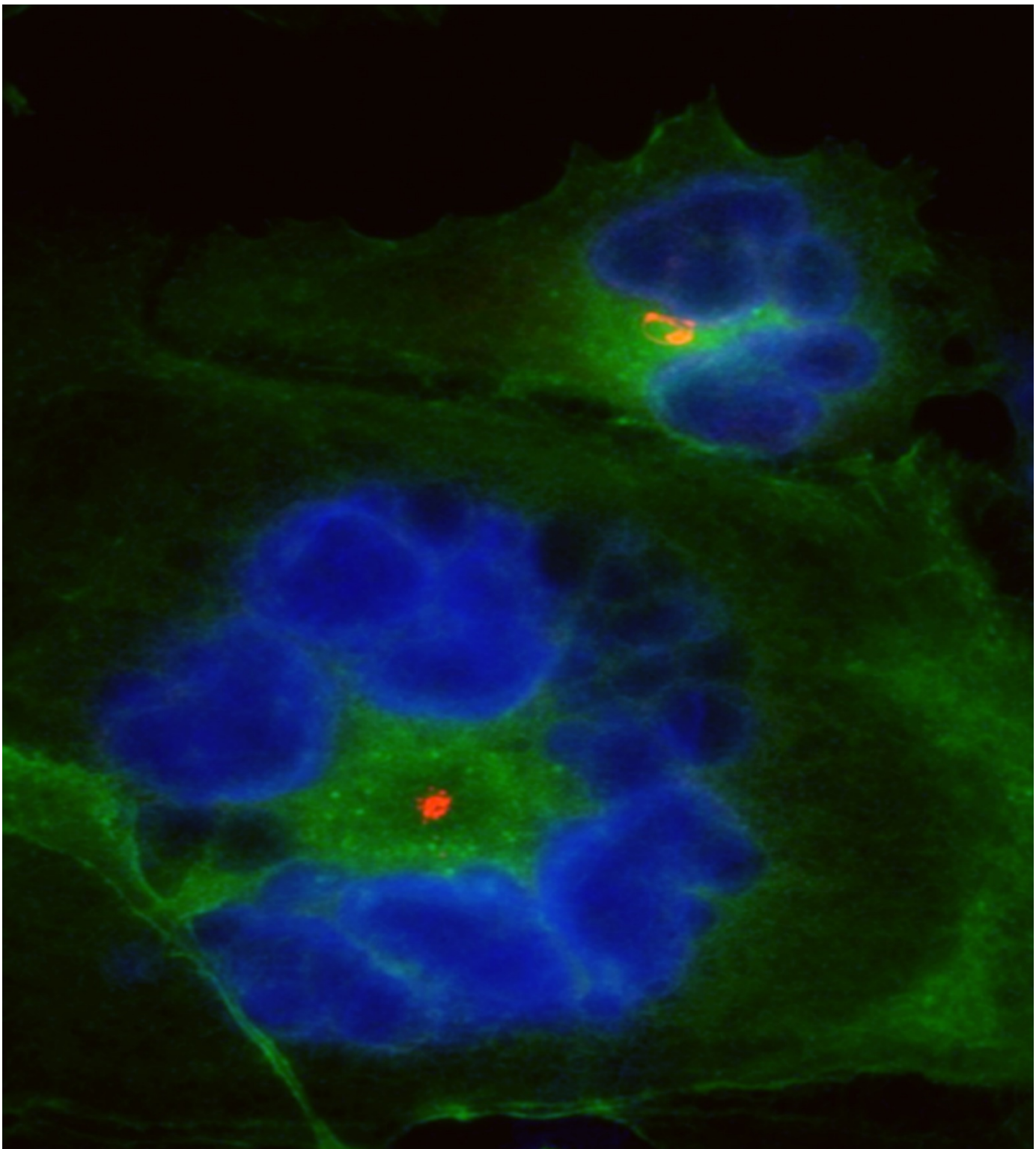
BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)



BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

Cover page: The Synthetic Lethal Rosette

Aberrant mitotic phenotype found in BRCA1-deficient cells treated with the PLK1 inhibitor Volasertib. Cells become giant and multinucleated and acquire a flower shape, with nuclei arranging in a circular disposition around a cluster of centrosomes. Blue (DAPI: nuclei), Green (FITC-phalloidin: actin cytoskeleton), Red (γ-Tubulin: centrosomes).

Author: María Laura Guantay (CONICET fellow; Director: Gaston Soria)

Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET), Facultad de Ciencias Químicas (Universidad Nacional de Córdoba).

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

MEMBERS OF THE SAIB BOARD

Silvia Belmonte
Auditor
IHEM CONICET
Facultad de Ciencias Médicas
Universidad Nacional de Cuyo - Mendoza

Romina Uranga
Auditor
INIBIBB CONICET
Universidad Nacional del Sur

DELEGATES OF SCIENTIFIC SESSIONS

Cell Biology
Javier Valdez Taubas
CIQUIBIC CONICET
Facultad de Ciencias Químicas
Universidad Nacional de Córdoba

Lipids
Nicolas Favale
IQUIFIB
Facultad de Farmacia y Bioquímica
Universidad de Buenos Aires

Plants
José M Estevez
FIL-IIBBA CONICET

Microbiology
Augusto Bellomio
INSIBIO-CONICET
Facultad de Bioquímica, Química y Farmacia.
Universidad Nacional de Tucumán

Signal Transduction
Vanesa Gottifredi
FIL-IIBBA CONICET

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

PABMB EXECUTIVE COMMITTEE

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

IN MEMORIAM

HORACIO G. PONTIS
(1928–2019)

Horacio Guillermo Pontis, born in Mendoza (1928), graduated in chemistry and obtained the Ph.D. (1953; Dir.: V. Deulofeu) from the University of Buenos Aires. After working for three years with Dr Luis F. Leloir—where he approached to carbohydrate metabolism—he stayed successively at King College (UK), Durham University (UK) and finally at Karolinska Institutet and University of Stockholm—where his attention turned to enzymology studies. After returning to Leloir´s lab (1960), he embarked on plant biochemistry studies. In his search for clues about fructans, Dr. Pontis´ lab synthesized not only UDP-fructose but also fructose-2-phosphate, which two decades later cleared the way for the chemical synthesis of fructose-2,6-bisphosphate (a key glycolysis modulator).

From 1968 to1977, he was the director of Dept. of Biology – Fundación Bariloche. In Nov. 1971, Bariloche hosted the SAIB Annual Meeting, being elected Dr. Pontis the President of SAIB (1972).

This reunion was followed by the Symposium "*Biochemistry of the glycosidic linkage*" with the presence of four Nobel Prizes (C. Cori (1947), G. Cori (1947), F. Lynen (1964), L. F. Leloir (1970)). However, his "*mi mejor experimento y experiencia de formación"* came to a halt-in when the Bariloche lab was closed (1977).

In 1979, Dr. Pontis moved to Mar del Plata where over time his outstanding capacity for innovation launched Instituto de Investigaciones Biológicas (IIB) – U. N. Mar del Plata, Fundación de Investigaciones Científicas (FIBA) and Centro de Investigaciones Biológicas (CIB).

In any site, Dr. Pontis maintained active research groups that trained graduate and post-graduate students generating a steady flow of important contributions to plant biochemistry. The research international community acknowledged these accomplishments, such as American Society of Plant Biologists that named him Correspondent Member. In his scientific activities, Dr. Pontis has been member of the National Research Council of Argentina (1961; CONICET), and Biochemistry Professor –at the UBA and at Universidad Nacional de Mar del Plata. The former and the later institutions recognized his academic performance designating him Emeritus Investigator and Emeritus Professor, respectively.

Dr. Pontis' story rose from limited beginnings—in Deulofeu´s and Leloir´s labs—to international scientific prestige. In this context, the challenge to overcome adversity during shameful periods in Argentine history honors not only his willingness but also his enthusiasm.

Ricardo Wolosiuk

Schedule	Tuesday November 5			Wednesday November 6	Thursday November 7	Friday November 8
8:30-9:00	**WORKSHOPS** **Workshop Accreditation**					
9:00-11:00	**Biochemistry Education Workshop**	**1st Workshop On Drug Discovery**		**Oral Communications** **Room Jacaranda** PL-C01, PL-C02, PL-C04 to PL-C06, PL-C09, PL-C12, ST-02, BT-C02 **Room Los Ceibos** CB-C01 to CB-C04, CB-C07 to CB-C10, ST-C01	**Conferences** ***Ro*om Lapacho** *Robert Gennis* *Francisco Barrantes*	**Oral Communications** **Room Jacaranda** PL-C03, PL-C07, PL-C08, PL-C10, PL-C11, PL-C13, PL-C14, PL-C15 **Room Los Ceibos** MI-C01 a MI-C06, BT-C01, CB-C06 **Room Lapacho** LI-C01 to LI-C05, ST-C03, CB-C11, CB-C05
11:00-11:30				**COFFEE-BREAK**		
11:30-12:30				**Plenary lecture** *Bruno Amati* **Room Lapacho**	**IUBMB Jubilee Lecture** *Philip D. Stahl* **Room Lapacho**	**"Hector Torres" Plenary Lecture** *Alejandro Colman Lerner* **Room Lapacho**
12:30-14:30				**LUNCH TIME**		
14:30-16:30			**Congress Accreditation**	**Symposia** *Lipids* **Room Jacaranda** *Plants* **Room Los Ceibos** *Signal Transduction* **Room Lapacho**	**Symposia** *Cell Biology* **Room Jacaranda** *RNA* **Room Los Ceibos**	**Symposia** *Microbiology* **Room Los Ceibos** *PABMB Young Investigators* **Room Jacaramda**
16:30-17:00				**COFFEE –BREAK**		
16:30-18:30			**Opening Ceremony** **Room Lapacho** ***In memoriam* of** Horacio Pontis **Room Lapacho**	**POSTERS** BT-P01 to BT-P06 CB-P01 to CB-P15 MI-P01 to MI-P18 PL-P01 to PL-P15 ST-P01 to ST-P13	**POSTERS** BT-P07 to BT-P12 CB-P16 to CB-P31 EN-P01 to EN-P11 MI-P19 to MI-P37 PL-P16 to PL-P32	**POSTERS** BT-P13 to BT-P19 CB-P32 to CB-P47 LI-P01 to LI-P15 MI-P38 to MI-P49 PL-P33 to PL-P48
18:30-19:30			**"Alberto Sols" Plenary Lecture** **Room Lapacho** *Encarnación Martinez Salas*	**EMBO Keynote Lecture** *F. Gisou van der Goot* **Room Lapacho**	**"Ranwel Caputto" Plenary Lecture** *Maria Elena Alvarez* **Room Lapacho**	**Closing Ceremony** **Room Lapacho**
			Cocktail 20:00 hs		**SAIB Assembly 19:45 hs**	**Dinner 20:00 hs**

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

SAIB 2019

TUESDAY November 5 2019

WORKSHOPS

WORKSHOP ON DRUG DISCOVERY

08:30-09:00 REGISTRATION

09:00-11:00 ORAL COMMUNICATIONS 1

Room Jacaranda

9:00-9:20
WELCOME BY ORGANIZERS

Ricardo Biondi
Instituto de Investigacion En Biomedicina de Buenos Aires - Instituto Partner De La Sociedad Max Planck (IBIOBA), Buenos Aires, Argentina
Hugo Gramajo
Instituto de Biologia Molecular y Celular de Rosario (IBR), Rosario, Argentina
Gaston Soria
Centro de Investigacion En Bioquimica Clinica e Inmunologia (CIBICI), Córdoba, Argentina

9:20 – 9:35
WS-C05
USE OF *IN VIVO* IMAGING SYSTEM FOR PRECLINICAL EVALUATION: EXAMPLES OF ITS APPLICATION IN DRUG DEVELOPMENT
Salinas FJ, Berengeno AL, Santiago G, Marelli BE, Baravalle ME, Salvetti NR, Ortega HH. Centro de Medicina Comparada, ICiVet-Litoral (UNL-CONICET), Esperanza, Santa Fe, Argentina. Facultad de Ciencias Veterinarias – Universidad Nacional del Litoral, Esperanza, Santa Fe, Argentina. E-mail: hhortega@fcv.unl.edu.ar

9:40 - 9:55
WS-C07
HYDROXYLAMINE CHEMICALLY ENGINEERED EXTRACTS AS SOURCE OF ANTIMYCOBACTERIAL COMPOUNDS
Ramallo IA[1], Parés VS[1], Gago G[2], Gramajo H[2], Furlan RLE[1]. [1]Fac. de Ciencias. Bioquímicas y Farmacéuticas, UNR-CONICET. [2]IBR-CONICET, Rosario. E-mail: aramallo@fbioyf.unr.edu.ar

10:00 – 10:15
WS-C06
VINARDO2: A NEW SCORING FUNCTION FOR MOLECULAR DOCKING WITH IMPROVED VIRTUAL SCREENING
Quiroga R, Villarreal MA. INFIQC-CONICET, Departamento de Química Teórica y Computacional - Facultad de Ciencias Químicas – UNC E-mail: rquiroga@unc.edu.ar

10:20-10:35
WS-C01
DRUG DISCOVERY OF TLS INHIBITORS TO SELECTIVELY TARGET CANCER CELLS WITH HOMOLOGOUS RECOMBINATION REPAIR DEFICIENCIES
García IA[1], Villafañez F[1]*, Quiroga R[2]*, Bocco JL[1], Villareal M[2], Soria G[1]. *These authors contributed equally to this work. [1]CIBICI-CONICET, [2]INFIQC-CONICET. Facultad de Ciencias Químicas. Universidad Nacional de Córdoba. E-mail: garcia.iris.alejandra@gmail.com*

10:40-10:55
WS-C03
EXPLORATION OF VITAMIN D RECEPTOR PHARMACODYNAMIC FEATURES UNDER TUMORAL AND NORMAL CELLS MICROENVIROMENTS: A MOLECULAR MODELING STUDY
Ribone SR[1], Cerutti JP[1], Vitale C[2], Fall F[3], Ferronato MJ[4], Curino AC [4], Facchinetti MM[4], Quevedo MA[1]. [1]UNITEFA-CONICET and Departamento de Ciencias Farmacéuticas, Facultad de Ciencias Químicas, UNC, [2]Laboratorio de Química Orgánica, INQUISUR, UNS, CONICET, UNS, Bahía Blanca, Argentina.[3]Departamento de Química Orgánica, Facultad de Química e IBI, Universidad de Vigo, Spain.[4]INIBIBB, UNS, CONICET, Departamento de Biología, Bioquímica y Farmacia, Bahía Blanca, Argentina. E-mail: sribone@fcq.unc.edu.ar

11:00-11:30 COFFEE BREAK

11:30-12:30 ORAL COMMUNICATIONS 2

ROOM JACARANDA

11:30-11:45
WS-C04
NOVEL FUNCTIONS OF THE UPS IN THE CONTROL OF TUMOR CELLS INVASIVENESS
Rossi FA[1], Enriqué Steinberg JH[1], Calvo Roitberg EH[1], Espinosa JM[2], Rossi M[1]. [1] Instituto de Investigaciones en Medicina Traslacional (IIMT), Universidad Austral, [2] Linda Crnic Institute for Down Syndrome and the Department of Pharmacology, University of Colorado, USA. E-mail: mrossi-conicet@austral.edu.ar

10:50 - 12:05
WS-C08
CHEMICAL BIOLOGY TO UNDERSTAND MOLECULAR MECHANISMS OF REGULATION AND AS POTENTIAL STARTING POINTS FOR DEVELOPMENT OF INNOVATIVE DRUGS
Sacerdoti M[1], Gross LZF[1], Suess E[2], Bollini M[3], Taylor M[1], Leroux AE[1], Biondi RM[1,2]
[1] IBioBA, CONICET, Partner Institute of the Max Planck Society. [2] Medizinische Klinik 1, Universitätsklinikum Frankfurt. [3]CIBION - CONICET. E-mail: rbiondi@ibioba-mpsp-conicet.gov.ar

12:10-12:30
WS-C02
ROCK INHIBITION INDUCES SYNTHETIC LETHALITY IN BRCA2-DEFICIENT CELL LINES
Martino J[1], Paviolo NS[1], Siri S[1], Pansa MF[2], Carbajosa S[2], Garro CA[2], Soria G[2], Gottifredi V[1]. [1]Fundación Instituto Leloir, Buenos Aires, Argentina, [2] CIBICI-Universidad Nacional de Córdoba, Córdoba, Argentina. E-mail: jmartino@leloir.org.ar

12:30-13:45 LUNCH AND POSTER SESSION

14:00-17:00 FOREIGN SPEAKERS

14:00 - 14:45

TARGET IDENTIFICATION AND TARGET VALIDATION BY CHEMICAL PROTEOMICS

Gerard Drewes

Senior Director and Head of Chemical Genomics at Cellzome, Cellzome - a GlaxoSmithKline company, EMBL campus, Heidelberg, Germany.
E-mail: gerard.drewes@cellzome.com

14:45 - 15:30

TAKING ACADEMIC DISCOVERIES INTO INNOVATIVE MEDICINES: THE EXPERIENCE AT LDC

Bert Klebl
Lead Discovery Center (LDC), Dortmund Germany
E-mail: klebl@lead-discovery.de

15:30-17:00 WORKSHOP DISCUSSION AND CLOSING

WORKSHOP
JOINT IUBMB/PABMB/SAIB BIOCHEMISTRY EDUCATION

ROOM LOS CEIBOS

"Helping Biochemistry Students Do Better Research"

Workshop Organizers:

Trevor Anderson, Ph.D.
Member of the IUBMB Education Committee, Head, Visualization in Biochemistry Education (VIBE) Research Group, Divisions of Chemistry Education and Biochemistry, Department of Chemistry, Purdue University, 560 Oval Drive West Lafayette, IN 47907-2084
E-mail: ander333@purdue.edu

Nancy Pelaez, Ph.D.
Fellow of the American Association for the Advancement of Science (AAAS), Chair-Elect AAAS Section Q Education, Biology Education Area (BEA) Convener, Department of Biological Sciences, Purdue University, Lilly Hall G-224, 915 West State Street, West Lafayette, IN 47907-2054
E-mail: npelaez@purdue.edu

R. Claudio Aguilar, Ph.D.
Assistant Head and Showalter Faculty Scholar
Treasurer of the Pan-American Association for Biochemistry and Molecular Biology
Department of Biological Sciences, Purdue University, Hansen Life Sciences Building, Room 321, 201 S. University Street, West Lafayette, IN 47907-2064, USA
E-mail: claudio@purdue.edu

08:30-09:00 REGISTRATION

09:00-11:00 SESSION 1

9:00 – 9:20
Participant Introductions
Workshop Chairperson:
Bianca Zingales, Ph.D.
Vice Chairman of the PABMB, Institute of Chemistry, University of São Paulo. São Paulo, Brazil

09:25 – 09: 50
The Key Interrelationship between Anticipated Learning Outcomes (ALOs), Teaching, Learning and Assessment in the Educational Process Presentation
Drs Nancy Pelaez and Trevor Anderson, Workshop Co-organizers

9:55 – 11:00 SESSION 2: ORAL COMMUNICATIONS

Teaching innovations from institutions in the PABMB/SAIB region

9:55-9:10
ED-01

MICROSCOPIO VIRTUAL: SIMULANDO LA REALIDAD PARA FORTALECER LA FORMACIÓN DE LOS FUTUROS BIOQUÍMICOS Y FARMACÉUTICOS

Nicolas Octavio Favale
Cátedra de Biología Celular y MolecularFacultad de Farmacia y Bioquímica
Universidad de Buenos Aires, Argentina
E-mail:nofaval@ffyb.uba.ar

9:10-9:25
ED-02

RENOVACIÓN DE LAS ACTIVIDADES DE ENSEÑANZA DE LA HISTOLOGÍA: EXPERIENCIAS EN CURSOS DE GRADO EN LA CARRERA DE DOCTOR EN MEDICINA

Julio C. Siciliano
Departamento de Educación Médica, Departamento de Histología y Embriología
Facultad de Medicina, Universidad de la República
Montevideo, Uruguay
E-mail: julio.c.siciliano@gmail.com

9:25-9:40
ED-03

REAL AND VIRTUAL LIVING BIOLOGICAL SCIENCE LABORATORY WITH "DO IT YOURSELF" (DIY), "DO IT WITH OTHERS" (DIWO) AND "BRING YOUR OWN DEVICE" (BYOD) TECHNOLOGIES

María Castelló
Departamento de Neurociencias Integrativas y Computacionales, Clemente Estable Biological Research Institute, Montevideo, Uruguay
E-mail: mcastello@iibce.edu.uy

9:40-9:55
ED-04

LOW-COST SPECTROPHOTOMETRY INVESTIGATIONS USING AN RGB LED AND A LIGHT DETECTOR.

