect CFS- PG 10x (of all strains) potential application for the prevention of green mold, assays were performed on lemons. For this, each lemon was wounded with a needle, 10µL of the different PG-CFS 10X were inoculated into each hole and fruits were air-dried for 1h. Then, 10µL of PD conidia suspension adjusted to 105 UFC/ml were added to each wound. After 7 d of incubation, the disease incidence was measured and the results showed that all de CFS-PG had a preventive action, being the CFS-PG of FZB42 the most active. Our work showed that the FZB42, CA1 and BQST713 and their metabolites evaluated in this study could be a new technology to be used as natural alternatives to synthetic fungicides in the control of postharvest diseases on lemons.

MI-25

Comparative proteomic analysis of wild type and mutant lacking histidine kinase BP1092 provide new insights into *Bordetella pertussis* virulence gene regulation

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Bordetella pertussis (Bp) is the etiological agent of whooping cough, a severe illness that is re-emerging even in highly vaccinated populations. Previous studies have shown that Bp survives inside human macrophages, which probably contributes to persistence and vaccine failure. Our group has shown that virulence factors play a key role in the Bp persistence inside host cells. Transcription of Bp virulence factors is controlled by the interrelated two-component systems (TCS) BvgAS and RisAK, which are involved in a phenotypic conversion between virulence and avirulence phases in response to environmental stimuli. Howerver, other yet unknown TCS could be involved in the adaptation of Bp to the intracellular environment. Through proteomics assays we show that BP1092 is a TCS histidine kinase with increased protein levels upon internalization by a human macrophage cell line. Here, we further characterized the regulatory contribution of BP1092 under virulent and avirulent conditions by global proteomics. To this end, we performed a mass spectrometric analysis of Bp Tohama I wildtype (wt), an isogenic BP1092 deficient mutant (ΔBP1092), and ΔBP1092 trans-complemented with BP1092 (ΔBP1092 pBBR-BP1092) under control (SS) and modulating (SS + 40 mM MgSO4) conditions.

We found ten proteins with altered abundance between ΔBP1092 and wt or the trans-complemented strain, respectively, under avirulent conditions but none under control conditions. Among the proteins with different abundance (fold change >1.5, q<0.05), response regulator BvgA and the protein BipA were detected with decreased levels in the mutant compared to wt. Interestingly, the levels of virulence factor as FhaS adhesin, CyaA toxin, and components of the type three secretion system showed increased trends in level in ΔBP1092 compared to wt under modulating conditions (fold change >1.5, q>0.05, p<0.05).

The impact of BP1092 knockout was additionally visible in increased biofilm formation and decreased intracellular survival compared to wt and ΔBP1092 pBBR-BP1092.

Thus, our comprehensive proteome data of *B. pertussis* infection-mimicking models help understand bacterial pathogenicity and reveal a new level of complexity of virulence regulations of Bp.

MI-26

Dual bacterial interactions between uropathogens co-isolated from "double double jJ" catheters

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Ureteral double-J (DJ) stents are extensively used in the management of upper urinary tract obstruction and prevention of complications in urological interventions. However, their use is associated with dysuria, hematuria, and lumbar or suprapubic pain. Furthermore, internal ureteral stents also offer an ideal surface for bacterial colonization and biofilm formation, features with an essential role in the pathogenesis of stent associated infections. A phenotypic characterization of uropathogens (UP) isolates obtained from DJ catheters removed from patients without primary symptoms of urinary tract infection (UTI) was previously carried out. Here, mixed planktonic cultures and biofilms derived from UP co-isolated from the DJ stents were investigated. Five co-isolated pairs isolated simultaneously from the same catheter sample were selected: *Enterococcus faecalis* EC2/*Escherichia coli* EC3 (pair 2/3), *E. faecalis* EC9/*Bacillus pumilus* EC10 (pair 9/10), *Staphylococcus epidermidis* EC15/*B. subtilis* EC16 (pair 15/16), *S. epidermidis* EC18/*B. subtilis* EC17 (pair 17/18) and *Klebsiella pneumoniae* EC24/*B. megaterium* EC27 (pair 24/27). Assays such as colony proximity in BHI agar plates and growth competition in static and shaked liquid medium were carried out to investigate the interaction and coexistence of the co-isolated pairs. In the colony interaction assay, after 72 h of incubation, *E. faecalis* strains from pairs 2/3 and 9/10 displaced their growth to the opposite periphery from the interaction zone with EC3 and EC10, respectively. In pairs 15/16 and 17/18, the presence of *Bacillus* spp. seems to inhibit growth of *S. epidermidis* in the interaction region. In regard to biofilm formation, there is a variation in the total biomass of the co-cultures when compared to single species cultures. No correlation was observed between biomass and number of viable cells in the mixed biofilms. It is worth to mention that a higher extracellular matrix production was observed in the polymicrobial condition compared to its single-species counterpart. In addition, to visualize the mentioned bacterial interactions, SEM was performed. The interactions studied in static or agitated liquid cultures showed variable results according to the pair under study. For instance, a lower number of S. epidermidis cells was observed in the co-culture of pairs 15/16 and 17/18, when compared to the axenic culture, while its pair (*B. subtilis*) showed a higher number of cells in the mentioned co-culture. The study of the microbial competitions, synergies or interferences between UP in the context of UTI could help to establish the real clinical role of these microorganisms in polymicrobial infections, as well as to gain further insight on how both pathogens interact with each other in the urinary tract.

MI-27

Pseudomonas fluorescens mutants affected in the bacteriocin production

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The bacteriocins are part of a diverse family of protein compounds characterized by their narrow killing spectrum; being toxic only to bacteria closely related to the producing strain. The rhizospheric strain *P. fluorescens* SF4c is known to produce phage tail-like bacteriocins, called tailocins. These bacteriocins are high molecular weight bactericidal protein complexes that resemble and are evolutionarily related to bacteriophage tails. SF4ctailocins SF4 tailocins have activity against phytopathogenic strains of the genera *Pseudomonas* and *Xanthomonas*. At present, the scientific interest is focused on the potential application of these antimicrobials in health, food, and agriculture. The SF4c tailocins regulation pathway is currently unknown. Recently, we have found a gene that is involved in the activation of these bacteriocins; however, the synthesis repressor gene is not yet known. Taking into account the aforementioned, we hypothesize that a mutation in the repressor gene of tailocins increases bacteriocin titers in cultures of *P. fluorescens* SF4c. To evaluate it, mini-Tn5-based transposon mutagenesis was used to generate random mutations. In our laboratory, we have a transcriptional fusion (pPROBE::Ptail) of the SF4c tailocin promoter to the green fluorescent protein (GFP) reporter gene. UV light-induced *P. fluorescens* SF4c (pPROBE::Ptail) strain cultures express the GFP protein, observing fluorescent colonies under blue light; this indicates that the promoter is being expressed, while under normal conditions, colonies do not fluoresce. A triparental conjugation was performed among a donor strain (*E. coli* CC118 λpir) containing the pUT::mini-Tn5 Km1 plasmid, a helper strain (*E. coli* HB101), and the receptor strain *P. fluorescens* SF4c. After, the mutants were picked and grown in minimum culture media supplemented with kanamycin (25 μg/ml) and tetracycline (10 μg/ml). Those mutants that exhibited enhanced fluorescence (increased expression of GFP) were selected. Promoter activity was quantified by fluorescence spectrometry using the proper positive and negative controls. Next, the presence of the mini-Tn5 Km1 transposon in the genome of the mutants was confirmed by PCR amplification of the transposon's kanamycin resistance gene. Finally, growth curve patterns and the tailocin production of the mutant strains were analyzed and compared with those of *P. fluorescens* SF4c (pPROBE::Ptail). The mutants 55, 174, 175, 233 y 253 showed statistically significant enhanced fluorescence; this means that there is an increase in the expression of tailocin promoter. Up to now, the results are promising in terms of obtaining information on the gene regulation system for *P. fluorescens* bacteriocins production.

MI-28

Antimicrobial activity of *lactobacillus Lactobacillus* spp against uropathogenic bacteria

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Urinary tract infections (UTI) remain a major medical problem in terms of the number of people afflicted each year. Although antimicrobial therapy is generally an effective way to eradicateseradicate these infections, a current problem is the increasing emergence of microbes resistant to antimicrobial agents. Uropathogens (UP) usually develop biofilms, resulting in persistent and chronic infections that are associated with resistance to antimicrobial therapy. The use of lactobacilli has been proposed as an alternative to prevent UTIs in the era of antibiotic resistance. Here, the potential antibacterial and antibiofilm activities of supernatants derived from five lactobacilli strains against different clinical urinary strains, isolated from patients of a public hospital of Tucumán, were investigated. First, *Lactiplantibacillus paraplantarum* CRL 1905, *L. brevis* CRL 1942, *L. fermentum* CRL 973, *L. helveticus* ATCC974 and *L. acidophilus* ATCC 4356 strains were grown in MRS for 24 h at 37 ºC; cell-free supernatants (CFS) were obtained by filtration. Inhibitory activities of these CFS were assayed by the agar well diffusion assay. Based on the inhibition halo, all CFS displayed a strong antimicrobial ability against the different UP strains, but with variable degrees. *Staphylococcus aureus* EC1 and *Escherichia coli* EC7 strains were selected for further studies. The minimum inhibitory volume (MIV) of the different CFS against the indicated strains were determined using a broth microdilution assay. The five CFS inhibited both pathogenic bacteria, with a MIV of 25 % (v/v). However, the minimal bactericidal volume (MBV) values of these CFS were ≥ 50 % (v/v), killing 99.99 % of the bacteria. The effect of all CFS against biofilm formation by EC1 and EC7 strains were determined. Dilutions up to 6 % of the tested CFS significantly inhibited biofilm formation, when compared with the untreated control, observing an antibiofilm activity in a concentration-dependent manner. In addition, the biofilm disassembly capacity of all CFS on 72 h- established biofilms of the UP strains was assessed. When compared with the untreated control, a reduction of the integrity of biofilms using 10-fold concentrated CFS was observed. Finally, to determine the nature of the antimicrobial compound/s, the different CFS were submitted to heat, proteinase K and tripsine treatment, or neutralization. It was observed that all CFS lost their antagonistic properties after pH neutralization, suggesting an acidic nature of the metabolite/s. Our results demonstrate that CFS obtained from five lactobacilli strains secrete products that inhibit growth, reduce biofilm formation and remove established biofilm of different UP, suggesting their potential application for controlling or preventing colonization in UTIs.

MI-29

The role of the com system in eDNA uptake mediated by the Type IV pilus in *Xanthomonas citri* subsp. *citri*

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Xanthomonas citri subsp citri is a bacterial phytopathogen that causes citrus canker disease, one of significant economic importance around the world. *X. citri* spreads from affected plants to healthy plants mainly through rain and wind and has the ability to survive for long periods on different surfaces usually due to the formation of biofilms. The bacterial biofilm is a highly regulated community of bacteria embedded in a polymer matrix composed of polysaccharides, proteins and extracellular DNA (eDNA) that provide physical support and protection against biotic and abiotic stresses. *X. citri* has been observed to produce a more robust mature biofilm in host plants than in non-host plants. Furthermore, the type IV pilus (T4P) plays an important role in the formation of biofilms, which highlights its importance in the bacterial life cycle. T4Ps are long, flexible filament located on the bacterial surface involved in a variety of important behaviours including twitching motility, surface adhesion, natural transformation, chemotaxis and biofilm formation. In some bacteria, T4P carry out the additional function of incorporating of eDNA into the cell by a mechanism coupled to the natural competence (com) system. In addition to the main components involved in T4P biogenesis and regulation, the *X. citri* genome also carries the com genes predicted to be involved in the internalization of exogenous DNA: comEA, comEC and comF. The main objective of this project is to investigate the interplay of the T4P, the com system and eDNA. Progress in this area will provide information of great interest for the understanding the phenomenon of T4P- and com-mediated eDNA uptake and signalling in biofilm formation and/or twitching motility; both mechanisms being involved in infection, horizontal gene transfer and bacterial dissemination and persistence.

MI-30

Bpp0974, an adhesin of the avirulent phase from *Bordetella parapertussis*

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B. parapertussis is an etiological agent of whooping cough, whose incidence has increased remarkably in the last years. This bacterium undergoes a phenotypic variation between the so-called virulent, intermediate and avirulent phases in response to environmental stimuli, such as temperature and exposure to modulating compounds. Due to the environmental conditions during transmission it has been suggested this pathogen reaches the host in avirulent phase, a phenotype that lack the expression of the main adhesin (filamentous haemagglutinin, FHA) required for bacterial attachment to host cells. Because bacterial attachment to the host cells is the first critical step of host colonization, bacterial adhesins expressed in avirulent phase might play a central role in *B. parapertussis* pathogenesis. In this study we searched for potential avirulent phase-expressed adhesins by means of comparative proteomics. We identified Bpp0974 expressed in avirulence, a protein that includes repetitive immunoglobulin-like domains, a von Willebrand Factor A domain, repetitive RTX motifs, a membrane retention domain, and a type I secretion target signal. All these features are characteristic of adhesins. The expression of Bpp0974 in the avirulent phase was confirmed by qRT-PCR and western blot. Interestingly, we observed that this protein remained highly expressed until the intermediate phase. We next built a *B. parapertussis* Bpp0974 in frame deletion mutant strain and checked it in attachment assays run in parallel with the wild type strain, both in avirulent and intermediate phases induced by modulating cultural conditions (high and intermediate concentrations of $MgSO_4$ or nicotinic acid). We found that Bpp0974 is involved in the attachment of *B. parapertussis* to human bronchial epithelial (16HBE14o-) cell line, both in avirulent and intermediate phases. In agreement with its adhesin role, Bpp0974 was found involved in *B. parapertussis* biofilm formation during avirulent and intermediate phases, as determined by microtiter crystal violet assays, and bacterial entry into the cell where it is able to remain alive for days, as determined for polymixyn B protection assays. Taken together these results suggest that Bpp0974 might play a central role in the early steps of infection enabling host colonization while protecting the bacteria from the innate immunity until the environmental conditions induce the virulent phase in the infecting bacteria.

MI-31

Toxigenic *Staphylococcus aureus* lineage belonging to Sequence Type (CC) 1 with ability to acquire antimicrobials resistance, as the main colonizer of Argentinean dairy cows.

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S. aureus colonizes and infects both humans and animals. It has the capacity to acquire virulence genes and antimicrobials resistance (AR), such as methicillin-resistance (MRSA) and the potential for spread in hospitals (HA), community (CA), livestock (LA) and in the animal–human interface, through clones with increased virulence, transmissibility and/or AR (high-risk clones, HRCs). *S. aureus* ST1/Clonal Complex (CC) 1 is associated with human infections and also is one of the major LA- lineages related to pigs and bovines in some European countries. There are not information in Argentina about the prevalence and molecular epidemiology of colonization in cattle.

The aim of this study was to investigate the prevalence of carriage, antimicrobial resistance profile and molecular types of *S. aureus* in bovines from dairy farms in a Humid Pampa region, the main agricultural and cattle area of Argentina, as a possible reservoir of HRCs. A prospective cross-sectional study was performed in four dairy farms from the area of Villa María city, Córdoba, Dec. 2019. Surveillance cultures for *S. aureus* from nasal (NS) and rectal (RS) swabs were obtained from 439 cows, in 56 of them milk samples (quarter-milk pool sample /QM) were also collected. A colonized cow had at least one positive sample for *S. aureus*. The AR was determined by diffusion/Vitek2, CLSI2019 and the presence of *mecA/C*, *ermA/C/B/T* and virulence genes by PCR. All isolates were genetically characterized by PFGE, spa typing and MLST.

Of the total positive samples, 77.5% (n: 55), 18.3% (n: 13) and 9.9% (n: 7) were from NS, QM and RS respectively. One lineage (CC) colonized 73.3% of the bovines positive (52/71), all in NS and/or RS: (47 NS, 4 NS and RS, 1 RS: 56 strains): ST1-PFGE-FF/t2207 *seh*+, *bsa*+, *lukED*+, *cna*+, *sek*+, with a major subtype FF11 (81%, n: 42/52) that also co-colonized 4 bovines in NS and RS. These strains showed a genetic feature

typical of animal adaptation such as the absence of Immune evasion cluster (IEC) genes *sak* and *scn*. Other four CCs were identified: 15.5% ST97-DD/t2297 (n: 11, 9 QM, 2 RS), 7.0% ST133-DDO/t17747 (n: 5, 4 QM and 1 RS) and 1.4% (all NS): ST398-/t1451, ST45-AA/t026 and ST8-USA300/t024. All were MSSA, 4/75 (5.3%) R to Erythromycin and Clindamycin: 2 MLSBc (ST1 ermC+) and 2 MLSBi (ST398 ermT+ and ST8 ermC+), 1.3% R to Rifampicin (ST1) and 1.3% R to Ciprofloxacin (ST45).

In conclusion, in Argentina, bovines are reservoir of a toxigenic *S aureus* HRC belonging to CC1, with ability to acquire AR, related to humans and adapted to animals, along with other minor CCs some of these related to human (CC45 and CC8) and others associated with cattle (CC97, CC133 and CC398). These data are important for the control of HRCs and AR transmission, and support the implementation of farm-level strategies to prevent spill-over.

MI-32

Cadherin–like domains in *Rhizobium* spp.: a phylogenetic and functional study.

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Bacterial cadherin-like domains (CHDLs) are ubiquitous among surface exposed and secreted proteins. They were first identified by *in silico* studies mostly associated to other domains with enzymatic activity or displaying adhesive properties. They may be present in one or few copies, or in numerous tandem repeats in large extracellular proteins. They show striking structural similarity to eukaryotic cadherins that mediate cell adhesion in Metazoa, but unlike these, CHDLs were scarcely studied. To gain insight into the biological role of bacterial CHDLs, we have previously characterized the domains present in the RapA2 lectin, an extracellular calcium binding protein acting in matrix assembly during biofilm formation by *Rhizobium leguminosarum*. We showed by structural and functional approaches that only the carboxy-terminal CHDL (C-CHDL) retain carbohydrate binding ability. In this work we have extended our analysis to other CHDLs, present in extracellular proteins of the Rap family (Rap for Rhizobium adhering proteins). First we performed a phylogenetic analysis of CHDL domains in Raps secreted by rhizobia, as a base to predict their functional properties. Then the domains were heterologously expressed in *Escherichia coli*

and purified as His-tag fusion proteins. The purified domains were analyzed in a functional test by means of a binding inhibition assay with the acidic exopolysaccharide produced by *R. leguminosarum*. Finally, the green fluorescent protein (GFP) fused to the C-CHDL of RapA2 was used to target the exopolysaccharide in an experiment of *Rhizobium* biofilm progression by confocal laser scanning microscopy (CLSM). The results show that CHDLs may exhibit greater functional diversity than first appreciated, and that some of them could be useful as novel fluorescent probes for exopolysaccharides produced by several rhizobia of agronomical interest.

MI-33

Genomic comparison of *S. meliloti* pSymA revealed a novel and functional conjugation system

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Rhizobia are soil bacteria capable of establishing a symbiotic relationship with legume plants. In this relationship, rhizobia reduce molecular N2 to ammonia through biological nitrogen fixation, and legumes supply nutrients to bacteria. This process has a significant ecological and economic importance since it avoids the use of contaminating and expensive nitrogenous fertilizers. One of the most studied rhizobia is *Shinorhizobium meliloti*, which forms symbiosis with *Medicago sativa* legume (alfalfa). *S. meliloti* strains always present a genome with three large replicons: the chromosome, and two plasmids designated pSymA and pSymB. These plasmids carry necessary genes for the establishment of a complete symbiotic state. Based on several studies, pSymA is the plasmid that most contributes to genetic variability. Thus, in this work, we propose to study the genetic variability of 27 pSymAs of *S. meliloti* strains whose genome was sequenced and separated in plasmids. First, to group pSymAs, we performed an alignment and then made phylogenetic trees according to several criteria (core genome, Average Nucleotide Identity). As a result, the trees obtained showed a similar topology, regardless of the grouping criteria used. In addition, since one of our research interests is plasmid conjugation, we investigate the conjugative systems present in these plasmids. Some studies have reported that the conjugative systems are composed of the DNA transfer and replication (Dtr) and Mating pair formation (Mpf) genes. These genes encode

proteins responsible for DNA processing and conjugative pore formation, respectively. The described conjugation system of pSymA is mediated by two genes: rctA and rctB. The rctA gene encodes a conjugation repressor and its effect is counteracted by protein encoded in rctB gene. Therefore, when the rctB gene is activated, the repressive activity of rctA decreases promoting conjugative transfer. Initially, we verified the presence of the Dtr and Mpf genes described in the 27 pSymAs. Next, we observed that the rctA repressor is present in all the pSymA unlike the rctB activator present in 10 of 27. It was interesting to find that 9 pSymAs presented a second DTR and MPF system. To characterize the new system, we built phylogenetic trees based on essential proteins of the conjugation systems: VirB4 (Mpf) and TraA (Dtr). Due to rctA-rctB system is repressed at laboratory conditions, we made conjugation test with the selected LPU88 strain. As a result, it was observed that the pSymA of the LPU88 strain moves with a frequency of 1*10-6. This work allowed us to globally compare the pSymAs of 27 strains of *S. meliloti* and the conjugative systems. In the future, we are interested in characterization of regulation of this novel conjugation system in pSymA.

MI-34

Genome characterization of *Enterococcus lactis* strain SU-B46 isolated from bovine mastitis

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Bovine mastitis is a disease that affects dairy cows causing reduction in milk production. Environmental bacteria, as streptococci and enterococci, are important agents responsible for bovine mastitis. The aim of this study was to assess a genome sequence analysis of a strain isolated from subclinical mastitis case. DNAg was isolated from pure cultures and quantified using Nanodrop-1000 and Quantus. gDNA library was constructed using Ligation Sequencing Kit (SQK-LSK109) and sequenced using Oxford Nanopore Technology platform on a MinION 1kb device. Basecalled RAW-sequences were filtered by quality/size using Filtlong and assembled by Trycycler pipeline. Final assemble was polished using

medaka and polypolish (https://github.com/rrwick/Polypolish) with Illumina paired-end reads. NCBI Prokaryotic Genome Annotation Pipeline (PGAP) was implemented for gene annotation and CheckM to obtain completeness metrics. For species classification, we used the Type (Strain) Genome Server (https://tygs.dsmz.de/) and Whole-genome Average Nucleotide Identity (ANI) (https://github.com/ParBLiSS/FastANI) analysis against selected reference genomes of *Enterococcus* species. Final genome was assembled in a single circular contig and one plasmid comprising 2.556.632 bp (GC% 38.60) (99.25% completeness) and 150.514 bp (GC 36%) respectively. A total of 2.379 predicted CDSs, 18 rRNA and 68 tRNA were annotated in the chromosome (~10% as hypothetical proteins) while 172 CDSs were predicted in plasmid. Whole-genome Average Nucleotide Identity (ANI) analysis showed highest values (99.37%) against the reference genome of *Enterococcus lactis* strain CX 2-6 (CP079880). These results were also confirmed by whole-genome sequence-based phylogeny where strain SU-B46 was closely clustered within the *Enterococcus lactis* reference genomes. Pairwise whole-genome alignment reveals a collinearity within the *E. lactis* strain CX 2-6 and *E. lactis* SU-B46. These results confirm the presence of *Enterococcus lactis* using a whole genome analysis approach. In recent years, *Enterococcus* species have emerged on some farms as a pathogen identified in chronic high SCC cows, even after treatment with intramammary antibiotics. This study shows that *Enterococcus lactis* is an etiological agent of bovine mastitis. Further studies should be conducted to understand the importance of this agent and warrant further investigation so that it can be managed in the best possible way especially within the dairy industry.

MI-35

Characterization of compost beds of a stable in the province of Córdoba

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Compost bed stables are a housing method for dairy cows. These systems have gained relevance in Argentina, and Córdoba is one of the provinces with the highest investment in these structures. According to experiences in other countries, these systems are sustainable, friendly to peri-urban areas and

favorable to animal welfare, with repercussions on the quality of the product obtained and on the perception of dairy consumers. The adoption of these systems would ensure the production of low levels of effluents and less water consumption than other systems improving the economic production. The objective of this work was to study the compost microbiota through metagenomic techniques, with sequencing and bioinformatic analysis to determine mastitis pathogens. Two areas of a stable in the province of Córdoba were selected: the cattle enclosure and the prepartum area for cows and heifers. Two independent compost samples were taken for each of the places and DNA extraction was performed. Sequencing was carried out using Illumina technique and the analysis of the crude readings obtained for the taxonomic assignment was carried out. In all samples, the percentage of fragments that could not be classified ranged from 58% to 65% of the total reads obtained. In addition, the assignment percentage for the Bacteria kingdom was about 40% in the four samples. A similar value was obtained for the eukaryota kingdom. Furthermore, *Microbacterium*, *Agromyces* and *Curtobacterium* were the main genera found in the cattle enclosure and the prepartum cove of cows, while *Sphingomonas*, *Novosphingobium* and *Rhizorhabdus* were identified in the prepartum cove of heifers. The bioinformatic analysis allowed to identify species of non-cultivable microorganisms in the analyzed samples. The present study demonstrates the advantage of using sequencing for the analysis of metagenome studies.

MI-38

A new c-di-GMP phosphodiesterase regulates motility and biofilm in *Bordetella bronchiseptica*

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Bordetella bronchiseptica is a respiratory pathogen that can infect a broad range of hosts. Our group has described that the second messenger c-di-GMP plays a role in the regulation of biofilm formation, motility and virulence in this bacterium. C-di-GMP intracellular levels are regulated by two kinds of proteins: diguanylate cyclases, which synthesize it, and phosphodiesterases, which degrade it. High concentrations of c-di-GMP are related to increased biofilm formation and decreased motility, whereas low concentrations are related to enhanced motility and lower biofilm levels.

In previous work, we described the *B. bronchiseptica* phosphodiesterase BB3116 which has a putative periplasmic CSS domain, similar to the one described by Hengge's group in 2018. CSS domain can

respond to redox stress, and consequently activate the phosphodiesterase domain. We noticed that the two highly conserved cysteines were present in our protein and hypothesized that this is a functional domain. In this work, we used *Escherichia coli* s17 and transformed it with an empty plasmid (s17pE) or one that overexpresses BB3116 (s17pBB3116). We tested biofilm formation and observed that s17pBB3116 forms 42% less biofilm than s17pE. Regarding motility, it was enhanced by BB3116 by 22%, as expected for a phosphodiesterase that degrades c-di-GMP. These results suggest that BB3116 is a functional phosphodiesterase.

We then used the same plasmids in *B. bronchiseptica*, in the background of the wild-type (WT) strain or the mutant lacking the BB3116 gene. We constructed 4 strains: WTpE, WTpBB3116, ΔBB3116pE and ΔBB3116pBB3116. When evaluating motility, ΔBB3116pE showed a decrease of 47% when compared to WTpE although the complemented strain ΔBB3116pBB3116 only partially restored the wild-type phenotype. This suggests that BB3116 is a phosphodiesterase that regulates motility in *B. bronchiseptica.*

Biofilm formation in *B. bronchiseptica* is regulated by the two-component system BvgAS. *Bordetella* has three distinct virulent phases and when BvgAS is partially activated, in the intermediate phase, biofilm formation is maximum. Nicotinic acid (AN) is used to modulate these phases *in vitro*. When testing the ability to form biofilm of our four constructed strains, we only saw differences in the virulent phase, when BvgAS is fully activated. Curiously, we noted that in this condition biofilm levels are 27% lower in the mutant strain ΔBB3116pE than in the wild-type one, contrary to expectations. This is an interesting finding, and further studies will be needed to elucidate how this regulation works.

Lastly, we used dithiothreitol (DTT), a reducing agent used in Hengge's work, and evaluated if the activity of BB3116 was enhanced in its presence by testing motility and biofilm formation. No differences were observed that account for a change in phosphodiesterase activity related to the presence of millimolar concentrations of DTT.

MI-39

Overexpressing the gene livK from *Rhizobium favelukesii* in *Escherichia coli*: imposible. Is it toxic?

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Leguminous plants can develop symbiotic interactions with rhizobia that culminate in the formation of root nodules. These symbiotic interactions play an essential role in agricultural-production systems through an enrichment of the soils with nitrogen that maximizes crop yields, thus decreasing the need for using chemical fertilizers that are harmful to the environment. Alfalfa is the most widely cultivated forage legume for cattle. The growth and persistence of alfalfa are impaired in moderately acid soils. *Sinorhizobium meliloti* is the efficient symbiont for biologic nitrogen fixation but is very sensitive to low pHs. Although alfalfa is a highly specific plant with respect to its symbiotic partner, rhizobial strains other than *S. meliloti* can also nodulate this plant species. *Rhizobium favelukesii* is an acid-tolerant rhizobium that nodulates alfalfa and other leguminous plants, though inefficient for nitrogen fixation. Hence, from a practical point of view, *R. favelukesii* provides a system for studying the natural mechanisms that bacteria could employ for pH resistance. Moreover, an understanding of the genetic response to acid stress in *R. favelukesii* constitutes a potentially powerful basis for effecting a rational improvement in *S. meliloti* performance at low pH for increasing biologic nitrogen fixation in acid soils. We have characterized the response to acid stress of *R. favelukesii* LPU83 through both the global response of the transcriptome and that of the proteome under acid stress conditions. It was shown that the response to acidity of *R. favelukesii* involves modifications in the cell envelope and, consequently, cell permeability, as well as in histidine biosynthesis and GABA metabolism. Strains of *R. favelukesii* were obtained in which the genes associated with GABA metabolism (livK, braD) were mutated; all the mutants evaluated showed an acid-sensitive phenotype. To evaluate whether livK over-expression allows obtaining strains of *S. meliloti* that have greater tolerance to acidity, we set out to clone livK with their own promoter in a replicative plasmid. The first step was a comparison of the region the upstream livK's ATG. We found that the region was only conserved near the *S. meliloti* livK TSS. To study the expression of livK from rhizobia, we tried to clone the complete livK gene in the broad-host-range intermediate-copy-number vector pBBR1-MCS5. Although both cloning orientations strategies were followed, we were systematically unable to obtain positive clones. Similar results were obtained by a "walking chromosome" approach using the high-copy-number vector pK18mob. To test the hypothesis that high livK gene dose could compromise cell viability in *E. coli*, we cloned the livK gene into the low-copy-number plasmid pLow. In contrast with our previous results, we obtained several positive clones using this vector, supporting the idea that overexpression of livK under its own promoter would induce toxicity in *E. coli*.

MI-40

Fructose and glucosamine metabolisms in *Rhodococci*: from basic knowledge to biotechnological applications

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The oleaginous behavior constitutes the basis for several rhodococcal species to be considered as potential biofactories for oiled compound productions. Oleaginicity analysis under different carbon and nitrogen supplies is a critical task to identify scenarios for the utilization of in the treatment of environmental pollutants. To elucidate *Rhodococcus jostii* and *Rhodococcus fascians* metabolic behavior we grew both bacteria in different sole carbon sources at different concentrations. We used minimal saline medium (MSM) complemented with ammonium chloride as nitrogen source, where MSM0, MSM0.1 and MSM1 contained no NH4Cl; 0.1 or 1g/L NH4Cl, respectively. It was extensively reported that high nitrogen availability (such as MSM1) increases biomass production, while lower nitrogen concentrations trigger lipid accumulation in Rhodococci. Amongst the different sugars analyzed as carbon sources, we found that both *R. jostii* and *R. fascians* grew efficiently in fructose (Fru) or glucosamine (GlcN). Thus, the aim of our current work is to deepen the understanding regarding the metabolic steps involved in Fru and GlcN metabolisms, and their possible interconnection.

Fru was assayed at 0.1, 1, 3 and 10 % w/v in either MSM0, MSM0.1 or MSM1 for *R. jostii*. No growth was detected in MSM0, demonstrating the essentiality of a nitrogen source. When grown in MSM0.1 with 0.1% w/v Fru, OD600 was 1.5, while with all other Fru concentrations, growing values reached OD600 of 6. On the other hand, when grown in MSM1, *R. jostii* reached OD600 units of 2, 11, 56 and 45 for 0.1, 1, 3 and 10 % w/v Fru, respectively. Results suggest an interplay between Fru consumption and nitrogen availability. *R. jostii* growth in 1, 3 y 10% w/v GlcN was between 6 and 8 in MSM, thus indicating that high biomass production seems to be linked to Fru metabolism. We then analyzed same Fru concentrations for *R. fascians* growth in MSM1. Curiously, the OD600 values were up to 10-11. Then, we are tempted to speculate regarding different regulatory mechanisms involved in Fru metabolism between different rhodococcal species. To understand the different behaviors described above, we approached the biochemical characterization of putative genes related to Fru and GlcN consumption. We focused on enzymes belonging to the Fru-6P/Fru-1,6-P2 interconversion node (e.g. PPi and/or ATP dependent phosphofructokinases and Fru-1,6-P2 phosphatase) and to the link between Fru-6P and GlcN-6P (GlcN-6P synthase and Glc-6P deaminase). Genes encoding the enumerated enzymes were amplified, cloned and expressed in *Escherichia coli* for their purification and kinetic characterization. These preliminary results provide new insights to further understand rhodococcal metabolism. The latter, would allow to harness their potential as microbial biofactories starting from agricultural disposals (generally rich in sugars like Fru), or as biocatalysts providers for biotechnological processes.

MI-41

Characterization of a local isolate of *Mannheimia haemolytica*

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Mannheimia haemolytica is a Gram-negative respiratory pathogen that is frequently isolated from Argentine feedlots. Symptoms are seen after a stressful situation such as transportation or diet changes. Infection by this bacterium causes weight loss and death in calves. Known virulence factors are capsular polysaccharides, lipopolysaccharide, adhesins, outer membrane proteins, iron-binding proteins, secreted enzymes and the ruminant-specific repeats-in-toxin (RTX), LktA.

In this work we present a strain of *M.haemolytica* isolated from the Buenos Aires province area. We performed a genome and phylogenetic analysis, and a search for the most preponderant virulence factors, such as the presence of the LktCABD operon, possible adhesins and outer membrane proteins.

In addition, we analyzed the formation of biofilm in glass tubes in static and shaking cultures. We observed a greater biofilm formation in the latter condition. We confirmed LktA presence in the strain purifying the toxin from the supernatant in an overnight culture.

Finally, a survival test was carried out, in which we used the nematode *Caenorhabditis elegans* as an infection model, observing that *M.haemolytica* presented a higher rate of death than the control.

Overall, we first molecularly characterized a local isolate of *M. haemolytica*, we characterized phenotypes important for virulence and we optimized a host model for the pathogen. This establishes a starting point to further analyze the role of the different virulence factors found in the genomic analysis.

MI-42

Get close but not too close. Microbial interactions between two sugarcane endophytic isolates

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Microorganisms interact with their host and with each other, establishing dynamic microbial community structures and functions. Microbial interactions take place at a chemical level, through the production of diffusible metabolites or by mechanisms involving direct contact. In this work, we study the interaction between two sugarcane endophytic isolates classified as BECA 336 and BECA 335 after genome sequencing. The two isolates were spotted on solid medium using a distance pattern that allowed observing growth effects at different ranges. When the two isolates were in close proximity, BECA 336 inhibited BECA 335. On the contrary, at greater distances BECA 336 stimulated BECA 335 growth. *In silico* analysis and *in vitro* assays appeared to indicate that growth-stimulating effect is mediated at least in part by the siderophore enterobactin, produced by BECA 336. The attempt of purification of the active compound using HPLC-UV-MS demostrated that the part of the extract that presented activity could correspond to DHBA-L-Ser. While growth stimulation showed an intensity gradient consistent with the diffusion of a metabolite, inhibition displayed an irregular pattern that rules out this possibility. Inhibition was enhanced under conditions favoring BECA 336 motility suggesting that the phenotype might be contact dependent. Unraveling how microorganisms interact may pave the way for the understanding of microbial community assembly.

MI-43

Dissecting the link between membrane and cell wall biosynthesis in *Bacillus subtilis*

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The plasma membrane and the peptidoglycan (PG) are essential constituents of the cell envelope in bacteria, so the control of cell size and growth is closely linked to the generation of both structures. Despite its relevance, it is still a mystery how the biosynthesis of these molecules is coordinated and the underlying molecular mechanisms of this coordination are superficially known. We have previously shown by microarrays analysis that inhibition of lipid biosynthesis by the antibiotic cerulenin induces yocH expression. This gene is under the control of the essential two component system (TCS) WalR (response regulator)-WalK (histidine kinase) and codes for the PG autolysin YocH, associated with the growth of cell wall. In order to study the role of this TCS on the induction of yocH expression upon inhibition of lipid synthesis we incorporated a PyocH-lacZ reporter fusion into a walRK conditional mutant. We determined that WalRK has an essential role in yocH induction upon lipid synthesis inhibition. It has recently been reported that the Ser-Thr kinase PrkC, can also phosphorylate WalR in Thr101 to control its function. In order to investigate if PrkC is also involved in yocH induction upon lipid starvation we analysed the expression of PyocH-lacZ in a ΔprkC background in the absence and presence of cerulenine. We found that both kinases, WalK and PrkC, are necessary for yocH induction during inhibition of lipid synthesis. Furthermore, using WalR punctual mutants, complementation tests as well as radiolabeling biosynthetic assays we are presently investigating the role of each signaling protein in the coordination between PG and membrane synthesis. This work is a first step towards elucidating the molecular basis of cell envelope homeostasis in Gram-positive bacteria, a crucial aspect for the survival of these microorganisms.

Dual- and mono-species biofilms formed by *Bradyrhizobium japonicum* E109 and *Azospirillum brasilense* Az39 show potential to improve biofertilization

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There is an actual demand for environmentally friendly agricultural practices, in which the use of fertilizers based on beneficial microorganisms becomes increasingly relevant. It is now well established that inoculation with plant growth-promoting rhizobacteria (PGPR) helps to preserve soil health and productivity. However there are several constraints affecting the effectiveness of current biofertilizers on crop yields, as survival in adverse environments or competition with native soil microbiota. In this regard, biofilms formed by PGPR could be envisaged as a means to develop a new generation of biofertilizers with higher efficiency and improved field results. Biofilms are composed of bacterial cells embedded in a self-produced polymeric matrix that may be attached to biotic or abiotic surfaces. Biofilms display a varied range of emergent properties that result in enhanced resistance to adverse conditions (UV light, pollutants, desiccation, etc.) and other activities significant for crop production (phosphorus solubilization, N_2 fixation). Interspecific interactions in multispecies biofilms add another level of complexity, but their study revealed synergism on biofilm biomass and functional changes that enhance a function or product. We selected *Bradyrhizobium japonicum* E109 and *Azospirillum brasilense* Az39 as model organisms to study biofilm formation, since they are commonly used in the formulation of biofertilizers in Argentina and neighbor countries. *B. japonicum* nodulates soybean roots carrying out N_2 biological fixation, while *A. brasilense* is a free-living N_2-fixing bacterium, that also stimulates root growth through auxin production. We have established conditions in which both strains can co-exist and also positively influence each other. Mono- and dual-species biofilms were visualized by confocal laser scanning microscopy. Biofilm dispersal assays allowed identification of major matrix components. Mixed Az39-E109 biofilms increased biomass from day 4, compared to mono-species biofilms. The presence of strain Az39 stimulated exopolysaccharide production by *B. japonicum* E109, an exopolymer of great importance for biofilm formation, root infection and nodulation. Moreover, *A. brasilense* Az39 increased morphological differentiation into cysts in the presence of strain E109. This phenomenon is of great interest since cyst formation and biofilm production were proposed mechanisms enabling free-living nitrogen-fixing bacteria to efficiently fix N2 in an aerobic environment. Our findings suggest synergism in microbial functions that stimulate plant growth, opening the way towards the idea of using mixed biofilms as biofertilizers.

MI-45

Discovering photoreceptors in *Staphylococcus aureus*

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Staphylococcus aureus, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* have been recognized by the World Health Organization and the Center for Disease Control and Prevention as critical human pathogens requiring urgent attention. They belong to the ESKAPEE group, for their hability to "escape" antibiotic treatment, thus increasing hospitalization, costs and mortality. Our group has demonstrated that these microorganisms sense and respond to blue light. Specifically, *S. aureus* USA300 senses and responds to light at 37ºC modulating important determinants of pathogenicity such as virulence and regulates persistence and metabolism. Detailed knowledge of the molecular mechanisms of light signal transduction is essential. The aim of this work is to identify and characterize the photoreceptors present in *S. aureus*. By analyzing the genome, we have identified three putative photoreceptors belonging to the GAF family, which are present in phytochromes and cyanobacteriochromes and are known to bind bilin cromophores (Chr). In two of these proteins, NreA and 7502, the GAF domain encompasses the complete sequence, but the third harbors an N-terminal GAF domain associated with a C-terminal histidine kinase, GAF-HK. The genomic environment of each protein was determined. Genes such as LuxR, involved in the regulation of quorum sensing is a regulator of bacterial motility response, virulence and biofilm formation, was found in the proximity of NreA and GAF-Hk. Moreover, we show that motility is modulated by blue light and by red and green light in *S. aureus*. Interestingly, some cyanobacteriochromes have been shown to absorb in different regions of the spectrum, compatible with our results.

Expression levels of 7502, GAF-HK and NreA in *S. aureus* USA300 cells grown in dark or under blue light, assessed by qRT-PCR, showed that GAF-HK is significantly more expressed in light. To evaluate their photochemical properties, we amplified the DNA fragments encoding these putative photoreceptors and cloned them into the pET-TEV expression vector. After induction, 7502 and NreA are in the soluble fraction, but GAF-HK is predominantly in the insoluble fraction. The interaction with different bilins was tested. The biliverdin (BV) was added exogenously to 7502 and a greenish protein solution was recovered after size separation Penevsky. To evaluate the Chr presence, we performed zinc gels in the presence and absence of β-mercaptoethanol (BME). 7502 binds BV in a non-covalent interaction since no fluorescence is observed after BME addition. We assayed co-induction with Phycocyanobilin and observed fluorescence

even in the presence of BME for 7502 and GAF-HK, indicating that these proteins can better accommodate the Chr. Overall, our results show that these putative photoreceptors can bind bilin Chr, compatible with the photoreceptor function of GAF proteins. Further experimentation will allow us to determine if their absorption spectra is sensitive to light.

MI-46

Infrared spectroscopy (FT-IR) used in microbiology to evaluate changes in bacterial communities within electrical fields during bioremediation

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Infrared spectroscopy is a technique based on the use of infrared (IR) radiation, which is obtained from the thermal emissions of appropriate sources. This kind of radiation stimulates certain molecular groups into an excited state where they vibrate differently, a phenomenon that only occurs at fixed wavelengths. This study used FT-IR to assess changes in bacterial communities within five spectral windows. Different molecular groups have prevailing influence in each window: Between 3000 and 2800 cm^{-1}, it is the functional groups in the membrane's fatty acids; between 1800 and 1500 cm^{-1}, the amide I and amide II groups in proteins and peptides; from 1500 to 1200 cm^{-1}, a mix of proteins, fatty acids and phosphate-carrying compounds; from 1200 to 900 cm^{-1}, mostly carbohydrates and polysaccharides. Finally, between 900 and 700 cm^{-1}, specific spectral patterns occur named "the true fingerprint". The bacterial communities (biofilms) were observed during electrobioremediation. A soil sample was placed into a 27 cm-long electrobioremediation glass cell. A 0.5 V cm^{-1} difference of potential was applied to the cell for 60 days, with polarity being rotated every four days to prevent abrupt changes in pH. A second cell to which no current was applied was used as the control. Changes were monitored by determining bacterial count, total pretroleum hydrocarbons (TPHs) and pH. The soil sample was prepared by suspending 1 g of soil in 10 ml of tryptein soy broth. The suspension was incubated for 24 ± 0.5 h at 30 ± 2°C, centrifuged at 4000 rpm and washed three times with saline. Then, 50 µL of 70% alcohol were added, and the sample was put

on a zinc selenide disc sample carrier and dried for 45 min at 42ºC. The transmission spectra of the resulting dry biofilms were recorded in the wavenumber region between 4000 and 700 cm-1, using a Varian 1000 spectrophotometer (120 scan, 4 cm^{-1} resolution). To ensure data reproducibility, a strict experimental protocol was followed regarding media preparation, incubation time and temperature, cell harvesting conditions, sample preparation and FT-IR measurements. The spectra were transformed (normalization, smoothing and second-derivation) and recorded in ASCII format to be analyzed on the PAST software. Thanks to the possibilities offered by FT-IR spectroscopy to study dynamic changes in bacterial populations, we observed that the communities were in different quadrants depending on whether electrobioremediation was applied, as well as on the nutrient concentration and the amount of moisture. In the absence of nutrients, the bacteria in the electroremediated soil were in a different quadrant than in the control. Electroremediation increased the degradation of TPHs but did not affect bacterial count (10^{-4} to 10^{-5} CFU/g). In general, FT-IR proved a useful tool to observe changes in these communities during electrobioremediation.

MI-47

Relationship between the triggering of the Stringent Response and the expression of the Type 3 Secretion System in *Bradyrhizobium diazoefficiens*

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Bradyrhizobium diazoefficiens USDA 110 is a Gram-negative soil bacterium which fixes nitrogen in symbiosis with soybean plants (*Glycine max*) in so-called root nodules. In return, the plant provides photo-assimilated products. In *B. diazoefficiens*, the Type 3 Secretion System (T3SS) is inducible by genistein, a soybean flavonoid that activates the transcriptional regulator TtsI. TtsI regulates the expression of the T3SS, including the structural gene rhcJ, and the effector proteins involved in the suppression of the host's defenses. Also, the T3SS induction is affected by the second messenger guanosine tetraphosphate and guanosine pentaphosphate variations (collectively referred as (p)ppGpp). In response to starvation, bacteria trigger the Stringent Response (SR) and (p)ppGpp is synthesized. A *B. diazoefficiens* rsh mutant, incapable of triggering the SR, establishes a defective symbiosis with soybean. In addition, this mutant induces far less the expression of the T3SS regulator ttsI

and the rhcJ structural gene in the presence of genistein compared to the induction of the wild-type and also, does not suppress host's defenses.

In order to find determinants linking SR and T3SS, we performed a comparative exoproteomic analysis. Extracellular proteins were obtained from wild-type (WT) and rsh mutant (MUT) strains, induced with genistein (G) or without induction (WI). These proteins were separated and identified by Orbitrap and the spectra were analyzed with DIA-NN software. We obtained about 1000 proteins per sample (WT G, WT WI, MUT G and MUT WI), from which different comparisons allow us to obtain differentially expressed proteins. As an example, in the WT G vs MUT G comparison, we found that the T3SS's effector protein NopE1 was overexpressed, indicating that T3SS is upregulated in the WT.

As a connection between SR and T3SS was described, we hypothesized if the nutritional stress that triggers SR might also trigger the expression of T3SS. For this purpose, we measured the transcriptional levels of the ttsI and rhcJ genes of the WT and rsh mutant subjected to nutritional stress by qRT-PCR. We incubated the WT and the rsh mutant with genistein, without induction as a control condition and with a C and N free solution, condition where the (p)ppGpp is accumulated in the WT. The transcript levels of genes ttsI and rhcJ were upregulated in the WT under a nutritional stress, in comparison with the rsh mutant. Even more, the expression of T3SS genes under stress condition was higher than the expression in presence of genistein. Under nutritional starvation, the symbiotic interaction is stimulated. Our results confirmed that in *B. diazoefficiens* USDA 110 there is a link between the T3SS expression and the nutrient starvation condition, and that the expression of T3SS is triggered by the SR, but further work will help to understand the complete pathways involved during this activation.

MI-48

TOWARDS DISSECTION OF IMMUNOGENIC AND IMMUNOMODULATORY PROPERTIES OF RSV SURFACE GLYCOPROTEIN

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Respiratory Syncytial Virus (RSV) is the primary cause of hospitalization for respiratory tract infection in young children. The attachment glycoprotein (G) is one of the three envelope proteins of RSV and is, undoubtedly, the most intriguing one. Its evolutionary origin is still unknown: it shows no sequence similarity with the envelope proteins of the other paramyxoviruses. Efforts to obtain its molecular structure have been sterile (except for the cysteine noose in the central conserved region), since most of the protein exhibits great flexibility and extensive glycosylation. In addition, G presents an alternative initiation codon that synthesizes a truncated version which is secreted, a property that has not been observed in other related viruses. On top of this, G has been shown to be extremely relevant in two manners: immunogenicity and immunomodulation. G epitopes account for half of the neutralizing antibodies after exposition to RSV, with all these epitopes focalized in the central conserved domain (CCD). The immune modulating effects are numerous: secreted G can act as an antigen decoy, reducing the efficacy of neutralizing antibodies; it has a chemotactic effect via mimicking a relevant cytokine (fractalkine), interacting with the receptor CX3CR1; it can down-regulate Toll like receptors; it inhibits the activation and maturation of dendritic cells. This work in progress aims to make a molecular dissection of these two properties (immunogenicity and modulation) and to use this knowledge to design a recombinant virus that carries single-residue substitutions that conserve the immune epitopes, while its immune modulating properties are disrupted. At this point we have identified, via a bioinformatic approach, three residues that appear to be very important for the interaction with the receptor CX3CR1. Importantly, none of these residues are considered to be key to maintain the protein structure, so the immunogenicity may not be altered. Moreover, we have seen that these residues are highly conserved in the RSV sequence in both strains (RSVA and RSVB). Currently, we are producing recombinant G protein in *E. coli*, inserting these point mutations and analyzing their relevance in cell culture and in mice.

MI-49

Volatile organic compound profiles in sudden death syndrome disease

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Fusarium tucumaniae (Ft) and *Fusarium crassistipitatum* (Fc) are fungus species that cause sudden death syndrome (SDS) in soybean crops in Argentina. These necrotrophic fungi infect root plants and generate foliar symptoms. Soil microorganisms and plant interaction is mediated by a wide range of chemical compounds. Published reports demonstrate that emitted volatile organic compounds (VOCs) can modulate plant growth and fungi virulence and that VOCs profiles can be altered by fungal growth conditions. In this work, we identified and compared VOC emission from Ft and Fc- infected soil and soybean roots as well as Ft and Fc grown in rich culture medium. For soybean infection, inoculum was prepared by culturing Ft and Fc strains on potato dextrose agar (PDA) in plastic Petri dishes for 1 week at 25 °C in the dark. Five agar plugs (6 mm diameter) with mycelium were added to autoclaved sorghum grains. The flask was incubated at 25 °C in the dark for 2 weeks. A nursery substrate was plugged in pots and a layer of infected sorghum were distributed in experimental pots and covered with the same substrate. Control pots contained non-inoculated sorghum grains. Five seeds of a susceptible SDS genotype were placed in each pot and covered with substrate. At 15 days after infection, VOCs from tap root areas contacting the sorghum seed sand rhizosphere soil were identified by head space solid phase microextraction (HS-SPME) coupled to gas chromatography-mass spectrometer (GC-MS). Toluene was used as an internal standard. In addition, VOCs from pure fungal cultures were analysed. PDA alone was used as a control sample. Identified VOCs were classified into the following biochemical groups: acids, terpenes, aldehydes, ketones and alcohols. Some of these differ between pathogen growth conditions, but not by any of the fungal pathogens used. More different types of alcohols were found in infected roots than in rhizosphere or in rich culture media. However, among these, 1-octen-3-ol accumulated only in rhizosphere and culture media compared with controls. More different types of terpenes were identified in rich media than in the other two conditions. Contrarily, aldehydes were more abundant in infected roots and rhizosphere compared with culture media. Among these, increased levels of benzaldehyde and 2-hydroxy benzaldehyde were found in root samples and rhizosphere due to the infection. Taken together, our results suggest that VOC profiles qualitatively and quantitative differ between samples. Identification of differential compounds may improve our understanding of plant-pathogen interaction and suggest that these chemical signals could be used as new strategies to estimate or control SDS disease severity.

MI-50

Probiotic potential of *Lactiplantibacillus plantarum* strains against *Enterococcus faecalis* infection in different experimental models.