Eduardo Galembeck
University of Campinas, Campinas, Brazil
E-mail: eg@unicamp.br

11:00-11:30 COFFEE BREAK

Exhibition of the innovative examples displayed by the four speakers

11:30-12:30 SESSION 3

11:30-12:20
Characterizing relevant ALOs for the above teaching innovations: Identifying research competencies
(Workshop in groups)

12:20–12:30
Report out by groups

12:30-13:45 LUNCH

Light lunch

14:00-15:30 SESSION 4

14:00 – 14:40

Assessing ALOs and confirming ALOs as Verified Learning Outcomes (VLOs) and identifying student difficulties
(Workshop in groups)

14:45 – 15:30

Aligning ALOs, assessments, and teaching approaches with student learning
Panel Discussion by the four invited speakers,
Moderators: Nancy Pelaez and Trevor Anderson

15:30-17:00 WORKSHOP CLOSING

Discussion of way forward and future networking with particpants

Chair: Bianca Zingales

Coffee breaks and a light lunch will be provided by the Workshop Facilitators, Nancy Pelaez and Trevor Anderson, sponsored by the National Science Foundation grant #1346567, which aims to establish a research coordination network among those who are Advancing Competence with Experimentation in Biology (ACE-Bio Network).

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

TUESDAY November 5 2019

SAIB-PABMB CONGRESS

14:30-17:30 REGISTRATION SAIB-PABMB CONGRESS

17:30-18:00 OPENING CEREMONY

Silvia Moreno
SAIB President

Hugo Maccioni
Past-Chairman PABMB

Room Lapacho

18:00-18:30 *In memoriam* of Horacio Pontis

Ricardo Wolosiuk
Investigador Emérito de la Fundación Instituto Leloir (FIL)

Room Lapacho

18:30-19:30 OPENING PLENARY LECTURE

"Alberto Sols"

RNA-BINDING PROTEINS MODULATING TRANSLATION CONTROL

Encarnación Martínez Salas

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

Chairperson:
Luis Alberto Quesada Allué

Room Lapacho

20:00 WELCOME COCKTAIL

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

WEDNESDAY November 6 2019

09:00-11:00 ORAL COMMUNICATIONS

Room Jacaranda
Plants, Signal Transduction and Biotechnology:
PL-C01, PL-C02, PL-C04, PL-C05, PL-C06, PL-C09, PL-C12, ST-02, BT-C02

Room Los Ceibos
Cell Biology and Signal Transduction
CB-C01 to CB-C04, CB-C07 to CB-C10, ST-C01

ROOM JACARANDA
Chairperson: Diego Fiol and Lorena Falcone

9:00-9:12
PL-C01
CONTRIBUTION OF FLAVODOXIN EXPRESSION IN POTATO PLANTS TO IMPROVED TOLERANCE AGAINST DROUGHT
Arce RC, Pierella Karlusich JJ, Zurbriggen MD, Hajirezaei M, Carrillo N. Instituto de Biología Molecular y Celular de Rosario, CONICET, Argentina.

9:13-9:25
PL-C02
NEW ROLES FOR OLD FRIENDS: A MICROTUBULE-LOCALIZED COP1-INTERACTING PROTEIN PROMOTES HYPOCOTYL ELONGATION IN THE DARK.
Arico DS[1], Wengier DL[1], Castro LM[1],Muschietti JP[1,2],Mazzella MA[1]. [1]INGEBI-CONICET, Argentina. [2]DBBE, FCEN, UBA, Argentina.

9:26-9:38
PL-C04
CONTRIBUTION OF THE DNA GLYCOSYLASE MBD4L TO DNA REPAIR DURING SEED GERMINATION
Lescano I, Nota MF, Torres JR, Cecchini NM, Álvarez ME. CIQUIBIC-CONICET, Dpto de Química Biológica Ranwel Caputto - Facultad de Ciencias Químicas – UN.

9:39-9:51
PL-C05
EXPRESSION OF NOS ENZYME FROM PHOTOSYNTHETIC MICROORGANISMS IN HIGHER PLANTS: A TOOL TO IMPROVE NITROGEN USE EFFICIENCY
Del Castello F, Nejamkin A, Foresi N, Lamattina L, Correa-Aragunde N. Instituto de Investigaciones Biológicas, Universidad Nacional de Mar del Plata.

9:52-10:04
PL-C06
MITOCHONDRIAL SMALL HEAT SHOCK PROTEIN AND CHILLING TOLERANCE IN TOMATO FRUIT
Escobar M[1], Herrfurth C [2], Boggio S[1], Feussner I[2], Valle EM[1]. [1]Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET-UNR), Ocampo y Esmeralda, Predio CCT, Rosario. [2]Georg-August-University Göttingen, Albrecht-von-Haller-Institute for Plant Sciences. Ernst-Caspari-Building, Dept. of Plant Biochemistry, Justus-von-Liebig-Weg 11, Göttingen, Germany.

10:05-10:17
PL-C09
PSI ACCEPTOR-SIDE STATUS CONTROLS CHLOROPLAST DEVELOPMENT AND PLANT GROWTH
Lobais C, Blanco NE. CEFOBI/UNR-CONICET, Rosario, Argentina.

10:18-10:30
PL-C12
PROLYL HYDROXYLATION IS NECESSARY FOR PROPER LOCALIZATION OF CELL WALL PROTEINS AND POLLEN GERMINATION IN *ARABIDOPSIS THALIANA*
Sede AR[1], Wengier D[1], Estevez JM[2,3], Muschietti JP[1,4]. [1] INGEBI–CONICET, Buenos aires, Argentina. [2] FIL-IIBBA-CONICET, Buenos aires, Argentina. [3] Centro de Biotecnología Vegetal (CBV), Facultad de Ciencias Biológicas, Universidad Andrés Bello, Santiago, Chile. [4]DBBE, FCEN-UBA, Argentina.

10:31-10:43
ST-C02
NITRIC OXIDE AND AUXIN REGULATE ROOT MERISTEM DURING GRAVITROPISM IN *ARABIDOPSIS THALIANA*
Vazquez M M[1], Goldy C[2], Rodríguez R[2,3], Casalongué C[1] and París R[1]. [1]IIB-CONICET-UNMdP, Mar del Plata, Argentina, [2] IBR-CONICET-UNR, [3]CEI-UNR.

10:44-10:56
BT-C02
IMPROVING POTATO YIELD THROUGH AN ENRICHED NITROGEN METABOLISM BY EXPRESSION OF NITRIC OXIDE SYNTHASE FROM *SYNECHOCOCCUS* PCC 7335
Nejamkin A [1], Foresi N [1], Del Castello F [1], Decima Oneto C [2], Correa-Aragunde N [1], Massa G [2,3] , Feingold S [2], Lamattina L [1]. [1]IIB-CONICET-UNMdP, Mar del Plata, Argentina, [2]Lab. Agrobiotecnologia. EEA INTA Balcarce, Argentina, [3]Facultad de Ciencias Agrarias, UNMdP, Balcarce, Argentina.

ROOM LOS CEIBOS

Chairpersons: Cecilia D'Alessio and Maria Teresa Damiani

9:00-9:12
CB-C01
ARCHITECTURE AND FUNCTION OF THE VACUOLE-MITOCHONDRIA MEMBRANE CONTACT SITE
González Montoro A, Auffarth K, Ungermann C. University of Osnabrueck.

9:13-9:25
CB-C02

GENETIC POLYMORPHISMS IN G-QUADRUPLEX AFFECT THE TRANSCRIPTION OF HUMAN DISEASE-RELATED GENES

Lorenzatti A[1], Piga, E[1], Margarit E[2], Gismondi M[2], Binolfi A[1], Calcaterra NB[1], Armas P[1]. [1]IBR – CONICET-UNR. [2]CEFOBI - CONICET-UNR.

9:26-9:38
CB-C03

INVOLVEMENT OF SUMO CONJUGATION IN snRNA BIOGENESIS

Bragado L, Magalnik M, Srebrow A. IFIBYNE-UBA-CONICET; Departamento de Fisiología, Biología Molecular y Celular, FCEyN, UBA.

9:39-9:51
CB-C04

INHIBITION OF TUMOR GROWTH, STEROIDOGENESIS, HORMONE AND DRUGS RESISTANCE BY AN ACYL-COA SYNTHETASE 4 NEW INHIBITOR

Castillo AF[1], Orlando UD[1], Maloberti PM[1], Prada JG[1], Dattilo MA[1], Solano AR[1], Szajnman S[2], Lorenzano Menna P[3], Gomez DE[3], Rodriguez JB[2], Podesta EJ[1]. [1]INBIOMED (UBA-CONICET), School of Medicine; [2]UMYMFOR (CONICET-FCEyN); [3]Lab. of Mol. Oncology, UNQ; Buenos Aires, Argentina.

9:52-10:04
CB-C07

IDENTIFICATION OF A MINIMAL SEQUENCE OF P21 THAT SENSITIZES TUMOR CELLS TO DNA-DAMAGING AGENTS

De la Vega MB, Mansilla SF, Bertolin A, Gottifredi V. Fundación Instituto Leloir- Instituto de Investigaciones Bioquímicas de Buenos Aires (IBBA).

10:05-10:17
CB-C08

GENETIC POLYMORPHISMS ON G-QUADRUPLEXES AS A CAUSE OF ONCOGENES TRANSCRIPTIONAL AND TRANSLATIONAL EXPRESSION VARIATIONS.

Piga E, Bezzi G, Lorenzatti A, Binolfi A, Calcaterra NB, Armas P. IBR– CONICET-UNR.

10:18-10:30
CB-C09

CONVERGENT APPROACH TO THE STUDY OF LONGEVITY OF *CERATITIS CAPITATA* AND *DROSOPHILA MELANOGASTER* MALES.

Rabossi A , Bochicchio PA , Quesada-Allué LA , Pérez , MM. IIBBA-Conicet, Depto. Química Biológica-FCEyN-UBA and Fundación Instituto Leloir.*

10:31-10:43
CB-C10

EVIDENCE OF ALTERED ENDOMEMBRANES IN FISSION YEASTS LACKING GLUCOSIDASE I, A MODEL FOR HUMAN CONGENITAL DISORDER OF GLYCOSYLATION CDG IIb

Valko A[1,2], Etchegaray E[2], Aramburu SI[1], González AC[2], Gallo GL[1], D'Alessio C[1,2]. [1]Fundación Instituto Leloir, IIBBA-CONICET and [2]FCEN-UBA.

10:44-10:56
ST-C01
ROLE OF THE P53 TARGET ICMT IN METASTASIS: POST-PRENYLATION PROCESSING AT THE CENTER OF THERAPEUTIC STRATEGIES IN CANCER
Borini Etichetti C[1,4], Cerri A [2], Arel Zalazar E[2], Larocca MC[1,4], Menacho-Márquez M[2], Porta E [3,4], Labadie, G[3,4], Girardini J[2,4]. [1]IFISE-CONICET, [2] IDICER-CONICET, [3]IQUIR-CONICET, [4] FCByF, UNR.

11:00-11:30 COFFEE BREAK

11:30-12:30 PLENARY LECTURE

Conference

GENOME RECOGNITION AND TRANSCRIPTIONAL REGULATION BY MYC: THE UNSOLVED EQUATION

Bruno Amati

European Institute of Oncology (IEO), Milan, Italy

Chairperson: Mario Rossi

Room Lapacho

12:30 -14:30 LUNCH

14:30-16:30 SYMPOSIA

LIPIDS SYMPOSIUM

"South Cone Symposium on Lipids"

Room Jacaranda

Chairpersons: Nicolás Favale and Ana Ves-Losada

Sayuri Miyamoto

MAPPING LIPID ALTERATIONS IN AMYOTROPHIC LATERAL SCLEROSIS THROUGH LIPIDOMIC ANALYSIS
Departamento de Bioquímica, Instituto de Química - Universidade de São Paulo, São Paulo, SP, Brazil.

Nelson Barrera

ROLE OF LIPID-MEMBRANE PROTEIN INTERACTIONS REVEALED BY MASS SPECTROMETRY AND MOLECULAR DYNAMICS SIMULATIONS

Faculty of Biological Sciences, Pontificia Universidad Católica de Chile.

Heinfried H. Radeke

REGULATION OF SPHINGOSINE-1-PHOSPHATE AND ITS ROLE FOR CHRONIC INFLAMMATION AND CARCINOGENESIS

Pharmazentrum frankfurt/ZAFES, Institute of General Pharmacology and Toxicology, Hospital of the Goethe University, Frankfurt am Main, Germany

Gerardo M. Oresti

BIOSYNTHESIS OF SPHINGOLIPIDS WITH VERY-LONG-CHAIN PUFA: A HALLMARK OF DIFFERENTIATING MALE GERM CELLS

INIBIBB, CONICET-UNS y Dpto. Biología, Bioquímica y Farmacia, UNS, Bahía Blanca, Argentina.

SIGNAL TRANSDUCTION SYMPOSIUM

"Understanding epigenetics: the contribution of different model organisms"

Room Lapacho

Chairpersons: Vanesa Gottifredi and Andrea Smania

Ivan Marazzi

THE PROTEOME'S DARK MATTER: ON THE ORIGIN OF NEOGENES AND NEOPROTEINS

Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA.

Paula Casati

SHEDDING LIGHT ON THE ROLE OF HISTONE CHAPERONES DURING DNA DAMAGE AFTER UV-B EXPOSURE IN PLANTS

Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI-CONICET), Universidad Nacional de Rosario.

Sophie Polo

EPIGENOME MAINTENANCE IN RESPONSE TO DNA DAMAGE

Laboratory of Epigenome Integrity, Epigenetics & Cell Fate Centre, CNRS/Université de Paris, France.

Pablo Strobl-Mazzulla

EPIGENETIC-microRNA CIRCUITRY REGULATING NEURAL CREST DELAMINATION AND CONDENSATION

INTECH (CONICET-UNSAM), Chascomús, Argentina.

PLANTS SYMPOSIUM

"Plant cell and molecular biology"

Room Los Ceibos

Chairpersons: José Estevez- Crisanto Gutierrez

Marisa Otegui

MEMBRANE REMODELING DURING ENDOSOMAL SORTING
University of Wisconsin-Madison, USA.

Elena Feraru

EMERGING ROLES OF PILS INTRACELLULAR AUXIN TRANSPORT FACILITATORS IN PLANT GROWTH AND DEVELOPMENT
BOKU-Universität für Bodenkultur, Vienna, Austria.

Enrique Rojo

UNRAVELING PLANT VACUOLAR TRAFFICKING THROUGH FORWARD GENETICS A RECIPE FOR SURPRISES
Dpto. Genética Molecular de Plantas.CNB-CSIC- Madrid, Spain.

Elizabeth Fontes

INVERSE MODULATION OF ANTIVIRAL AND ANTIBACTERIAL IMMUNITY BY THE RECEPTOR-LIKE KINASE NIK1
Dept of Biochemistry and Molecular Biology and National Institute of Science and Technology in Plant Pest Interactions, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.

16:30-18-30 COFFEE BREAK

POSTER SESSION

ST-P01 to ST-P13
BT-P01 to BT-P06
CB-P01 to CB-P15
MI-P01 to MI-P18
PL-P01 to PL-P15

18:30-19:30 PLENARY LECTURE

"EMBO KEYNOTE LECTURE"

DYNAMIC CONTROL OF PROTEIN FUNCTION BY PALMITOYLATION

F. Gisou van der Goot

School of Life Sciences, EPFL, Lausanne Switzerland

Chairperson: María Corvi

Room Lapacho

THURSDAY November 7 2019

09:00-11:00 PLENARY CONFERENCES

09:00-10:00

STRUCTURE OF THE ALTERNATIVE COMPLEX III IN A SUPERCOMPLEX WITH CYTOCHROME OXIDASE

Robert B. Gennis

Department of Biochemistry, University of Illinois, Urbana, IL, U.S.A.

Chairperson: Viviana Rapisarda

10:00-11:00

4 BILLION-YEAR-OLD NANOSCALE INTIMACY BETWEEN ION CHANNELS AND STEROL-LIKE MOLECULES: AN EVOLUTIONARY DIALOGUE

Francisco Barrantes

Laboratory of Molecular Neurobiology, Institute of Biomedical Research, UCA-CONICET, Buenos Aires, Argentina.