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The increase in multiresistant bacteria due to the misuse of antibiotics is a growing threat to health worldwide. *Enterococcus faecalis* belongs to a group of high priority bacteria resistant to antibiotics as defined by the World Health Organization. This pathogen has little pathogenic potential in the normal host, but in the elderly and immunocompromised patients it causes opportunistic infections. Because of their increasing frequency and the challenging treatment, there is a need for alternative strategies to control it. The aim of this work was to study the ability of *L. plantarum* MPL16 and CRL1506 strains to control *E. faecalis in vivo*. First, we tested the effect of the feeding of *Caenorhabditis elegans* with LAB on its longevity. Usually, when nematodes are fed with *E. coli* OP50 they live 21-25 days but when they received *L. plantarum* MPL16 or CRL1506, their lifespan increased ~20%. When nematodes were fed with *E. faecalis*, they suffered intestinal distention and their progeny was affected: most of the eggs did not hatch and the larvae did not develop normally. Further, the survival decreased [lethality of 50% (L50) = 7 days]. When they received the pre-treatment with the CRL1506 and MPL16, the nematodes reached the L50 24 h and 48 h later, respectively, than the infection control. Further, 1 log reduction in enterococci CFU/mL of MPL16-treated nematodes was observed. The strain also restored the reproductive capacity lost by enterococcal infection. In contrast, *L. plantarum* CRL1506 did not reduce the bacterial load nor restored fertility. In addition, the effect of LAB on resistance to *E. faecalis* infection in mice immunosuppressed by malnutrition was studied. BALB/c malnourished mice were on a hypoprotein diet for 21 days, then, they were re-nourished for 7 days with a conventional balanced diet alone or supplemented with LABs. After renourishment, they were challenged with the *E. faecalis* 102. The infection was evaluated 48 h post-infection, by counting the CFUs in the intestinal lavage, feces, liver, spleen, and blood. The supplementation with LAB to the conventional diet increased resistance against infection by *E. faecalis* 102, which was confirmed by a significant decrease in the CFU counts in the tissue samples. In conclusion, the MPL16 and CRL1506 strains have probiotic potential to prevent or improve *E. faecalis* infections in both a vertebrate and an invertebrate model. Since the probiotic effect was only observed *in vivo*, we postulate that it may be related to the modulation of innate immunity and/or competition for nutrients and adhesion sites, but not to a direct antimicrobial action of the LAB. These strains have a great potential to be used for prevention of *E. faecalis* infections in susceptible hosts.

MI-51

Structure-based drug discovery in the lipoic acid salvage pathway against MRSA

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of healthcare-related infection worldwide. The increasing emergence of multidrug resistant strains urgently requires novel therapeutic approaches in order to keep the drug discovery pipeline filled. Lipoic acid (LA) is a universally conserved sulfur-containing cofactor required for intermediary metabolism, that is either synthesized de novo or acquired from environmental sources. In the model Gram-positive bacterium *Bacillus subtilis* LA synthesis involves four protein activities, instead of the two enzymes necessary in the Gram-negative bacterium *Escherichia coli*. First, the octanoyl-acyl carrier protein (ACP): protein-N-octanoyltransferase, LipM, transfers the octanoyl moieties to GcvH, the H subunit of the glycine cleavage system. Then, the lipoate synthase LipA inserts sulfur atoms into C6 and C8 of the octanoyl moieties. Finally, the amidotransferase LipL transfers the lipoyl side chain from GcvH to the E2 subunits of dehydrogenase complexes. Since lipoate ligase, LplJ, can only transfer exogenous lipoate to GcvH and E2o (the lipoylable subunit of oxoglutarate dehydrogenase), LipL is also required for modification of the remaining E2s. The bacterial pathogen *S. aureus* also employs this "lipoyl-relay" pathway for *de novo* biosynthesis and salvage. Furthermore, it encodes two additional proteins: a secondary lipoate ligase, LplA2, and a protein similar to GcvH, named GcvH-L, that which are sufficient for LA salvage during infection. Due to its essentiality for cell viability and virulence, interfering with LA synthesis represents a promising approach for treating *S. aureus* infections. In this work, we performed a phenotypic screen of different molecules that were identified by a virtual screen against *S. aureus* enzymes involved in LA salvage, LplA1 and LplA2. We selected a compound, lpl-004, that caused a marked growth inhibition of the WT strain. This effect was less severe in ΔlplA1 or ΔlplA2 single mutants. Furthermore, growth of the double mutant ΔlplA1 ΔlplA2 was not affected in the presence of the compound. Similar results were obtained using the LA analogue selenolipoate, a compound reported to block dehydrogenase activity in *E. coli*. Using protein extracts of different mutants of *S. aureus*, deficient in LA synthesis and uptake, we determined that lpl-004 would be bound to E2s. We can conclude that, by the sequential action of staphylococcal lipoate ligase and amidotransferase, lpl-004 would be accepted as a substrate and transferred to E2s, eventually impairing dehydrogenase activity. This compound would be useful for further drug development against this pathogenic bacterium.

MI-52

MUTAGENESIS INDUCED BY CONFLICTS BETWEEN REPLICATION AND TRANSCRIPTION MACHINERIES: CONTROL BY THE MISMATCH REPAIR SYSTEM IN *Bacillus subtilis*

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DNA replication and transcription use the same template and occur concurrently in bacteria. The lack of temporal and spatial separations of these two processes leads to conflicts between them. The transcription-replication conflicts (TRCs) have detrimental consequences on replication and cell viability, as well as they promote mutagenesis in highly transcribed genomic regions. The TRCs-induced mutagenesis is produced by the DNA synthesis catalyzed by low fidelity DNA polymerases (LF-Pols), which are call to action by the Mfd factor. This protein recognizes RNA Pols stalled at a DNA template lesion, and subsequently displaces it from DNA. This exposes the offending lesion to the base and nucleotide excision repair systems (BER and NER) that generate a gap, which is fill in by LF-Pols. Our previous findings demonstrated that the Mismatch Repair protein, MutS, controls the mutagenesis produced when LF-Pols perform translesion synthesis by regulating their access to replication sites through the processivity beta clamp factor in *Pseudomonas aeruginosa*. In the present study, we analyzed if this novel MutS-dependent mechanisms modulates the TRCs-mutagenesis induced by LF-Pols in *Bacillus subtilis*. With this aim, mutation rates in endogenous genes with low (thyA) and high (rpoB and rpsL) transcription levels were estimated in a mutSβ strain, which expressed a MutS mutant that does not bind to β clamp and therefore does not control LF-Pols, compared to the wild type (WT) strain. We found a significant increase in the mutation rates to resistance to rifampicin (target gene: rpoB, rifR) and streptomicine (target gene: rpsL, smR) in the mutSβ strain relative to the WT strain. In contrast, both strains showed similar mutation rates to trimetoprim resistance (target gene: thyA, tmpR). Then, we tested if the mutator factor Mfd and the LF-Pols, Pol I, PolY1 and PolY2, are involved in the increased mutation exhibited by the highly transcribed genes in the mutSβ strain. Inactivation of Mfd and Pol I specifically decreased mutation rates to rifR and smR but not to tmpR in the mutSβ genetic background. We also analyzed if the UvrA factor, which initiates the NER

pathway, is implicated in the increased mutagenesis observed in mutSβ. Deletion of the gene encoding this NER protein had no effect on the mutagenesis levels of the highly transcribed rpoB and rpsL genes. Similar results were obtained with the exogenous thyP3 reporter gen, which was placed under an IPTG-inducible promoter. In conclusion, these results suggest that MutS regulates the action of the low fidelity Pol I in the Mfd-dependent mutagenesis resulting from TRCs. Currently, we are analyzing the molecular signatures of this process by determining the mutation spectra of the endogenous (rpoB) and exogenous (thyp3) highly transcribed genes.

MI-53

BfmRS encodes a regulatory system involved in light signal transduction in the human pathogen *Acinetobacter baumannii*

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We have extensively shown that *Acinetobacter baumannii* senses and responds to light. Particularly, we have accumulated a large amount of evidence about the light signal transduction and physiological responses at moderate temperatures such as 23ºC, which are mainly but not only governed by the blue light using FAD (BLUF)-type photoreceptor BlsA. We have also shown that *A. baumannii* responds to light at 37ºC modulating virulence in an epithelial infection model using human keratinocytes in culture, as well as quorum sensing, through a BlsA-independent mechanism. However, the light signal perception and transduction components operating at this temperature were not yet identified.

BfmRS is a two component system described in *A. baumannii*, showing atypical characteristics mainly because the sensor, BfmS, appears to antagonize the functioning of the response regulator, BfmR, by dephosphorylation in response to yet unknown signals. Recent results suggest that phosphorylated BfmR (BfmR~P) is the active form of this response regulator that directly influences gene expression. BfmR can autophosphorylate *in vitro* using small phosphodonors such as acetylphosphate. BfmS contains two

membrane-spanning regions common to sensor kinases, and a C-terminal, cytoplasmic histidine kinase catalytic domain. The N-terminal extracellular region has no conservation to known sensors.

Several physiological responses have been shown to be modulated by BfmRS. BfmR controls pellicle and biofilm formation. BfmS supports increased tolerance to carbenicillin and controls production of outer membrane vesicles (OMVs) and OMV-mediated host cell cytotoxicity. BfmRS has been also shown to be involved in negative regulation of the CDI system. BfmR has been also shown to control tolerance to desiccation and BfmRS controls oxidative and osmotic stress, at the level of the cell envelope and resistance to different antibiotics. From analyses of ours as well as other researchers' data we observed that the BfmRS system and light integrate signals into the same pathways in *A. baumannii*. Furthermore, it has been shown that BfmR interacts with BlsA mostly in the dark. The overall evidence prompted us to study whether a connection exists between light modulation and the BfmRS system. In this work, we show that both components of the BfmRS system are involved in light modulation of motility at 37ºC in *A. baumannii* V15. On the contrary, light modulates desiccation tolerance through a BfmR-dependent BfmS independent pathway, both at moderate (23ºC) as well as at the normal temperature in mammals (37ºC). The overall data indicate that BfmRS is involved in light signal transduction in *A. baumannii*. Interestingly, this system inhibits blsA expression at 37ºC, thus governing the response to light at 37ºC. BfmRS also represses blsA expression at 23ºC, but to a lesser extent. Neither BfmR nor BfmS contain a traditional photoreceptor domain, and thus the most plausible possibility is that the system is sensing light indirectly, most probably as a result of differential metabolism arising under blue light vs. dark conditions.

Furthermore, we present evidence indicating that the BfmR~P form represses motility and mediates dessication tolerance in the dark, since mutation D58A in BfmR, which prevents it from becoming activated by phosphorylation, is unable to inhibit motility or tolerate desiccation in contrast to the wild type allele, when expressed from an inducible plasmid in a ΔbfmRS background.

MI-54

INCREASED INVASIVENESS AND PERSISTANCE OF HYPERMUTATOR *Pseudomonas aeruginosa* WITHIN LUNG EPITHELIAL CELLS

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Pseudomonas aeruginosa is an opportunistic pathogen that chronically infects the airways of patients with cystic fibrosis (CF). One mechanism that could contribute to the persistence and survival capacity of *P. aeruginosa*, in a changing and heterogeneous environment like CF´s lungs, is the ability to thrive in the intracellular environment of the eukaryotic cell. Despite *P. aeruginosa* is considered an extracellular pathogen, numerous studies showed that *P. aeruginosa* can be internalized in different cell types, including epithelial and endothelial cells. Also is important to mention that major traits such as biofilm growth mode and hypermutability are considered a source of adaptive phenotypes providing increased *P. aeruginosa* tolerance and resistance. Here we performed a long-term evolution experiment with hypermutator and wild-type strains of *P. aeruginosa* by carrying out successive reinfection assays, which consisted in using intracellular bacterial cells, recovered after antibiotic exclusion assays from A549 lung epithelial cells, as the inoculum for the next round of infection. In this way we performed 10 successive infection assays to evaluate and compare the ability of *P. aeruginosa* to invade and persist in the intracellular milieu of eukaryotic cells. Interestingly, we observed that after round 4 of infection, the recovery of intracellular hypermutator but not wild-type bacterial cells began to increase uninterruptedly until round 10.

The localization of bacteria inside eukaryotic cells was confirmed by laser scanning confocal microscopy. Furthermore, the use of flow cytometry and high-content imaging analyses showed an increase in the invasive capacity as we progress in the number of rounds of infection which was more pronounced in the hypermutator strain compared to the wild-type. Flow cytometry also allowed the evaluation of cytotoxicity by using a viability dye to label dead cells. Finally we characterized 10 different isolated clones of the hypermutator and wild-type strains to evaluate the diversity of the evolved population recovered from Round 10 by measuring the invasive capacity of each individual isolate. These results suggest that hypermutability plays an important role in this progressive adaptive process of *P. aeruginosa* to the intracellular milieu of eukaryotic cells. Further experiments such as whole-genome sequencing will be required to explore the molecular bases of this adaptive process, which might play a role in the evolution of chronic infections in the airways of CF patients.

MI-55

Growth medium Pi concentration affects stress resistance and metabolic traits of *Lactiplantibacillus plantarum* CRL 1905

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Lactic acid bacteria (LAB) are a group of microorganisms that have multiple biotechnological applications. LAB used in food production or as probiotics are subjected to harsh conditions derived from industrial processes or from the passage through the gastrointestinal tract. Thus, stress adaptation and survival play a crucial role in the robustness of strains. Polyphosphate (polyP) is a linear polymer formed by orthophosphate (Pi) residues, which is involved in response to stressful conditions in bacteria. The ability of *Lactiplantibacillus plantarum* CRL 1905 to maintain high levels of polyP in stationary phase was previously evidenced in a chemically defined medium (CDM) containing high Pi content (60 mM). In this condition, cells showed an increased viability through time, as well as an up-regulation of proteins involved in the main metabolic pathways, indicating an improved metabolism in late stationary phase. The aim of this study was to evaluate whether media Pi concentration affects metabolites production and stress resistance in *L. plantarum* CRL 1905. A HPLC analysis on supernatants obtained from cells grown for 24 and 48 h in CDM supplemented with 2 and 60 mM of Pi (CDM-P and CDM+P, respectively) was carried out, showing a higher lactic acid accumulation, as well as a faster glucose consumption by cells grown in the high Pi condition when compared to those grown in CDM-P. In addition, 48 h-cells grown in both media were subjected to several stressors (NaCl, H_2O_2, freeze-drying, and gastrointestinal tract simulation solutions). In all cases, an enhanced survival of the cells grown in high Pi medium in respect to those grown in CDM-P was observed. The intracellular polyP levels in CDM+P decreased after the exposure to different stressors, an already known survival strategy for bacteria. It is worth to mention that after freeze-dried process, CDM+P cells maintained their antimicrobial activity against pathogenic bacteria. Our results demonstrate that variations in media Pi concentration alter the production of main organic acids and resistance to different stressful conditions in CRL 1905 strain.

MI-56

THE ROLE OF THE HTRA PROTEASE IN THE PNEUMOCOCCAL SURVIVAL MECHANISM IN HOST CELLS

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Streptococcus pneumoniae is an important human pathogen and the causal agent of a variety of diseases, such as otitis media, sinusitis, pneumonia, and meningitis, especially among children. While causing infection, the pneumococcus encounters oxygen and its reactive derivatives at different concentrations depending on the stage of the infection process. Understanding

how the bacterium copes with reactive oxygen species in the host could facilitate the development of anti-infective agents against S. pneumoniae. HtrA (high-temperature requirement A) is a heat shock-induced serine protease that has both chaperone and proteolytic activities. It has been reported that HtrA could also have a role in the oxidative stress response of S. pneumoniae. Strains lacking the gene that encodes for HtrA showed decreased sensibility to exogenous hydrogen peroxide exposure, and this phenotype could be restored with antioxidants. To further investigate the role of HtrA in the oxidative

stress response, we tested its capacity to survive the oxidative stress found during intracellular survival in host cells. We created the ΔhtrA mutant using deletion mutagenesis, and we confirmed that the mutant ΔhtrA was more sensitive to exogenous H2O2 than the wild-type strain, as reported, being HtrA involved in the oxidative stress response of S. pneumoniae. Moreover, it has been described that CiaRH, a pneumococcal two-component system, is the main regulator of HtrA. To determine the effect of oxidative stress in the induction of htrA, qPCR was used to quantify the relative expression level of this gene in both the wild-type and ΔciaR strains. When exposed to 20mM H2O2, htrA transcripts showed a 3-fold increment in the wild-type strain. Although the ciaR mutation negatively impacted the expression of htrA compared to the wild-type strain, it still retained the capacity to increase its expression in response to H2O2, suggesting additional mechanisms involved. On another hand, intracellular survival of pneumococcal mutants was measured in A549 pneumocytes and RAW 264.7 macrophages. We found significantly decreased survival of the ΔhtrA and ΔciaR mutants in both cell types, being even more predominant in RAW 264.7 cells, probably because ROS levels are much higher in macrophages than in pneumocytes. When A549 and RAW 264.7 cells were previously treated with NAC (a known ROS inhibitor), the survival of ΔhtrA was similar to the wild-type strain, suggesting that this protein plays a key role in the defense of the oxidative stress response in S. pneumoniae. We propose that HtrA, which is part of the oxidative stress response in S. pneumoniae, has a relevant role in the pneumococcal survival mechanism in host cells. Thus, we propose that it could be a potentially good candidate for the development of novel therapeutics in the fight against pneumococcal disease.

MI-57

The use of a machine learning classification approach to predict plant tropism in model gammaproteobacteria

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The genus *Pantoea* has been described as a group of gammaproteobacteria isolated from a wide range of environments from terrestrial to aquatic, and in association with plants, fungi, insects, animals, and humans. *Pantoea* spp. show broad and different phenotypic characteristics within the genus, with species that can establish pathogenic or symbiotic relationships depending on the host with which they interact. The versatility and ubiquity of bacteria of this genus make it a group of interest to explore bacterial adaptation to specific niches and opportunism.

In our laboratory, bacteria of the genus *Pantoea* were isolated both from seeds and vegetative tissue of alfalfa plants. Several isolates had the ability to produce auxins, cellulases, siderophores, and also to generate phosphorus solubilization. With these studies as a cornerstone, our group already characterized the colonization of alfalfa by *Pantoea* sp. LPU 12 isolated from seeds.

Seeking to get a first insight into the genetic traits involved in the interaction of *Pantoea* with plants and making use of the large amount of information available in databases together with bioinformatics tools we decided to perform: (A) a taxonomic analysis of the genus level, and (B) a supervised machine learning (ML) classification of *Pantoea* to try predicting whether strains are potentially able to colonize plants. To perform both studies we downloaded 274 genomes and 143 metagenome-assembled genomes (MAGs) from the Integrated Microbial Genomes and Microbiomes System. We also included the genome of our model bacteria into this dataset. The genomes were classified (labeled) according to their isolation niche, and a phylogenetic tree was constructed with the GTDB-Tk tool. A trend for most plant-isolated isolates to be located in common or in neighbor clades was observed. Based on this observation, we decided to explore the performance of a ML approach to predict the plant vs non-plant tropism in the *Pantoea* genus. To do this, we first built up binary matrixes (presence or absence) and quantity matrixes (total number of particular genome features) describing for each genome its composition of COGs, KOs

and PFAMs (total of 6 matrixes). These matrixes together with an array of labels corresponding or not to isolates recovered from plants were used to train Random Forest Classification models. Hyperparameters were optimized to achieve the highest f1 scores, and the best model was determined resulting in a predictive power characterized by an accuracy and f1 score both ≥ 0.92 (COGs binary matrix). This work enabled us to select the best classifier features (specific PFAM domains, COGs and KOs that could potentially be involved in the ability to colonize plants) that will be used in forthcoming analysis to explore their functional role and ubiquity in another plant associated bacteria.

MI-58

Microbial community structure and CAZyome of kelp-amended intertidal sediments

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Intertidal sediments host highly diverse microbial communities, mostly living in biofilms on the surface of the sediment particles. These microbial communities play key roles in the carbon cycle of coastal ecosystems, including the degradation of the beached kelp biomass. Approximately 50 % of the dry weight of kelp biomass are polysaccharides, mostly fucoidans and alginates. The goal of this work was to explore the CAZyome profile of metagenomes from intertidal sediment from two sites of the Patagonian coast amended with kelp biomass. Triplicate experimental systems were built containing sediments of Ushuaia Bay with blade fragments of the native kelp species *Macrocystis pyrifera* (M) or sediments of Nuevo Gulf with blade fragments of the exotic species *Undaria pinnatifida* (U), in sterile seawater. These kelp species are abundant at each site. Metataxonomic analyses (16S rRNA gene fragments) showed a rapid reduction in richness (~ 40 %) and diversity (~ 23 %) indices, when compared with experimental controls without kelp addition. The microbial community structure was enriched in members of the Proteobacteria, Bacteroidetes and Verrucomicrobia phyla. Two metagenomes were generated by shotgun sequencing from M and U amended sediments. The metagenomes (908,438 and 1,006,370 protein coding sequences for M and U metagenomes, respectively) were analyzed using the Conserved Unique Peptide Patterns (CUPP) tool, resulting in the identification of 23,517 (2.6 %) and 21,501 (2.1 %) sequences of putative CAZymes from the following classes: Auxiliary Activity (AA), Carbohydrate

Esterase (CE), Glycoside Hydrolase (GH), Glycosyl Transferase (GT) and Polysaccharide Lyase (PL). Overall, 257 and 272 CAZy families were represented in M and U metagenomes, respectively. GH was the most abundant class in the metagenomes, with 43.21 % (M) and 48.41 % (U) of the sequences annotated into CAZy families. This class included 469 (M) and 453 (U) sequences assigned to families related to fucoidan degradation, mostly from the GH29, GH95, GH107 and GH168 families. The taxonomic assignment of these sequences showed that the majority probably originated in members of the *Flavobacteriaceae* family, although the PVC group (Planctomycetes, Verrucomicrobia and Kiritimatiellaeota phyla) was well represented in U-amended metagenomes. The PL class represented 6.16 % (M) and 5.50 % (U) of the annotated sequences, and included 13 of the 14 families containing alginate lyases (504 and 478 sequences for M and U, respectively). The sequences, mostly from the PL6, PL7 and PL17 families, were assigned to the Flavobacteriaceae, to Gammaproteobacteria and to the PVC and Terrabacteria groups. Overall, these results show the high diversity of enzymes encoded in the genome of bacteria with the potential to degrade fucoidans and/or alginates, and the taxa potentially involved in this process.

MI-59

TAM system is involved in permeability 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). Our previous work showed that the TAM (Translocation and Assembly Module) system is required for the integrity of OM of *Brucella suis*, an intracellular pathogen that belongs to the Alphaproteobacteria group. The aim of this work is to give insight into the role of the TAM system in the cell envelope biogenesis of Alphaproteobacteria plylum, using as model bacterium the opportunistic pathogen *Ochrobactrum anthropi*. A mutant in the tamB homologue locus of *O. anthropi* (Oant_0054) was generated and several cell envelope related phenotypes were analyzed. While some phenotypes were similar to those of the *Brucella* tamB mutant, others were different. The *O. anthropi* tamB mutant showed enhanced sensitivity to SDS 0.5% and a marked susceptibility to lysozyme. However, no differences were detected between the tamB mutant and the wild type (wt) strain after Triton X-100, DOC or EDTA treatment. Surprisingly, while the *Brucella* tamB

mutant had shown a marked sensitivity to polymyxin B, the *O. anthropi* mutant was even more resistance to polymyxin B compared to the wt and the complemented strain. Moreover, transmission electron microscopy (TEM) was performed in both strains and showed different structural characteristics of the membrane. While the wild-type strain exhibited defined edges of the OM, the mutant had a looser appearance. Taken together, these results show that while it is clear that the TAM system is crucial for the cell envelope biogenesis and homeostasis in Alphaproteobacteria, the impact of the absence of a functional TAM system depends on the characteristics of the cell envelope of each species, and in particular to those of the OM.

MI-60

STUDY OF BACTERIAL ISOLATES OF REGIONAL INTEREST AS POTENTIAL BIOCONTROL AGENTS AGAINST *Sclerotinia sclerotiorum*

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Sclerotinia sclerotiorum (Lib.) de Bary is a necrotrophic pathogen of great agricultural concern. It is known to be the cause of disease on more than 400 plant species, including many crops of economic importance. Particularly on soybean (*Glycine max*), it causes the stem rot disease and could be a determinant factor on significant yield losses. Biological control has been considered a promising approach for the treatment of diseases caused by certain phytopathogens. Antagonistic bacteria are known as biocontrol agents for decreasing soil born diseases of plants. In this study, an isolate of *S. sclerotiorum* was obtained from sclerotia collected from diseased plants of a productive field in the northwestern region of Argentina. Then, 60 bacterial strains isolated from soil and rhizosphere were screened as potential biological control agents against *S. sclerotiorum in vitro*. Using dual culture methods, 7 isolates all belonging to the genus *Bacillus* showed inhibitory activity against *S. sclerotiorum*. Nonvolatile compounds of all promising isolates could reduce the mycelial growth. On the other hand, no considerable effect was seen using cell-free supernatants nor testing for volatile compounds produced

by any of the studied isolates. Complementarily, biochemical traits associated with plant growth promotion were evaluated in order to further characterize the isolates that showed positive antagonistic activity against the pathogen. This study intends to find suitable bacterial candidates and set the basis for the development of an effective biocontrol agent against *S. sclerotiorum*.

MI-61

Outer membrane vesicles as vaccines candidates against Brucella suis infection

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Gram-negative bacteria produce outer membrane vesicles (OMVs) that contain biologically active proteins and perform diverse biological processes. In the last few decades the OMVs have been used in the development of acellular vaccines in a variety of pathogens both for human and veterinary use. *Brucella* is an intracellular pathogen capable of produce acute and chronic infections in wide variety of mammals and it causes a worldwide spread zoonosis called brucellosis. Our previous work showed that the TAM (Translocation and Assembly Module) inner membrane component (ΔmapB) is required for the integrity of the OM of *Brucella suis* and production of higher amount of proteins associated to OMVs fraction. In this work, we characterized the OMVs produced by *B. suis* M1330 (wt) and *B. suis* ΔmapB and evaluated their immunogenicity. OMVs were obtained by ultracentrifugation of clarified culture supernatants from both strains. The size of the vesicles was analysed by dynamic light scattering (DLS) and we found that they were similar (around 40 nm diameter) and stable when stored at 4ºC. In order to characterize the protein composition and differences in abundance, LFQ proteomic assay was achieved. 104 hits were detected in total and the vast majority corresponded to OM and periplasmic proteins. In particular, OMVΔmapB showed 14 hits differentially expressed which showed high predicted antigenicity. Female Balb/c mice were immunized intramuscularly (i.m.) with OMVwt, OMVΔmapB or saline at 0 and 30 days. One week after last immunization serum, bronchoalveolar lavage, feces and saliva samples were

obtained to measure OMV-specific antibodies. Vaccination with both OMVs induced serum specific IgG. Sera from OMVΔmapB vaccinated animals reached higher IgG titers than OMVwt group. In addition, OMVΔmapB mice showed high levels of serum specific IgG1, IgG2a and IgA, while in OMVwt vaccinated animals only low levels of specific IgA were detected. Serum specific antibodies from OMVwt and OMVΔmapB vaccinated mice reduced *B. suis* adherence and invasion to A549 cells, indicating the ability of the antibodies to neutralize *Brucella* infection. Moreover, serum obtained from both groups showed opsonizing capacity of antibodies since when preincubated with *Brucella*, CFU/ml obtained from lysates of infected macrophages was increased. Taken together these results show that vaccination with *B. suis* wt and ΔmapB OMVs induced systemic and mucosal specific humoral immune response, which may contribute to prevent *Brucella* mucosal entry and its dissemination.

MI-62

ANTIBACTERIAL ACTIVITY OF PYRIMIDINE DERIVATIVES WITHOUT DETECTABLE RESISTANCE

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Antibacterial resistance is an urgent concern in health care due to the lack of effective antibacterial therapeutic entities. The development of new antibiotics, particularly those with novel mechanisms of action (MOA) and that are active against multidrug-resistant pathogens, is of major interest in this context. Pyrimidine and its derivatives are known to have versatile pharmacological activities, especially as potential antibacterials. In this work, the antibacterial activity of a series of 28 synthetic pyrimidine derivatives was evaluated. These derivatives had been synthesized with a p-chlorophenyl or a naphthyl group as the hydrophobic tail, a pyrimidine unit as the backbone, a 1-phenylpropenone or a piperazine ring or an aminophenyl fragment as the linker chain joint to a quinolone moiety as the polar head. The antibacterial activity was determined against two Gram-positive bacteria: *Staphylococcus aureus* methicillin sensitive and resistant (MSSA and MRSA) and *Bacillus subtilis*; and two Gram-negative bacteria: *Pseudomonas aeruginosa* and *Escherichia coli*. Compounds 7b, 5b and 10a showed to be

effective against MSSA, MRSA and *B. subtilis*, being 5b the most potent against MSSA (MIC = 4 μg/mL) and *B. subtilis* (MIC = 8 μg/mL). Compounds 7b and 10a also inhibited *E. coli* growth, being 7b the most active (MIC = 16 μg/mL). Time kill curves were performed in MSSA and a bactericidal effect was observed for the compounds. The checkerboard assay was used to detect combination effects of active compounds and commercially available antibiotics against MSSA. Gentamicin and 5b combination resulted in synergistic antibacterial effects against this strain. Bacterial cytological profiling was performed in order to identify the MOA of these compounds. Bacteria treated with 7b showed smaller cells and a more condensed chromosome compared to untreated cells. Cell membrane staining in these cells was uneven showing increased fluorescence intensity in the cell poles. Also, membrane integrity was compromised since SYTOX Green fluorescence signal was detected. These cytological profiles resemble cells treated with compounds that disrupt cell membranes. Finally, it was found that these compounds could avoid development of resistance. This observation was supported by the fact that no resistant mutants of MSSA were isolated after 14 days of experimental evolution under the presence of different concentrations of each compound. These results suggest that compounds 5b, 7b and 10a could serve as promising leads for the development of potential antibacterial agents with low-level of resistance that probably affect the cell membrane.

MI-63

Molecular and biochemical characterization of an emerging pathogen in walnut (*Juglans regia* cv Chandler)

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The walnut represents one of the most valuable plants with an important role in the economy. However, different diseases can cause an important reduction of quality and production of walnut. In Argentina, cracks were observed in the bark of walnut trees (*Juglans regia* cv Chandler) in the province of Catamarca (27°53'57.30"S, 65°50'37.80"W) during the summer of 2020-2021.The cracks, found both on

trunks and branches, were accompanied by a soft rot with exudate. Intense leaf and shoot blight was also observed on the affected branches, which eventually died. On the trunks, the lesions reached different depths but managed to affect the plants' vascular system. A bacterial strain was isolated from the symptomatic tissues and named S2-FC. In the present study, the pathogenicity of this strain was investigated by injecting bacterial suspensions (10^8 CFU/ml from a 28 h LB culture) into the mesocarp of disinfected walnut fruits and under the bark of walnut branches. Non-inoculated fruits and branches served as the control. All the treatments were covered with polyethylene bags for 5 days to maintain high humidity, and kept under greenhouse conditions at temperatures ranging from 25 to 35ºC. Necrosis and reddish brown exudate started to appear at the sites inoculated with the strain as early as 3 days after inoculation. No symptoms were detected in the controls. Reinfection tests (following Koch's postulates) were carried out on immature fruits and on branches, and confirmed the strain's pathogenicity. S2-FC was classified as a facultatively anaerobic gram-negative rod, nonfluorescent on King's B medium. It elicited a hypersensitive response in geranium and formed white, mucoid colonies with well-defined edges on Tryptic Soy Agar medium (TSA). It tested negative for the hydrolysis of gelatin and starch, as well as for production of indole, urease, and H_2S. It was able to use glucose, sucrose, mannitol, lactose, raffinose, cellobiose and citrate as sources of carbon, and proved to have oxidative and fermentative metabolism. Furthermore, it could partially grow in media containing xylose, maltose and glycerol as sources of carbon, both under aerobiosis and micro-anaerobiosis. Pathogenicity and pectolytic enzymes activity tests were carried out on carrot and potato discs. They showed that S2-FC is highly virulent: maceration areas were visible at the infection sites 24 hours after initial infection. For genetic identification, a 1509-bp fragment of the strain's 16S rRNA gene was sequenced. The obtained sequence was compared to the reference sequences retrieved from GenBank. The results, which indicated a 99.7% similarity to *Pectobacterium carotovorum* subsp. carotovorum strain JR1.1 (CP034237.1), were later confirmed through their fatty acid profiles and bio-typed by MALDI-TOF. Additional epidemiological studies are being carried out to gain further knowledge about its behaviour in the areas where symptoms have been observed.

Lipids

LI-01

STARD3 and cholesterol transport in *Caenorhabditis elegans*

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Cholesterol is an essential metabolite present in virtually all eukaryotic organisms. It mediates highly relevant biological processes such as the regulation of membrane fluidity and the synthesis of steroid hormones and bile acids. Therefore, proper cholesterol trafficking to different subcellular locations is crucial for cell viability and proper organism functioning. Non-vesicular cholesterol transport is mediated by a multi-domain membrane protein called STARD3 that binds cholesterol through its cytosolic domain (START). Recent studies have reported that STARD3 co-localizes and interacts with methionine sulfoxide reductase A, an enzyme that reduces methionine sulfoxide side-chains, suggesting that methionine oxidation could modulate the sterol binding properties of the START domain of STARD3 and hence cholesterol transport. Our goal is to understand the regulation of non-vesicular cholesterol transport employing the nematode *Caenorhabditis elegans* as a model organism. By applying a set of biophysical and structural biology methodologies, we have characterized the cholesterol-binding properties of the START domain of C. elegans STARD3 and solved its crystal structure in the absence and presence of cholesterol, providing a high-resolution picture of a cholesterol-START complex for the first time. We propose that a conserved Met residue is involved in lipid binding. Further studies aimed to characterize the interaction between STARD3 and MSRA in vitro and in vivo will shed light on the possible role of this Met residue as a redox regulatory switch for cholesterol trafficking.

LI-02

A premetazoan origin of plasmalogen biosynthesis in animals

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Plasmalogens are glycerophospholipids with a vinyl ether bond at sn-1 position, which have unique physical-chemical properties. They are involved in the regulation of membrane functions and in important signaling pathways. Its synthesis evolved first in anaerobic bacteria but did not persist in facultative and aerobic bacteria that appeared after the rise of oxygen in the primitive Earth's atmosphere. Plasmalogen biosynthesis, now requiring molecular oxygen, later reappeared in animals (vertebrates and invertebrates), some protists and mixobacteria, but not in plants and fungi. The final and key step in plasmalogen synthesis is catalyzed by a 1´alkyl desaturase (PDES1). This, till very recently

orphan enzyme, was shown to be encoded by the *CarF* and *TMEM189* genes in mixobacteria and vertebrates, respectively. *Capsaspora owczarzaki* belongs to the clade *Filozoa* (*Filasterea, Choanoflagellata and Metazoa*), and is one of the closest unicellular relatives of animals. Its life cycle alternates between filopodial (adherent), aggregative and cystic stages. In this work we analyzed the fatty acid profile of each stage. This analysis also showed a progressive increase in the content of plasmalogen-derived aldehydes from the filopodial to the cystic stage. We found a highly reliable ortholog of PDES1 in *Capsaspora* but not in other members of *Opisthokonta*. A phylogenetic analysis revealed that the *Capsaspora* desaturase clusters together with *TMEM189*, indicating a single and unicellular origin for the reappearance of plasmalogen in animals, probably in the common ancestor of *Filozoa*. The finding of CarF/TMEM189-like desaturases and plasmalogen synthesis in *Amoebozoa* and *Excavata* protists should be ascribed to independent acquisition processes. This is the first report of plasmalogens in a non-metazoan *Filozoa*.

LI-03

PTEN knockdown or inhibition induces aberrant lumens at the lateral domain of renal epithelial cells

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Epithelial cell differentiation, characterized by apicobasal polarization and asymmetric expression of the proteins and lipids of the membrane, is essential for the development of functioning epithelial renal tubules. MDCK cells are a prototype of renal epithelial cell line widely used to study the polarized trafficking machinery used by epithelial cells to distribute their plasma membrane proteins into apical and basolateral domains. In these cells, the apical accumulation of the marker gp135 is necessary for a correct lumen formation. We have previously demonstrated that the correct localization of PTEN, a key enzyme in the metabolism of polyphosphoinositides, depends on glycosphingolipid metabolism. In this study, we used immunofluorescence and phalloidin-FITC stain to analyze by confocal microscopy the distribution of gp135 and f-actin in cells cultured under hypertonicity (inductor of cell differentiation) in the presence of SF1670, a selective PTEN inhibitor, or siRNA specific for PTEN. MDCK cells cultured under hypertonicity 48 h post-confluence developed a differentiated phenotype with typical cobblestone-like morphology defined by cortical actin fibers and apical accumulation of gp135. Cells cultured under hypertonicity treated with SF1670 revealed aberrant lumen formation between neighboring cells. Instead of at the

apical membrane, gp135 was found localized to these uncharacteristic lateral lumens, that were also enriched with F-actin. Similar results were observed in PTEN - knockdown cells. Confocal images showed that SF1670 - treated and PTEN - knockdown cells displayed elongated morphology and dissipation of actin cortex with evident stress fibers at the lower plane. Normally, MDCK cells organize their lumen at the apical surface, in contrast to hepatocytes, which form lateral lumen known as bile canaliculi. Either PTEN inhibition or depletion induces the mislocalization of gp135 at the lateral lumens, thus shifting the kidney epithelial phenotype of MDCK cells to hepatic polarity and denoting an important role of PTEN activity in MDCK cell differentiation. Due to GSLs synthesis being essential for the correct localization of PTEN, our study suggests that sphingolipid polarity and protein polarity are highly interconnected to accomplish apical-basal polarization of epithelial cells.

LI-04

Is the lipid metabolism involved in renal cell adaptation to damage by oxalate?

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Renal collecting ducts, involved in the urine concentration mechanism, are exposed to wastes coming from blood filtration, which includes several nephrotoxic agents, such as antibiotics, diuretics, antineoplastic and cytostatic agents, and renal stones. In this way, calcium oxalate stones are the most common type of kidney stone. Crystal aggregates are harmful to epithelial renal cells and tubular structures, and that damage could lead to the development of chronic kidney disease. Our previous results showed that differentiated renal cells treated with oxalate (Ox) for 24 h lost the typical epithelial cobblestone morphology and showed a spindle-shaped morphology characteristic of an epithelial-mesenchymal transition. After 48 h of Ox, cells started to recover their morphology and after 72 h of Ox, the epithelium was almost reestablished. Hence, the present study seeks to evaluate whether lipid metabolism is altered in Ox-exposed epithelial renal cells and its involvement in the epithelium restitution after 72 h. To do that, renal epithelial cells MDCK were grown in a hyperosmolar environment (512 mOsm/kg H2O) for 72 h to get a differentiated epithelium, and further were incubated for 24, 48, or 72 h in the absence or presence of 1.5 mM Ox. After treatment, cells were harvested, counted, and viability, morphology, and cellular lipid content and composition were evaluated. After 24 h, both phospholipid (PL) endogenous content and synthesis significantly increased in Ox-treated cells, while a tendency

towards higher values was observed in cholesterol (Cho) endogenous content and both triacylglycerides (TAG) endogenous content and synthesis. However, at longer times of exposure, no significant differences were observed neither in endogenous content nor lipid synthesis in Ox-treated cells with respect to the control condition. Microscopy images showed that Ox significantly increased the number of lipid droplets (LD) per cell after 24 h of incubation, but surprisingly these were more smalls. Whereas at longer times of exposure, Ox did not alter the number per cell nor mean area of LD. Taken together, obtained results indicate that the lipid metabolism would be involved during the first 24 h of Ox exposure. Nevertheless, further experiments are needed to follow deepening the study of such alterations and its impact on Ox-mediated damage.

LI-05

Sphingosine-1-phosphate receptor 2 (S1PR2) is essential in epithelial renal cells adaptation and differentiation

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Renal collecting duct cells are physiologically subject to an interstitial hypertonic environment, which is a key signal for renal physiology. High interstitial osmolality contributes to the differentiation of tubular structures and to the maturation of the urinary concentrating system in the developing kidney. We have demonstrated that, in hypertonic media, epithelial collecting duct cells (MDCK) acquire a fully differentiated epithelial cell phenotype. Epithelial cell differentiation is a process that involves mesenchymal-epithelial transition (MET) and includes cell cycle arrest, cell-cell junction maturation and changes in cell migration capacity. Sphingosine 1-phosphate (S1P) is a bioactive sphingolipid resulting from the phosphorylation of sphingosine by sphingosine kinases (SKs) which is involved in processes such as cell proliferation, growth, migration and differentiation. S1P can act both intracellularly as a second messenger or extracellularly as a ligand of 5 different G protein-coupled receptors (S1PR1-5). Only S1PR1, 2 and 3 are expressed in renal epithelial cells. In the present work, we evaluated the role of the S1P/S1PR2 pathway MDCK cell adaptation to hypertonic media and MET/differentiation. First, we evaluated the expression and localization of S1PR2 during epithelial differentiation and adaptation to hypertonic media. We found an increase in the expression of S1PR2 RNA and protein levels in differentiated cells (subjected to hypertonicity). Moreover, immunofluorescence studies showed that S1PR2 was progressively enriched on the plasma membrane during cell differentiation. We then evaluated the effect of S1PR2 antagonist JTE-013 on the acquisition of an epithelial differentiated phenotype. Immunofluorescence studies showed

that S1PR2 (but not S1PR1 or S1PR3) prevented adherens junction maturation and F-actin cortex formation, with a decrease in the protein levels of E-cadherin, beta-catenin, and alpha-catenin. Furthermore, cells were unable to adapt to hypertonic stress. Later we used a specific S1PR2 siRNA to corroborate our results. We observed that knockdown resembled the effect observed with the antagonist. In order to corroborate these results, we obtained a MDCK-S1PR2-KO line by CRISPR/Cas9 technology. MDCK-S1PR2-KO cells were unable to acquire a differentiated phenotype or adapt to a hypertonic medium. For these reasons, we performed a recovery assay where MDCK-S1PR2-KO cells were transfected with a S1PR2-EGFP plasmid. We observed that transfected cells recovered their adaptive capacity and presented a differentiated phenotype. In addition, recovery was blocked by JTE-013. These findings highlight the central role of S1P/S1PR2 in renal epithelial cell differentiation and tissue preservation in hypertonic environment-induced stress.

LI-06

The role of PLA2-COX2-PGE2 axis on renal epithelial restitution after calcium oxalate damage.

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The renal inner medulla is responsible for the hydro-saline equilibrium maintenance through water and electrolyte excretion in urine. The collecting ducts, involved in the urine concentration, are immersed in an extracellular matrix with the highest body osmolarity and are exposed to wastes coming from blood filtration. There are several nephrotoxic agents such as antibiotics, diuretics, antineoplastic and cytostatic agents, and renal stones. Calcium oxalate stones are the most common type of kidney stone. The crystal aggregates are harmful to epithelial renal cells and tubular structures, and that damage could lead to the development of chronic kidney disease. Our previous results showed that renal differentiated cells treated with oxalate (Ox) for 24 showed a spindle-shaped morphology characteristic of an epithelial-mesenchymal transition. After 48 h of Ox, cells started to recover their morphology and after 72 h of Ox, the epithelium is almost restituted. We also observed that Ox treatment modulates mRNA and protein Cyclooxygenase-2 (COX-2) expression and that the inhibition of COX2 with 10 μM NS398 does not allow epithelial restitution. The aim of the present work is to evaluate the role of the PLA2-COX2-PGE2 axis on

damage and the restitution of the renal differentiated epithelial after Ox damage. To do that, the renal epithelial cells MDCK were grown in a hyperosmolar environment (512 mOsm/Kg H2O) for 72 h to get a differentiated epithelium, and then subjected to 1.5 mM Ox for 24, 48, and 72 h. To inhibit COX2, 10 µM NS398 was added 30 min before Ox treatment; and to bypass the inhibition, 10^{-6} M PGE2 was added 30 min after Ox addition. After treatments, cell number and viability were evaluated, and epithelial morphology was assessed by fluorescence microscopy. The treatment with 10 µM NS398 + Ox caused a slight decrease in cell numbers at 24 h but not at 48 h. 10^{-6} M PGE2 addition did not affect cell numbers at 24 and 48 h. Cell viability did not change after all treatments. NS398 induced COX2 expression and the addition of PGE2 slightly decreased it. Cell treated with 10 µM NS398+Ox showed a cobblestone morphology, but gaps in the monolayer were observed. PGE2 addition to cells treated 10 µM with NS398 + Ox did not allow the EMT at 24 and 48h. Furthermore, PGE2 treated cells showed a morphology characteristic of renal epithelium (cobblestone). As PGE2 is the final product of PLA2-COX pathway PLA2 expression, COX and PLA2 activity were evaluated. The levels of PGE2, the main COX product in renal cells, increased significantly after 24 h of Ox treatment. Then, PGE2 levels started to decrease reaching control values at 72 h post Ox. The same profile was observed for PLA2 activity. The cPLA2 expression was also modulated by Oxa. The results showed that PLA2-COX2-PGE2 may be implicated in the restitution of the differentiated epithelia damaged with oxalate, but further experiments are needed to elucidate the molecular mechanisms involved.

LI-07

Effect of phosphatidylcholine on NSCs differentiation and proliferation under stress conditions

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Neuroinflammation is a common feature of many neurodegenerative diseases, and causes an imbalance in neural stem cells (NSCs) growth and differentiation, preventing important processes such as neurogenesis. Thus, it is essential to generate a favourable condition for NSCs and conduct them to differentiate towards functional neurons. Here, we show that inflammation has no effect on NSCs proliferation but induces an aberrant neuronal differentiation that gives rise to dystrophic, non-functional neurons. This is perhaps the initial step of brain failure associated to many neurological disorders.

Interestingly, we demonstrated that phosphatidylcholine (PtdCho)-enriched media enhances neuronal differentiation even under inflammatory stress by modifying the commitment of post-mitotic cells. The pro-neurogenic effect of PtdCho increases the population of healthy normal neurons. In addition, we provide evidences that this phospholipid ameliorates the damage of neurons and, in consequence, modulates neuronal plasticity. These results contribute to our understanding of NSCs behavior under inflammatory conditions.

LI-08

miR-33 alters cholesterol transport between astrocytes and neurons in aging.

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The brain is the most cholesterol-rich organ in the human body, containing about 25% of the body's total cholesterol. In neurons, cholesterol has been shown to play a critical role in neurite growth, synaptogenesis, and the proper function of pre and post-synaptic compartments. Thus, the cholesterol homeostasis has to be tightly regulated in the brain in order to avoid potential imbalances which will have severe consequences to brain performance.

Previous reports from our laboratory indicate that the decrease in neuronal cholesterol levels in aging would be related to the development of cognitive problems. Due to the incapacity of cholesterol to cross the blood-brain barrier, the brain cholesterol homeostasis is strictly controlled through synthesis *de novo*, mainly carried out by glial cells. Mature neurons depend mainly of cholesterol synthesized by astrocytes, which is imported in the form of ApoE-Cholesterol complexes. Once endocytosed, the cholesterol is released from the endolysosomal system by the cooperative action of the Niemann-Pick Type C proteins 1 and 2 (NPC1 and NPC2), which allow the incorporation of this lipid into the intracellular pool.

In this work we show that aging results in increased miR33 which triggers a Niemann Pick phenotype in senescent astrocytes which accumulate cholesterol in lysosomal compartments. Furthermore using astrocyte-neuron cocultures we found that the cholesterol delivery from astrocytes to neurons is also impaired in astrocytes aged in vitro. Interestingly, cholesterol accumulation in aged astrocytes could be alleviated by endocannabinoid treatment. We believe that understanding these mechanisms will allow

the identification of new targets for therapies or prevention of central nervous system pathologies associated with aging.

LI-09

Oxidative stress and lipolysis: new insights in fat metabolism

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Prolonged oxidative stress (OS) directly affects fat metabolism, with implications in the onset of obesity, insulin resistance, and type 2 diabetes. Particularly in adipocytes, it is well known that OS participates in several mechanisms related with proliferation and differentiation. Our aim was to study the signaling events underlying lipolysis triggered by OS. For this purpose, we worked with different adipocyte in vitro cultures (differentiated 3T3L1 and mesenchymal stem cells) and with an in vivo model, all subjected to iron-induced OS.

3T3L1 adipocytes challenged with ferric ammonium citrate (FAC, 500-1000 μM) displayed augmented lipid peroxides and membrane permeability when compared with non-treated cells. The increase in OS markers observed in 3T3L1 adipocytes was coincident with a rise in glycerol release to the medium. These results were also corroborated in the in vivo model, where a decreased neutral lipid content in gonadal adipose tissue of iron-treated mice was observed. In addition, iron-treated animals presented a different architecture of gonadal fat characterized by cell shrinkage, decreased volume tissue, and fibrosis.

Lipolysis in the white adipose tissue of humans and rodents is a step-wise process regulated by adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoacylglycerol lipase. Exacerbated lipolysis was accompanied by the upregulation of β-catenin expression in 3T3L1 and in gonadal adipocytes. To ascertain the role of this signaling pathway in OS-induced lipolysis, we worked with adipocytes differentiated from primary mesenchymal stem cells with wild type expression or deletion of β-catenin gene. To this end, stem cells were isolated from outer ears of β-catenin fl/fl mice and after differentiation to adipocytes, gene deletion was induced with adenoviral Cre recombinase. The expression of the lipolytic enzymes, *ATGL* and *HSL*, was evaluated by qRT-PCR in wild type and β-catenin knock out (β-catenin KO) adipocytes exposed to vehicle or FAC. In wild type adipocytes, iron exposure increased *ATGL* and *HSL* mRNA levels, whereas lipolytic enzyme expression remained unchanged in β-

catenin KO cells. Our results demonstrate that iron-induced OS is able to activate lipolysis through a mechanism involving the β-catenin pathway in fat cells.

LI-10

Excess lipids effect on mitochondrial function in inflammatory cells

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Mitochondrial–nuclear signalling plays a crucial role in cellular homeostasis, and the disruption of the interplay between mitochondria and nucleus contributes to ageing, age-related disease, and chronic inflammatory diseases. We reported that cholesterol mediates the mitochondria-to-nucleus retro-communication as ROS and Ca^{2+} do. Under stress, the enrichment in the number of contact zones between the nucleus and mitochondria results in an increased amount of cholesterol transported by TSPO (the Translocator protein), stabilising NFkB and its pro-survival response and so allowing the evasion of cell death and mitophagy. On the contrary, TSPO deficiency significantly decreased the mitochondrial membrane potential, mitochondrial oxidative phosphorylation (OXPHOS) and ATP production. TSPO is widely used as a biomarker of inflammation in the nervous system based on its marked upregulation in activated microglia. Moreover, TSPO deficient microglia cells are less prone to be stimulated by LPS or IL4.

Later, we evaluate how intracellular fatty acid accumulation affects mitochondrial functioning and mitochondria-to-nucleus retro-communication. Fatty acids (FA) are essential regulators of mitochondrial structure and function because they are structural components in mitochondria membrane phospholipids, oxidative substrates (beta-oxidation), inhibitors of pyruvate dehydrogenase, and ligands for nuclear receptors that regulate the expression of mitochondrial proteins. They also up-regulate the expression of genes involved in mitochondrial fatty acid metabolism. Lipid accumulation results from an increased *de novo* synthesis, an increased lipid uptake, or lipolysis regulation. We use an in vitro cellular model for RAW 264.7 cells, which emulated foam cells. Since free fatty acids are toxic, their

concentration was optimised to reduce their toxicity. We tested oleic acid (w-9 monounsaturated) at different combinations and timing. In addition, we evaluated cellular and ROS biology changes at different fatty acid treatments. Finally, we followed the changes in morphology using flow cytometry on Scatter and Fluorescent signals, oil red absorbance at 492 nm, protein expression by western blot and gene expression for mitophagy precursors.

LI-11

Glycerol-3P-acyltransferase 2 counteracts the apoptotic effect of arachidonic acid and modulates the expression of genes that influence cell migration and invasion

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Glycerol-3P-acyltransferase 2 (GPAT2) features the most dissimilar expression profile among all other GPAT enzymes found in mammals, restricting its normal function mainly to sperm cells and away from classic lipogenic tissues, in contrast to GPAT1, 3, and 4. Surprisingly, we also found a role for our gene of interest in cellular models of breast and colon cancer, where it contributes to the establishment of a more tumorigenic scenario through the stimulation of cell proliferation, anchorage-independent growth, and tumorigenic features both in-vitro and in mouse experiments. We have also shown that GPAT2 gene is able to act via the modulation of several cancer-related metabolic pathways and both short and long non-coding RNAs.

With the aim of getting new insights into the cellular mechanisms linking high GPAT2 expression to increased tumor-related phenotype on MDA-MB-231 human triple-negative breast cancer cells, we studied the changes in invasion and migration behaviors induced by this gene at the cellular and genetic levels. Because the increase in the apoptotic index in response to arachidonic acid (AA) is one of the most relevant modifications after GPAT2 silencing, we analyzed those pathways implicated in AA usage and investigated the modulation of the activity of caspases belonging to extrinsic and intrinsic apoptotic pathways.

We found an alteration in the expression of genes involved in extracellular matrix degradation and cytoskeleton organization which can explain, at least partially, the increase in invasion and migration

properties for cells expressing GPAT2 at a higher level. Our results also show a modulation on the expression levels of genes coding for AA metabolism and apoptosis-related enzymes that correlate to a diminished apoptotic effect of AA supplementation for those cells expressing higher GPAT2 amount.