Chairperson: Ernesto Podesta

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

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

"IUBMB Jubilee Lecture"

EXOSOMES: THE RISE OF A NEW PARADIGM IN INTERCELLULAR COMMUNICATION

Philip D. Stahl

Washington University School of Medicine, St Louis, MO, USA

Chairpersons: Luis Mayorga and Hugo Maccioni

Room Lapacho

12:30-14:30 **LUNCH**

14:30-16:30 **SYMPOSIA**

CELL BIOLOGY SYMPOSIUM

"Intracellular Traffic and Organelle contact-sites"

Room Jacaranda

Chairpersons: Javier Valdez and Philip Stahl

Maria Carolina Touz

SORTING WITHOUT A GOLGI COMPLEX: THE ENDOPLASMIC RETICULUM AS THE SORTING CORE FACILITY IN THE PROTOZOAN *GIARDIA LAMBLIA*

Instituto de Investigación Médica Mercedes y Martín Ferreyra, INIMEC – CONICET – Universidad Nacional de Córdoba, Argentina.

Elizabeth Conibear

THE VPS13 FAMILY OF LIPID TRANSPORT PROTEINS

Department of Medical Genetics, University of British Columbia, Vancouver, Canada.

Christian Ungermann

MECHANISM OF ORGANELLE IDENTITY WITHIN THE ENDOLYSOSOMAL PATHWAY

University of Osnabrück, Department of Biology/Chemistry, Osnabrück, Germany.

Luis Mayorga

IN SILICO TESTING THE FUNDAMENTAL MECHANISMS AND LOGIC OF INTRACELLULAR TRAFFIC

IHEM -CONICET, Universidad Nacional de Cuyo, Facultad de Ciencias Exactas y Naturales, Mendoza, Argentina.

RNA SYMPOSIUM

"Insights on the biogenesis, functions and regulation of RNA"

Room Los Ceibos

Chairpersons: Anabella Srebrow and Paula Portela

Ariel A Bazzini

TRANSLATION AFFECTS mRNA STABILITY IN A CODON DEPENDENT MANNER IN HUMAN CELLS AND ZEBRAFISH EMBRYOS

Stowers Institute for Medical Research, Kansas City, USA, Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, USA.

Graciela Lidia Boccaccio

DYNAMICS AND FUNCTIONAL RELEVANCE OF RIBONUCLEOPROTEIC MEMBRANE-LESS ORGANELLES

Instituto Leloir (FIL) and IIIBBA-CONICET, Argentina.

Juan Pablo Tosar

FRAGMENTATION OF EXTRACELLULAR RIBOSOMES AND tRNAs SHAPES EXTRACELLULAR SMALL RNA PROFILES.

[1]Institut Pasteur de Montevideo, Uruguay. [2]Facultad de Ciencias y [3]Facultad de Medicina, Universidad de la República, Uruguay. [4]Brigham and Women's Hospital, Harvard Medical School, USA.

Anabella Srebrow

STORIES ON DENGUE VIRUS AND HOST CELL pre-mRNA PROCESSING

IFIBYNE-UBA-CONICET; Depto.FBMC, FCEyN, UBA, Argentina.

Javier Martinez

ANGEL2, A MEMBER OF THE CCR4-NOT FAMILY OF DEADENYLASES, IS A MAMMALIAN 2',3'-CYCLIC PHOSPHATASE

Max Perutz Labs Vienna, Medical University of Vienna and Vienna BioCenter, Vienna, Austria.

16:30-18-30 COFFEE BREAK

POSTER SESSION

BT-P07 to BT-P12
CB-P16 to CB-P31
EN-P01 to EN-P11
MI-P19 to MI-P37
PL-P16 to PL-P32

18:30-19:30 PLENARY LECTURE

"Ranwel Caputto"

CHROMATIN AND EPIGENETIC ALTERATIONS AFFECTING PLANT IMMUNITY

CIQUIBIC-CONICET, Dpto Química Biológica Ranwel Caputto, FCQ, Universidad Nacional Córdoba, Argentina

Maria Elena Alvarez

Chairperson: Paula Casati

Room Lapacho

19:45 SAIB Assembly

FRIDAY November 8, 2019

09:00-11:00 ORAL COMMUNICATIONS

Room Jacaranda
Plants
PL-C03, PL-C07, PL-C08, PL-C10, PL-C11, PL-C13, PL-C14, PL-C15

Room Los Ceibos
Microbiology, Biotechnology and Cell Biology
Mi-C01 to Mi-C06, BT-C01, CB-06

Room Lapacho
Lipids, Cell Biology, Biotechnology and Signal transduction
LI-C01, LI-C02, LI-C03, LI-C05, ST-C03, CB-C11, CB-C05

ROOM JACARANDA

Chairperson: Maria Elena Alvarez and Anabella Lodeyro

9:00-9:12
PL-C03

DYNAMIC REGULATION OF CHROMATIN TOPOLOGY BY INVERTED REPEAT-DERIVED SMALL RNAS IN SUNFLOWER

Gagliardi D, Cambiagno DA, Arce AL, Tomassi AH, Giacomelli JI, Ariel FD, Manavella PA. Instituto de Agrobiotecnología del Litoral (IAL CONICET-UNL).

9:13-9:25

PL-C07

A TALE OF TWO PROTEINS: GAINING INSIGHT INTO GRF/GIF REGULATORY NETWORK

Hedin N[1], Perrone AP[1], Ferella A[1], Ercoli MF[1], Rodriguez R[1], Palatnik J[1]. [1]Instituto de Biología Molecular y Celular de Rosario (IBR) – CONICET – UNR.

9:26-9:38

PL-C08

UNDERSTANDING THE EXPANSION OF microRNA NETWORKS IN PLANTS

Larran AS, Hedin N, Rodriguez RE, Debernardi J, Palatnik JF. Instituto de Biología Molecular y Celular de Rosario (IBR) – CONICET – UNR.

9:39-9:51

PL-C10

IDENTIFICATION AND FUNCTIONAL STUDIES OF THE REQUIREMENTS FOR EMBRYONIC NADP-MALIC ENZYME GENE TRANSCRIPTION

Gismondi M[1], Souza D[2], Permingeat H[2], Borras, L[2], Andreo CS[1], Drincovich, MF[1], Grotewold E[3], Saigo M[1]. CEFOBI-CONICET, Rosario, Santa Fe, Argentina. [2]IICAR-CONICET, Zavalla, Santa Fe, Argentina, [3]Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI, USA.

9:52-10:04

PL-C11

THE *ARABIDOPSIS* TRANSCRIPTION FACTOR ATHB40 INHIBITS ROOT ELONGATION AND THE RESPONSE TO GRAVITROPISM

Mora CC, Ribone PA, Ariel F, Chan RL[1]. [1]IAL, UNL, CONICET, FBCB, Santa Fe, Argentina. E-mail: cmora@santafe-conicet.gov.ar

10:05-10:17

PL-C13

STUDY OF RALF4/19 PEPTIDES ROLE DURING POLLEN TUBE GROWTH IN *ARABIDOPSIS THALIANA*

Somoza SC, Boccardo NA, Sede AR, Wengier DL, Muschietti JM
[1]Instituto de Investigaciones en Ingeniería Genética y Biología Molecular (INGEBI) [2] DBBE, FCEyN, UBA.

10:18-10:30

PL-C14

THE TRANSCRIPTION FACTOR AtPHL1 MODULATES SUCROSE TRANSPORT AFFECTING LIPID CONTENT IN *ARABIDOPSIS* SEEDS

Spies FP[1], Raineri J, Chan RL[1]. [1]Laboratorio de Biotecnología Vegetal - Instituto de Agrobiotecnología del Litoral – UNL – CONICET.E-mail: fspies@santafe-conicet.gov.ar

10:31-10:43
PL-C15
FUNCTIONAL CHARACTERIZATION OF AN ERF TRANSCRIPTION FACTOR WHICH MODULATES ABA SENSITIVITY AND JA-RELATED RESPONSES IN *ARABIDOPSIS*
Mengarelli DA, Osella AV, Valle EM, Zanor MI. Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET-UNR). E-mail: mengarelli@ibr-conicet.gov.ar

ROOM LOS CEIBOS
Chairpersons: Eleonora García Véscovi and Paula Vincent

9:00-9:12
MI-C01
RETHINKING THE MECHANISM OF ACTION OF CLASS II BACTERIOCINS: A COMPARATIVE STUDY THROUGH THE USE OF SUICIDE PROBES.
Rios Colombo NS, Chalón MC, Dupuy FG, Bellomio A. Instituto Superior de Investigaciones Biológicas (INSIBIO), CONICET-UNT and Instituto de Química Biológica "Dr. Bernabé Bloj", Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán. Argentina.

9:13-9:25
MI-C02
BVGR REGULATES THE TRANSCRIPTION OF A WIDE VARIETY OF GENES IN *BORDETELLA BRONCHISEPTICA*, INCLUDING VIRULENCE FACTORS
Gutiérrez MP[1], Wong TY[2], Sen-Kilic E[2], Damron FH[2], Fernández J[1], Sisti J[1].[1]IBBM-CCT-CONICET-La Plata, Dto Cs Biológicas, FCE, UNLP. [2]West Virginia University, USA.

9:26-9:38
MI-C03
CHARACTERIZATION OF *BORDETELLA BRONCHISEPTICA* DIGUANILATE CYCLASE BdcB
Belhart K, Sisti F, Fernández J.IBBM-CCT-CONICET-La Plata, Dto de Cs Biológicas, FCE, UNLP, La Plata, Argentina.

9:39-9:51
MI-C04
A PUTATIVE PAUSE ENCODED IN THE *ureA* mRNA OF *ASPERGILLUS NIDULANS*, MIGHT PLAY A ROLE IN THE CORRECT RECOGNITION BY FACTORS AT EARLY BIOGENESIS
Sanguinetti M[1], Iriarte A[1], Amillis S[2], Musto H[1], Marin M[1], Ramón A[1]. 1-Faculty of Sciences, UdelaR, Montevideo, Uruguay; 2-Faculty of Biology, University of Athens, Greece.

9:52-10:04
MI-C05
REPURPOSING TRICLABENDAZOLE AND CLOFAZIMINE AS PUTATIVE ANTI-TOXOPLASMIC COMPOUNDS.
Ganuza A[1], Alberca LN[2], Dietrich RC[2], Gavernet L[2], Talevi A[2], Corvi MM[1]. [1]INTECH, CONICET-UNSAM.[2]LIDeB, Universidad Nacional de La Plata, Argentina.

MI-C06
IDENTIFICATION OF A *SALMONELLA* PHOP/PHOQ SYSTEM INHIBITOR FROM A DYNAMIC COMBINATORIAL LIBRARY.
Lobertti, CA. [1]; Cabezudo, I. [2]; Furlán, RLE. [2]; García Véscovi, E. [1]. [1]Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET, [2]Área de Farmacognosia, Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR).

10:05-10:17
BT-C01
IDENTIFICATION AND CHARACTERIZATION OF NOVEL B-GALACTOSIDASES FROM A SEQUENCE-BASED METAGENOME ANALYSIS OF STABILIZATION PONDS
Eberhardt, MF, Irazoqui, JM, Amadio, A. INTA EEA-Rafaela – CONICET.

10:18-10:30
CB-C06
CHMP4B IS REQUIRED FOR THE EFFICIENT REPLICATION OF *TOXOPLASMA GONDII* IN DENDRITIC CELLS
Croce C[1] , Mayorga LS[1] , Blanchard N[2] , Cebrián I[1]. [1]IHEM-CONICET, Facultad de Ciencias Médicas, UNCuyo, Mendoza, ARGENTINA. [2]CNRS-INSERM-Universitéde Toulouse-UPS, CPTP, Toulouse, FRANCIA.E-mail: croce.cristina@gmail.com

ROOM LAPACHO
Chairpersons: Cecilia Casali and Andrea Rópolo

9:00-9:12
LI-C01
α-SYNUCLEIN AND LIPID METABOLISM: INTERSECTING PATHWAYS
Alza, NP[1,2], Conde, MA[1,3], Scodelaro Bilbao PG[3,4], González Pardo V[2], Salvador GA[1,3]. [1]INIBIBB-CONICET, [2]DQ-UNS, [3]DBByF -UNS, [4]CERZOS-CONICET, Bahía Blanca, Argentina.

9:13-9:25
LI-C02
LIPID DROPLETS POPULATIONS IN THE INSECT VECTOR OF CHAGAS DISEASE (*TRIATOMA INFESTANS*)
Girotti JR[1], Borús DL[1], Scelsio NS[1], Favale NO[2,3], Ves-Losada A[1,4]. [1]INIBIOLP-CCT-La Plata-CONICET-UNLP, [2]Cat Biol Cel Mol, FFB, UBA, [3]IQUIFIB-CONICET, [4]Dep. Cs Biol. FCE, UNLP, Argentina.

9:26-9:38
LI-C03
THE REGULATION OF PROTEINS 14-3-3 AND THE HIPPO VIA AFFECT THE ADIPOGENESIS OF 3T3-L1
Del Veliz S[1,3], Uhart M[1], Lim Gareth E[3], Bustos Diego M[1,2], IHEM[1] (CONICET-UNCuyo), FECEN[2], UNCuyo, Argentina. CRCHUM[3], Canada.

9:39-9:51
LI-C05
SPHINGOSINE KINASE 2 AS REGULATOR OF LIPID DROPLETS BIOGENESIS
Santacreu BJ, Romero, DJ, Tarallo E, Otero D, Sterin de Speziale NB; Favale NO. Facultad de Farmacia y Bioquímica, Cátedra de Biología Celular y Molecular, UBA, Argentina. IQUIFIB-CONICET Buenos Aires, Argentina.

9:52-10:04
CB-C05
MULTIPLE REACTION MONITORING (MRM): CHALLENGES IN MASS SPECTROMETRY BASED PROTEIN QUANTIFICATION
Zieschang S[1], Macur K[1], Lei S[1], Harwood E[1], Lech K[1,2], Jaquet S[1] , Morsey B[1] , Fox H[1] , Ciborowski P[1]. [1] University of Nebraska Medical Center, Omaha, NE [2] Faculty of Chemistry, Warsaw University of Technology, Warsaw, Poland [3] Intercollegiate Faculty of Biotechnology of University of Gdansk and Medical University of Gdansk, University of Gdansk, Gdansk, Poland.

10:05-10:17
ST-C03
HISTONE POST-TRANSLATIONAL MODIFICATIONS IN THE PROTOZOAN PARASITE *GIARDIA LAMBLIA*
Salusso A[1], Jaquet S[2], Diaz Perez L[1], Ciborowski P[2], Rópolo AS[1]. [1]INIMEC-CONICET-UNC, Córdoba, Argentina. [2]Dept. of Pharmacology and Exp. Neuroscience, University of Nebraska Medical Center. Omaha, USA.

10:18-10:30
CB-C11
THE FLIGHT RESPONSE INDUCES THE RELEASE OF AN ILP FROM THE INTESTINE TO INHIBIT CYTOPROTECTIVE MECHANISMS IN *C. ELEGANS*
Veuthey T , Giunti S , De Rosa MJ , Rayes D. Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB) (CONICET–UNS)/ DBByF-UNS.

11:30-12:30 PLENARY LECTURE

"Hector Torres"

ROBUSTNESS AND INFORMATION PROCESSING IN A CELL FATE DECISION SYSTEM

Alejandro Colman Lerner

Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE), CONICET-UBA, Buenos Aires, Argentina

Chairperson: Eduardo Ceccarelli

Room Lapacho

12:30 -14:30 LUNCH

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

14:30-16:30 SYMPOSIA

YOUNG INVESTIGATORS SYMPOSIUM
PABMB

Room Jacaranda

Chairpersons: Gabriela Salvador and Gustavo Chiabrando

Ignacio E. Schor

THE IMPACT OF NON-CODING GENETIC VARIATION ON PROMOTER FUNCTION

. Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE, UBA-CONICET). Departamento de Fisiología, Biología Molecular y Celular (FCEyN, UBA), Argentina

Valentina Parra

DYSFUNCTIONAL MITOCHONDRIAL FUNCTION AND DYNAMICS IN HUMAN DOWN'S SYNDROME INDUCED PLURIPOTENT STEM CELLS

[1]Advanced Center for Chronic Diseases; [2]Autophagy Research Center and University of Chile, Santiago, Chile.

Pablo Smircich

DARK: DEEP ANNOTATION OF REPRESENTATIVE KINETOPLASTIDS

Universidad de la República / Facultad de Ciencias – UdeLaR. Uruguay

María Guadalupe Vizoso Pinto

PLATFORM FOR PROTEIN SURFACE DISPLAY WITH MULTIPLE BIOTECHNOLOGICAL PURPOSES

INSIBIO - CONICET, Tucumán, Argentina

Mariano Bisbal

A CDC42 SIGNALING PATHWAY REGULATES MITOCHONDRIAL FISSION

Laboratorio de Neurobiología. Instituto Ferreyra (INIMEC-CONICET-UNC). Córdoba, Argentina.

MICROBIOLOGY SIMPOSYUM
Room Los Ceibos

Chairpersons: Augusto Bellomio and Conrado Adler

Viviana Andrea Rapisarda

BACTERIA RESPONSES TO HIGH ENVIRONMENTAL PHOSPHATE: PHYSIOLOGICAL ASPECTS AND POTENTIAL BIOTECHNOLOGICAL APPLICATIONS

INSIBIO (CONICET-UNT) and Fac. de Bioquímica, Química y Farmacia (UNT), Tucumán, Argentina.

Lici A. Schurig-Briccio

ROLE OF RESPIRATORY NADH OXIDATION IN THE REGULATION OF *STAPHYLOCOCCUS AUREUS* VIRULENCE

Dpt. of Biochemistry UW-M, WI, USA.

Manuel Espinosa-Urgel

MULTICELLULAR LIFE OF *PSEUDOMONAS PUTIDA*: FROM METABOLIC SIGNALS TO GLOBAL REGULATORS

Estación Experimental del Zaidín. CSIC, Spain

Nicolas Tomasini

DNA SEQUENCE DIVERSITY OF THE MINICIRCLE HYPERVARIABLE REGION OF *TRYPANOSOMA CRUZI*

Instituto de Patología Experimental (CONICET-unas, Salta, Argentia)

Marcelo E. Guerin

STRUCTURAL BASIS OF GLYCOGEN BIOSYNTHESIS REGULATION IN BACTERIA

Structural Biology Unit, CIC bioGUNE, Spain

16:30-18-30 COFFEE BREAK

POSTER SESSION

BT-P13 to BT-P19
CB-P32 to CB-P47
LI-P01 to LI-P15
MI-P38 to MI-P49
PL-P33 to PL-P48

18:30-19:30 CLOSING CEREMONY AND AWARDS

20:00 CLOSING PARTY

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

ABSTRACTS:

All abstract will be published in:
BIOCELL 2019

available online at: www.saib.org.ar
and
http://www.techscience.com/biocell/index.html.