Altogether, these results provide new data supporting the consideration of GPAT2 expression as a poor prognostic factor for breast cancer patients.

LI-12

Role of acyl-Coa synthetase 4 in epithelial ovarian cancer

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Acyl-CoA synthetase 4 (ACSL4) is an enzyme taking part in the fatty acid metabolism. ACSL4 plays a key role in arachidonic acid metabolism and in steroidogenesis. We and others have described the role of ACSL4 in breast and prostate cancer. Particularly, in triple-negative breast cancer (TNBC) and in castration-resistance prostate cancer (CRPC), increased ACSL4 levels are associated to the promotion of a highly aggressive tumoral phenotype. We developed a specific ACSL4 inhibitor, PRGL493, and characterized its inhibitory effect in TNBC and CRPC in steroidogenesis, chemotherapeutic resistance and tumor growth. Since epithelial ovarian cancer (EOC) is often diagnosed in advanced stages, the available treatments are limited and the prognosis is poor. EOC is the third cause of gynecologic malignancy but the first one of dead. Of the total of the ovarian primary tumors, the 90% corresponds to EOC. Sex-steroid hormones may have a relevant role in the development and progression of EOC. Given the need to develop new effective therapeutic strategies, the aim of this work is to study the role of ACSL4 in EOC. In previous works we have studied the gene expression signature of ACSL4 by RNA-seq, finding that ACSL4 regulates genes associated with an aggressive phenotype such as invasion, migration, proliferation, drug resistance and signal transduction. Therefore, we performed a bioinformatics analysis comparing ACSL4 signature with EOC genetic signature of patient samples obtained from public databases. Cross analysis showed a positive correlation coefficient higher than 0.5 for 38 of 42 the genes, with the correlation coefficient being 0.46 for drug resistance-associated protein genes. Immunohistochemistry was performed on biopsies obtained from patients with EOC. The analysis showed increased levels of ACSL4

in the EOC human tissue samples compared to normal tissue samples. Subsequently, the expression of ACSL4 was compared between EOC cell lines (A2780, OV-90 and SKOV-3) and the non-tumor cell line HOSE. Western blot analysis showed an increased levels of ACSL4 in EOC cell lines relative to HOSE cells. Then, the inhibitory effect of PRGL493 was tested on EOC cell lines by performing MTT and BrdU proliferation assays. Incubation in the presence of PRGL493 produced a significant decrease in cell proliferation in A2780, OV-90 and SKOV-3 cell lines compared with the incubation with vehicle as a control

The IC50 value of PRGL493 was approximately 40 µM for EOC cell lines, being similar to the IC50 value previously obtained for breast and prostate cancer cell lines. These results allow us to conclude that ACSL4 is involved in EOC ovarian tumor biology and also allow us to conclude that ACSL4 could be a therapeutic target in EOC ovarian cancer.

LI-13

Inhibitors of photosystem II increase the expression of chloroplast diacylglycerol acyltransferase-3 and promote triacylglycerol accumulation in *Chlamydomonas reinhardtii*

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Considerable progress has been made towards the understanding of triacylglycerol (TAG) accumulation in algae. A key aspect is finding conditions that trigger TAG production without reducing cell division. We identified a soluble diacylglycerol acyltransferase (DGAT), exclusive to the green lineage and moderately related to plant DGAT3, with heterologous DGAT activity. We demonstrated that DGAT3 localizes to the chloroplast in the model green alga *Chlamydomonas reinhardtii* and that its expression is induced by light in the presence of acetate, consistent with TAG accumulation. The light dependence and the presence of an iron-sulfur cluster-binding domain (2Fe-2S) in the sequence of DGAT3 indicates that this protein could accept electrons, directly or indirectly, from the photosynthetic machinery. The aim of this study was to investigate the relationship between DGAT3 expression and photosynthetic electron transport. With that purpose, we incubated *C. reinhardtii* wild type cc-125 cells with two photosystem II (PSII) inhibitors, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and 2,5-dibromo-6-isopropyl-3-

methyl-1,4-benzoquinone (DBMIB), and one photosystem I (PSI) inhibitor, N,N′-dimethyl-4 4′-bipyridinium dichloride (paraquat). Both *Dgat3* mRNAs and TAGs increased in DCMU and DBMIB-treated cells as early as 15 minutes after initiating the experiment, whereas no significant variations were observed in paraquat-treated cells in the course of a 3-h incubation period. Our results suggest that DGAT3 expression and TAG biosynthesis increase when PSII is over-reduced in order to avoid photodamage, as TAG is an adequate molecule to store excess electrons. PSII over-reduction occurs naturally in situations in which light absorption is higher than the rate of photosynthesis (e.g. during illumination at high light intensity) or artificially in chemically-altered PSII centers. Currently, this hypothesis is being evaluated in mutants that have deficiencies in the function of PSI, PSII or the cytb6f complex.

LI-14

Nuclear Lipid droplets in Oenocytes cells from *Triatoma infestans* insects are a dynamic nuclear organoid

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The objective of this work was to characterize nuclear populations of Lipid Droplets (nLD) in *Triatoma infestans* (vinchuca) under different development conditions. This hematophage insect is one of the main vectors of the parasite *Trypanosoma cruzi*, the causative agent of Chagas disease in Argentina and the Americas. The cuticle (C) is the insect most external structure, which protects against physical, chemical (dehydration, etc.) and biological (infections, etc.) external factors. Oenocyte cells (OE) are involved in the anabolism of C hydrophobic molecules (hydrocarbons, alcohols, waxes, glycerides, fatty acids, etc). The fat body (FB) is the organ that regulates the entire insect metabolism. The information on the lipid metabolism of the insect will allow the acquisition of new tools to control the vector. Taking into account the scarce information on OE from C in *Triatoma infestans*, the aim of the work was to characterized the LD populations in these cells as organoids involved in the genesis of the cuticle. Previously, we demonstrated that in liver, LD populations are dynamic organelle where neutral lipids are stored, mainly located in the cytosol (cLD) and in a small proportion in the nucleus (nLD). For this purpose, protocols were developed and optimized to identify and characterize LD populations in the different cells beneath the cuticle. We examined and characterized the LD populations of OE cells from fifth instar nymphs of the insect that were feed or starved for 1 month. Light field microscopy and fluorescence

(epifluorescence and confocal) and hematoxylin / Oil Red and DAPI / BODIPY stains were used, respectively. In OE cells the main LD population is located in the cytosol and a small population within the cell nucleus (nLD) in both conditions, feed and starved insects. These results would confirm the role assigned to OE to actively participate in the anabolism of the cuticle components.

In conclusion, *Triatoma infestans* cuticular oenocytes were characterized as cells that have a very varied morphology, depending on the development state of the insect, and are larger than the surrounding epithelial cells. The OEs have two LD populations in both conditions tested, a main cytosolic and a nuclear one. These are the first results where nLDs are described in insects.

LI-15

Progressive accumulation of n-9 PUFAs in testicular lipids during *ex vivo* tissue maintenance

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Spermatogenesis has been achieved in vitro using a gas-liquid interphase culture system. In this setting, it is possible to follow lipid metabolism to know its role during the spermatogenic process, thus gathering potentially useful information for ex vivo spermatogenesis biotechnology. We observed a relationship between the progression of spermatogenesis in both, in vivo and *ex vivo*, at cytological and histological level and the gene expression of enzymes involved in fatty acid desaturation, elongation and transport. The aim of this study was to examine and extend whether developmental changes that occur in vivo in testicular lipids with long-chain (C18-C22) and very long-chain (≥ C24) polyunsaturated fatty acids (PUFA) also occur *ex vivo* in explants of neonatal testes in culture. Explants from 6-day old mice cultured for 22 days showed that differentiation proceed from spermatogonial stem cells up to haploid round spermatids. Notably, after 44 days in culture we were able to detect some spermatozoa, although still scarce. Interestingly, like *in vivo*, total lipids from explants increased their proportion of PUFA during the period in culture. In addition to the common n-6 and n-3 PUFAs (22:5n-6, 22:6n-3, 24:4 and 24:5) we observed that unusual n-9 PUFAs (20:3n-9 and 22:4n-9) were accumulated in the explants. The 22:5n-6/20:4n-6 ratio in the glycerophospholipids was increased during the first 22 days associated to the appearance of haploid spermatids and then it remains unchanged until day 44. In addition, we noted that in culture the testicular tissue accumulated neutral lipids, mainly, triacylglycerides (TAG) and cholesterol esters (CE) that contained a high proportion of n-9 PUFAs. *In vivo*, the increase of neutral lipids

occurs in testicular tissue associated with spermatogenesis impairment. The presence of the unusual fatty acids of the n-9 serie in the explants suggests that the culture system does not ensure the provision of essential fatty acids to the tissue. Addressing this point could improve the rate of gamete production in this system. Supported by SGCyT UNS-PGI-UNS [24/B272 to GMO], FONCyT, [PICT2017-2535, PICT2020-02056 to GMO].

LI-16

HDAC2 mediated repression of BDNF in old hippocampus triggered by cholesterol loss

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Aging is associated to epigenetic alterations which lead to diminished expression of memory-related genes. One of the main alterations in the aging brain is cholesterol loss. This lipid is capable of interacting both with sphingolipids and proteins and thus play a key role in Membrane Lipid Rafts formation (MLR), key structures for proper function of some receptors such as the NMDAR. The cholesterol loss during aging reduces MLR formation and impairs proper NMDAR activation and synaptic-activity dependent transcription of memory-genes. Contributing to this process, a decrease in histone residues acetylation due to Histone Deacetylase 2 (HDAC2) accumulation has been observed. This particular event is determinant for memory loss during aging. In this work, we found that aging triggers the accumulation of HDAC2 in promoters II and VI of the BDNF gene, a key transcription factor for synaptic plasticity, learning and memory formation. We found that the transcriptional co-repressor Chromodomain Y like protein (CDYL), which interacts with HDAC2 in hippocampal extracts, is accumulated in the nucleus of old neurons. In addition, the co-accumulation of CDYL and HDAC2 was observed in neurons of transgenic Thy-1(GFP) mice brain slices and after cholesterol oxidase treatment in 14-DIV rat hippocampal neurons. Taking into account that has been reported that CDYL degradation is triggered by synaptic activity, and we observe a decrease in CDYL mean fluorescence intensity after NDMA stimulation, we propose that CDYL accumulation can occur as a consequence of impaired NMDA receptor activation due to reduced MLR formation. The findings of this work contribute to the understanding of the epigenetic mechanisms underlying synaptic impairment during aging.

ST-05

Study of PDK1 regulation by metabolites and interaction with kinase substrates

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Protein kinases are very important ON-OFF switches in cells. Dysregulation of their activities often lead to diseases, such as cancer or diabetes. Therefore, protein kinases are tightly and selectively regulated. Phosphoinositide-dependent protein kinase 1 (PDK1) is a master AGC kinase of the PI3K signalling pathway that phosphorylates at least other 23 AGC kinases in mammals, including isoforms of Akt/PKBs, S6Ks, SGKs, PKCs, PRKs, among others. However, PDK1 is present not only in mammals but throughout the eukaryotic evolution with orthologs in plants, yeasts and protozoa. Over the years, our laboratory used a chemical and structural biology approach to study and characterize in detail the regulation of PDK1. We demonstrated an allosteric regulation within the protein kinase domain, from a regulatory site termed PIF-pocket to the active site, as well as the existence of the reverse process. This bidirectional allosteric mechanism of regulation between both pockets can be modulated by small molecules that bind to the active orthosteric site and either enhance or inhibit the interaction at the PIF-pocket allosteric regulatory site. As a probe directed to the PIF-pocket, we employed the polypeptide PIFtide, which corresponds to the last 23 residues of the protein kinase PRK2. We showed that the metabolite adenosine can enhance the interaction of PDK1 with PIFtide and therefore asked ourselves if other metabolites could bind at the active site of protein kinases and physiologically regulate the formation of protein kinase complexes. In this work we present the effect of diverse metabolites and compounds on the allosteric interaction of PDK1 with the polypeptide probe PIFtide and the interaction between PDK1 and PDK1 substrates. We also validate and analyze the binding mode of such metabolites by obtaining the crystal structures of the catalytic domain of PDK1 in complex with them. Finally, we will discuss the potential impact of the studies on new

mechanisms by which metabolites binding at the active site could allosterically modulate protein kinase conformations, formation of protein kinase complexes and thereby, be modulators of cellular signalling.

ST-06

S-palmitoylation: a new layer of regulation for Akt

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Akt/PKB protein kinase is involved in a wide variety of physiological processes, including cell metabolism, proliferation, and survival, as well as pathological processes such as viral infection and malignant transformation. Thus, it is not surprising that different clinical trials are underway to test the efficacy of a variety of inhibitors of the Akt pathway as anticancer and antiviral treatments. In recent years, new Akt post-translational modifications (PTMs) have been found, which have been reported to affect its activity. However, it is not fully understood how these modification patterns affect certain key features of Akt such as its target specificity, localization, and function. Our hypothesis is that the molecular code of Akt, that is, the profile of Akt PTMs, can determine its subcellular localization and vice versa, regulating Akt function.

In the present work, we experimentally demonstrate that Akt can undergo S- palmitoylation, a PTM related to protein sorting through subcellular membranes, which has been shown to affect several oncogenic proteins. Using a strategy that combines the use of palmitoylation-deficient Akt mutants, palmitoylation inhibitors and fluorescence imaging of human cell lines, we show that this PTM affects Akt localization as well as its activation. On the other hand, by using an in vitro adipocyte cell model, we demonstrate that S-palmitoylation is involved in the differentiation of these cells. Finally, by using flow cytometric techniques, we show that blockade of S-palmitoylation affects essential cellular processes such as death and survival in different human cell lines.

Understanding the relationship between the molecular code and the cell's decision-making brings us closer to understanding how these PTMs influence the development and progression of diseases such as cancer. In particular, our data support a role for Akt S palmitoylation in the phosphorylation, localization, and function of this kinase, influencing key processes such as cell differentiation and death.

ST-07

Amino acids shorten the chronological longevity of yeast through changes in the accumulation of storage carbohydrates

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In most living organisms, aging is characterized by a deterioration in physiological integrity and an increase in susceptibility to disease and death. Aging is the main risk factor for most diseases and conditions that limit longevity. Nutrient metabolism plays an important role in longevity. Caloric restriction is one of the non-genetic interventions that extends longevity in a variety of species. It is a type of dietary intervention in which calories are reduced while maintaining an adequate level of macronutrients. In yeast, caloric restriction, which is achieved by decreasing the concentration of glucose in the culture medium, also increases longevity. When the intervention being performed involves fasting other macronutrients such as protein, the intervention is called dietary restriction. In particular, reducing the availability of amino acids in the diet also increases longevity in yeast. The aim of this work was to study the mechanism by which dietary amino acids shorten longevity. Previous results showed that Gcn4, a central transcription factor in the general amino acid control pathway, promotes longevity by maintaining not only cellular proteostasis but also modifying cellular carbon flux.

We analyzed mitochondrial activity in yeast cells grown in the absence or presence of amino acids. To do this, cells were treated with the MitoTracker Red CMXRos reagent and visualized using confocal fluorescence microscopy. The accumulation of the mitochondrial marker did not vary significantly with the addition of amino acids, suggesting that they do not generate changes in mitochondrial activity.

We also measured glucose consumption and ethanol production in cells under both growth conditions. While the presence of amino acids caused a decrease in glucose consumption, the ethanol formation did not change. On the other hand, we also measured the glycogen accumulated in stationary phase cells. We found that amino acids lead to a decrease in this storage carbohydrate accumulation. Measuring these same parameters in cells deficient in Gcn4, we found that this mutant consumes glucose to the same extent as the wild type; however, it produces significantly less ethanol, accumulates

less glycogen, and has increased respiratory activity. Storage carbohydrates, mainly glycogen and trehalose, are used as energy sources during the stationary phase. As a consequence, mutants unable to accumulate or utilize them have a significantly shorter lifespan longevity. The decreased ability to accumulate glycogen generated by the addition of amino acids to the growth medium, as well as by the lack of Gcn4, could be the cause, or at least one of them, determining the short longevity.

ST-08

DOWNREGULATION OF KLF6 TUMOR SUPPRESSOR IN CANCER CELLS EXPRESSING MUTATED RAS AS ONCOGENIC DRIVER INCREASE THEIR VULNERABILITY TO CHEMOTHERAPY BASED ON ROCK INHIBITION

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Early induction of KLF6 tumor suppressor following mitogenic stimulation mediated by growth factors and/or oncogene activation works as negative feedback to attenuate the extent of growth-promoting signaling. This function is characterized by a cell cycle arrest mediated by KLF6 which contributes to a decrease in both, cell proliferation and oncogenesis triggered by activated Ras, functions compatible with a tumor suppressor. However, during the cell cycle arrest imposed by KLF6, cells are more resistant to cell death mediated by chemotherapy drugs based on DNA damage agents or on mitotic stress, suggesting that alleviation of the early KLF6 induction would be required for efficient toxicity of this type of chemotherapy drugs. Accordingly, KLF6 silencing in cancer cells expressing mutated Ras as oncogenic driver (HCT116), increases the susceptibility to fasudil, a chemotherapy drug for cancer treatment based on inhibition of the Rho-associated protein kinases (ROCK1/2), enzymes playing a key role in cytokinesis and cancer progression. Additionally, KLF6 silencing per se leads to the accumulation of DNA damage, increased p53 expression, and signs of genomic instability. Hence our hypothesis is that KLF6 downregulation following its early induction will enhance cell vulnerability upon cell treatment with fasudil. More importantly, tumor cells expressing mutated Ras as oncogenic driver should be more

susceptible than normal cells due to the proliferation stress, enhanced glycolisis and overload of the DNA repair system imposed by the mutated Ras signaling.

Here we show that high doses of Vitamin C, in addition to produce oxidative stress, is able to downregulate KLF6 protein expression, increasing the vulnerability of HCT116 cancer cells to fasudil. These results suggest that the combined treatment with Vitamin C and fasudil could lead to increased toxicity of cells expressing mutated Ras, which is accompanied by a decrease in KLF6 protein level. Given no efficient therapy targeting mutated Ras has been successful to date, due to its non-pharmaceutical target status, these results open possibilities for the design of new therapeutic strategies, based on the downregulation of a tumor suppressor, such as KLF6, along with ROCK inhibition.

ST-09

AN INTEGRATED BIOINFORMATICS APPROACH STUDY OF 25-D 1 ALPHA-HYDROXYLASE REGULATION IN PROSTATE CANCER

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Prostate cancer, the second most common cause of cancer-related death in men, is an heterogeneous disease, evident in the spectrum of molecular anomalies and their variable clinical course. The role of 25-D 1-alpha hydroxylase (CYP27B1) in this cancer, as well as the levels of it in this pathology, are controversial. This work aimed to evaluate the gene expression of CYP27B1 in prostate cancer cells of the patients of the PRAD project (Prostate Adenocarcinoma TCGA, Cancer Genome Atlas), and unravel its regulation. To achieve these goals, a systems biology approach was carried out combining differential expression analysis, enrichment in canonical biological pathways, protein-protein interaction (PPI) and regulatory network inference with differentially expressed genes. As well as identification of kinases associated with key genes in the regulation of CYP27B1. Moreover, information was collected on prostate cancer pathways, with frequently altered genes, namely: point mutations and variation in the number of copies (CNV) in them.

Exploration of all the patients showed a significant increase of the CYP27B1 in patients with cancer concerning the controls. When it was analyzed the distribution of values of CYP27B1, it was possible to

describe an exponential distribution. For a first exploration, the patient samples were divided according to their CYP27B1 level into Low(L) and High-Medium(H-M) groups, with the 1st and 2nd quartiles approximately as limits. Differential expression analysis was performed finding a total 813 genes differentially expressed in group H-M and 374 genes in group L. The enrichment analysis in Kegg pathways and GO terms showed remarkable differences in the number of genes mapped and the routes represented. For the L group we found that it was only significantly enriched in the Kegg term serotonergic synapses. For the H-M, instead, we found genes were mainly enriched in kegg pathways related to Drug metabolism, chemical carcinogenesis, and cell cycle. In order to gain insight into the CYP27B1 regulation, a gene regulatory network was induced with Genie3 with the H-M genes as input. The last network was split out in Clusters with MCL algorithm. Of the five obtained, annotation was carried out with gprofiler. Of these, cluster 2 hosts CYP27B1 and was enriched in Kegg terms associated with Cardiac muscle contraction and cardiomyopathy. Cluster 3 in turn, was rich in genes involved in cell cycle, oocyte maturation and progesterone-mediated oocyte maturation. Based on its annotation and bibliographic support, this cluster could be considered a driver-subnetwork cancer, besides, many of the genes included in it, in turn stimulates the activity of CYP27B1. With the analysis centered on the CYP27B1 node, it could distinguish two groups of genes based on positive or negative correlation with CYP27B1 transcript level: Activators and Repressors. The validity of the assignments was made by the search of Transcription Factors (TF) binding sites discriminated by prediction, experimental determination, and literature. Of them, is important to highlight the stimulatory axes (FOXM1-MYBL2, FOXM1-EZH2) and the inhibitory (FOXM1-SNAI2) axis, that have as central actor FOXM1, which noteworthy also regulates several genes of the cell cycle. The framework used, allows us to propose a top-down model which includes driving pathways like PKI3A-AKT (among others), TFs, CNV, and point mutation of the most important of the CYP27B1 regulatory genes.

ST-10

A subcellular atlas of Akt as a predictive tool of its physiological and pathological functions

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Regulation of protein kinase AKT activity is associated with a diversity of processes, including cell metabolism, proliferation, differentiation and survival, as well as pathological processes such as viral infection and cancer development. AKT is a therapeutic target for cancer treatment and it is known to be regulated through numerous posttranslational modifications (PTMs) as well as to be recruited to different subcellular compartments. However, little is known about how a cell determines which substrates and functions AKT should regulate. Our hypothesis is that the profile of AKT PTMs can determine the subcellular localization of AKT, and vice versa, thus establishing the subset of AKT target substrates and the set of functions that AKT displays in response to each stimulus and each particular cellular context. The aim of this work is to develop an atlas of AKT subcellular localizations as a predictive tool of AKT physiological and pathological functions using bioinformatic and experimental tools. Using a combination of different bioinformatic resources, we performed an analysis of the AKT interactome, which allows us to explain and even predict the functional and subcellular code of AKT.

The analysis of the AKT interactome showed an endomembrane system-enriched domain, associated with biological processes such as cell proliferation, cell death, cellular response to stress, and autophagy, and associated with different types of cancer. We experimentally tested these predictions by performing quantitative analysis of fluorescence microscopy images and we found a tight coregulation of Akt recruitment to endoplasmic reticulum, Golgi and lysosome membranes. Particularly, we show that Akt is recruited to these membranes in response to stressing signals like serum starvation and oxidative stress, in association with biological processes such as autophagy and cell death, as predicted.

Furthermore, our bioinformatic analysis revealed a nuclear speckle-enriched domain on the AKT interactome, associated with RNA splicing, and diseases related to congenital malformations. However, to date, AKT has not been reported to be recruited to this subcellular compartment. Indeed, fluorescence microscopy experiments showed that both AKT and phosphorylated AKT substrates colocalize with nuclear speckles.

These results shed light into the role of AKT recruitment to different cell compartments and the specific targets and physiological as well as pathological functions triggered by AKT in different subcellular localizations.

ST-11

“4HER” PROJECT: HER ONCOGENES FAMILY INTERACTION IN TRASTUZUMAB RESISTANT BREAST TUMORS

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INTRODUCTION: In breast cancer (BC), resistance to Trastuzumab (TZM) is the main cause of death in HER2 patients, occurring in 30% of primary and up to 70% of metastatic tumors. We propose that this happens, at least in part, due to the synergic functioning of HER2 with the other 4 members of the oncogene family. These receptors can compensate the inhibition of one of them by forming heterodimers among themselves, and generating resistance. We’ve previously developed a tool based on MLPA to determine the CNV of the 4oncogenes. Our analyses indicate that 15-30% of HER2 tumors present co-amplification with another family members. Our actual goal is to explore in vivo (patients) and in silico the association of co-amplified HERs with TZM response. OBJECTIVES: 1-Clinical study: To analyze in 50 HER2 breast tumors the correlation between the TZM response and CNV of the 4 HER oncogenes. 2- In silico study: To analyze in HER2 tumors from TCGA the clinical response variables associated with co-amplification of HER members, and identify the genes associated with a worst outcome.

METHODS: Clinical study: 50 HER2 patients treated in CEMIC (CABA) were included (9 neoadjuvant and 41 adjuvant), and DNA was extracted from FFPE tissue. MLPA assay was performed with the developed X026 probemix for HER oncogenes (MCR Holland). Pathologic complete response and recurrence was registered. In silico study: XENA/TCGA database was used to select the 67 HER2 patients (PAM50RNAseq phenotypic variable) from whom RNAseq data was obtained.

RESULTS: Clinical study: To the date, we have analyzed by MLPA 27/50 tumors, and 24 were confirmed as HER2 (1 was discarded and 2 were HER2- by MLPA).). We found that 9/24 samples (37%) presented co-amplification of HER2 with at least another HER oncogene (7/24 with 1 and 2/24 with 3 or more). So far, we have collected the clinical data of 19/50 patients, of which 3 (15,7%) presented disease recurrence. By MLPA we could determine that 1 was HER2- (and presumed to be refractory to TZM treatment), whereas the other 2 were HER2+ with HER3 co-amplification. In silico study: TCGA data revealed that BC patients presenting high co-expression of HER2/HER3 have the worst overall survival (OS) among all HER combinations. In a differential gene expression analysis, we found 57 upregulated and 71 downregulated genes in high HER/HER3 expressing tumors. By filtering genes with the strongest correlation (r>0,48) and worst OS, we identified 4 upregulated genes related to treatment resistance, and among 10 downregulated genes associated with immune infiltration.

CONCLUSION: Our results suggest that the co-amplifications of HER family members can affect the outcome of HER2 BC patients, by affecting the regulation of other genes involved in resistance. Further studies are required to better understand how these oncogenes "work together for the family".

ST-12

APOLIPOPROTEIN-AI INDUCE p62 EXPRESSION VIA Nrf2-DEPENDENT

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Apolipoprotein A-I (apoA-I) is the major protein component of high-density lipoproteins (HDLs). The apoA-I interaction with cells produces a complex intracellular signal transduction system activation that leads cholesterol endogenous pools movilization and anti-inflammatory respons. We have performed a proteomic profile in THP-1 cells treated with apoA-I in order to study differences in the level of total protein expression compared to controls without protein treatment. The total protein extract was analyzed in an orbitrap/quadrupole and processed by two different software to obtain robust data. Several proteins with a significant level of expression ($p \leq 0.05$) in cells treated with apoA-I were identified, such as the protein Sequestosome-1 (P62). P62 is a multifunctional chaperone that transports non-functional macromolecules to autophagosomes for degradation and could be regulated by the nuclear factor-erythroid 2-related factor 2/Kelch-like ECH-associated protein 1 (Nrf2/keap1) antioxidant response system. Also, we have observed a significant increase in the heme oxygenase1 (HO1) expression levels, a key enzyme transcribed by Nrf2. When Nrf2 was inhibited by retinoic acid (ATRA), P62 expression levels decreased by 40-50% in treated cells (ATRA+ ApoA-I). We conclude that the P62 expression increase in apoA-I cells treatment would be related to the activation of the Nrf2/keap1 antioxidant system.

ST-13

ANTI-ANGIOGENIC AND APOPTOTIC ACTIONS OF THE NATURAL FLAVONOID QUERCETIN IN A CELULAR MODEL OF KAPOSI'S SARCOMA

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Quercetin (QUE) is a flavonoid that belongs to the family of phytoestrogen and exhibits anticancer properties in multiple types of solid tumors; nevertheless, its effect on virally oncogenic transformed cells is less studied. The viral G Protein-Coupled Receptor (vGPCR) is one of the molecules from the lytic phase of human herpesvirus-8 able to induce cellular modifications through a paracrine oncogenic signaling cascade in Kaposi's sarcoma. We preliminary showed that QUE exerts antiproliferative effects on endothelial cells that stably express vGPCR. In this work, we further explore the mechanism of QUE-induced cell death in vGPCR cells. First, the IC50 of QUE was calculated by crystal violet technique after the treatment of SVEC and vGPCR cells with different concentrations of QUE (1-100 μM) or vehicle (0.1% DMSO) for 48 h. We found that SVEC cells (IC50 = 14.09 μM) were more susceptible to QUE treatment than vGPCR cells (IC50 = 30.078 μM). Herein, 30 μM of QUE was selected to further characterize the cell death of vGPCR cells. Cell cycle analysis revealed that QUE increase sub G0 phase and reduce S phase of vGPCR cells treated with QUE for 24 h presuming an apoptotic event. Annexin V/PI stain and caspase-3 activity confirmed that apoptosis takes place in vGPCR cells after QUE treatment. The vGPCR activates and controls the HIF-1α transcription factor promoting the expression of pro-angiogenic molecules such as VEGF. Consistently, qRT-PCR studies indicated that QUE downregulates the expression of HIF-1α and VEGF mRNA in a concentration dependent manner. In conclusion, our findings from this study suggest that QUE promotes its anticancer effects triggering both, anti-angiogenic and pro-apoptotic, programs to induce the cell death of the vGPCR cells.

ST-14

Effects of autocrine expression of sex pheromones in the yeast S. cerevisiae

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In Saccharomyces cerevisiae, mating between cells from the two mating types, MATa and MATα, is initiated when secreted pheromones (a- and alpha-factor) activate specific GPCR receptors in each partner, Ste2 and Ste3, respectively. GPCR activation triggers a prototypical signal transduction MAP kinase cascade that drives mating behavior, including cell cycle arrest, chemotropic growth and large changes in gene expression.

The magnitude of the response in MATa cells is proportional to the extracellular concentration of alpha factor. Thus, ectopic expression of alpha-factor by MATa cells should lead to an exacerbated response through autocrine stimulation of Ste2. However, in our lab, using various assays, including fluorescent transcriptional reporters, we found that in these conditions yeast cells shut down the pheromone response pathway. Here, we studied the mechanism behind this unexpected response. We found that desensitization takes place at the level of the receptor, but is not mediated by its endocytosis, since cells expressing Ste2 point mutations that prevent it are still desensitized when alpha factor is expressed. Rather, fluorescence microscopy revealed that Ste2 is unable to reach the plasma membrane when alpha factor is co-expressed and that it is re-routed to the vacuole. In addition, we found that the three alpha arrestins, Rog1, Rod3 and Ldb19, involved in Ste2 recycling, do not mediate this effect. Finally, we show that the signal for re-routing is present in the C-terminal tail of Ste2, since truncation mutants are resistant to alpha-factor.

Our results shed light on the mechanisms by which cells prevent activation of signaling by ectopic activation of their GPCRs. This mechanism resembles that used by the quality control of protein folding machinery to dispose of mutant membrane proteins.

ST-15

Gene expression is robust to variation in transcription factor abundance in the S. cerevisiae pheromone response pathway

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Cells sense signals from the environment and integrate their information to make cell fate decisions. In doing so, they process that information using diverse molecular systems, responding at various levels, from changes in molecular states, activating or inactivating proteins, to changing the concentrations of components in those systems via transcriptional responses. The specific architecture of the system that decodes those signals into the cell responses determines, among other things, to which extent extrinsic noise, in the form of variability in the system elements concentrations, affect them and, conversely, how robustly cells can respond to the signals they're sensing despite the existing natural variability in protein numbers. S. cerevisiae, or baker's yeast, has two mating types, MATα and MATa, and in its haploid stage can reproduce asexually or respond to the other mating type's pheromone, initiating mating behavior to reproduce sexually. The pheromone response pathway, which starts with a pheromone-binding GPCR that triggers a signal transduction cascade, causes, finally, a transcriptional response. We have previously described how, in this system, cell response to external pheromone concentration at the level of scaffold protein recruitment to the cell membrane is robust to variation in the receptor's concentration, being exclusively determined by the fraction of active receptors and not their absolute number, and thus being capable of robustly responding to the signal's magnitude, exerting what we termed "ratiometric control" (RC). Using fluorescence microscopy to measure the pheromone dose-response curves in single cells, we've found that the transcriptional activity from several of the pathway's promoters is also robust to over-expression of the pheromone response pathway transcription factors, while other cellular responses are augmented with this TF over-expression. We're currently exploring the mechanisms that could enable this transcriptional robustness.

ALLOSTERY TO DISRUPT PROTEIN-PROTEIN INTERACTIONS WITH SMALL MOLECULES.

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It is commonly considered that protein-protein interactions are extremely difficult to target using small molecules. Our group uses a chemical biology approach to study the allosteric, regulatory, mechanisms of protein kinases involved in growth factor signaling downstream of PI3-kinase. Over the years we have described small molecules that target a regulatory site in AGC kinases called "PIF-Pocket", and allosterically affect the active site, ATP-binding site. We also have shown that molecules that bind to the active site of protein kinases can stimulate or inhibit protein-protein interactions at the PIF-pocket regulatory site, by a mechanism that we have termed "reverse allostery". The reverse allosteric effects are widely produced by protein kinases inhibitors that target the ATP-binding site, although the phenomenon has been almost completely unperceived by pharmaceutical industries. The presence of a reverse allosteric effect is also the cause of the paradoxical effects on the protein kinases signal transduction detected for certain inhibitors. Thus, using the knowledge of the molecular mechanism involved in the bidirectional allostery, it becomes possible to exploit it to break protein-protein interactions between protein kinases in their protein complexes (Trends Biochem Sci 45(1):27-41, 2020). We will summarize our detailed research on the protein kinase PDK1 as a model of allosteric protein and will analyze the results in the context of the modern models of allostery, "conformational selection" and "population shift". We suggest that the principles of allostery should be used to rationalize new approaches to push forward the discovery of novel drugs that break protein-protein interactions. In line with the allosteric mechanism to disrupt protein kinase interactions, we also hypothesized that such a mechanism could also be used to break the interactions between other proteins, for example, between the angiotensin converting enzyme II (ACE2) and SARS-CoV-2 spike protein, which could be used as treatment against coronavirus infection (ChemMedChem. 15(18):1682-1690, 2020). Current studies confirm that compounds with allosteric mechanism can indeed disrupt the interaction between ACE2 and Spike.

Enzymology

EN-04

Cloning and expression of a GH10 xylanase from *Cellulomonas* sp. B6 for application in lignocellulosic biomass deconstruction

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The abundant availability of lignocellulosic biomass from agricultural waste has promoted numerous studies focused on its potential for industrial applications. Lignocellulose contains mainly the polysaccharides cellulose, hemicellulose and the polymer lignin. In Nature, bacteria and fungus secrete enzymes that can deconstruct these polymers. The efficient and complete bioconversion of lignocellulose is currently a major goal to produce chemicals, platform molecules, fuels and energy to enhance the development of a sustainable bio-economy. Xylanases (EC 3.2.1.8) are a group of depolymerizing enzymes that catalyze the hydrolysis of the β-1,4 glycosidic linkage in the backbone of xylan (XYN) (the main component of hemicellulose in terrestrial plants) and long xylo-oligosaccharides (XOS). These enzymes are classified in CAZy database as glycoside hydrolases (GH) in several families, mainly GH10 and GH11, and can include one or more carbohydrate binding modules (CBM), that may have specificity for different polysaccharides. The main objective of this work was to characterize the activity of *Cs*Xyn10C, a GH10 xylanase from *Cellulomonas* sp. B6, a cellulolytic soil strain with high potential for lignocellulose deconstruction and biomass valorization. *Cs*Xyn10C was identified in the supernatant of *Cellulomonas* sp. B6 cultures, using lignocellulosic biomass as sole carbon source. The sequence has an aminoterminal (N-terminal) signal peptide (absent in the secreted mature protein), a GH10 catalytic domain and a CBM13 (which may be related in binding to xylan). We amplified the coding sequence for the mature protein from total genomic DNA, with and without the sequence coding for the CBM13: *Cs*Xyn10C$_{CBM}$ and *Cs*Xyn10C$_{cat}$, respectively, and cloned them as amino-terminal His-tagged fusions. Both enzymes were expressed in *Escherichia coli* Arctic Express and purified by Niquel- affinity chromatography in a soluble form. We then characterized their xylanolytic activity to determine the potential of the enzyme for bioprocesses and evaluate the contribution of the CBM13 to its activity. Both forms of the enzyme were active towards beechwood xylan although *Cs*Xyn10C$_{cat}$ showed higher specific activity (6.75 and 12.46 IU/nmol for *Cs*Xyn10C$_{CBM}$ and *Cs*Xyn10C$_{cat}$, respectively). Both showed the highest activity at 50 °C (while active in a range from 40 °C to 55 °C) and at close to neutral pH, displaying more than 50% activity in a wide range of pH (4 to 9). Regarding thermal stability, at 50 °C, *Cs*Xyn10CCBM was more stable than *Cs*Xyn10Ccat, suggesting that the CBM might stabilize the protein. Nevertheless, at 45 °C no differences

were observed as the two forms of the enzyme retained above 60% activity after 4 hours of preincubation. Overall, *Cs*Xyn10C is an attractive candidate to be assayed in xylan-valorization bioprocesses that require mild temperature conditions in a wide range of pH.

EN-05

Application of xylanases and debranching enzymes of *Cellulomonas* sp. B6 for plant polysaccharides deconstruction into compounds with prebiotic and biopharmaceutical potential

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Xylan is the most abundant hemicellulose in nature, composed by a backbone of β 1-4 xylose unites and a diversity of substituent groups (arabinose, acetyl groups, glucuronic acids, feruloyl acids). The efficient degradation of xylan is of particular biotechnological interest since the hydrolysis products are desirable for a broad range of applications: short xylo-oligosaccharides (XOS) are emerging prebiotics, with demonstrated beneficial effects on health. Xylose, arabinose and glucuronic acids are precursors of biopharmaceuticals, food additives and biofuels.

Cellulomonas sp. B6 is a soil bacterium with high xylanolytic potential, since it secretes a repertoire of xylanases by growth on biomass and encodes for diverse debranching enzymes active on hemicellulose.

In this work we describe the catalytic potential of two xylanases from glycosode hydrolases (GH) families 10 and 11 (r*Cs*Xyn10A, r*Cs*Xyn11A), a GH62 α-L- arabinofuranosidase (r*Cs*Abf62A) and a GH67 α-glucuronidase (r*Cs*Agu67A), expressed and purified as recombinant enzymes. Additionally, we studied the effect of combining these xylanases and debranching enzymes on biomass conversion to XOS and monomeric sugars. The hydrolysis products were analyzed by thin layer chromatography (TLC), high-performance anion-exchange chromatography/pulsed amperometric detection (HPAEC-PAD) and mass spectrometry (Maldi-TOF).

The two recombinant xylanases showed different performance on commercial arabino- and glucuronoxylan, the main hemicelluloses present in plant biomass. The most active and thermostable was r*Cs*Xyn10A, which achieved a maximal activity above 400 U/mg on arabinoxylan at pH6 and 50ºC, releasing short xylooligosaccharides (XOS) and xylose. Meanwhile, r*Cs*Xyn11A hydrolyzed xylans to xylose-free XOS reaching 65 U/mg in glucuronoxylan at 30ºC, pH6. Debranching enzymes showed the expected activity: r*Cs*Abf62A released arabinose from monosubstituted (α-1,2 and α-1,3) side-groups in arabinoxylooligosaccharides (AXOS) and arabinoxylan while r*Cs*Agu67A was only active on external substitutions of glucuronic acid from glucuronoxylooligosaccharides (GXOS) but was not active on glucuronoxylan.

Combined activity of each xylanase with r*Cs*Abf62A improved the deconstruction of wheat arabinoxylan and wheat bran (a complex biomass composed of glucans, arabinoxylan, and lignin) to shorter XOS and xylobiose (X2). Besides, there was also a slight increase in the yield of xylose released by r*Cs*Xyn10A.

r*Cs*Agu67A was only active on oligosaccharides released by r*Cs*Xyn10A from glucuronoxylan-rich biomasses, obtaining glucuronic acid and unsubstituted XOS as products.

Therefore, the individual or concerted activity of the tested enzymes has biotechnological potential for biomass hydrolysis into value added products. We will keep working in the combination of them and other enzymes in order to produce an enzyme cocktail with high activity and selectivity on hemicellulose.

EN-06

Post-translational modulation of sucrose-phosphate synthase from Nitrosomonas europaea

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The β-proteobacteria *Nitrosomonas europaea* is a chemolithoautotroph that oxidizes ammonia to nitrite, which enables its biotechnological use for industrial and sewage waste treatment. Recently, much effort has been advocated for genome-based studies to decipher *N. europaea* biochemistry, biology, and

physiology. However, our knowledge regarding the regulation of metabolic routes in response to different growing conditions remains limited. In this study, we performed a comparative analysis of the growth of Nitrosomonas under heterotrophic (2% w/v fructose) or chemolithoautotrophic (air-supplemented) conditions and evaluated their impact on sucrose metabolism by determining the levels of the putative bifunctional sucrose-phosphate synthase (SPS) protein, the main enzyme involved in sucrose synthesis. SPS has a glucosyl-transferase domain connected to a sucrose phosphatase (SPP) domain by a linker composed of 47 amino acids. Studies performed with anti-SPP antibodies revealed that the full-length SPS was only present in extracts from *N. europaea* grown under heterotrophic conditions. In contrast, chemolithoautotrophic cultures produced a discrete band corresponding to the SPP domain, suggesting the occurrence of a putative proteolytic event within the linker region. An *in silico* approach revealed that the SPS linker has a putative recognition site (RLRR, score 0.9/1) for a protease from the S8 family (PS8). Using recombinant enzymes, we confirmed in vitro the cleavage of *N. europaea* SPS by PS8. Moreover, recombinant SPS and a protein extract from the bacterium grown on chemolithoautotrophic conditions also showed proteolysis of the SPS substrate. Then, we studied the putative effect of the proteolytic event on SPS activity by separately producing the glucosyl-transferase domain (SPS-S) from the SPP domain (SPS-P). While the full-length SPS exhibited values of 0.065 and 0.012 U/mg for the synthase and the phosphatase activities, the separated SPS-S and SPS-P proteins displayed activities that were 3- and 230-fold higher, respectively. Overall, our results pose proteolysis as a post-translational modification that could enhance SPS activity and thus carbon flux to sucrose metabolism. Moreover, we found that almost 7% of the total *N. europaea* proteome (~2000 proteins) has the consensus RXRR/RXKR site for SP8 recognition, which opens multiple possibilities for future research in proteome reshaping mediated by these and other proteases that are expressed in specific growth conditions.

EN-07

In situ substrate generation for kinetic analysis of the GlgE glucan:maltosyl-transferase from (Actino)bacteria.

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Natural occurring polysaccharides (starch, cellulose, chitosan) are widely distributed in the biosphere and can be considered as important biomass resources, constituting molecules of potential utility as biomaterials. Currently, glucans such as starch and glycogen are substrates for modifications and substitutions that allow their use in different biomedical applications, or in the food, pharmaceutical and cosmetic industries. Bacteria are a sustainable source for obtaining glucans to be modified, instead of using those sources for food, such as plants and/or animal tissues.

In our group we have studied the kinetic, regulatory and structural aspects of several enzymes of bacterial carbohydrate metabolism, particularly the classic pathway for glycogen synthesis. The latter uses ADP-glucose (ADP-Glc) as the glucosyl donor to elongate the glucan in one single moiety. In recent years, an alternative route where glycogen is elongated into two glycosidic subunits from the donor maltose-1P was described. The key enzyme in the pathway is the maltosyl-transferase GlgE (EC 2.4.16.99), which was predicted to be in 14% of known bacterial genomes, coexisting with the classical pathway. The maltosyl donor can be synthesized by a the GT4-type glycosyl-transferase, GlgM (EC 2.4.1.142), that synthesizes maltose-1P from ADP-Glc and Glc-1P. So far, GlgM and GlgE have been characterized only from mycobacteria and the behavior of the homologous enzymes from other sources is unknown. Thus, we focused in to increase the availability of kinetic data for these enzymes, studying the GlgM and GlgE from bacteria of biotechnological interest, such as *Streptomyces coelicolor* (*Sco*) and *Rhodococcus jostii* (*Rjo*). To do so, we addressed the joint analysis of GlgE with GlgM together as a single catalytic block. This strategy allowed us to analyze a possible channeling of substrates, since it has been reported that the intracellular accumulation of maltose-1P can be toxic. As well, the coupled assay for in situ maltose-1P generation resulted in the development of a molecular tool for GlgE studies circumventing the scarce availability of the substrate.

A derivation of the results obtained from the characterization of the GlgM/GlgE pair was the identification of a combination of biocatalysts (*Rjo*GlgM/*Sco*GlgE) which were incorporated to a cell-free synthesis methodology of novel glycans. In that regard, we exploited the ability both GlgM to use different hexose-1Ps and the promiscuity of the GH13 family of glycosyl transferases, to which GlgE belongs. Thus, we propose the GlgM/GlgB block may be located as a cornerstone in the development of strategies for the production of biopolymers with potential use as biomaterials.

Biotechnology

BT-02

PRODUCTION OF CYCLIC BETA-1,2-GLUCANS IN HCDC OF RECOMBINANT *E. coli*.

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Bacterial polysaccharides are molecules with interesting biotechnological applications. Many bacterial carbohydrates have been purified from natural producers while others, particularly those where natural producers or isolation methods were undesirable, have been obtained by means of heterologous gene expression. Cyclic beta-1,2-glucans (CBG) are bacterial cyclic homopolysaccharides consisting in 17 up to 40 beta-1,2 linked glucoses. These ring-shaped molecules have a hydrophilic surface that confers high solubility and a hydrophobic cavity able to include poorly soluble molecules. Several studies demonstrate that unsubstituted CBG and many derivatives can be applied in drug solubilization and stabilization, enantiomer separation, catalysis, synthesis of nanomaterials and even as immunomodulators, making these molecules interesting targets for commercial/industrial development. Nowadays, there is no method to produce CBG by chemical synthesis. Furthermore, naturally synthesized CBG differ in the distribution of the degree of polymerization and in the presence and nature of chemical substituents in the final product, changing the properties of the molecule depending on the bacterial host. Finally, bacteria that synthesize CBG, mostly *Rhizobiaceae* and *Brucellaceae* members, are slow-growing or even pathogenic, which makes the scaling up of the process more difficult and expensive. Therefore, scalable production and purification methods are needed to afford the demand. Here, we present the production of CBG in a specially designed *E. coli* strain by means of the deletion of intrinsic polysaccharides biosynthetic genes and the heterologous expression of *B. abortus* enzymes involved in CBG synthesis. This strain synthesizes and secretes unsubstituted CBG with a degree of polymerization of 17 to 24 glucoses. Through high cell density culture (HCDC) in a 7L stirred tank bioreactor we obtained 6,9 g/L of crude product (total reducing sugars) in culture medium within 48 h culture. By Size Exchange Chromatography purification, we recovered 4,5 g CBG/L of culture (94 $mgL^{-1}h^{-1}$), the highest volumetric productivity reported to our knowledge. With this new approach, we are expanding the use of bacteria as a platform for the recombinant synthesis of polysaccharides such as the CBGs.

BT-03

Optimization of Human Papillomavirus type 18 E6 recombinant oncoprotein expression in HEK293T cells

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Human papillomavirus (HPV) is a small, non enveloped double-stranded DNA virus. More than 150 HPV types have been identified, and at least 12 of these have been defined as cancer causing. HPV16 and HPV18 variants are together responsible for 70% of all cervical cancers. The E6 and E7 oncoproteins are responsible for the onset and maintenance of the cell transformation state by causing uncontrolled cell division and genome instability. They are the main targets for vaccine development and for diagnostic and therapeutic tools design. Several failed attempts have been made to produce recombinant full-length E6 protein in its native and soluble form in *Escherichia coli*. Given the difficulties found to express the HPV E6 protein in a prokaryotic host, we aimed at establishing an optimized recombinant HPV18 E6 mammalian expression system in order to purify this oncoprotein, which can potentially be used in the development of diagnostic tests.

An increase in protein production in mammalian cells can be achieved by optimizing the culture medium or by the addition of small molecule enhancers (SME) to the media. HEK293T HPV18 E6 expressing cells were obtained by transient transfection with pcDNA3.1 (+) vector containing HPV18 E6 coding sequence fused to an N-terminal secretory signal peptide and a C-terminal 6xHis tag. First, we assessed whether modifications in the culture medium could have an effect on HPV18 E6 secretion in transfected HEK293T cells. Under serum depletion conditions, we observed an upregulation of HPV18 E6 protein levels in the supernatant of HEK293T cells, with a maximum at day 3 of incubation. Next, we tested whether addition of SME could further induce HPV18 E6 secretion to the supernatant in serum-starved HEK293T cells. Optimization of HPV18 E6 protein secretion was accomplished with 2 mM and 4 mM sodium butyrate treatment in serum-free media incubation for 3 days. Addition of rapamycin or hypotonic shock treatment did not enhance HPV18 E6 production in serum starved HEK293T cells. Cell viability in HPV18 E6 HEK293T expressing cells in the presence of sodium butyrate was evaluated by Alamarblue reduction assay. We observed that 2 mM sodium butyrate had a protective effect in HEK293T cells incubated in serum-starved media. Finally, HPV18 E6 oncoprotein was purified by nickel affinity chromatography from

the supernatant of transiently transfected HEK293T cells exposed to 2 mM sodium butyrate and incubated in serum-free media for 3 days. The identity of the purified HPV18 E6 protein was confirmed by immunoblotting. Additionally, we performed a cross-linking assay using different glutaraldehyde concentrations, which resulted in the formation of a predominant complex with a molecular mass compatible with a dimeric form.

The obtained HPV18 E6 recombinant oncoprotein is a reliable biological tool that can potentially be applied in the development of diagnostic tests to prevent cervical cancer.

BT-04

DEVELOPMENT OF HIGHLY SPECIFIC CAPTURE IMMUNOASSAYS FOR THE EARLY DETECTION OF ZIKA VIRUS INFECTION

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Zika virus (ZIKV) is a flavivirus that is primarily transmitted from bites of infected *Aedes aegypti* or *Aedes albopictus* mosquitoes. There are currently no vaccines to prevent ZIKV infection nor commercially available clinical diagnostic tests demonstrated to identify ZIKV without cross-reactive interference of other related flaviviruses. In this context, the development of sensitive and accurate diagnostic methods is urgently needed for the early detection of ZIKV. Nonstructural protein 1 (NS1) is a highly conserved glycoprotein that is secreted as a hexamer and circulates at high levels in the bloodstream of acute patients. These properties turn ZIKV NS1 (ZNS1) into a good diagnostic marker, allowing early detection and diagnosis of ZIKV infection. In order to develop a highly specific and sensitive capture ELISA, we aimed at obtaining monoclonal antibodies (mAbs) against hexameric ZNS1 protein. We selected 6F6 specific hybridoma clone, which binds to a ZNS1 linear epitope. Cross-reaction studies through Western blotting, indirect ELISA and immunofluoresence staining indicated that 6F6 specifically recognizes ZNS1, and does not cross-react with the NS1 protein from other related flaviviruses. The 6F6 mAb enabled the development of a sensitive, reliable and reproducible capture ELISA with a limit of detection (LOD) of 10.8 ng/ml and a limit of quantification (LOQ) of 29.4 ng/ml. The accuracy of the 6F6 sandwich ELISA was

assessed by spike-and-recovery tests, obtaining average recoveries between the ideal range from 80 to 120%. A quantitative capture ELISA was conducted using concentrated and inactivated supernatants of A549 cells infected with molecularly defined ZIKV or DENV1-4 isolates. We only detected 625 ng/ml of ZNS1 protein in the supernatant of A549 ZIKV infected cells, with no cross-reaction with DENV NS1 proteins. A customized lateral flow assay (LFA) kit was used to evaluate the suitability of the 6F6 mAb to perform as the capture and detection antibody in a sandwich LFA strip. The applied sample was normal human sera spiked with different concentrations of ZNS1. The minimum ZNS1 concentration which could be detected by the 6F6 LFA strip was 250 ng/ml. In conclusion, we established valid capture immunoassays that allow the detection and quantification of small amounts of ZNS1 in human sera, which constitute promising bioanalytical methods for control strategies and the prevention of ZIKV propagation.

BT-05

Development of a molecular point of care detection kit for *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae (NG) is the etiological agent of gonorrhea, one of the four most prevalent sexually transmitted diseases worldwide. The diagnosis of this pathogen is carried out mainly by PCR methodologies, because it is accurate, sensitive and fast. However, it is an expensive technique that is difficult to perform in every health center. Thus, the development of rapid and affordable molecular point-of-care tests (POCT) is very important because it can be applied in isolated places or low complexity centers. Nucleic acid diagnostics generally have three steps: extraction, amplification and visualization. To solve the two last steps, we decided to use an isothermal nucleic acid amplification system coupled to a pH-sensitive dye. In our laboratory (LIGBCM - Laboratory of Genetic Engineering and Cellular and Molecular Biology, of the National University of Quilmes), an isothermal loop-based nucleic acid amplification methodology called Easy Loop Amplification (ELA) was developed. Its application for the diagnosis of both human and veterinary pathogens, showed a great adaptability to different organisms. One of our developed kits was for the detection of SARS CoV-2.