Lectures
Lectures L01 to L08

Symposia
Cell Biology: CB-01 to CB-04
Lipids: LI-01 to LI-04
Microbiology: MI-01 to MI-05
Plants: PL-01 to PL-04
Signal Transduction: ST-01 to ST-04
RNA: RN-01 to RN-05

Oral Communications:
Biotechnology: BT-C01 to BT-C02
Cell Biology: CB-C01 to CB-11
Enzymology: EN-C01 and EN-C02
Lipids: LI-C01 to LI-C05
Microbiology: MI-C01 to MI-C05
Plant PL-C01 to PL-C15
Signal Transduction: ST-C01 to ST-C03

Posters:
Biotechnology: BT-P01 to BT-P19
Cell Biology: CB-P01 to CB-P47
Enzymology: EN-P01 to EN-P11
Lipids: LI-P01 to LI-P15
Microbiology: MI-P01 to MI-P49
Plant: PL-P01 to PL-P48
Signal Transduction: ST-P01 to ST-P13

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

LECTURES AND SYMPOSIA ABSTRACTS

TUESDAY November 5, 2019

LECTURE

L-01
RNA-BINDING PROTEINS MODULATING TRANSLATION CONTROL

Martínez-Salas, E
Centro de Biología Molecular Severo Ochoa (CSIC-UAM. E-mail: emartinez@cbm.csic.es

RNA-binding proteins (RBPs) perform a pivotal role in gene regulation in all organisms, governing RNA fate from its synthesis to translation and decay. The initiation of mRNA translation is finely controlled in response to distinct signals. Beyond the well-established cap-dependent translation initiation mechanism, internal ribosome entry site (IRES) elements govern protein synthesis in a wide variety of RNA viruses and specific subsets of cellular mRNAs. IRES elements differ in primary sequence, RNA structure, and requirement of initiation factors (eIFs) and RBPs to recruit the translation machinery. Recent RNA-capture approaches using IRES elements identified classical RBPs, such us PTB, PCBP2, or G3BP1, which perform different roles in RNA-dependent events, including translation control. In addition, these approaches identified novel factors, exemplified by Rab1b, ARF5, or Gemin5; evidence of their multitasking role presumably due to the capacity to recognize numerous partners. While Rab1b and ARF5 colocalize with IRES-RNA on the ER-Golgi compartment, Gemin5 negatively modulates translation. This protein, initially described as the RBP of the survival of motor neuron (SMN) complex, is mainly located outside of the SMN complex. The protein contains three separate functional domains. At the N-terminus, WD repeats recognize snRNAs targeting them for snRNPs assembly. In addition, this region interacts with the ribosome through L3 and L4 ribosomal proteins. The C-terminal region harbors a non-canonical bipartite RNA-binding site (RBS1-RBS2). RBS1 exhibits robust affinity for RNA, and has an intrinsically disordered structure, while RBS2 negatively regulates IRES activity. Identification of the RBS1 cellular targets using CLIP-based approaches revealed that this domain establishes a feedback loop with its mRNA counteracting the negative effect of Gemin5 in translation. In contrast, the central domain does not bind RNA; it folds into an extended tetratricopeptide-like domain that self-assembles into a canoe-shaped dimer. Interestingly, Gemin5 cleavage in picornavirus-infected cells renders a non-repressive stable product (p85), encompassing the TPR-like domain, RBS1 and RBS2. Mass spectrometry analysis showed that the dimerization module is functional in living cells, driving the interaction with the full-length Gemin5, whereas disruption of the dimerization surface prevents the interaction and abrogates the translation enhancement induced by p85. In summary, work carried out with IRES elements revealed that distinct RBPs are involved in translation modulation through diverse mechanisms, highlighting the importance of exploring novel roles of ribonucleoprotein complexes for gene expression control.

WEDNESDAY November 6, 2019

L-02
GENOME RECOGNITION AND TRANSCRIPTIONAL REGULATION BY MYC: THE UNSOLVED EQUATION

Amati B
European Institute of Oncology (IEO), Milan, Italy. E-mail: bruno.amati@ieo.it

The transcription factor MYC orchestrates complex transcriptional programs that foster cell growth and proliferation in either normal or cancer cells. MYC binds DNA with a preference for the E-box consensus sequence CACGTG but, when expressed at high levels, can be detected in virtually all active promoters and enhancers in the genome (a phenomenon known as “invasion”). Yet, MYC activation leads to up- and down-regulation of discrete sets of genes. We and others addressed the relationship between DNA binding and gene regulation by MYC in various cell types. Altogether, the stronger predictor of transcriptional responses was the relative efficiency in MYC binding at promoters: MYC-induced loci showed the highest gains in binding and rapid loading of RNA Polymerase II (Pol2), while down-regulated loci showed poor MYC binding and no recruitment – or even loss of Pol2. Hence, rather than being actively repressed by MYC, the latter loci may suffer from a competitive disadvantage in Pol2 recruitment. Mathematical modeling of our data confirmed that the main regulatory step modulated by MYC was Pol2 loading, with ancillary effects on pause-release. In order to study the role of sequence recognition by MYC, we mutagenized its DNA-binding domain by substituting residues involved in base-specific contacts. The mutant protein retained non-specific DNA binding activity and associated with active chromatin domains when overexpressed in cells but failed to discriminate between E-box containing and non-containing sites, and was unable to sustain proper transcriptional activity and cell growth. Thus, E-box recognition is essential for MYC’s transcriptional and biological activities.

L-03
DYNAMIC CONTROL OF PROTEIN FUNCTION BY PALMITOYLATION

van der Goot FG
School of Life Sciences, EPFL, Lausanne Switzerland. E-mail: gisou.vandergoot@epfl.ch

The diversity of the genetic code is massively amplified at the post-translational level through chemical modification of proteins. Amongst the five most abundant modifications is S-palmitoylation, the addition of a medium length acyl chain to cytosolic cysteines through a thioester bond. The human genome encodes 23 S-palmitoyltransferases of the DHHC family. Depalmitoylating enzymes appear to be present in fewer numbers. They include Acyl Protein Thioesterases 1 and 2. APT1 was recently found to localize mainly to mitochondria, while APT2 is cytosolic, and therefore involved in controlling the dynamic cycle of DHHC-mediated S-palmitoylation. I will present our recent advances in understanding palmitoylation, its dynamics and its consequences on target proteins. A special focus will be on depalmitoylation and the APT2 enzyme, removal of the palmitate indeed being key to the regulatory system. Integrating X-ray crystallography, molecular modeling and simulation, mutagenesis and *in vitro* and *in vivo* experiments, we found that interaction of APTs with membranes occurs in a two-step mechanism, first via a membrane-binding event, followed by DHHC-mediated palmitoylation at the N-terminus on Cys-2. Single point mutations in the membrane interacting region drastically inhibited palmitoylation. Membrane interacting capacity, via both mechanisms, was found to have a drastic effect on protein turn over and thereby on cellular APT2 activity. Soluble, non-membrane bound, APT2 undergoes rapid ubiquitination on a single Lysine followed by targeting to the proteasome, suggesting that freely diffusing APT2 in the cytosol is actively prevented by the cell. We also identified a conserved hydrophobic pocket within APT1 that accommodates palmitate. Using mutagenesis, we found that distorting the equivalent pocket in APT2 abolishes its depalmitoylating activity, both *in vitro* and *in vivo*. Altogether we have characterized the molecular mechanisms by which APT2 binds to membranes to encounter its palmitoylated targets, and how it removes palmitate from the membrane by incorporating it into a hydrophobic pocket followed by hydrolysis. Thus, APT2 not only depalmitoylates proteins but ensures that the freed palmitate does not remain in the membrane, ensuring membrane integrity.

SYMPOSIA

LI-01
MAPPING LIPID ALTERATIONS IN AMYOTROPHIC LATERAL SCLEROSIS THROUGH LIPIDOMIC ANALYSIS

Chaves-Filho AB, Pinto IFD, Yoshinaga MY, Miyamoto S
Departamento de Bioquímica, Instituto de Química - Universidade de São Paulo, São Paulo, SP, Brazil. E-mail: miyamoto@iq.usp.br

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects upper and lower motor neurons leading to muscle paralysis and death. While a link between dysregulated lipid metabolism and ALS has been proposed, precise and comprehensive information about lipid species and lipid pathways involved in ALS disease progression is still lacking. Aiming to map lipidome changes we have recently established a robust global lipidomic analysis workflow based on liquid chromatography coupled to high-resolution mass spectrometry. Using a semi-quantitative discovery lipidomics approach we were able to map major lipid alterations in the motor cortex, spinal cord and blood plasma of ALS rat model overexpressing mutant human SOD1 gene (SOD1-G93A). For the analysis, we used tissues and blood plasma collected at asymptomatic (70 days) and symptomatic (120 days) stages and compared the results age-matched controls. Interestingly, lipidome alterations found in the motor cortex were mostly associated with the age of the animals, while drastic lipidome changes linked to disease evolution were mostly observed in the spinal cord. Of note, symptomatic ALS rats showed a six-fold increase in cholesteryl ester species and a decrease in cardiolipin content compared to WT animals, reflecting increased lipid droplet accumulation and mitochondrial dysfunction. Additionally, our data also showed dysregulated sphingolipid metabolism in both the spinal cord and the blood plasma of ALS rats. Among sphingolipids, hexoxylceramides and acylceramides were markedly elevated in blood plasma at the symptomatic stage. Collectively, our results shed light on some important dysregulated lipid pathways linked to ALS disease progression. Although the mechanisms involved in lipid alterations found in ALS are still unclear, our study provides interesting insights into potential lipid targets for future studies of ALS.
Acknowledgements: CEPID-REDOXOMA (FAPESP 2013/07937-8), CNPq (424094/2016-9), CAPES and PRPG-USP.

LI-02
ROLE OF LIPID-MEMBRANE PROTEIN INTERACTIONS REVEALED BY MASS SPECTROMETRY AND MOLECULAR DYNAMICS SIMULATIONS

Barrera NP
Faculty of Biological Sciences, Pontificia Universidad Católica de Chile. E-mail: nbarrera@bio.puc.cl

Membrane proteins play versatile functions ranging from sensing extracellular environment to triggering complex intracellular transduction pathways. Nevertheless, elucidation of their atomic structure is usually impaired by the amphipathic nature, which has continuously stimulated novel complementary techniques to tackle their molecular architecture. Recently, several structural breakthroughs, via native mass spectrometry (nMS) approaches that allow to preserve lipid-membrane protein interactions in vacuum, have been developed to understand how lipids can regulate membrane proteins structure and function. However, as these measured interactions occur within the hydrophobic protection governed by

detergent micelles or lipid bilayers, mechanistic insights into the detection of these processes in vacuum are still not clear. By combining nMS on membrane transporters, such as EmrE and Sav1866, and molecular dynamics simulations, we have been able to characterize the phosphatidylethanolamine and cardiolipin binding to specific regions of the transporters. In addition, via nanomechanical simulations of the collision events between lipid-transporter complexed to detergent micelles and gas molecules in vacuum, we can propose the interaction energy pattern associated with detection of the lipid-transporter complexes. Taken together, these data lead to a molecular mechanism of the release of lipid-membrane proteins in the gas phase and highlights the role of lipid binding to control protein stability. *Funded by Millennium Science Initiative P10-035F and Wellcome Trust Programme #088150/Z/09/Z grants.*

LI-03
REGULATION OF SPHINGOSINE-1-PHOSPHATE AND ITS ROLE FOR CHRONIC INFLAMMATION AND CARCINOGENESIS

Radeke HH
Pharmazentrum frankfurt/ZAFES, Institute of General Pharmacology and Toxicology, Hospital of the Goethe University, Frankfurt am Main, Germany. E-mail: radeke@em.uni-frankfurt.de

For the last 15 years, sphingolipids (SphL) have been the focus of the research in my group. SphL enzymes generating sphingosine-1-phosphate (S1P), dephosphorylating and degrading it, as well as S1P receptors have been analyzed mainly in immune cells *in vitro*. The understanding of the *in vitro* function of S1P but moreover its role *in vivo* in chronic inflammation has been boosted by the success but also adverse effects of fingolimod (FTY720-phosphate) a modulator of 4/5 S1P receptors. SphL and its main representative S1P are basic lipid mediators ascribed to cellular functions like survival, proliferation, and migration, with a most prominent role as immune modulators and targets of clinical relevance in autoimmunity and chronic inflammation. Currently, new functions of S1P in basic cellular energy metabolism and carcinogenesis start to be addressed. Initially, the S1P receptor 1 (S1PR1) was the dominant therapeutic target. Meanwhile, more detailed knowledge gathered recently about the other four S1P receptors 2-5 (S1PR2-5) by us and others unraveled a vastly more complex picture of S1Ps actions and possibly, new therapeutic immune-modulatory applications. In this presentation, these newly defined actions will be covered in some detail rather than the "S1PR1-dependent lymphocyte sequestering effect". In addition, new findings of immune relevance regarding sphingolipid enzymes and transporters will be included and are briefly mentioned. New concepts of the spatial organization of adaptive immune cells, central memory versus local, tissue-resident memory lymphocytes, are arising in the field of immunology and clearly challenging the textbook concepts. Their meaning for the concepts of SphL and S1P immune function and subsequent possible new therapeutic targets will be discussed. In conclusion, the immune cell type- and their differentiation status-dependent expression of S1P receptors, the regulation and the activity of SphL enzymes, and transporters of the SphL pathway, vastly extend the scope of therapeutic options of SphL-targeting modulators in chronic inflammation, fibrotic diseases, and carcinogenesis.

LI-04
BIOSYNTHESIS OF SPHINGOLIPIDS WITH VERY-LONG-CHAIN PUFA: A HALLMARK OF DIFFERENTIATING MALE GERM CELLS

Oresti GM, Santiago Valtierra FX, Aveldaño MI
INIBIBB, CONICET-UNS y Dpto. Biología, Bioquímica y Farmacia, UNS, Bahía Blanca, Argentina. E-mail: gmoresti@criba.edu.ar

The sphingomyelins (SM) and ceramides (Cer) of rodent spermatogenic cells contain very-long-chain (C28-C32) polyenoic fatty acids (VLCPUFA), in non-hydroxy (n-V) and 2-hydroxy (h-V) forms. The SM and Cer species with n-V, present in meiotic spermatocytes, become in part h-V species in post-meiotic spermatids. In each of these cells, the mentioned species are located in the non-raft fraction of the plasma membrane. The enzymes required for PUFA elongation to n-V, Elovl5, Elovl2 and Elovl4, and the fatty acid 2-hydroxylase (Fa2h) that converts n-V to h-V, are expressed in germ cells, with Elovl4 and Fa2h protein levels being highest in spermatocytes and spermatids, respectively. The Cer and SM species with n-V and h-V are biosynthesized *de novo* in a germ cell type-specific and steroid hormone-dependent manner. CerS3, which specifically N-acylates VLCPUFA to sphinganine, is highly expressed in meiotic cells. The Elovl4 and CerS3 protein expression prevails in spermatocytes, is seminiferous stage-specific, and is mostly concomitant. In spermatids, the Fa2h protein appears concentrated in late stages, especially when they elongate and their heads change shape. The unique sphingolipid species with n-V and h-V, as well as the enzymes involved in their biosynthesis, are useful biomarkers for investigating normal and pathological aspects of germ and sperm cell functions. *Supported by SGCyT UNS-PGI-UNS (24/B272 to GMO and 24/B218 to MIA), FONCyT (PICT2017-2535 to GMO).*

PL-01
MEMBRANE REMODELING DURING ENDOSOMAL SORTING

Otegui MS
University of Wisconsin Madison, USA. E-mail: otegui@wisc.edu

Endocytosis and endosomal trafficking control the turnover of plasma membrane proteins, a critical process for cell survival, development, and physiological responses. Ubiquitylated plasma membrane proteins are internalized by endocytosis and delivered to endosomes, where they are sorted by the ESCRT (Endosomal Sorting Complex Required for Transport) machinery into endosome intraluminal vesicles for their final degradation in the vacuole. Besides their role in endosomal sorting, ESCRT proteins play other essential functions, by remodeling cellular membranes during cytokinesis, plasma membrane wound repair, nuclear envelope reformation after mitosis, and autophagy. I will discuss the diversification and functional specialization of plant ESCRT proteins and a novel membrane remodeling mechanism operating in plant endosomes.

PL-02
EMERGING ROLES OF PILS INTRACELLULAR AUXIN TRANSPORT FACILITATORS IN PLANT GROWTH AND DEVELOPMENT

Feraru E
Department of Applied Genetics and Cell Biology (DAGZ), BOKU University, Vienna, Austria. E-mail: elena.feraru@boku.ac.at

Environmental fluctuations are important determinants of plant growth and development, but the underlying mechanisms are largely elusive. We identified the family of PILS auxin transport facilitators at the endoplasmic reticulum, which limits the nuclear availability and thereby the signaling output of auxin. Here, I will discuss recent findings on how external and internal signals impact PILS function to steer organ growth rates.

PL-03
UNRAVELING PLANT VACUOLAR TRAFFICKING THROUGH FORWARD GENETICS, A RECIPE FOR SURPRISES

Ruano G [1], Delgadillo MO [1], Zouhar J [1], Sauer M [1], Shen J [2], Jiang L [2] and Rojo E [1]
[1]Centro Nacional de Biotecnología-CSIC, Madrid, España, [2] School of Life Sciences, The Chinese University of Hong Kong.
E-mail: erojo@cnb.csic.es

A unique characteristic of plant cells is the presence of very large vacuoles that allow for energetically-cheap cell expansion, maintain cellular homeostasis and sequester toxic compounds. In addition, vacuoles have crucial functions in biotic stress responses and are involved in fine-tuning signaling pathways, mainly through the degradation of receptor complexes. Hence, proper operation of the vacuole is required for many, if not all, cellular, developmental and physiological processes of the plant. In our lab, we are focused on unraveling the molecular mechanisms that operate the trafficking pathways responsible for delivering the membranes and contents of the vacuole, and thus ultimately for the biogenesis and function of this critical compartment. To this end, we have undertaken a forward genetic dissection of vacuolar trafficking in Arabidopsis. Here, we will report our latest results on the characterization of 17 novel vacuolar trafficking mutants and discuss some intriguing data on the localization of the trafficking factors identified.

PL-04
INVERSE MODULATION OF ANTIVIRAL AND ANTIBACTERIAL IMMUNITY BY TRANSMEMBRANE IMMUNE RECEPTORS

Ferreira MA [1], Li B[2], Teixeira RM [1], Martins LGC [1], Fontes EPB [1]
[1]Bioagro, Universidade Federal de Viçosa (INCTIPP/Bioagro/UFV), Brazil, [2]Huazhong Agricultural University, China
E-mail: bbfontes@ufv.br

Plants deploy various immune receptors to recognize pathogens and defend themselves. Crosstalk may happen among receptor-mediated signal transduction pathways in the same host during simultaneous infection of different pathogens. However, the related function of the receptor-like kinases (RLKs) in thwarting different pathogens remains elusive. In the current study, we demonstrate that NUCLEAR SHUTTLE PROTEIN-INTERACTING KINASE 1 (NIK1), which was previously shown to positively regulate plant antiviral immunity, acts as an important negative regulator of antibacterial immunity. Plants with nik1 null alleles exhibit dwarf morphology, enhanced disease resistance to the bacteria *Pseudomonas syringae* pv. *tomato* and *P. syringae* pv. *Maculicola* and enhanced pathogen-associated molecular pattern (PAMP) - immune response, a phenotype reversed by NIK1 complementation. Furthermore, NIK1 interacts constitutively with BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED KINASE 1 (BAK1) and FLAGELLIN SENSING 2 (FLS2), negatively regulates the formation of the FLS2/BAK1 complex and flg22-mediated responses. NIK1 interaction with the immune complex is strengthened by the PAMP flg22. Fgl22 treatment induces NIK1 phosphorylation through the activation of the FLS2/BAK1 complex and induces activation of the NIK1-mediated antiviral signaling; thereby increasing resistance to both DNA and RNA viruses. In contrast, virus infection increases resistance to bacteria as it alleviates the NIK1 negative impact in antibacterial immunity through viral suppressors of the NIK1 kinase activity. We will present additional data showing that nucleic acids may function as PAMPs to activate the NIK1-mediated signaling pathway, which also affects the dynamics of immune complex formation.

ST-01
THE PROTEOME'S DARK MATTER: ON THE ORIGIN OF NEOGENES AND NEOPROTEINS

Ho JSY[1,2], Marazzi I[1,2]
[1]Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA. E-mail: ivan.marazzi@mssm.edu

The emergence of new genes plays a critical role at cellular and organismal levels by influencing response and adaptation to the environment. Identifying processes that trigger the creation and fixation of new genes, in both somatic and germ cells, is important in understanding how cells adapt to stress in normal and diseased states. Here, we expand the concept of "one gene, many isoforms" to "one gene locus is a multitude of genes and isoforms". We will show how transcriptional and epigenetic mechanisms induced by stress can, in the absence of duplication, give rise to

novel genes (neo-genes) and proteins (neo-proteins). Mechanistically, neo-gene formation occurs via alternative splicing, transcriptional readthrough, hybrid-RNA formation, genomic overprinting, and altered translation. These events occur pervasively in virally infected cells as well as in cells undergoing an oncogenic transformation or being exposed to drugs. Unsurprisingly, viruses have co-opted many of these mechanisms to further expand their genetic repertoire during infection. Making new genes from pre-existing ones increases proteomic diversity and provides fodder for the emergence of new biological functions. This has important implications for a given organism within timescales encompassing both its lifespan (short term) and its evolutionary history (long term). In the long term, new beneficial alleles are fixed and passed through the generations. In the short term, neo-genes and neo-proteins contribute to the establishment of, and the response to, disease states associated with clonal expansions.

ST-02
SHEDDING LIGHT ON THE ROLE OF HISTONE CHAPERONES DURING DNA DAMAGE AFTER UV-B EXPOSURE IN PLANTS

Casati P
Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI-CONICET), Universidad Nacional de Rosario. E-mail: casati@cefobi-conicet.gov.ar

Terrestrial life evolved only after the stratospheric ozone layer formed and could absorb most of the damaging UV-B (280-315 nm) in solar radiation. The strong absorption of UV-B by biological molecules, particularly DNA, makes this radiation extremely dangerous. Because plants must absorb photons to power photosynthesis, they are inevitably exposed to damaging UV-B. Chromatin remodeling in response to UV-B has been implicated in plants. In particular, the package of DNA in the chromatin affects the structure and accessibility of DNA, and therefore the velocity of formation and repair of damage in DNA molecules. Thus, we explored the role of chromatin proteins in DNA damage and repair after UV-B exposure. In particular, we investigated the role of histone acetylation and the participation of histone chaperones in DNA repair and UV-B damage responses, and the function of chromatin proteins in the regulation of the cell cycle after exposure. Our results indicate that chromatin remodeling is a key process in DNA repair after a UV-B treatment and that lines deficient in this process are more sensitive to UV-B.

ST-03
EPIGENOME MAINTENANCE IN RESPONSE TO DNA DAMAGE

Polo SE
Laboratory of Epigenome Integrity, Epigenetics & Cell Fate Centre, CNRS/Université de Paris, France.
E-mail: sophie.polo@univ-paris-diderot.fr

The response to DNA damage in the cell nucleus proceeds on a chromatin substrate, whose integrity is central to cell functions and identity. The coordinated maintenance of genome stability and its organization into chromatin when challenged by genotoxic stress is thus critical. Yet, the underlying mechanisms are largely unknown, and how much the DNA damage response impacts the chromatin landscape is poorly understood. We approach these issues by investigating alterations in histone variant patterns at sites of DNA damage in mammalian cells. By combining *in vivo* tracking of newly synthesized histones and localized UVC damage, we have uncovered histone deposition pathways involved in restoring chromatin structure and transcriptional activity in response to genotoxic stress. We have also set up an innovative system for simultaneous visualization of new and parental histone dynamics at sites of DNA damage, which provided interesting insights into how the original information conveyed by chromatin may be preserved and unveiled a reshaping of histone variant patterns during chromatin repair. I will present our latest findings on these topics and discuss their implications for the maintenance of chromatin integrity following DNA damage.

ST-04
EPIGENETIC-microRNA CIRCUITRY REGULATING NEURAL CREST DELAMINATION AND CONDENSATION

Sánchez-Vásquez E[1], Bernardi YE[1], Strobl-Mazzulla PH[1]
INTECH (CONICET-UNSAM), Chascomús, Argentina. E-mail: strobl@intech.gov.ar

MiR-203 is a tumor-suppressor microRNA with known functions in cancer metastasis. Here, we explore its normal developmental role in the context of neural crest development using chick embryos. During the epithelial-to-mesenchymal transition of neural crest cells to emigrate from the neural tube, miR-203 displays a reciprocal expression pattern with key regulators of neural crest delamination - Phf12 and Snail2 - and interacts with their 3′UTRs. We show that maintenance of miR-203 expression inhibits neural crest migration in chick, whereas its functional inhibition using a 'sponge' vector or morpholinos promotes premature neural crest delamination. Bisulfite sequencing further shows that epigenetic repression of miR-203 is mediated by the *de novo* DNA methyltransferase DNMT3B, the recruitment of which to regulatory regions on the miR-203 locus is directed by SNAIL2 in a negative feedback loop. Later during neural crest migration, the miR-203 locus is actively demethylated and re-expressed at the time of their coalescence to form peripheral ganglia. Experiments of gain and loss of mir203-function result in premature condensation or disrupted ganglion formation, respectively. These findings reveal a dynamic epigenetic-microRNA regulatory network that influences the delamination and condensation of neural crest cells.

THURSDAY November 7, 2019

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

LECTURES

L-04
STRUCTURE OF THE ALTERNATIVE COMPLEX III IN A SUPERCOMPLEX WITH CYTOCHROME OXIDASE

Gennis RB
Department of Biochemistry, University of Illinois, 600 S. Mathews Street, Urbana, IL 61801, U.S.A. E-mail:r-gennis@illinois.edu

Alternative Complex III (ACIII), like Complex III, catalyzes the oxidation of membrane-bound quinol and reduction of cytochrome c but is structurally unrelated to Complex III. We solubilized the ACIII directly from membranes of *Flavobacterium johnsoniae* using styrene–maleic acid (SMA) copolymer in the absence of traditional detergents. The ACIII was isolated as a functional 1:1 supercomplex with an aa_3-type cytochrome c oxidase (cyt aa_3) within SMA copolymer nanodiscs. We determined the structure of the ACIII component of the supercomplex to 3.4 Å resolution by cryo-EM and constructed an atomic model for its six subunits, two of which are anchored to the lipid bilayer with N-terminal triacylated cysteine residues, resolved here for the first time. The structure also contains a [3Fe-4S] cluster, a [4Fe-4S] cluster, and six hemes *c* along with 11 phospholipid molecules. The ACIII is in direct contact with subunit III of the cyt aa_3 component of the supercomplex. The structure revealed that this subunit is structurally modified from the canonical form of subunit III to facilitate association with ACIII, suggesting a specific role of the supercomplex in the respiratory system of this bacterium. *This work was accomplished as a collaboration with the groups of Dr. John L. Rubinstein (University of Toronto, Canada) and Dr. Emad Tajkhorshid (University of Illinois).*

L-05
4 BILLION-YEAR-OLD NANOSCALE INTIMACY BETWEEN ION CHANNELS AND STEROL-LIKE MOLECULES: AN EVOLUTIONARY DIALOGUE

Barrantes FJ
Laboratory of Molecular Neurobiology, Institute of Biomedical Research, UCA-CONICET, Av. A. Moreau de Justo 1600, 1107 Buenos Aires, Argentina. E-mail: francisco_barrantes@uca.edu.ar

Neurotransmitter receptors belonging to the pentameric ligand-gated ion channel superfamily share structural motifs, which appear not only to be conserved among the "contemporary" proteins in our brains and other organs, but also extend to prokaryotic homologs in bacteria. The phylogenetically conserved motifs include domains of the ion channels embedded in the lipid bilayer, and particularly consensus sites for sterols in eukaryotic proteins and for hopanoids in bacterial channels, which do not synthesize cholesterol. This evolutionarily conserved structural trait suggests that the sterol surrogate molecules in bacteria and cholesterol in eukaryotes play similar roles in stabilizing transmembrane polypeptide chains. Our work on cholesterol-nicotinic acetylcholine receptor (nAChR) interactions has further established the functional crosstalk between the two molecules. I will illustrate some dynamic aspects of this dialogue, focusing on the translational motion of the nAChR, an important factor for determining receptor number and stability at the synapse and hence, synaptic efficacy. We combine single-molecule STORM superresolution localization microscopy of the nAChR with single-particle tracking, mean-squared displacement, turning angle, ergodicity, and clustering analyses to characterize the diffusional properties of individual molecules and their collective behavior in living cells. nAChR diffusion is highly heterogeneous: a mix of anomalous subdiffusive, Brownian, and superdiffusive. At the single-track level, the free walks of an individual trajectory are transiently interrupted by confinement sojourns in small nanodomains (~50 nm radius), with millisecond-long lifetimes. Millisecond-long confinement sojourns and s-long reversible nanoclustering affect all trajectories and determine the resulting macroscopic motional regime and the breadth of the heterogeneity in the ensemble population. The emerging picture is that the nAChR operates in a complex variety of motional regimes, including anomalous diffusion, and that these are subject to cholesterol modulation at the cell surface.

L-06
EXOSOMES: THE RISE OF A NEW PARADIGM IN INTERCELLULAR COMMUNICATION

Stahl PD
Washington University School of Medicine, St Louis, MO 63110 USA. E-mail: pstahl@wustl.edu

Over the last decade, a virtual tsunami of reports has focused attention on intercellular communication by exosomes and microvesicles, collectively referred to as extracellular vesicles (EVs). The concept rests on three pillars, the discovery that cells secrete small vesicles by fusion of multivesicular endosomes (MVEs) with the plasma membrane (exosomes), the observation that antigen-presenting cells package MHC-antigen complexes into intraluminal vesicles (ILVs) that, following their secretion, present antigen to receptive immune cells (indicating that a cell signaling event has occurred) and lastly, a set of observations that cells can package mRNA and miRNA into EVs, which can travel to cell targets where content is delivered in a functional form. It is now known that all eukaryotic cells secrete EVs, including plant cells. Thus, EV secretion and communication is an inter-kingdom phenomenon that will impact both the practice of medicine and agriculture. With the discovery of the ESCRT and other vesicle assembly pathways, much has been learned about how MVEs form and how they are secreted. However, the molecular mechanisms by which specific signaling content is packaged into nascent exosomes (or microvesicles) and how secretion is regulated remain to be elucidated. Moreover, how vesicles recognize their cognate targets and deliver content is poorly understood. Nevertheless, elucidating the cell biology and physiology of EVs is predicted to have a significant impact of our understanding of the pathophysiology of disease including of

diagnosis and therapy. The broad collection of homeostatic pathways that EVs participate in and regulate can be loosely described as one of the following: commingling, seeding, and signaling. Commingling refers to the transfer of cellular entities from one cell to another (e.g., regulatory molecules such as snoRNAs), seeding refers to the secretion of EVs with enzymatic activities that can influence or modify the microenvironment, and signaling occurs where specific signaling entities are selectively transferred from one cell to another. At this time, most examples of EV-dependent communication appear to be within local environments where different cell types, within a tissue setting, cross-talk with each other by EV-dependent transport of proteins and miRNAs. Although many obstacles remain, the emerging field of EV biology holds great promise to expand our understanding of all aspects of multicellular life.

L-07
CHROMATIN AND EPIGENETIC ALTERATIONS AFFECTING PLANT IMMUNITY

Alvarez ME
CIQUIBIC-CONICET, Dpto Química Biológica Ranwel Caputto, FCQ, Universidad Nacional Córdoba, Argentina
E-mail: malena@fcq.unc.edu.ar

Plants use sophisticated strategies to cope with microbial infections. This includes physical and chemical barriers that restrict a wide range of invaders, and a complex surveillance system that detects adapted pathogens that overcome the mentioned defenses. The plant innate immune system is based on a large family of pattern recognition receptors (PRR) and nucleotide-binding leucine-rich repeat proteins (NLR) that recognize microbial components at the cell surface or the intracellular level, respectively, to then activate defense networks. Over the past years, we have studied early defense responses induced by stimulation of PRR/NLR in the *Arabidopsis–Pseudomonas syringae* pv. *tomato* (*Pst*) pathosystem. Among them, we were recently interested in the epigenetic control of plant immunity. Our finding that *Arabidopsis* tissues infected with *Pst* lose basal pericentromeric heterochromatin compaction led us to evaluate the causes and consequences of such a response. Normally, these regions are kept condensed by the accumulation of repressive epigenetic marks, such as 5-methyl cytosine (5-mC) and dimethylated H3 histone lysine 9 (H3K9me2). We found that after infection, 5-mC is reduced at CG, CHG, and CHH sites of centromeric repeats and pericentromeric transposable elements (pTE). This occurs without replication suggesting that active demethylation causes chromatin relaxation. Furthermore, several studies reported that infection triggers de-repression of TEs with consequent activation of proximal PRR/NLR genes, and genome-wide methylation analysis described the multiple alterations of DNA methylation that occur in infected cells. However, the impact of DNA methylation on plant immunity is complex and requires further study. A clear example is limited information that exists on the behavior of pTE, which are the majority in the genome, under biotic stress. We found that *Pst*-infection triggers early expression of pTE and their subsequent re-silencing by small RNAs (sRNAs) through RNA-directed DNA methylation (RdDM). Interestingly, these sRNAs also map to PRR/NLR genes that are distal from pTEs. Moreover, *Arabidopsis* mutants that lose repression of pTEs and accumulate homologous sRNAs constitutively express distal PRR/NLR genes. Our findings indicate that the epigenetic state pTEs affects plant immune responses, and the de-repression of these elements leads to the activation of immune receptor genes by *trans*-acting mechanisms. This type of regulation has been recently suggested for other pathosystems, and could still be replicated in other stress conditions, suggesting new roles of pTEs in adaptation.

SYMPOSIA

CB-01
SORTING WITHOUT A GOLGI COMPLEX: THE ENDOPLASMIC RETICULUM AS THE SORTING CORE FACILITY IN THE PROTOZOAN *GIARDIA LAMBLIA*

Touz MC, Zamponi N
Instituto de Investigación Médica Mercedes y Martín Ferreyra, INIMEC – CONICET – Universidad Nacional de Córdoba, Argentina
E-mail: ctouz@immf.uncor.edu

Our understanding of protein and lipid trafficking in eukaryotic cells has been challenged by the finding of different forms of compartmentalization and cargo processing in protozoan parasites. *Giardia lamblia* is a parasitic organism that belongs to the Excavata, an early diverging group within Eukarya. Both early divergence (contingency) and parasitism (selective pressure) are evolutionary forces that have given rise to the unique feature of this organism. *Giardia* lacks many organelles that are considered hallmarks of the eukaryotic organization, such as mitochondria, peroxisomes, endosomes, and the Golgi apparatus. Moreover, the *Giardia* genome is small, has very few introns, and lacks many genes that are considered essential for the maintenance of the eukaryotic cellular machinery. Our studies showed that, in the absence of a Golgi compartment in *Giardia*, proteins destined for secretion are directly sorted and packaged at specialized ER regions enriched in COPII coatomer complexes and ceramide. We also demonstrated that ER-resident proteins are retained at the ER by the action of a KDEL receptor, which, in contrast to other eukaryotic KDEL receptors, showed no interorganellar dynamic but instead acts specifically at the limit of the ER membrane. Our results suggest that the ER-exit sites and the perinuclear ER-membranes are capable of performing protein sorting functions. These findings support the vision that *Giardia* adaptation represents an extreme example of reductive evolution without loss of function.

CB-02
THE VPS13 FAMILY OF LIPID TRANSPORT PROTEINS

Conibear E

Department of Medical Genetics, University of British Columbia, Vancouver, Canada. E-mail: conibear@cmmt.ubc.ca

Mutations in each of the four human VPS13 (VPS13A-D) proteins are associated with distinct neurological disorders: chorea-acanthocytosis, Cohen syndrome, early-onset Parkinson's disease, and spastic ataxia. Recent evidence suggests that the different VPS13 paralogs transport lipids between organelles at different membrane contact sites. However, how each VPS13 isoform is targeted to these different sites is not known. Yeast has a single Vps13 protein whose localization depends on developmental stage or nutrient conditions. We have found that the membrane localization of yeast Vps13 requires a conserved six-repeat region, the Vps13 Adaptor Binding (VAB) domain, which binds to organelle-specific adaptors. Our results suggest that all adaptors compete for a single binding site in the VAB domain. Using a systematic mutagenesis strategy to define the contribution of each repeat, we have identified the putative adaptor binding site. Importantly, a missense mutation in VPS13D that causes spastic ataxia is predicted to impact this binding site, suggesting a conserved adaptor binding role for the VAB domain. Current efforts are focused on identifying novel VAB binding partners in both yeast and humans.