In this way, the main objective of this research project is the development of a molecular POCT kit for NG with no need of complex equipment.

The development of this kit for NG started with a bioinformatic analysis that allowed the molecular target selection from the genome of NG and afterwards the primer design. With one of the selected target sequences, a positive control was constructed de novo and used for the optimization of the isothermal amplification system. Different reaction parameters were evaluated and optimized (temperature, magnesium concentration, etc.). The specificity and detection limit of ELA were evaluated. The *in silico* analysis of the primers showed 100% specificity and the detection limit (analyzed by a calibration curve) resulted in less than 1000 molecules.

ELA diagnostics of NG showed similar parameters to those found with qPCR techniques. This amplification method could be used with a portable device, allowing the diagnosis in low complexity centers and highlighting its capability to be exploited as a POCT system.

BT-06

Sustainable extraction of antioxidant and antimicrobial proteins and peptides from brewer's spent grain (BSG).

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Brewer's spent grain (BSG) is the main by-product of brewing production since it constitutes 85% of the total waste generated in this process. BSG is a lignocellulosic material with a high content of sugars, proteins, and minerals. Additionally, BSG has been reported as a source of bioactive compounds, with antioxidant and antihypertensive activities. A common practice is to dispose of this by-product in landfills or use it as animal feed. Therefore, the environmental perspective is necessary to generate new sustainable products that increase BSG's economic value. This work aims to standardize and optimize the extraction conditions of water-soluble proteins and peptides with antimicrobial and antioxidant activities,

using environmentally sustainable extraction conditions. Total protein extraction conditions were optimized using four factors with three possible values, following a Box- Behnken experimental design. These factors were pH (5, 7, and 9), temperature (30ºC, 45ºC, and 60ºC), homogenization time (1 min, 2 min, and 3 min), and $CaCl_2$ concentration (0, 5, and 10 mM). The amount of protein extracted varied between 0.5 and 6 g per 100 g of BSG (dry weight). SDS- PAGE analysis of the protein profiles showed that all BSG extracts obtained contain a majority protein with a molecular weight (MW) of 55 kDa, approximately. This molecular weight value would correspond to proteins like hordeins type C. Protein bands with a molecular weight estimated between 20 and 24 kDa, were detected in extracts obtained at pH 5 and 9, and 60 °C. These molecular weight values correspond to proteins like barley glutelins. The antioxidant activity was analyzed by reducing power, DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals scavenging activity, and superoxide radical scavenging assay by the antioxidant enzyme superoxide dismutase (SOD). Results show that the maximum value of DPPH radical scavenging, 70 %, was obtained in the extracts with the highest protein content. On the other hand, no antioxidant activity was detected when the BSG was incubated at 37 °C overnight. However, SOD activity was detected in BGS extract after 37 ºC overnight incubation. Antimicrobial activity was detected in BSG extracts toward *E. coli* culture, in a protein content-dependent manner. The results herein obtained optimize a new protocol to obtain antioxidant and antimicrobial proteins and peptides from BSG in a sustainable way.

BT-07

Influence of culture conditions on the antifungal performance of an Antarctic fungus (*Cadophora malorum*)

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The ability of fungi to produce molecules with biological activity is confirmed by studying the growing number of fungal sequenced genomes, which showed that their metabolic potential has been vaguely

explored. These sequences also showed that many of the fungal genes involved in secondary metabolites synthesis are clustered, being the expression of many of them silent under standard culture conditions. In that way, stress, or changes in growth conditions, can result in a significantly different physiological behavior, with consequent differential metabolite production.

Filamentous fungi from regions with extreme conditions present physiological adaptations that result in metabolic pathways able for producing novel molecules with antimicrobial activity encoded in gene clusters that are silent and whose expression can be triggered by exposure to physicochemical, mechanical, or biological stress. In this work, the effect of a different kind of stress on the secondary metabolites production profile of a strain of the Antarctic fungi *Cadophora malorum* was evaluated. The fungus was cultivated in potato dextrose medium (PD) in solid (PDA), liquid (PDB), and inert solid (Polyutertane foam) under different temperatures (5, 10, and 15ºC) and agitation conditions (static and 200 rpm). Additionally, the effect of mechanical damage to the mycelia was evaluated. After 28 days of culture, the biomass-free culture supernatant was extracted with ethyl acetate; the organic phase was evaporated and resuspended in methanol to evaluate their antifungal activity against a set of filamentous fungi and yeasts of clinical relevance using the Kirby-Bauer technique. Also, RP-HPLC analysis of the extracts was done to compare metabolite profiles. Different levels of antifungal activity, expressed as inhibition diameter (ID, cm), were observed. The highest inhibition was obtained from cultures growing under static conditions in liquid media at 15ºC, showing activity (ID) against *Candida albicans* (2,23±0.08), *C. haemulonii* (1.03±0.08), *Aspergillus fumigatus* (1,32±0.09), and *A. lentulus* (1,11±0.12). As a control, 20 µl of Cycloheximide (10 mg/ml) was used showing the following ID (1.08±0,04, 1.98±0,11, 2.95±0.70, 3.1±0.5 respectively). Mechanical damage to the mycelia did not change the antifungal performance of *C. malorum*. When the HPLC profiles were analyzed, several changes in the variety and size of peaks were observed. *Rhizopus* sp showed no inhibition when challenged with extracts obtained from all the tested conditions.

These results highlighted the relevance of exploring fungal culture and environmental conditions when looking for particular bioactivity. The procedure present in this summary is going to be applied to continue exploring the metabolic diversity of Antarctic fungi.

BT-08

Antarctic recombinant proteins: Cloning and characterization of a recombinant thermolabile uracil DNA glycosylase for its application in One step reactions

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Recombinant protein production continues to be one of the most significant contributions of modern biotechnology. They represent a fundamental part in countless processes, being an indispensable tool in the scientific, technological and industrial fields. The impact of recombinant proteins can be seen in numerous areas, and this market niche is continuously growing and the possibility of generating new variants is an area of great interest.

Uracil DNA glycosylases (UDG) are a highly conserved and specific type of DNA repair enzymes that catalyze the release of uracil molecules in DNA by hydrolyzing the N- glycosidic bond, leaving a basic site. The most popular biotechnological applications are the elimination of carryover contamination in real time PCR reactions and the adapter ligation for some massive sequencing technologies.

Although the incorporation of UDG into nucleic acid amplification systems has solved the huge problem of contamination caused by traces of products from previous PCR reactions in a rather simple way, certain drawbacks can also be found in its use. Uracil DNA glycosylases from mesophilic microorganisms, such as *E. coli*, have the particularity of retaining their activity even up to temperatures close to 85ºC. This represents a challenge when working with One Step or isothermal systems, where a UDG that maintains activity at the temperature at which the amplification reaction occurs would imply the degradation of the desired product. Therefore, a thermolabile UDG would solve this problem. As they are inactivated at low temperatures (>42º C), they can be coupled to both One Step RT- qPCR and isothermal systems, without affecting the amplification reaction. The main objective of this project is to obtain a thermolabile uracil DNA glycosylase, which can be coupled to one step RT-PCR and isothermal amplification systems. In order to do this, sequences that could meet the characteristics sought were selected from a gene database through bioinformatic analysis. A sequence from a psychrophilic microorganism isolated in the Argentine Antarctic territory was selected. Subsequently, different genetic modifications necessary to optimize its synthesis, cloning, expression and subsequent purification of the expression product were designed.

The purified protein was characterized for its uracil DNA glycosylase activity by real-time PCR. The enzyme showed an improved UDG activity between 10ºC and 25ºC, in comparison to other commercial enzymes. On the other hand, the thermostability tests showed that the enzyme is completely inactivated at 42ºC. This feature would allow the use of this protein in integrated enzyme systems, that allow many stages of

a biotechnological process to be carried out at different temperatures in a single reaction tube, reducing the time per reaction and optimizing the workflow.

Obtaining this tool is of high impact in the field of molecular biology, and has great potential as a biotechnological product.

BT-10

Thermostable phospholipase C from *Thermococcus kodakarensis* suitable for oil degumming.

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During the last decades, the replacement of chemicals used in industrial processes with enzymatic alternatives has become a general trend. Compared to classical chemical catalysts, enzymes offer high specificity; work under mild conditions of temperature and pH; avoid the use of toxic and deleterious substances and the production of toxic end products providing cleaner technologies. Hence, traditionally used chemicals have been replaced by novel enzymatic alternatives in a wide variety of industrial-scale processes.

Phospholipid removal of crude oils or oil degumming, is the first step of the oil refining process. Enzymatic oil degumming exploits the conversion catalyzed by phospholipases to remove vegetable crude oils' phospholipids. This enzymatic method reduces the gums' volume and increases the overall oil yield. A

thermostable phospholipase would be highly advantageous for industrial oil degumming as oil treatment at higher temperatures would save energy and increase the recovery of oil by facilitating the mixing and gums removal. Hyperthermophiles, predominantly distributed in the domain Archaea, are an unlimited source of thermostable enzymes since they are adapted to high temperatures. In this work, a bioinformatic search on genomes of thermophile species was performed in order to identify a thermostable phospholipase suitable for the high-temperature oil degumming process. As a result, a thermostable phosphatidylcholine (PC) (and phosphatidylethanolamine (PE))- specific phospholipase C from *Thermococcus kodakarensis* (*Tk*PLC) was identified. *Tk*PLC was successfully expressed and purified as a recombinant protein in *Pichia pastoris* (*Komagataella phaffii*). The enzyme completely hydrolyzed PC and PE in crude soybean oil degumming reactions at 80 °C. Thereby, *Tk*PLC is a promising new candidate for high-temperature enzymatic degumming, an environmentally improved and more profitable industrial scale oil degumming process.

BT-11

A highly stable Zn(II) dependent phospholipase C obtained by consensus design

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Proteins' extraordinary performance in recognition and catalysis have led their use in a range of applications. But proteins obtained from natural sources are oftentimes not suitable for direct use in industrial or diagnostic setups. Natural proteins, evolved to optimally perform a task in physiological conditions, usually lack the stability required to be used in harsher conditions. Therefore, the alteration of the stability of proteins to address critical environmental and industrial challenges is commonly pursued in protein engineering studies. In particular, the enzymatic removal of phospholipids from crude

vegetable oils, or enzymatic oil degumming, by phospholipases is a key enabling technology to obtain cleaner and more efficient methods. Nonetheless, advanced enzymes are needed for their widespread adoption.

In this work, in order to thermally stabilize a bacterial phospholipase C by consensus sequence design, several sequenced homologs from different sources, selecting a subset of examples for expression and characterization were retrieved and analyzed. As a result, a non-natural consensus sequence, called ChPLC, showed the highest stability and activity among those tested. Comparison of activity and stability parameters between this stabilized mutant with other natural variants bearing similar mutations allowed the characterization of the sites most likely to be responsible for the enhancement. In addition, an enzymatic degumming process based on ChPLC was developed. This new degumming process does not require modifications in current soybean oil factories, avoiding upfront investments, and is four times faster than the enzymatic processes available in the market. A techno-economic analysis predicts a ~60 % cost reduction for a base-case scenario, establishing the largely expected conditions to promote the global adoption of enzymatic oil degumming by refineries of all sizes.

BT-12

Evaluation of antioxidant capacity of purple carrot extracts in cell cultures of rat aorta

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In the cells, oxidative stress is produced when an excess of free radicals coincides with an insufficient amount of antioxidants. Excess of oxidative stress can lead to damage of cell components and impair normal cell functioning Dietary approaches to increase antioxidant levels include raising the consumption of fruits and vegetables with high content of antioxidant phytochemicals. Purple carrots are rich in anthocyanins, and also accumulate varying quantities of carotenoids and phenolic compounds, all with known beneficial health properties, including antioxidant activity.

The present work evaluated the antioxidant capacity of purple carrot extracts with low, medium, and high anthocyanin concentration in cultures of 'vascular smooth muscle cells' (VSMC) isolated from rat aorta. Angiotensin II (AngII) was used as an inducing agent of oxidative stress followed by the addition of the carrot extracts (as a potential protective agent). Negative controls with AngII but without the carrot extracts were included. To estimate oxidative stress, the level of superoxide production in the cell cultures was quantified by the cytochrome C reduction assay, as a measurement of the total content of reactive oxygen species (ROS). All the carrot extracts had protective effects on the oxidative stress of the cells, exhibiting significant ($p<0.001$) reductions of ROS levels, as compared to the negative controls. The extent of ROS reduction was directly and strongly correlated with anthocyanin concentration in the carrot extracts, high anthocyanins content reduces ROS by 84.7% relative to the AngII treatment ($r=0.96$), suggesting that these flavonoid pigments have antioxidant properties and are involved in the observed protective effects. Ongoing work in our lab focuses on the gene expression underlying these effects.

These results support studies related to the nutraceutical benefits of consuming vegetables with a high content of anthocyanins.

BT-13

Identification of immune systems with potential biotechnological application in bacteria of the genus *Acinetobacter*

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Bacteria and archaea are constantly exposed to the rampant invasion of phages or plasmids, generically known as mobile genetic elements (MGEs). In response, they have evolved diverse, complex defense systems that limit the intrusion of these foreign elements.

Many new types of defense systems have recently been discovered by studying the genomic 'dark matter' of defense islands using a blame-by-association approach: uncharacterized genes that commonly reside alongside genes from known phage defense systems. they often encode new defense systems. As more genomic data is deposited in sequence databases, so are renewed efforts to comprehensively identify and characterize known defense systems.

We propose to evaluate the heterogeneity of two recently developed tools: Prokaryotic Antiviral Defense LOCator (PADLOC) and DefenseFinder of in silico prediction of phage defense systems from NCBI genomes.

From a Unix command line we used the rsync program and downloaded from RefSeq release 212, 8468 genomes of bacteria of the genus *Acinetobacter*. We make the predictions locally and compare them with each other.

We believe that the integration of PADLOC and DefenseFinder is necessary since both tools are valuable for the identification and future characterization of defense systems against phages in bacteria of the genus *Acinetobacter*. No single best approach was identified. The definition of the "defense islands" on each genome will provide new regions to identify potentially new systems.

BT-14

Biosurfactant production and characterization from Antarctic bacteria for enhancing bioremediation of hydrocarbon-contaminated soils

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Introduction. Hydrocarbon contamination is a global problem, also affecting Antarctica. Bioremediation, particularly biostimulation, is an adequate tool for the recovery of contaminated soils. Nonetheless, a certain portion of the contaminants are recalcitrant and other strategies are required. Biosurfactant-producing strains provide the advantage of increasing the bioavailability of hydrophobic substrates such as total petroleum hydrocarbons (TPH) through the production of amphiphilic molecules -i.e., surfactants. Objective. To identify suitable Antarctic microorganisms for biosurfactant production. Characterization and quantification of the produced surfactant activity. Methods. 28 microbial strains were isolated from hydrocarbon-contaminated soils near Carlini Station (Antarctica, Argentina) and identified up to Genera using the 16S (bacteria) or 18S (for fungi) RNA genes. CTAB/Methylene Blue agar plates were used as a screening method for biosurfactant production. Growth kinetics were followed by optical density determinations at 600nm. Surfactant activity was assayed by emulsification index 24 hrs. (EI24),

hemolytic assay, drop collapse test, and oil displacement test. Results. 3 out of the 28 strains resulted positive for surfactant production in the CTAB/MB plates. 2 of those strains belonged to *Pseudomonas* (strains 9 and 14), while the remaining one belonged to the *Rhodococcus* genus (named ADH). Emulsification indexes (24) up to 58% (*Pseudomonas*) and 63% (*Rhodococcus*) were determined at cultivations days 5 and 7, respectively. Stationary phases were achieved after 24 and 48 hrs, respectively. The three strains resulted in hemolytic when assayed on blood agar plates. In the drop collapse test and oil displacement, strains 9 and 14 resulted in positive. Conclusion. Three strains (two *Pseudomonas* sp. and one *Rhodococcus* sp.) were isolated and identified as promising biosurfactant producers with potential application in bioremediation and MEOR (Microbiological enhanced oil recovery)

BT-15

The IS6770 insertion sequence modifies the promoter region structure of the gene *kup* encoding for the potassium symporter in *Enterococcus faecalis* JH2-2.

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Enterococcus faecalis is a ubiquitous colonizer of the gastrointestinal tract of animals, however, this microorganism evolved in the last decade from an avirulent commensal to nosocomial multiresistant bacteria. The intrinsic robustness of *E. faecalis*, which allows it to tolerate different environmental stress conditions, is directly related to the pathogenic potential of this bacterium. In our laboratory demonstrated that Kup and KimA proteins, two members of the K+ transporter Kup family, catalyze the uptake of K+ inside the cells and are involved in the response to osmotic stress in *E. faecalis*. These genes are located in a chromosomal region of 16 kbp, including the *opuABCD* operon, which encodes for putative osmolyte transporters, and two putative cation transporters. Insertion Sequences (IS), which codify for transposases, are mediators of genetic diversity in prokaryotes. They could induce rearrangements including deletions, duplications, inversion and activation of genes. The IS6770 is composed of a unique Orf that codifies for a putative transposase (member of the IS30 element) and 30

nucleotides of the imperfect inverted repeat. Even though, IS6770 was identified in *E. faecalis* strain associated to clinical isolated, in the vancomycin resistant strain V583 is absent. In the laboratory strain JH2-2 we detected 12 copies of the IS6770, two of them located downstream of both the *kup* and the *kimA* gene forming a genetic structure that constitutes a putative compound transposon. The presence of the IS6770, located 79 bp upstream of the *kup* gene initiation, modified the structure of the promotor region. To analyze whether the presence of IS6770 in the JH2-2 strain modified the transcriptional expression of the *kup* gene, transcriptional fusions of *kup* upstream regions of different sizes with a fluorescent reporter gene were performed, and then compared with transcriptional fusions of the promoter region of the V583 strain (IS6770 deficient strain). It was observed that while the promoter region amplified from the V583 strain allowed the expression of the fluorescent protein, the upstream region amplified from JH2-2, which includes part of the IS6770, showed no activity. However, the region containing part of the *kup* promoting region and a full copy of IS6770 from JH2-2 did show activity. Thus, these experiments suggest that the promoter region required for *kup* gene expression was either displaced by the IS6770 insertion or replaced by an internal promoter region located in the IS. Finally, in silico analyses suggest a recent formation of this composite transposon with the potential to transfer genes involved in osmotic resistance, since transposition of this putative element was not found in other localization of the bacterial genome.

BT-16

EXPRESSION AND CHARACTERIZATION OF RECOMBINANT CAZYME IN *LACTOCOCCUS LACTIS* NZ9000 TO ENHANCE SILAGE QUALITY.

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The main components of plant cell walls that constitute forages include cellulose, lignin, and hemicellulose (which is primarily formed by xylan). These are the building blocks for livestock feed. Silage fermentation is crucial for agroindustry and society because ruminants can generate meat and milk from plant biomass that is unsuitable for human consumption. However, how efficiently plant cell walls could be digested has a significant impact on how successful this process is. In hardwoods and grasses, xylan,

which is composed of beta-1,4-linked xylopyranosyl residues, is the second most prevalent polysaccharide. It is hydrolyzed by Xylanases (EC 3.2.1.8) present in many fungi, yeasts, as well as bacteria. By enhancing fermentation and digestibility, increasing metabolizable energy, and causing a shift in structural carbohydrates, the incorporation of enzymes in the silage promotes the degradation and also is beneficial once the silage reaches the rumen. One of the most widely common lactic acid bacteria used in the manufacturing of fermented foods is *Lactococcus lactis*, which is generally regarded as safe (GRAS). Thus, its incorporation into biotechnological procedures and the manufacture of commercial enzymes could simplify the downstream processing while reducing contamination hazards. In this context, the aim of this work was the over-expression of the XynA xylanase in *L. lactis* NZ9000 strain and its biochemical characterization to assess its potential for ensiling improvement. The *xyn*A gene from *Bacillus subtilis* was codon-optimized, synthesized, and cloned in the pNZ8048 plasmid under the control of the Pnis promoter. Protein over-expression was detected, in medium supernatant. XynA was purified to homogeneity by Ni-affinity chromatography and its biochemical properties were characterized. Xylanase activity was examined by the DNS assay, by measuring the amount of reducing sugars liberated from solubilized beechwood xylan. We found that XynA activity is maximum at 50ºC however the enzyme is stable up to 40ºC thus defining the optimal temperature to 40ºC. Concerning pH dependance, maximum activity was found between pH 5 and 6 with a stability range between pH 4,5 - 8. These characteristics are consistent with what has been reported so far about numerous xylanases (XynA) from several organisms. The measured parameters for the purified XynA protein are consistent with the pH and temperature found in silage practices.

BT-17

Development of technologies for detection of GMOs tolerant to herbicide glyphosate

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In the last decades, agrobiotechnology has been favored by the advance of genetic engineering and molecular biology techniques that allow the genetic modification of plant organisms. These modifications consist in the introduction of genes that confer specific improvements to plants, such as resistance to insect damage, tolerance to herbicides or to different environmental stress factors, and as a result, generate genetically modified organisms (GMOs). Argentina is one of the major producers of GMOs, and the largest production volume correspond to crops that carry herbicide tolerance genes, especially those that confer resistance to glyphosate. To regulate GMOs production and commercialization, it is necessary to determine special cultivation conditions and quality control systems to ensure the agro-ecological balance. Therefore, the use of appropriate detection methods is required. Despite the great demand generated by the massive production and commercialization of transgenic crops in Argentina, national diagnostic tools have not yet been developed and most of the ones currently available involve expensive and imported reagents. Our aim is to develop technologies to identify genetically modified plants tolerant to herbicides, based on "*in house*" produced biomolecules. These technologies will allow detecting the presence of GMOs in the main crops produced and commercialized by Argentina.

In this study we report the production of biomolecules (antigens and antibodies) and the development of a capture ELISA (Enzyme-Linked ImmunoSorbent Assay) to detect the transgenic protein that confers tolerance to glyphosate: CP4 EPSPS. We produced and purified the recombinant protein from E. coli and obtained monoclonal and polyclonal antibodies against it. With these antibodies we developed a capture ELISA and optimized it using different combinations of them to capture the recombinant CP4 EPSPS. The results showed that the system is able to detect low protein concentrations. Moreover, we analyzed the ability to capture the native protein in two plant species: Alfalfa (*Medicago sativa* L.) and Soybean (*Glycine max* L.). Our results indicated that the developed technology allows to detect the presence of GMOs in different crop samples, with high sensitivity and specificity.

BT-18

DESIGN OF AN ORAL VACCINE AGAINST CHAGAS DISEASE COMPOSED BY A MULTICOMPONENT ANTIGEN AND A GRAS BACTERIUM

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Chagas disease (CD) is a neglected disease, endemic in Argentina, caused by a flagellar protozoan named *Trypanosoma cruzi* for which neither prevention nor treatment is available by a vaccine. One of our perspectives in this regard is the development of a multi-component vaccine. Previously, we constructed a chimeric fusion protein based on *T. cruzi* antigens named NTc52/TSKB20 able to control parasitemia levels caused by *T. cruzi* in a mouse model. In this work, we present an approach that combines the adjuvant properties of a generally recognized as safe (GRAS) bacterium with the immunogenicity of NTc52/TSKB20 antigen. We developed a genetic construction between the chimeric antigen and the surface layer protein of *Lactobacillus acidophilus* SlpA to obtain a system that enables the antigen-self-assembly and adhesion on *L. acidophilus* surface. The gene encoding to SlpA was amplified by PCR from and fused to the gene encoding to NTc52/TSKB20 in an asymmetric PCR. After cloning, the recombinant plasmid derived of pRSETa containing the hybrid gene 6His-NTc52/TSKB20-SlpA was inserted into *Escherichia coli* BL21[DE3]. The expression was carried out by the addition of IPTG and cells were lysed by repetitive cycles of sonication, freezing and thawing. Subsequently, the lysate was centrifuged and the pellet, containing the protein in inclusion bodies, was resuspended in Urea 8M. The protein was purified through a Ni-NTA resin and dialyzed against PBS. The purified protein was added to *L. acidophilus* cells devoid of its S-layer by LiCl pre-treatment thus obtaining the vaccine formulation named lactos- NTc52/TSKB20-SlpA. The formulation was treated with digestive enzymes from mice to determine its endurance in a first approach to potential oral delivery. lactos-Tc52/TSKB20-SlpA diminished its ability to form colonies after treatment but chimeric antigen NTc52/TSKB20-SlpA kept up its integrity. An immunization scheme in mice was diagrammed to prove the potential activity of formulation by oral in comparison with subcutaneous administration. Animals were inoculated 3 times separated between 21 days. Blood was collected before each dose and 21 days after last dose and mice were challenged with a lethal dose of *T. cruzi* trypomastigotes. Parasitemia were analyzed twice weekly for 25 days for tested vaccine efficiency and then animals were sacrificed. Samples were taken to assed the immune response.

BT-20

AN EXPERIMENTAL MUCOSAL VACCINE FOR HEPATITIS E VIRUS

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The Hepatitis E virus (HEV) causes hepatitis; its principal port of entry is the gastrointestinal mucosa. Nowadays, there are no vaccines globally available. Our group has been working on a platform to display antigens on the surface of so-called Bacterium-like-particles (BLP), which are non-live immunomodulatory bacteria with adjuvant and carrier properties. The main target for an HEV vaccine is ORF2, the capsid protein. We cloned ORF2 most immunogenic domain (O2P2) fused to a LysM domain, which in nature mediates attachment of enzymes to bacterial peptidoglycan, as anchor to expose the antigen on the surface of the BLPs. To evaluate the experimental vaccines, we immunized mice (n=5) as follows: three oral immunizations with a) the chimeric protein LysM5O2P2, b) the chimeric protein displayed on the surface of BLPs (LysM5O2P2-BLP) or c) BLP. In a second schedule, we administered the first dose subcutaneously followed by two oral boosting of d) LysM5O2P2 or e) LysM5O2P2-BLP. The immunizations were performed every 14 days and blood samples were taken each time. Ten days after the last dose, mice were euthanized, and blood and gut fluid were collected to evaluate the humoral response by ELISA. Spleen and Peyer's patches (PP) were taken to isolate mononuclear cells for an ex vivo stimulation with the capsid antigen, after which, cytokines were measured by a commercial ELISA on the culture supernatant. Both groups that received oral immunizations produced specific IgG 2 weeks after the first immunization, but then, the antibodies were no longer detectable. In contrast, the levels of specific anti-HEV IgG in mice receiving one subcutaneous dose followed by oral immunizations increased after each dose. Specific IgA was detectable in every group, but it was higher in the mice that received oral doses. In every case, the co-administration of the chimeric protein and BLP induced a stronger humoral response. Regarding the cellular response, we measured higher amounts of INF-γ, TNF-α, IL-4 and IL-17 in the groups that received LysM5O2P2-BLP orally or under the mixed schedule. We conclude that the oral schedule induced a known phenomenon called immune tolerance. This was avoided by giving the first immunization subcutaneously. Comparing all groups, the highest humoral response was found in those groups which received LysM5O2P2-BLP under a mixed schedule. The groups that received LysM5O2P2-BLP orally or combining subcutaneous and oral administration reached higher levels of every cytokine measured in comparison with those that received just LysM5O2P2, proving the adjuvant effect of BLPs. Here with, we present a promising experimental vaccine that stimulates systemic as well as mucosal humoral and cellular response against the main HEV antigen.