CB-03
MECHANISM OF ORGANELLE IDENTITY WITHIN THE ENDOLYSOSOMAL PATHWAY

Ungermann C
University of Osnabrück, Department of Biology/Chemistry, Osnabrück, Germany. E-mail: cu@uos.de

Eukaryotic cells have an elaborate endolysosomal system of interconnected organelles, much similar to our digestive tract. Proteins, in particular, membrane proteins such as nutrient transporters, which need to be degraded during cellular adaptation, travel along this pathway from the plasma membrane by being incorporated into endocytic vesicles to the early endosome. These then mature into late endosomes, where intraluminal vesicles form to remove selected proteins from the endosomal surface. These vesicles are then degraded with their entire luminal content, once late endosomes fuse with the lysosome, called vacuole in yeast. Likewise, autophagosomes are formed during autophagy as a major catabolic pathway and deliver proteins and organelles in double-membrane vesicles by fusing directly with lysosomes. This poses the question, how each organelle gains identity to become fusion competent. Using yeast as a model system, we have now evidence that both endosomes and autophagosomes use comparable mechanisms to acquire the machinery to fuse with vacuoles. A first requirement during this process is the recruitment of the small Rab7 GTPase to the surface of each organelle. Rab GTPases can be kept soluble in the GDP-bound form in the cytosol. They require a guanine nucleotide exchange factor (GEF) for their membrane localization and activation into the active GTP-form. The principles of GEF localization to the right membrane are mostly unknown. We have identified and characterized the Rab7 GEF, called Mon1-Ccz1, and I will present evidence on its function and regulation. Once on the membrane, Rab7 binds in its GTP-form to effectors, in our case to the HOPS tethering complex. This large complex acts like a bridge between endosomes (or autophagosomes) and lysosomes, but can also catalyze fusion. This unique ability is due to one of its subunits, which can assemble SNAREs as the fusion machinery present on both endosomes (and autophagosomes) and lysosomes. I will present a working model, how both processes—endosome and autophagosome fusion with lysosomes—can be studied *in vivo* and *in vitro*. Our analysis provides, in addition, insights into the regulation of metabolic adaptations of yeast cells—and these also occur in human cells.

CB-04
IN SILICO TESTING THE FUNDAMENTAL MECHANISMS AND LOGIC OF INTRACELLULAR TRAFFIC

Cebrián I, Pavarotti M, Mayorga LS
IHEM -CONICET, Universidad Nacional de Cuyo, Facultad de Ciencias Exactas y Naturales, Mendoza. E-mail: lmayorga@fcm.uncu.edu.ar

Intracellular traffic is a central process in cellular physiology. Numerous macromolecules must be transported in the endocytic and exocytic pathways for the correct function of eukaryotic cells. However, the way by which macromolecules are transported between compartments is still a matter of intense debate. Our group has developed a simulation platform, based on a combination of agent-based modeling and ordinary differential equations, for processes that occur in dynamic organelles that merge, divide, and change position and shape, while altering their composition by complex networks of molecular interactions and chemical reactions. We have already described how this modeling strategy successfully reproduces transport in the endocytic pathway. Our next objective is to apply this modeling approach to the trafficking within the Golgi apparatus. It is worth mentioning that several hypotheses regarding this issue are still in conflict, despite the abundance of experimental results and the development of ingenious probes to assess transport. Interestingly, our modeling strategy is flexible enough to simulate all these hypotheses and test the agreement between the different models and experimental data. At present, we have successfully modeled the transport of small and large membrane-associated cargos using the "cisternal progression/maturation" hypothesis. We expect that the active dialogue between simulations and experimental results will foster our understanding of the logic underlying the transport mechanisms that efficiently sort a large number of macromolecules to their final destination inside and outside the cell.

RN-01
TRANSLATION AFFECTS MRNA STABILITY IN A CODON DEPENDENT MANNER IN HUMAN CELLS AND ZEBRAFISH EMBRYOS

Wu Q [1], Medina S [1], Gopal Kushawah[1], DeVore M [1], Castellano L [1], Hand J [1], Wright M [1], Bazzini AA [1,2]
[1]Stowers Institute for Medical Research, 1000 East 50th Street, Kansas City, MO 64110, USA,[2]Department of Molecular and Integrative Physiology, University of Kansas Medical Center, 3901 Rainbow Boulevard, Kansas City, KS 66160, USA. E-mail: arb@stowers.org

mRNA translation decodes nucleotide into amino acid sequences. However, translation has also been shown to affect mRNA stability depending on codon composition in model organisms, although the universality of this mechanism remains unclear. Our results show that in human cells, translation strongly affects mRNA stability in a codon dependent manner, impacting the homeostatic mRNA and protein levels. Using three independent approaches to measure exogenous and endogenous mRNA decay, we defined the regulatory identity of the 61 coding codons in 4 different human cell lines. We demonstrate that the regulatory information affecting mRNA stability is encoded in codons and not in nucleotides. We found that stabilizing human codons (optimal codons) tend to be associated with higher tRNA levels and higher charged/total tRNA ratios. These results suggested that the 'tRNA ready to go' level (quantity and quality) may be serving as a determinant of codon optimality. The molecular mechanism is still unclear, and while we observe that in human lines the poly(A)-tail length correlates with the codon-mediated mRNA stability (similarly to other species), we demonstrate that the poly(A)-tail is not required by this mechanism in both human and zebrafish embryos. And likely, the shortening of the poly(A)-tail in genes enriched in non-optimal codons is an indirect consequence of decreased stability rather than a required step in the codon-mediated mechanism. This mechanism depends on translation; however, the number of ribosome loads into an mRNA modulates the codon-mediated effects on gene expression. Therefore, this result leads us to explore that *trans*-regulatory elements and physiological conditions where mRNA translation is globally affected, may also impact the codon-mediated effects on gene expression. In sum, our work provides definitive evidence that translation strongly affects mRNA stability in a codon-dependent manner in human cells.

RN-02
DYNAMICS AND FUNCTIONAL RELEVANCE OF RIBONUCLEOPROTEIC MEMBRANE-LESS ORGANELLES

Boccaccio GL
Instituto Leloir (FIL) and IIIBBA-CONICET. E-mail: gboccaccio@leloir.org.ar

The dynamic formation of stress granules (SGs), processing bodies (PBs) and related RNA membrane-less organelles, regulates diverse cellular processes, including the coordination of functionally linked messengers, and the translational regulation at the synapse among others. The formation of these cellular bodies is governed by liquid-liquid phase separation (LLPS) processes, and their dysregulation may provoke pathogenic aggregation. LLPS *in vitro* depends on the thermal diffusion of macromolecules, which is limited inside cells, where the condensation and dissolution of membrane-less organelles (MLOs) would be helped by energy-driven processes. We found that the active transport by the retrograde motor dynein helps SG assembly, whereas the anterograde motor kinesin mediates SG dissolution, and a tug of war between these molecular motors allows transient SG formation. As in the case of PBs, SGs contain repressed mRNAs but are not required for their silencing, and the contribution of SGs to the protective response triggered upon stress remains elusive. In addition to SGs and PBs, several RNA granules and related MLOs are present in neurons. We found that distinct subsets of PBs and additional RNA bodies located at dendrites and synapses respond selectively to specific synaptic stimuli, which promote their rapid assembly or disassembly, thus controlling the release of bound mRNAs. This modulates the local transcriptome and allows fine-tuning of the translation at the post-synapse. More recently, we focused on Smaug MLOs. Smaug orthologs are highly conserved in the animal kingdom and recognize a wide variety of stem-loops termed Smaug Recognition Elements (SREs), which are present in a large number of mRNAs including nuclear transcripts that encode mitochondrial enzymes. We performed time-lapse confocal microscopy and found that Smaug1 MLOs are highly motile and frequently contact mitochondria, speculatively coordinating the transport and/or the translation of nuclear-encoded mRNAs at the mitochondrial periphery.

RN-03
FRAGMENTATION OF EXTRACELLULAR RIBOSOMES AND tRNAs SHAPES EXTRACELLULAR SMALL RNA PROFILES

Tosar JP[1,2], Gámbaro F[1], Fagúndez P[2], Segovia M[1,3], Hill M[1,3], Ivanov P[4], Cayota A[1,3]
[1]Institut Pasteur de Montevideo, Uruguay. [2]Facultad de Ciencias y [3]Facultad de Medicina, Universidad de la República, Uruguay [4]Brigham and Women's Hospital, Harvard Medical School, USA. E-mail: jptosar@cin.edu.uy

Extracellular RNAs have attracted increasing interest in recent years. This is explained by their role in intercellular communication and their use as biomarkers in minimally invasive liquid biopsies. Most studies performed thus far have focused on extracellular microRNAs and their presence inside extracellular vesicles (EVs), of which exosomes are the most renown type. It is generally accepted that the mechanisms for sorting small RNAs to the extracellular milieu are selective (i.e., RNAs harboring specific sequence motifs are recognized by molecular machinery that induces their release from cells). However, studies from our group have challenged this model. Our work suggests that RNAs are mainly released as a consequence of their steady-state levels inside the cells and that RNA intracellular and extracellular stability is a key variable influencing this process. Identification of RNAs capable of forming oligomeric structures that render them resistant to extracellular RNases encouraged us to characterize the extracellular non-vesicular RNaome, both in the presence and absence of externally added ribonuclease inhibitor. Inhibition of extracellular RNase A-family members enabled us to identify and purify extracellular tRNAs, ribosomes and even polysomes. Density gradient centrifugation provided robust separation between EV-associated and extravesicular tRNAs. The latter being highly sensitive to fragmentation in an anticodon sequence-dependent manner and are probably the source of highly abundant non-vesicular tRNA halves identified by us and others, both in cell culture and human biofluids such as plasma, serum, saliva, urine, and cerebrospinal fluid. Thus, relative extracellular enrichment of these fragments can be explained by a combination of their differential extracellular stability and the differential sensitivities of their parental

tRNAs to extracellular fragmentation (in contrast to selective secretion of these small RNAs directly from cells). In cell culture, dead cells seem to be the source of extracellular ribosomes and tRNAs. Nevertheless, these RNAs form complexes which, *in vitro*, are potent activators of dendritic cells. We speculate that extracellular tRNAs and ribosomes might function as damage-associated molecular patterns in necrotic tissue. Taken together, our work introduces the concept of extracellular RNA metabolism and points out to the extracellular biogenesis of certain small RNA types. Moreover, it highlights that EV-RNA profiles might be contaminated with co-purifying extravesicular RNAs (especially when EVs are obtained by ultracentrifugation or size-exclusion chromatography) and, by identifying RNAs highly resistant to extracellular degradation, provides several sequences which are predicted to accumulate in biofluids and thus show biomarker potential.

RN-04
STORIES ON DENGUE VIRUS AND HOST CELL pre-mRNA PROCESSING

Pozzi B, Torti MF, Vaz-Drago R, Gaioli N, García Solá M, Gebhard LG, Iglesias NG, Gamarnik A, García C, Srebrow A[1]
[1] IFIBYNE-UBA-CONICET; Depto.FBMC, FCEyN, UBA. E-mail: asrebrow@fbmc.fcen.uba.ar

Dengue virus is a highly prevalent human pathogen transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes. This virus presents a sense and single-stranded RNA genome that encodes for a limited set of proteins, depending on the host cell machinery for productive replication. While eukaryotic cells rely on innate immune responses to protect from viral infections, viruses have evolved sophisticated mechanisms of evasion that counteract the antiviral response. A few years ago, in collaboration with the Gamarnik laboratory, we reported a link between dengue infection and host cell splicing machinery, mainly the intrusion of the viral protein NS5 in the cellular spliceosome. In RNA-seq experiments from human cells infected with dengue, we observed severe alterations of the splicing process, basically an enrichment of intronic sequences and changes in alternative splicing patterns. In particular, we noted that the transcript of the SAT1 gene, of well- known antiviral action, evidences higher inclusion of alternative exon 4 in infected cells. This exon4-containing SAT1 mRNA isoform is targeted for degradation by non-sense mediated decay, whereas the exon 4-lacking variant codes for a spermidine/spermine acetyl-transferase enzyme that decreases the reservoir of these polyamines in the cell, limiting viral replication. Delving into the molecular mechanism responsible for the alternative splicing change in SAT1 pre-mRNA upon viral infection, we observed that this condition decreases protein levels of RBM10, a splicing factor responsible for SAT1 exon 4 skipping. We found that the dengue polymerase NS5 interacts with RMB10 and triggers its degradation in a proteasome-dependent manner. Moreover, RBM10 over-expression in infected cells prevents SAT1 splicing change and also decreases viral replication, while knock- down of this splicing factor not only enhances the splicing change but also benefit viral replication. These results lead us to propose an anti-viral role for RBM10. In addition, RBM10 depletion attenuates the infection-triggered transcriptional induction of interferon and pro-inflammatory cytokines. Currently, we hypothesize that RBM10 may be exerting its antiviral role not only via its already well-documented activity as a splicing regulator but also by modulating the innate immune response through impinging the signaling pathway downstream of the viral sensor protein RIG-I. Undoubtedly, understanding the molecular mechanisms underlying the complex host-pathogen interplay is of paramount importance for the proper design of antiviral therapies.

RN-05
ANGEL2, A MEMBER OF THE CCR4-NOT FAMILY OF DEADENYLASES, IS A MAMMALIAN 2',3'-CYCLIC PHOSPHATASE

Pinto PH., Kroupova A, SchleifferAS, Mechtler K, Jinek M, Weitzer S and Martinez J.
Max Perutz Labs Vienna, Medical University of Vienna and Vienna BioCenter, Dr. Bohr-Gasse 9/3, 1030 Vienna, Austria.
E-mail: Javier.Martinez@meduniwien.ac.at

The termini of cellular RNA molecules are frequently modified with a 2',3'-cyclic phosphate (2',3'>P) group that arises as a result of endonuclease cleavage, exonuclease trimming or *de novo* synthesis by the RNA 3'-terminal phosphate cyclase (RTCA). RNAs displaying 2',3'>P are essential intermediates in mammalian cells as they are substrates for the tRNA ligase complex during pre-tRNA processing and *XBP1* mRNA splicing in the Unfolded Protein Response (UPR). Terminal 2',3'>P can be hydrolyzed to yield 2'-phosphate (2'-P), 3'-hydroxyl (3'-OH), or 2'-OH, 3'-P. Only T4 polynucleotide kinase-phosphatase (T4 PNK) is known to convert 2',3'>P into 2',3'-OH, but such activity has not been detected in eukaryotic cells. Here we identified ANGEL2, a member of the CCR4-NOT family of proteins and a predicted deadenylase, as a human RNA 2',3'-cyclic phosphatase, through activity-guided purification from HeLa cell extracts. ANGEL2 is a monomer that converts 2',3'>P and 2'-P in single and double-stranded RNA termini into 2',3'-OH, indicating that the removal of the 2',3'>P group occurs via a 2'-P intermediate. The crystal structure of ANGEL2 suggests a plausible reaction mechanism and shows close similarities with true deadenylases such as CCR4a and CCR4b. ANGEL1, a paralog of ANGEL2, displays a much weaker, RNA 2',3'-cyclic phosphatase activity. As 2',3'>P is the preferred substrate of the human tRNA ligase complex, we show that mis-expression of ANGEL2 affects the efficiency of pre-tRNA splicing and *XBP1* mRNA splicing during UPR. Our results indicate that ANGEL proteins may be generally involved in RNA pathways that rely on the ligation or hydrolysis of 2',3'>P.

FRIDAY November 8, 2019

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

LECTURES

L-08
ROBUSTNESS AND INFORMATION PROCESSING IN A CELL FATE DECISION SYSTEM

Colman Lerner A
Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE), CONICET-UBA, Buenos Aires, Argentina
E-mail: colman-lerner@fbmc.fcen.uba.ar

Cell behavior requires the ability to detect and respond to extracellular signals. We use the response to mating pheromone in the budding yeast *Saccharomyces cerevisiae* as a model system. I will present recent work that addresses two general problems signaling pathways must solve: eliciting robust responses despite large variability in cellular components and appropriate integration of extracellular and intracellular signals. Robustness: The effect of ligands usually depends on the amount of the ligand–receptor complex. Thus, changes in receptor abundance should have quantitative effects. However, the response to pheromone in yeast is robust (unaltered) to variation in the abundance of the GPCR receptor, Ste2, responding instead to the *fraction* of occupied receptor. To learn how *fractional occupancy* is measured by the cell, we developed a mathematical model of GPCR activation, which suggested that the ability to compute fractional occupancy depended on the physical interaction between the inhibitory RGS, Sst2, and the receptor. I will present here the experimental evidence that supports this model and explain how it might apply to other GPCR systems and to signal transduction pathways in general. Signal integration: The pheromone pathway has an antagonistic relationship with the cell cycle. The pathway MAPK (Fus3) arrests the cell cycle by activating a CDK inhibitor. Conversely, when cells pass START, the CDK (Cln2-Cdc28) blocks pheromone response by phosphorylating the scaffold protein Ste5, which prevents its association with the plasma membrane. We found that efficient Cln2-Cdc28 inhibition of Ste5 requires the help of Fus3. This is surprising since previous literature suggested that CDK should be able to do the blocking by itself. However, our results thus show that Fus3, which usually serves to promote mating, also serves to block mating during the wrong part of the cell cycle. We made this discovery thanks to our ability to perform fast single-cell measurement of Ste5 membrane recruitment in a large number of mutant strains. This approach, in combination with analyses of Ste5 phosphorylation via gel mobility assays and quantitative mass-spectrometry, led us to propose a detailed molecular model that I will present here to explain this unexpected MAPK-CDK collaboration.

SYMPOSIA

MI-01
BACTERIA RESPONSES TO HIGH ENVIRONMENTAL PHOSPHATE: PHYSIOLOGICAL ASPECTS AND POTENTIAL BIOTECHNOLOGICAL APPLICATIONS

Rapisarda VA
INSIBIO (CONICET-UNT) and Fac. de Bioquímica, Química y Farmacia (UNT), Tucumán, Argentina. E-mail: vrapisarda@fbqf.unt.edu.ar

In the last decade, our research has focused on the physiological changes associated with environmental phosphate (Pi) and intracellular polyphosphate (polyP) in bacteria of clinical, agronomic, and biotechnological interest. PolyP, a polymer of orthophosphate residues, has a crucial role in bacteria survival, stress responses, biofilm formation, and virulence factors production. We found that *Escherichia coli* grown in media with >37 mM Pi maintained a high polyP level in late stationary phase, which could account for changes in gene expression and enzyme activities that enhance its stationary-phase fitness. Indeed, maintenance of high polyP levels in high Pi medium improved bacterial survival and tolerance to stress agents when compared to cells grown in sufficient Pi medium. Once accumulated in high Pi, polyP degradation by PPX (an exopolyphosphatase) mediated copper tolerance with the participation of the low-affinity inorganic phosphate transport system. Moreover, in media with sufficient Pi (< 25 mM), polyP degradation during the stationary phase triggered biofilm formation via a LuxS quorum-sensing system. Indeed, PhoB was activated by acetyl phosphate when high polyP levels were maintained in the stationary phase, with the consequent repression of biofilm formation owing to the inhibition of c-di-GMP synthesis and autoinducer-2 production. In uropathogenic *E. coli* isolates causative of prostatitis, environmental Pi differentially affected the virulence phenotypes (biofilm formation, curli, cellulose production, and expression of virulence factors). However, in spite of the complexity of UPEC responses to environmental Pi, polyP degradation induced biofilm formation in all the assayed clinical isolates. The plant growth-promoting bacterium *Gluconacetobacter diazotrophicus* accumulated polyP and degraded it in high Pi medium, thereby improving survival, tolerance to stressors, biofilm formation capacity, and competence as a promoter of plant growth. In *Herbaspirillum seropedicae*, another plant growth-promoting bacterium, Pi concentration changes intracellular polyP levels, generating a differential expression of 620 genes, being 53% repressed and 43% induced in high Pi condition. In the lactic acid bacterium *Lactobacillus paraplantarum*, the proteins involved in glycolysis, pyruvate metabolism, and pentose phosphate pathway were up-regulated in high Pi stationary phase cells, implying an active metabolism similar to that of exponential phase cells. Contrary, stress-related chaperones were repressed in high Pi medium, inferring that high polyP levels in the stationary phase may provide chaperone capacity. Together, environmental Pi concentration modulates polyP levels in bacteria, affecting their gene expression profile, cellular fitness, and biofilm formation. Accordingly, media Pi concentration results critical for some bacteria and should be considered in physiological studies and in the development of strategies to benefit their biotechnological potential.

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

MI-02
ROLE OF RESPIRATORY NADH OXIDATION IN THE REGULATION OF *STAPHYLOCOCCUS AUREUS* VIRULENCE

Schurig-Briccio LA[1], Parraga Solorzano PK[2], Lencina AM[1], Radin JN[2], Chen GY[3], Sauer J-D[3], Kehl-Fie TE[2], Gennis RB[1].
[1]Dpt. of Biochemistry, [2]Microbiology, UIUC, IL, [3]Medical Microbiology & Immunology, UW-M, WI, USA. E-mail: lschurig@illinois.edu

The success of *Staphylococcus aureus* as a pathogen is due to its capability of fine-tuning its cellular physiology to meet the challenges presented by diverse environments, which allows it to colonize multiple niches within a single vertebrate host. Elucidating the roles of energy-yielding metabolic pathways could uncover attractive therapeutic strategies and targets. In this work, we seek to determine the effects of disabling NADH-dependent aerobic respiration on the physiology of *S. aureus*. Differing from many pathogens, *S. aureus* has two type-2 respiratory NADH dehydrogenases (NDH-2s) but lacks the respiratory ion-pumping NDHs. Here, we show that the NDH-2s, individually or together, are not required either for respiration or growth. Nevertheless, their absence eliminates biofilm formation, production of α-toxin, and reduces the ability to colonize specific organs in a mouse model of systemic infection. Moreover, we demonstrate that the reason behind these phenotypes is the alteration of fatty acid metabolism. Importantly, the SaeRS two-component system, which responds to fatty acids regulation, is responsible for the link between NADH-dependent respiration and virulence in *S. aureus*.

MI-03
MULTICELLULAR LIFE OF *PSEUDOMONAS PUTIDA*: FROM METABOLIC SIGNALS TO GLOBAL REGULATORS

Barrientos-Moreno L, Molina-Henares MA, Huertas-Rosales Ó, Travieso ML, Ramos-González MI, Espinosa-Urgel M
Estación Experimental del Zaidín. CSIC. E-mail: manuel.espinosa@eez.csic.es

Biofilm formation is a common persistence strategy in many bacterial species. These multicellular communities associated with solid surfaces are considered the predominant lifestyle of bacteria in the environment. The process of biofilm development on abiotic and biotic surfaces is driven by complex regulatory mechanisms that our group has been studying for several years in the plant-beneficial bacterium *Pseudomonas putida* KT2440. Two large, secreted adhesins, LapA and LapF, and four exopolysaccharides are the main structural components involved in surface colonization and biofilm formation by *P. putida*. LapA is essential for efficient attachment, and LapF participates in cell-cell interactions, whereas the role and relevance of each exopolysaccharide may vary depending on the environmental conditions. As in many other bacteria, the intracellular second messenger cyclic diguanylate (c-di-GMP) modulates the transition between planktonic and sessile lifestyles: high levels of this molecule favor bacterial adhesion to surfaces and the establishment of biofilms, whereas low levels promote biofilm dispersal. We have identified several elements controlling the turnover of c-di-GMP in *P. putida* and directly or indirectly regulating the expression of adhesins and exopolysaccharides at different levels. They include transcriptional factors and global post-transcriptional regulators of the CsrA/RsmA (acronyms for carbon storage regulator and regulator of secondary metabolism) family. These are small sequence-specific RNA-binding proteins that usually repress gene expression (although sometimes they function as activators) by altering transcript elongation, translation, and/or RNA stability. The genome of *P. putida* encodes three Rsm homologs named RsmA, RsmE, and RsmI. The deletion of the three genes causes increased biofilm formation and reduced motility, associated with changes in c-di-GMP levels and expression of LapF and exopolysaccharides. While the precise environmental signals triggering the switch between bacterial lifestyles remain poorly characterized, we have obtained recent evidence of a regulatory network connecting the metabolism of certain amino acids with c-di-GMP signaling.

MI-04
DNA SEQUENCE DIVERSITY OF THE MINICIRCLE HYPERVARIABLE REGION OF *TRYPANOSOMA CRUZI*

Tomasini N, Rusman F, Floridia-Yapur N, Diosque P
Instituto de Patología Experimental (CONICET-UNSa) Email: patricio.diosque@unsa.edu.ar

Trypanosoma cruzi, the protozoan causative of Chagas disease, is classified into six main lineages: TcI-TcVI. This parasite, as all the kinetoplastids, has a unique and large mitochondrion that contains a complex network of DNA, the kinetoplast DNA (kDNA). The kDNA represents approximately 20–25 % of the total cellular DNA in *T. cruzi* and consists of two kinds of circular DNA molecules: maxicircles and minicircles. Maxicircles contain mitochondrial genes characteristic of other eukaryotes. Minicircles are present in tens of thousands of copies. Each minicircle is organized into four highly conserved regions located 90° apart of each other, and an equal number of hypervariable regions (mHVRs) interspersed between the conserved regions. mHVRs code for short RNAs called guide RNAs (gRNAs). gRNAs are involved in the edition of several mitochondrially encoded mRNAs. Until now, the genetic diversity of the mHVRs was virtually unknown. However, cross-hybridization assays using mHVRs showed hybridization only between isolates belonging to the same lineage or even between genetic groups at the intra-lineage level. We developed an amplicon deep sequencing approach that allows an accurate knowledge of the sequence diversity of the hypervariable region of kDNA minicircles in representative strains of the six main lineages of *T. cruzi*. This approach could be also used as a typing method for hundreds of samples at a time. Our results also allow the identification of the mHVR sequences coding for the gRNAs needed for the edition of mitochondrially encoded mRNAs. The developed approach provides us also with the tools to further investigate other still unanswered questions, such as: What is the reason for strong variations in mHVR diversity among strains? How do hybrids deal with the biparental inheritance of minicircles? what is the function, if any, of the mHVRs that do not code for gRNAs? How does the mHVRs repertoire evolve? Why is such an expensive mRNA editing mechanism maintained?

MI-05
STRUCTURAL BASIS OF GLYCOGEN BIOSYNTHESIS REGULATION IN BACTERIA

Guerin ME
Structural Biology Unit, CIC bioGUNE, Spain. E-mail: mrcguerin@cicbiogune.es

ADP-glucose pyrophosphorylase (AGPase) catalyzes the rate-limiting step of bacterial glycogen and plant starch biosynthesis, the most common carbon storage polysaccharides in nature. A major challenge is to understand how AGPase activity gets regulated by metabolites in the energetic flux within the cell. Here we report the first crystal structures of the paradigmatic homotetrameric AGPase from *Escherichia coli* in complex with its physiological positive and negative allosteric regulators, fructose-1,6-bisphosphate (FBP) and AMP, and sucrose in the active site. FBP and AMP bind to partially overlapping sites located in a deep cleft between glycosyltransferase A-like and left-handed beta-helix domains of neighboring protomers, accounting for the fact that sensitivity to inhibition by AMP gets modulated by the concentration of the activator FBP. Single point mutations of key residues in the AMP-binding site decrease its inhibitory effect but also clearly abolish the overall AMP-mediated stabilization effect in wild-type EcAGPase. Single point mutations of key residues for FBP binding did not revert the AMP-mediated stabilization. Strikingly, an EcAGPase-R130A mutant displayed a dramatic increase in activity when compared with wild-type EcAGPase, and this increase correlated with a significant increment of glycogen content *in vivo*. Altogether, we propose a model in which the energy reporters regulate EcAGPase catalytic activity by intra-protomer interactions and inter-protomer crosstalk, with a sensory motif and two regulatory loops playing a prominent role.

YI-01
THE IMPACT OF NON-CODING GENETIC VARIATION ON PROMOTER FUNCTION

Schor IE.
Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE, UBA-CONICET). Departamento de Fisiología, Biología Molecular y Celular (FCEyN, UBA). E-mail: ieschor@fbmc.fcen.uba.ar

All known biological species show genetic variation, which accounts for heritable phenotypic differences among the individuals. On the large genomes of metazoans, most of the natural genetic variants occur at non-coding positions. Yet, many of these variants are not neutral but have the potential to affect gene expression by modifying regulatory elements such as promoters, enhancers, or non-coding RNA genes. We have shown that a large proportion of gene promoters that are active during *Drosophila* embryonic development harbor functional genetic variants that affect transcription. Effects might vary between modulation of start site usage or changes in promoter strength, and their frequency depends on the promoter class. We have also uncovered the presence of cryptic effects of promoter variants on transcriptional noise, which are often alleviated by their genetic context. We are now starting to apply these concepts to characterize the impact of natural promoter variants on gene regulatory networks during mammalian development, as well as the possible role of non-coding somatic mutations on the phenotypic characteristics of cancer cells.

YI-02
DYSFUNCTIONAL MITOCHONDRIAL FUNCTION AND DYNAMICS IN HUMAN DOWN'S SYNDROME INDUCED PLURIPOTENT STEM CELLS

Hernández-Fuentes C[1], Gómez-Contreras A [1], Arias-Durán C [1], Bravo F [1], Gómez W [1], Leiva-Navarrete S [1], Parra V [1,2,3]
[1]Advanced Center for Chronic Diseases; [2]Autophagy Research Center and [3]Center for Exercise, Metabolism and Cancer Studies, Faculty of Chemical and Pharmaceutical Sciences, University of Chile. Sergio Livingstone 1007, Independencia, Santiago, Chile.
E-mail: vparra@ciq.uchile.cl

Mitochondria are highly dynamic organelles that undergo different processes of fusion, fission, biogenesis, and mitophagy. Perturbation in either direction in normal mitochondrial dynamics can lead to the accumulation of damaged and inefficient organelles. The gene DSCR1 or RCAN1, on the critical Down syndrome (DS) region 1, is an inhibitor of the phosphatase calcineurin that promotes mitochondrial fission through DRP1 dephosphorylation. We used advanced imaging techniques and siRNA to dissect the role of RCAN1 in mitochondrial dynamics and function of DS induced pluripotent stem cells (iPSCs). Trisomic iPSCs showed a decrease in the mitochondrial number per cell, along with an increase in the mitochondrial mean volume in comparison to disomic cells, which is consistent with lower rates of mitochondrial fission. Moreover, DS iPSCs had an increased basal and proton leak-induced oxygen consumption, as well as decreased levels of PINK1 (a protein involved in mitophagy) compared to control iPSCs. The decreased mitochondrial fission and PINK1 protein levels of the DS iPSCs were rescued with an RCAN1 siRNA. These data suggest that RCAN1 decreases mitochondrial fission and mitophagy by the inhibition of the calcineurin-dependent activation of DRP1, thus increasing mitochondrial network connectivity. *Funding: FONDECYT 1190743, ACT172066, and FONDAP 15130011.*

YI-03
DARK: DEEP ANNOTATION OF REPRESENTATIVE KINETOPLASTIDS

Radío S, Garat B, Sotelo-Silveira J, Smircich P
Universidad de la República / Facultad de Ciencias – UdeLaR. Uruguay

Since the completion of the genome sequencing of the model trypanosomatids in 2005, we have faced the problem of the huge percentage of predicted proteins not functionally annotated. Although this is a frequent feature of the genomes of many organisms, the phenomenon accentuates in trypanosomatids. This is possibly due to their early divergence from the rest of the eukaryotes, which hinders the commonly used technique of annotation by sequence similarity. To help mitigate this problem and improve the annotation of the kinetoplastid genomes, we performed a new genomic annotation by homology prediction using highly sensitive statistical techniques. In this way, we were able to assign functional annotation to several hundred proteins previously classified as hypothetical. On the other hand, we generated a free tool that allows easy access to this information.

YI-04
PLATFORM FOR PROTEIN SURFACE DISPLAY WITH MULTIPLE BIOTECHNOLOGICAL PURPOSES

Vizoso Pinto MG
INSIBIO - CONICET, Tucumán

Peptidoglycan is the main component (90%) of Gram-positive (G+) bacteria. This polymer consisting of sugars and amino acids is essential for bacterial shape and resistance. Further, it is recognized by immune effectors to mount an immune response to G+ bacteria. The lysin motif (LysM) is a ubiquitous motif across kingdoms, which in bacteria allows proteins involved in cell growth and division to bind non-covalently to peptidoglycan. This property can be exploited for building recombinant fusion proteins that can be exposed to G+ bacterial or peptidoglycan-coated surfaces. This research line aims to establish a platform for displaying proteins on the surface of G+ bacteria and look for possible biotechnological applications such as vaccines, protein purification methods, detection and enrichment of G+ bacteria. We have identified, cloned and characterized two novel LysM domains from *Lactobacillus fermentum* to use them as an anchor for displaying antigens on G+ bacteria without modifying them genetically. We selected the best domain and constructed a destination vector for bacterial expression compatible with recombinational cloning for tagging proteins with the LysM motif. We prepared bacterium-like-particles from a different strain of *Lactobacillus* with an acid and heat treatment that kills bacteria, exposes peptidoglycan and releases cytoplasmatic content as checked with transmission electron microscopy. We constructed a fusion protein consisting of the yellow fluorescent protein Venus fused to the selected LysM. The recombinant protein was expressed in *Escherichia coli* Rosetta using standard procedures, and the supernatant containing the fusion protein was incubated in PBS with BLPs for binding at room temperature. (i) First, we evaluated the effectiveness of binding by fluorescent microscopy and SDS–PAGE. After binding, we evaluated if the recombinant protein could be eluted from the complex and if this could be used as a protein purification method. (ii) Second, we tested if the LysM5–Venus–IBLP027 complex can be used as a nasal mucosal vaccine. For this, we have immunized Balb/c mice with three doses of the complex and measured specific systemic (IgG) and local (respiratory IgA) antibodies increases in vaccinated mice. (iii) In on-going work, we fused LysM to viral antigens from the Hepatitis E virus and HSV-2 to evaluate the immunogenicity of these complexes when administered orally and nasally, respectively. Further, we are testing if these complexes can also be used for the detection of specific antibodies in patient sera. In conclusion, we present a versatile platform for displaying several antigens on the surface of G+ bacteria and show some of its possible applications.

YI-05
A CDC42 SIGNALING PATHWAY REGULATES MITOCHONDRIAL FISSION

Bisbal M.
Laboratorio de Neurobiología. Instituto Ferreyra (INIMEC-CONICET-UNC). Córdoba, Argentina.

Mitochondria are key organelles involved in several homeostatic processes within eukaryotic cells, which are crucial for cell viability. Mitochondria exhibit a complex morphology that consists of interconnected tubules forming a highly dynamic network that is constantly remodeled by fission and fusion events. The frequency of these events is under tight regulation, and any alteration of this equilibrium impacts mitochondrial structure and function. The interaction between the cytoskeleton and the mitochondrial network has been strongly associated with the structural plasticity of the organelle. Several works have shown that the contact sites between the endoplasmic reticulum (ER) and the mitochondrial network predict mitochondrial fission events. This process requires the activity of INF2, an ER-associated actin-nucleating protein, as well as other cytoplasmic and mitochondrial components, such as DRP1 and its receptors. This is how the ER generates an initial constriction that reduces the mitochondrial tubule diameter, which leads to the accumulation of DRP1 receptors and the subsequent recruitment of DRP1, ultimately leading to the excision of the mitochondrial tubule. In addition, it was recently described that fission events also require the activity of Dynamin 2. On the other hand, it was demonstrated that the actin microfilaments can accumulate specifically in the outer mitochondrial membrane and that this accumulation correlates with an increase in the fragmentation of the organelle in an INF2-ER independent manner. These results suggested that the actin assemblies induce a mitochondrial deformation, promoting Drp1 recruitment and subsequent mitochondrial fission. Although the evidence clearly points to a leading role played by the actin cytoskeleton in mitochondrial dynamics, the signaling pathways that regulate this process are still unknown. Here, using live-cell imaging and confocal microscopy in primary mouse embryonic fibroblast cells (MEFs), we show a novel Cdc42 signaling pathway involving Wasp, Arp2/3, Cortactin, Dynamin 2 and Drp1 that regulates mitochondrial fission and therefore mitochondrial structural plasticity.