BT-21

Antimicrobial activity of silver nanoparticles supported on S-layer proteins from *Lentilactobacillus kefiri*

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Research on nanoparticles obtained on biological supports is a topic of growing interest in nanoscience. The S-layer is a bidimensional proteinaceous structure that covers the surface of several prokaryotic species. Since isolated S-layer proteins (SLP) can reassemble in solution, we have recently synthesized and characterized silver nanoparticles (AgNP) supported on SLP from *L. kefiri* (Lk), which showed excellent performance as catalysts in the reduction of a dangerous pollutant such as *p*-nitrophenol. In this work, we aim to evaluate the antimicrobial activity of AgNP supported on SLP from different Lk strains against Gram-positive and Gram-negative pathogens. To synthesize the AgNP/SLP-Lk systems, different SLP-Lk solutions (1 mg/mL) were mixed with a 0.85 mg/mL AgNO3 solution for 24 hs at 25ºC. After that, two different reduction strategies (using H2 or NaBH4, respectively) were applied, and six AgNP/SLP-Lk systems were obtained on three different SLP (Lk5, Lk8, and Lk1). Antimicrobial activity was assessed against Staphylococcus aureus ATCC 6538 and *Escherichia coli* ATCC 25912. Bacteria were incubated in Luria-Bertani broth at approximately 2x1010 cfu/ml and 4x109 cfu/ml, respectively, in absence or presence of two different concentrations of AgNP/SLP-Lk for 24 hs at 37ºC using 96-well plates. Then, a 10 ul volume of each culture was seeded in nutritive agar and incubated at 37ºC for 24 hs to evaluate colony growth. Bacteria incubated with each SLP-Lk (without AgNP) were included as controls. A bactericidal effect on both pathogens was observed with the highest concentration of all AgNP/SLP-Lk systems studied. However, at the lowest concentration, the AgNP/SLPs obtained by reduction with NaBH4 were not able to inhibit *S. aureus*, whereas the bactericidal activity against *E. coli* was observed for the three AgNP/SLP-Lk systems. To note, none of the SLP-Lk contributed to the bactericidal effect observed at any condition. These results revealed that these nanosystems obtained at eco-friendly conditions could be interesting tools for environmental decontamination.

BT-22

Analysis and quantification of compounds from microalgae used in the food industry.

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Microalgae are simple unicellular or multicellular microorganisms spread throughout the planet, occupying both aquatic and terrestrial ecosystems. In general, they are highly efficient in CO_2 fixation and utilization of solar energy to produce biomass, with an efficiency four times higher than that of plants. The composition of microalgae (lipid, carbohydrate and protein content) depends on the species considered and, even among the same species, on the system and cultivation conditions. In addition to providing proteins and carbohydrates, microalgae are an important source of polyunsaturated fatty acids, which are essential for humans. Their consumption as food is restricted to a few species due to strict food regulations. The food market is dominated by *Chlorella vulgaris*, *Dunaliella salina* and *Spirulina platensis* in either tablet or powder form. This work presents the optimization of an economical culture medium for the growth of *Tetraselmis chuii* (a strain recently approved for human consumption) considering a specific light intensity and CO_2 injection. Besides, the measurements of photosynthetic efficiency, at fixed and variable irradiance, as well as the quantities of proteins, carbohydrates, lipids and chlorophylls at different days of culture at laboratory scale were studied for this algae, *S. platensis* and *C. vulgaris*.

All strains showed a significant amount of proteins in their different growth phases (50 to 100 mg/ mg dry biomass), with *S.platensis* standing out in exponential phase with an accumulation twice as high as the other two strains. *C.vulgaris* had a higher carbohydrate accumulation. *S.platensis* and *C.vulgaris* accumulated 50 % more lipids than *T.chuii* in their late growth phases.

The analysis of the electron transport efficiency of the three strains allowed us to conclude that neither *S.platensis* nor *T.chuii* were inhibited at high radiation, whereas *C.vulgaris* was inhibited at 1200 PAR (Photosynthetically active radiation). *S.platensis* presented the highest photosynthetic capacity, followed by T.chuii and finally C.vulgaris.

The results allow us to conclude that *T.chuii* is a good strain for mass production in photobioreactors because no photoinhibition was observed, and the quantum yields were similar to those of *C.vulgaris*, a

strain currently used in current markets. In addition, the protein yield of this strain is expected to be similar to *S. platensis*, which could be of interest, both for human and aquaculture consumption or for obtaining amino acids to produce biofertilizers.

BT-24

SYNERGIA BETWEEN *Trichoderma harzianum* AND PLANT EXTRACTS FOR THE GENERATION OF AGROECOLOGICAL BIOINOCULANTS

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In order to reduce losses in crop productivity and ensure the quality of the grains, the control of diseases caused by pathogens is one of the main factors to be considered. One of the strategies currently used for disease management is the use of synthetic agrochemicals, which is controversial due to their harmful effects in human health and the environment. In this sense, the use of natural agents and compounds for the biological control of plant diseases is increasingly in demand. Several beneficial microorganisms, such as *Trichoderma*, and different plant-derived extracts have individually shown antagonistic effects against different phytopathogenic organisms. In addition, some of them have shown they have an impact on crop productivity. The aim of this work was to evaluate the synergistic effect of a coculture of *Trichoderma* and vegetal extracts in the reduction of plant diseases and tomato and soybean growth promotion. For this purpose, three minimal media were prepared as follows: 1- vegetal extract of *Larrea nitida* Cav. (Zygophyllaceae); 2- strain of *Trichoderma harzianum* ($1x10^6$ conidia/mL); and 3- a mix of the components used for the conditions 1 and 2. After incubation at 28°C under agitation for 7 days, the cultures were vacuum filtered through a 0.20 µm membrane to eliminate the fungal biomass. The filtrates obtained were used to evaluate the antifungal activity against two fungal phytopathogens

(*Fusarium oxysporum* and *Diaporthe caulivora*) and also to evaluate plant growth promotion by inoculating the filtrates in soybean and tomato seeds. Subsequently, chromatographic separation was carried out to obtain the metabolic profile of the organic extractions. The results obtained indicated a greater inhibition of the growth of *F. oxysporum* and *D. caulivora* in the presence of the filtrate of *T. harzianum* in association with the plant extract, evidencing an activity-enhancing effect, compared to using each component separately. Similar results were observed for the tests of plant growth promotion in soybean and tomato, showing greater germination of seeds, plant development and biomass of aerial parts. Regarding the metabolic profile, there were differences in the chromatographic pattern between the *Trichoderma* filtrate alone and in the presence of plant extract, with some bands being observed with greater intensity under ultraviolet light at 365 nm and others with less intensity under ultraviolet light at 254 nm. These results show an enhancing effect of the activity of the consortium formed by *Trichoderma* and the plant extract for the control of phytopathogenic fungi, the promotion of plant growth, and the agronomic performance of tomato and soybean, suggesting its potential use as an agricultural bioinoculant.

BT-25

Plant HSP90s as novel adjuvants in the SARS-CoV2 model: humoral response analysis and antibody neutralization assay in mice

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Heat shock proteins of 90 kDa (HSP90) have been proposed as adjuvants in the design of new vaccine formulations. In our laboratory, two cytosolic isoforms of plant HSP90 (AtHsp81.2 from *Arabidopsis thaliana* and NbHsp90.3 from *Nicotiana benthamiana*) demonstrate to have adjuvant properties against parasitic infections such as Toxoplasmosis and Neosporosis. We showed that plant HSP90 fused or mixed with the antigen of interest can enhance and modulate the immune response. To evaluate the adjuvant properties of plant HSP90 in other infectious diseases poorly characterized, we proposed to use the receptor binding domain (RBD) of the Spike protein of SARS-CoV2, and the main candidate in the design of subunit vaccines. Initially, we evaluated the humoral response against RBD using the strategy protein mixture (Adjuvant + Antigen). Thirty C57 mice (male and female) were randomly separated into 7 groups and intramuscularly immunized with AtHsp81.2, NbHsp90.3 or RBD as control groups, rRBD + rAtHsp81.2, rRBD + rNbHsp90.3 or rRBD + Alum as vaccinated groups. A PBS group immunized with PBS 1x buffer was also included. Mice received a two-dose schedule delivered in 21-day intervals, and the sera were obtained at 0, 21-, 42-, 63- and 84-days post-immunization (dpi). The analysis of the humoral response showed a significant increase in anti-RBD IgGt, which persisted between 21 and 42 dpi. In addition, the RBD + AtHsp81.2 group showed a Th2 profile with a significant increase of anti-RBD IgG1 like the control RBD + Alum group. By contrast, the RBD + NbHsp90.3 group showed a significant increase in anti-RBD IgG2b towards a Th1 profile. Finally, we evaluated the capacity of the sera to neutralize lentiviral vectors pseudotyped with Spike glycoprotein. Serum-neutralizing antibody titers were determined by the 50% inhibitory dilution. The sera obtained at 42 dpi from mice immunized with rRBD + rAtHsp81.2 and rRBD + rNbHsp90.3 showed the potential to neutralize viral infection. In addition, the differential profile of both isoforms in the triggered humoral response offers an advantage of rapid and safe response in pandemic situations such as those experienced by SARS-CoV2.

Neurosciences

NS-03

Effects of an environmental enrichment protocol on epigenetic modifications in a rat transgenic model of Alzheimer's disease-like amyloid pathology

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Alzheimer's disease (AD) is characterized by massive accumulation of amyloid β (Aβ) in the cortex and hippocampus. Previous results in a transgenic (Tg) mice model of AD showed in hippocampus and cortex a decreased global DNA methylation levels together with a hypomethylation in the promotor of a critical enzyme for Aβ generation (BACE-1). Moreover, in human brains greater methylation in the BACE-1 promoter was associated with lower β-amyloid load in subjects with AD dementia, suggesting that epigenetic factors play a relevant role in AD amyloid pathology. Recently, it was shown that hydroxymethylation of cytosine residues (5hmC) is much more abundant in the brain than in other tissues. However, this epigenetic mark has been little studied in AD brains. The aim of the present work was to determine the patterns of histone H3 methylation and acetylation, and DNA hydroxymethylation in the hippocampus of 9-month-old Tg rats that recapitulate early stages of the AD-like amyloid pathology, with intraneuronal Aβ accumulation in the absence of amyloid plaques (pre-plaque stage). Our results showed that there were not differences neither in the histone H3 methylation nor in the histone H3 acetylation levels between wild-type (WT) and Tg rats. Furthermore, given that epigenetic factors can be modified by environmental stimulation, we aimed to study whether an environmental enrichment (EE) protocol can reverse cognitive impairment and DNA hydroxymethylation patterns in Tg rats. To this end, WT and Tg rats were raised in standard cages and from 6 to 9 months of age subgroups of both genotypes were exposed to bigger cages (100 x 50 x 50 cm) that provided an environment of greater motor, cognitive, and social stimulation (EE). At approximately 8 months of age, spatial memory was assessed using the Morris Water Maze test. Then, rats were sacrificed and hippocampi were dissected and stored at -80 °C until further processing. Hippocampal DNA were isolated and sonicated, in an ultrasonic bath sonicator, in approximately 200-700 bp fragments. The size of the fragments was verified in a 2% agarose gel and concentration and quality of DNA was measured in a NanoDrop spectrophotometer. Next, fragments were immunoprecipitated in order to obtain DNA fragments enriched in 5hmC and RT-qPCR were performed to measure the expression of Bdnf transcripts from promoters I and IV. Results showed that spatial memory was impaired in Tg rats compared to WT rats, and that the EE protocol was able to prevent this cognitive decline. Real Time qPCR for BDNF promoters I and IV were fine-tuned in control samples that were not immunoprecipitated and in the samples that were immunoprecipitated. The main objective of the present work is to perform next-generation sequencing (NGS) in order to identify differentially hydroxymethylated regions between the different treatments (genotypes and environmental protocols). This study was supported by PICT-2019-2019-00940 (ANPCyT) to PG and MCD.

NS-04

L-Dopa incorporation into tubulin permanently alters the detyrosination/tyrosination cycle, microtubules dynamics and neuronal differentiation

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Microtubules are key elements of the eukaryotic cytoskeleton composed of α- and β-tubulin heterodimers. Differential expression of tubulin isotypes and a variety of post-translational modifications constitutes the tubulin code that modulates the properties and function of microtubules. One of the first described tubulin post-translational modifications is the cyclic removal and re-addition of the tyrosine (Tyr) residue encoded at the C-terminal end of the α-subunit, known as detyrosination/tyrosination cycle. This modification of α-tubulin involves different enzymes: the tubulin tyrosine ligase and the detyrosinase complexes composed of a vasohibin (VASH1 or VASH2) and a small vasohibin-binding protein (SVBP). Recently, MATCAP, a third tubulin carboxypeptidase, has been described. The role of this post-translational modification in cell physiological processes remains poorly understood; nevertheless, there is increasing evidence of its importance in specialized microtubule functions.

In previous studies, we have demonstrated that L-3,4-dihydroxyphenylalanine (L-Dopa) is incorporated into α-tubulin at the same position as Tyr, both in vitro and in living cells. However, using soluble brain extracts, we found that L-Dopa was not released by the endogenous carboxypeptidase under the conditions that allow rapid release of Tyr from tubulin. In addition, we reported that L-Dopa treatment impairs mitochondrial transport along the axon and that the molecular motor KIF5B shows a reduced affinity for L-Dopa-microtubules. Now, we analyzed the interaction between the VASH-SVBP complex and microtubules enriched in L-Dopa-tubulin in vitro, using single-molecule TIRF and immunofluorescence assays. We found a reduction in both the capacity of the complex to bind L-Dopa-microtubules and its

carboxypeptidase activity, supporting the idea of L-Dopa irreversible incorporation into tubulin. Additionally, we examined in mouse hippocampal neurons the effect of L-Dopa treatment on neuronal differentiation in vitro. We observed a delay in the establishment of polarity and transition to stage III in WT neurons, but not in neurons without carboxypeptidase activity (SVBP KO). Based on our results, we hypothesize that the irreversible incorporation of L-Dopa into tubulin and microtubules permanently alters the detyrosination/tyrosination cycle and modifies microtubules dynamics with a direct implication in neuronal differentiation.

NS-05

Association of microbiome, metabolome and cognitive performance: An exploratory multi-omics approach in a rat model of cerebral amyloidosis.

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Emerging literature indicates that gut microbiota could impact on the development of different pathologies including Alzheimer's disease (AD), the leading cause of dementia in older adults. The intestinal microbiota is a complex ecosystem composed of more than 1014 type of bacterias, mainly from the phyla Bacillota, Bacteroidetes and Firmicutes of which some are considered pathogenic while most of them are involved in homeostatic processes promoting beneficial health effects. Modulation of the host capacity for energy uptake and storage, and increment of gut permeability and inflammation are proposed mechanisms of microbiota activity. Evidence using animal models indicates that the gut microbiota can communicate with the central nervous system (CNS), influencing brain function and behavior, via 2 pathways: the vagus nerve and the transmission of signaling molecules through the circulatory system crossing the blood-brain barrier. However, the mechanisms by which microbiota may impact and generate an effect on AD have not been elucidated.

Here we studied behavior, neuroinflammation, microbiota and metabolome of McGill-R-Thy1-APP rats, a transgenic (Tg) model of early stages of AD-like amyloid pathology. Morris water maze (MWM) test was

assessed for spatial learning and memory evaluation. Brains, stool and plasma samples were collected from 9 month-old male control (Wistar) (n=20) and Tg rats (n=23). Performance in spatial learning was similar between groups, however impairment in spatial reference memory was detected in Tg animals. Neuroinflammation was assed by immunohistochemistry (using anti-iba 1) and high-sensitivity multiplexed ELISA (MSD) that quantifies 9 cytokines (IFN-γ, IL-4, IL-1β, IL-5, IL-6, KC/GRO, IL-10, IL-13, TNF-α). Microglia activation was similar in WT and Tg brains, however Tg rats showed a loss of correlation between hippocampal number of iba-1 (+) cells and plasma levels of IL-10 (anti-inflammatory) suggesting that amyloid deposition can alter the inflammatory interplay between CNS and the periphery. Fecal samples were split in 2 and one part (n=43) was sent to StarSEQ GmbH (Germany) for 16S microbial rDNA genotyping using the 16S platform (Illumina) and the other one (n=30) to Metabolon (USA) for untargeted UPLC/MS-MS metabolomics to characterize the fecal and plasma metabolome. We obtained information on 189 OTUs, 702 metabolites in feces and 686 in plasma. Together, these data were curated and analyzed using the R packages: mixOmics, Metaboanalyst and Fella to define the type of microbe and the secreted compound that best discriminates Tg rats. On an exploratory basis, presence of Turicibacter and Lactobacillus (phylum Bacillota and Firmicutes respectively) and lack of Gastranaerophilales (phylum Melainabacteria), Muribaculaceae, Paraprevotella and Alloprevotella (phylum Bacteroidetes) in Tg rats were observed. We explored the correlations ($r > 0.80$) of this OTUs and rats metabolome and identified more than 10 metabolites which in turn are associated with the pathways of fatty acid metabolism, urea cycle and purine metabolism. Mann-Whitney U test was performed to compare the means of the variables highlighted in both groups of rats considering $p < 0.05$ as statistically significant. This work represents a first step towards understanding the association of peripheral inputs (gut microbiota and the intestinal metabolome) on neuroinflammation and its relationship with memory, a topic of which very little is known to date.

NS-06

Induction of stress granules in ganglion cells and the inner nuclear layer in a model of retinal degeneration caused by constant LED low-intensity light.

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The retina is a specialized light perception tissue that has evolved in an environment in which light intensity values vary daily by several orders of magnitude as a consequence of the day/night cycle. During the day the retina is prepared to deal with the deleterious effects of light, but during the night, these protective mechanisms are diminished. Artificial light and the use of devices with luminous LED screens -such as cell phones, monitors or TVs- expose our retinas to light at times when the phototoxic effect cannot be avoided. In our laboratory we have developed a model of retinal degeneration caused by constant low intensity LED light in rats, in which accumulation of reactive oxygen species (ROS) and progressive cell death of cones and rods is observed. In the present work, considering that cellular stress induces the formation of stress granules (SGs), and that they have a protective role, we analyzed the presence of these ribonucleoprotein biocondensates in rat retinas maintained under constant light. SGs are formed in the cytoplasm as part of the integrated stress response, in which global protein synthesis is inhibited so that translation initiation complexes accumulate and give rise to liquid-liquid phase transitions giving origin to these biocondensates. Since they had not been described in this tissue, we first characterized them by immunohistochemistry (IHC) using two different antibodies (against eIF3 and G3BP1 markers) and by Fluorescence in situ hybridization (FISH), in control retinas and retinas treated with hydrogen peroxide or sodium arsenite (oxidative stress). We also did it on primary cultures of total retina. Once we were certain that we were detecting SGs, we proceeded to quantify them by IHC in the different retinal layers in control animals (kept under a 12 h light-12 h dark cycle, LD) and rats kept under constant LED light (LL) for 2-8 days (model of retinal degeneration). We found that retinas subjected to LL presented a significantly larger number of SGs. Notably, ganglion cells presented the highest number of SGs, cells of the inner nuclear layer also presented several granules, while the photoreceptor layer (cones and rods) presented almost no SGs. That is to say that the neurons that manage to survive during the first 8 days of LL are the ones that form more SGs, coinciding with the protective role attributed to them. This correlation has allowed us to propose the hypothesis that SGs would favor neuronal survival in the context of retinal photodamage.

NS-07

INVESTIGATING THE ROLE OF MIR-340 AS A POST-TRANSCRIPTIONAL REGULATOR OF NEURONAL MIGRATION IN THE MAMMALIAN DEVELOPING CORTEX

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Neuronal migration during corticogenesis is one of the central mechanisms underlying the wiring of the brain and failures in this process can lead to defective cortical connectivity and subsequent neurodevelopmental disorders. A growing body of evidence suggests that a class of small noncoding RNAs, the microRNAs, have a crucial role in mammalian cerebral development, however, how individual microRNAs influence neuronal migration remains largely unknown. To address this, we have performed an in vitro screening to identify candidate microRNAs altering cellular movement, where miR-340 emerged as a top candidate altering both the speed and total distance of mammalian cell migration. Using qRT-PCR profiling of developing cortices, we confirmed that miR-340 has a dynamic expression pattern during cortical development coinciding with the peak of neurogenesis in the murine cortex. To assess the role of miR-340, we performed loss-of-function experiments using CRISPR/Cas9 genome editing in vivo. For this, we have established an in utero electroporation set-up that allowed us to target either the developing cortex or the ganglionic eminences, to assess radial and tangential migration respectively. Our preliminary results show a tendency for miR-340 KO neurons to migrate further than non-edited control cells. Interestingly, we found that a higher proportion of miR-340 KO neurons express doublecortin (DCX), a marker of neuronal differentiation and microtubule organization when compared to the non-edited control. Altogether, this data suggests that miR-340 could act as a negative regulator of neuronal differentiation and migration in the mammalian developing cortex. Further studies will allow us to address the regulatory interactions underlying the observed miR-340 phenotypes in vivo.

Structural Biology

SB-1

Prediction of epitopes recognized by primary response IgM antibodies to chicken egg lysozyme by means of homology modeling and docking experiments

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Studies of IgM-antigen (Ag) interaction focused on epitope mapping and cross-reactions are important issues in the design of vaccines and diagnostic kits. In previous research, it was observed that IgM isotype antibodies (Ab) are encoded by germline genes with few or no mutations, being of low to medium affinity compared to IgG isotype Abs. In this work, chicken egg lysozyme (HEL) as Ag model has been used, since it was widely studied, from the structural and physicochemical point of view, both in its free form and bound to Ab. Our goal is to characterize the structure of the epitopes recognized by anti-HEL monoclonal (Mabs) IgM Fvs, on HEL surface. Two populations of anti-HEL IgM were obtained: one by intraperitoneal immunization with complete Freund's adjuvant and another by intrasplenic immunization (without adjuvant). In both groups, the average of affinity constants obtained by means biosensor SPR experiments, was about 106 M-1 (published in previous presentations). To identify residues that are part of putative epitopes recognized by both IgM groups, we use immunoinformatic techniques such as: homology modeling of the Fvs IgM and docking experiments. Primary sequences of anti-HEL IgM VH and VL regions were modeled by homology modeling at three web server portals: AbodyBuilder, abYmod and SI Repertoire Builder. The assessment of obtained models was performed by QMEAN-Disco and SAVESv6.0 online servers. Docking experiments were performed at HADDOCK, ClusPro and AbAdapt servers. As a first approach, we studied a set of anti-HEL IgM that, according to ELISA and Biosensor SPR competitive assays, would recognize the same epitope or epitopes with a high overlapping degree on HEL surface. Such epitope would be the same or in the vicinity of that recognized by the anti-HEL IgG F.9.13.7 and the recombinant ScFv F1F9, which contains the HEL catalytic site. The epitope or epitopes near the catalytic site present certain rigidity since are formed mainly by alpha helix secondary structures. Also, adjacent to that epitope a second epitope is recognized by IgG D44-F10 and IgM antibodies such as HEL2. This particular epitope involves beta strands and disordered structures. The identification of the residues that comprise the first epitope formed mainly by alpha helices was easier to perform than those from the

second epitope. However, it was observed that the results obtained with the docking servers depend on the structural conformation of IgM Fvs used in the in silico experiments. Better results, consistent with those obtained in ELISA and SPR biosensor experiments have been acquired with modeled IgM Fvs by the SI Repertoire Builder server more than with those modeled by AbBuilder platform. At the same time, better results were obtained by performing in silico experiments with servers HADDOCK and AbAdapt than ClusPro. Further experiments with the models from all anti-HEL IgM Fvs will give statistically more confident results.

SB-2

Mass spectrometry analysis of calcineurin-PERK interface

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A common event in many neurodegenerative diseases and brain injuries, such as ischemic stroke, is the accumulation of misfolded proteins in the lumen of the ER. This causes ER stress, and activates the Unfolded Protein Response (UPR), a complex signaling pathway that can lead either to cell recovery or to programmed cell death if the damage cannot be resolved. A key ER stress sensing protein and activator of the UPR is PERK, an ER transmembrane kinase. It´s activation leads to a global reduction in protein synthesis. PERK is inactivated if the ER stress is resolved in the acute phase. However, in chronic ER stress PERK remains active, and contributes to the transition to apoptosis.

Calcineurin (CN) is a heterodimeric calcium/calmodulin-dependent phosphatase. Previous results from our lab showed a non-canonical cytoprotective function of CNAβ/B in astrocytes, by direct interaction with PERK. We have observed that the cytoprotective effect of CNAβ/B takes place during the acute phase of ER stress, and it is independent of CN phosphatase activity. This non-canonical function is related to PERK activity regulation. The aim of this work is to identify the interface between CN and PERK binding, using crosslinked recombinant proteins (6xHis-CNAβ/B and GST-cPERK) and mass spectrometry (MS) analysis. Our long-term goal is to design peptides derived from the contact surface of CNAβ/B that can modulate PERK signaling. MS spectra of tryptic fragments after reaction with crosslinker disuccinimidyl suberate (DSS) shows 3 main products suggesting inter-molecular links between lysine pair of CN-Aβ/B

and cPERK fragments. The analysis suggests that disordered regions of both proteins form part of the interface and that the calmodulin-binding domain of CN may interact with the juxtamembrane domain of PERK. These findings give insights into the structural features of the CN/PERK complex, and help characterize the non-canonical function of CN.