ORAL COMMUNICATIONS

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

BIOTECHNOLOGY

BT-C01
IDENTIFICATION AND CHARACTERIZATION OF NOVEL B-GALACTOSIDASES FROM A SEQUENCE-BASED METAGENOME ANALYSIS OF STABILIZATION PONDS

Eberhardt MF, Irazoqui JM, Amadio A.
INTA EEA-Rafaela – CONICET. E-mail: eberhardt.maria@inta.gob.ar

Whey, a by-product of the dairy industry, especially of cheese production, is composed of lactose (4.5–5 % w/v), proteins (0.6–0.8 % w/v), lipids (0.4–0.5–5 w/v), and mineral salts (8–10 % of dried extract). It represents an environmental problem because of the high BOD, COD and the large volume of production. The worldwide production of whey is currently estimated at nearly 180–190 million tons/year. Alternatively, it can be used as an attractive raw material for value-added products through physicochemical or enzymatic treatments. For example, Galactooligosaccharides (GOS) are non-digestible carbohydrates derived from lactose, composed of galactose monomers and a terminal glucose unit, varying in chain length and type of linkage. It has been demonstrated to have prebiotic properties, related to their impact on the composition and activity of the intestinal microbiota. GOS can be produced from lactose by the transgalactosylation activity of β-galactosidases (EC 3.2.1.23). Here, we report the identification, production, and characterization of novel genes encoding the putative lactose-modifying enzyme β-galactosidase from whole-genome shotgun metagenome from stabilization ponds of two small dairy companies in the central region of Santa Fe. The genes were identified via the conservation of catalytic domains, compared against the CAZy database. All hits corresponding to families with β-galactosidases activity (GH1, GH2, GH35, and GH42) were selected, using 70% identity and 70% sequence coverage as thresholds. These enzymes were taxonomically classified using the closest BLAST hit against the nr database. 189 candidate genes were found for GH1, GH2, GH35, and GH42 families. All sequences identified corresponded to bacteria. Candidates per family differ significantly: GH1 is the most represented with 100 candidates, while GH2 has 60, GH42 has 8 and GH35 only 6. Twelve candidates were selected for cloning and expression in heterologous systems. Candidate genes were amplified and cloned into plasmid vectors for inducible protein expression. Five putative β-galactosidase proteins were efficiently expressed. Clones allowed *Escherichia coli* DH5α to use lactose as a sole carbon source and hydrolyze 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-gal) on agar plates. Four affinity-purified enzymes cleaved efficiently ortho-Nitrophenyl-β-galactoside (*O*NPG) substrate at physiological pH (5–7) with optimal temperatures between 30–37 °C. In conclusion, we successfully identified and characterized a novel group of mesophilic β-galactosidases from diary stabilization ponds metagenomes. Optimization of expression in heterologous systems, purification, and hydrolysis reaction conditions would allow evaluating its use for the production of lactose-free products or whey value-added by-products, such as GOS.

BT-C02
IMPROVING POTATO YIELD THROUGH AN ENRICHED NITROGEN METABOLISM BY EXPRESSION OF NITRIC OXIDE SYNTHASE FROM *SYNECHOCOCCUS* PCC 7335

Nejamkin A [1], Foresi N [1], Del Castello F [1], Decima Oneto C [2], Correa-Aragunde N [1], Massa G [2,3] , Feingold S [2], Lamattina L [1]
[1]Instituto de Investigaciones Biológicas, CONICET-UNMdP, Mar del Plata, Argentina, [2]Laboratorio de Agrobiotecnologia. EEA INTA Balcarce, Argentina, [3]Facultad de Ciencias Agrarias, UNMdP, Balcarce, Argentina. E-mail: anejamkin@gmail.com

Nitric oxide (NO) is a signal molecule that participates in various processes of development and stress tolerance in plants. NO participates in defense responses to pathogens, resistance to abiotic stress, tropic and developmental processes triggered by phytohormones. In animals, the enzyme nitric oxide synthase (NOS) catalyzes the biosynthesis of NO from arginine, but it was not found yet in higher plants. Recently we identified and characterized a novel NOS type from the cyanobacteria *Synechococcus* PCC 7335 (*SyNOS*). *SyNOS* has a similar structure to animal NOS with both oxygenase and reductase domains. However, *SyNOS* presents an additional domain in the amino-terminal, which encodes a globin. The globin domain of *SyNOS* seems to function into the hemoglobin/NO cycle, in which NO is oxidized to NO_3^- and oxyhemoglobin is converted to methemoglobin. Then, NO_3^- would enter into the nitrogen (N) assimilation pathway producing amino acids and proteins. Since arginine is the main organic storage N source in plants, SyNOS expression would allow the remobilization of N from Arginine into NO_3^-. Transgenic potato plants expressing SyNOS under the constitutive promoter 35S has an increased shoot growth when they are grown under poor nutritional conditions (5 L pots containing 66 % p/p vermiculite and 33% p/p of soil). In this condition, a 30% increase in chlorophyll content was detected in transgenic SyNOS potato plants, indicating a better state of nitrogen. On average, SyNOS transgenic lines produced 50% more tubers than wild type under sufficient and deficient nutrient conditions. Our results suggest that SyNOS transgenic plants might be more efficient in the use of nutrients, requiring fewer fertilizers for tuber production and would be able to increase potato yields in poor soils.

CELL BIOLOGY

CB-C01
ARCHITECTURE AND FUNCTION OF THE VACUOLE-MITOCHONDRIA MEMBRANE CONTACT SITE

González Montoro A, Auffarth K, Ungermann C.
University of Osnabrueck. E-mail: ayelen.gonzalez-montoro@biologie.uni-osnabrueck.de

Membrane contact sites (MCSs) are regions where the membranes of two organelles are closely apposed and establish a direct communication platform. The network of MCSs in the cell is arising as essential for the transport of lipids and signaling molecules and the regulation of organelle dynamics. The yeast vacuole forms a contact site with the mitochondria, called vCLAMP (vacuolar and mitochondrial patch), which is regulated by carbon source metabolism. The formation of vCLAMPs involves the vacuolar Rab GTPase Ypt7 and the Ypt7-interacting Vps39 subunit of the HOPS tethering complex. We have found that the general preprotein translocase of the outer membrane (TOM) subunit Tom40 is the direct binding partner of Vps39 on mitochondria. We have generated a mutant version of Vps39 that is functional as a subunit of the HOPS complex but is unable to establish vCLAMPs. We have used this mutant to address the effects of vCLAMP disruption. We have found that cells that cannot establish vCLAMPs are hypersensitive to metal toxicity pointing to a defective vacuole function. Additionally, we have found a functional connection to the nuclear vacuolar junction: Cells in which both contacts are simultaneously disrupted display impaired growth and defects in both vacuole and mitochondrial morphology and function. These results support a model of a network of partially redundant and interconnected membrane contact sites that play a major role in organelle biogenesis and function.

CB-C02
GENETIC POLYMORPHISMS IN G-QUADRUPLEX AFFECT THE TRANSCRIPTION OF HUMAN DISEASE-RELATED GENES

Lorenzatti A[1], Piga, E[1], Margarit E[2], Gismondi M[2], Binolfi A[1], Calcaterra NB[1], Armas P[1].
[1]IBR – CONICET-UNR. -[2]CEFOBI - CONICET-UNR. E-mail: lorenzatti@ibr-conicet.gov.ar

G-quadruplexes (G4) are secondary structures that can be transiently folded within proximal promoter regions (PPRs) during transcription when single-stranded G-rich regions are exposed. Genomic scale association studies by massive DNA sequencing revealed that single nucleotide polymorphisms (SPNs) associated with human diseases are mainly present nearby transcription start sites (TSS) within promoters and 5' untranslated regions. The goal of this work was to identify SNPs associated with human diseases that are located in PPRs (here defined as the -1000 bp from TSS) and may affect the folding of putative G4 (PG4), hereafter called SNP-PG4. First, we performed a bioinformatic analysis by downloading (using Ensembl Biomart tool) the flanking sequences (+/− 50 bp) of each SNP located within the PPR associated with human diseases from COSMIC, ClinVar, dbSNP and HGMD. Nearly 3% of the 427479 sequences with at least two variants contained PG4s. To identify those SNPs most affecting G4 folding, we selected sequences containing at least one PG4 in either the reference or the variant versions, i.e., SNPs that disrupt or promote the G4-folding. Then we used a novel G4 folding predictor based on a large-scale machine learning from an extensive experimental G4-formation dataset (Quadron) to choose for those PG4s with the higher scores. SNP-PG4 within the PPRs of the genes *GRIN2B*, *F7*, *CSF2*, and *SIRT1* were further analyzed. Spectroscopic analyses by Circular Dichroism (CD) demonstrated that the of the SNP-PG4 identified *GRIN2B* and *F7* fold *in vitro* as G4 and that SNPs cause quantitative spectral changes. Moreover, 1D ^{1}H NMR spectroscopy confirmed the formation of the G4s identified for *GRIN2B* and *F7* and that SNPs induce quantitative and qualitative changes. qPCR stop assays and CD melting assays indicated that SNPs induce G4 stability changes. Finally, PG4s cloning into the pGL3 promoter vector revealed that firefly luciferase reporter activity was altered by SNPs when transfected into HEK293 cells. *GRIN2B* codes for a subunit of glutamate receptor and has been reported related to Parkinson's, Huntington's, and Alzheimer's diseases and a broad spectrum of neurodevelopmental disorders. *F7* codes for coagulation factor VII, a member of the coagulation cascade which miss-regulation causes rare severe bleeding disorders known as congenital factor VII deficiency. Results gathered in this work suggest that SNPs in the PPRs of these genes may alter G4 folding thus modifying transcriptional activity. In spite of this, SNP-PG4s should be considered as a novel molecular etiology mechanism for the predisposition or establishment of human diseases, as well as potential targets for chemotherapeutic treatments.

CB-C03
INVOLVEMENT OF SUMO CONJUGATION IN snRNA BIOGENESIS

Bragado L, Magalnik M, Srebrow A.
IFIBYNE-UBA-CONICET; Departamento de Fisiología, Biología Molecular y Celular, FCEyN, UBA. E-mail: laureanobragado@gmail.com

In addition to protein-coding genes, RNA polymerase II (Pol II) transcribes numerous genes that correspond to non-coding RNAs, including those of small nuclear RNAs (snRNAs). snRNAs are not only a fundamental component of the spliceosome, but also some of them are necessary for the transcriptional activity of Pol II, as well as for maintaining cellular homeostasis. Although the functions of snRNAs are well understood, the regulation of their biogenesis is still a matter of deep investigation. snRNA genes share common features with protein-coding genes, including the relative positioning of elements that control transcription and RNA processing. However, there are important differences in the set of proteins required for the proper expression and metabolism of these two gene types. SUMOylation is a reversible post-translational modification consisting in the conjugation of SUMO (small ubiquitin-related modifier) to different target proteins. It mainly regulates intra- and inter- molecular interactions, and consequently, the function of a great variety of cellular proteins. A few years ago, we reported the influence of SUMOylation of spliceosomal protein components on spliceosome assembly and catalytic activity. Currently, we are studying the involvement of SUMO conjugation in snRNA biogenesis. So far, we have observed that modifying the levels of global SUMOylation in cultured mammalian cells alters

the proportion of nascent vs. mature snRNAs measured by RT-qPCR. Furthermore, we have shown that the transcription factor SNAP43 and the 3'-end processing factor INTS11, both required for snRNA biogenesis, are modified by SUMO, and we have identified the target residues of this modification, allowing us to generate SUMOylation deficient mutants of these two factors. The SNAP43 SUMOylation mutant fails to activate transcription of snRNAs and, intriguingly, leads to cell death in a dominant-negative manner. On the other hand, the INTS11 mutant is unable to achieve 3' end processing of precursor snRNAs. Thus, SUMO conjugation clearly is involved in the regulation of snRNA biogenesis, warranting further investigation.

CB-C04
INHIBITION OF TUMOR GROWTH, STEROIDOGENESIS, HORMONE AND DRUGS RESISTANCE BY AN ACYL-COA SYNTHETASE 4 NEW INHIBITOR

Castillo AF[1], Orlando UD[1], Maloberti PM[1], Prada JG[1], Dattilo MA[1], Solano AR[1], Szajnman S[2], Lorenzano Menna P[3], Gomez DE[3], Rodriguez JB[2], Podesta EJ[1].
[1]INBIOMED (UBA-CONICET), School of Medicine; [2]UMYMFOR (CONICET-FCEyN); [3]Lab. of Mol. Oncology, UNQ; Buenos Aires, Argentina. Email: castillofernanda@yahoo.com

Acyl-CoA synthetase 4 (ACSL4) is an isoenzyme of the fatty acid ligase-coenzyme A ligase family that takes part in arachidonic acid metabolism. This enzyme is involved in cancer development, particularly in breast and prostate cancers, where a correlation between its high expression and tumor cell aggressiveness has been found. Functionally, it was shown that ACSL4 is part of the mechanism responsible for increased breast and prostate cancer cell proliferation, invasion, and migration. ACSL4 is involved in the regulation of several signal transduction mechanisms, including the mTOR pathway, and furthermore increases the expression of proteins involved in drug resistance. Another relevant property of this enzyme is its participation as an essential protein in the activation of cholesterol transport from the external to the internal mitochondrial membrane, the regulatory and rate-limiting step in the syntheses of all steroids. The development of selective inhibitors for ACSL4, which may inhibit tumor growth and steroidogenesis, may be an important tool in the prevention and treatment of breast and prostate cancers expressing ACSL4. In this work, we have developed and characterized a new ACSL4 inhibitor, PRGL493. An ACSL4 homology model was generated by MODELLER, and a docking based virtual screening was performed. The selected compound was modified to improve particularly solubility properties and fully characterized by nuclear magnetic resonance and mass spectroscopy. We demonstrated that PRGL493 was effective in reducing AA-CoA levels, as a product of ACSL4 activity, in a cell-free assay system using recombinant protein and in intact cells using cell models for steroidogenesis and tumor growth. Our compound inhibited cell proliferation and migration of highly aggressive human breast and prostate cancer cell lines (MDA-MB-231 and PC-3 respectively) and *in vivo* using the Chick Embryo Chorioallantoic Membrane model assay. In addition, PRGL493 inhibited steroid synthesis both *in vitro* in Leydig, adrenal, and PC-3 prostate cancer cells and *in vivo* in a mouse model, inhibiting cholesterol transport to the inner mitochondrial membrane and thus preventing steroid accumulation. Moreover, the inhibitor produced sensitization to hormonal and chemotherapeutic treatment. The combination of PRGL493 with tamoxifen, cisplatin, doxorubicin, or paclitaxel in MDA-MB-231 cells, or with docetaxel in PC-3 cells, showed a synergistic effect on the inhibition of cell proliferation. The results show that ACSL4 is an essential target for breast and prostate cancer therapy. These findings open an important route to treat these tumors that may lead to the development of rational cancer treatment and continue to lead to the introduction of the combination of chemotherapy to specific other agents.

CB-C05
MULTIPLE REACTION MONITORING (MRM): CHALLENGES IN MASS SPECTROMETRY BASED PROTEIN QUANTIFICATION

Zieschang S[1], Macur K[1], Lei S[1], Harwood E[1], Lech K[1,2], Jaquet S[1], Morsey B[1], Fox H[1], Ciborowski P[1].
[1] University of Nebraska Medical Center, Omaha, NE [2] Faculty of Chemistry, Warsaw University of Technology, Warsaw, Poland [3] Intercollegiate Faculty of Biotechnology of University of Gdansk and Medical University of Gdansk, University of Gdansk, Gdansk, Poland.
Email: pciborowski@unmc.edu

Macrophages are essential cells of the innate immune system. Exposure to toxic substances such as methamphetamine (Meth) impairs their protective function. We used a targeted proteomics approach to determine the effects of Meth exposure on infected macrophages. Our model uses human monocyte-derived macrophages (hMDM) *in vitro* infected with HIV-1 (CIC), exposed to Meth after infection (CIM), and exposed to Meth before and after infection (MIM). The trypsin digested samples were analyzed with UPLC-MS/MS using a multiple reaction monitoring (MRM) mode. Proteins selection was based on previous studies of the HIV-1 infected hMDMs proteome. Five replicate injections of three concentrations of whole-cell lysates were measured for three conditions CIC, CIM, and MIM. The spiked-in BSA peptides showed no interferences in the presence of the complex biological matrix in contrast to endogenous β-actin, galectin-1, and galectin-9. Galectin-1 expression increased in MIM at all tested concentrations when compared to either CIC or CIM. Experimental verification of *in silico* predefined peptides of two randomly selected proteins present in the investigated cell lysates, clathrin heavy chain 1 and chitinase-3-like protein 1, showed that only 19.4% and 8.3% were high-responding peptides, respectively. BSA peptides spiked-in to CIC, CIM, and MIM-treated hMDM whole-cell lysates show concentration-dependent linearity in the highly complex biological matrix, unlike the peptides from endogenous proteins. Multiple concentrations injections are therefore needed to rule out false positives. Only few peptides are high-responding for MRM.

CB-C06
CHMP4B IS REQUIRED FOR THE EFFICIENT REPLICATION OF *TOXOPLASMA GONDII* IN DENDRITIC CELLS

Croce C[1], Mayorga LS[1], Blanchard N[2], Cebrián I[1].
[1]IHEM-CONICET, Facultad de Ciencias Médicas, UNCuyo, Mendoza, ARGENTINA.
[2]CNRS/INSERM/Universitéde Toulouse-UPS, CPTP, Toulouse, FRANCIA. E-mail: croce.cristina@gmail.com

Toxoplasma gondii is an apicomplexan parasite responsible for toxoplasmosis, a disease that can be lethal to immunocompromised individuals. *T. gondii* is an intracellular parasite that resides within a parasitophorous vacuole (PV), where it grows and replicates. It is thought that *T. gondii* can interact with the host cell cytoplasm through the intravacuolar tubulo-vesicular network, a membranous structure connected to the PV membrane. In this work, we focus on the interaction between the parasite and dendritic cells (DCs), the most potent antigen-presenting cells capable of triggering CD4+ and CD8+ T cell responses. First, we found that DC treatment with U-18666A, an inhibitor of multivesicular bodies (MVBs) formation, significantly inhibited the antigen presentation ability of *T. gondii*-derived antigenic peptides in the context of both, MHC-I and MHC-II molecules. Nevertheless, the treatment with this drug did not affect the infection rate of DCs. By indirect immunofluorescence and confocal microscopy, we observed clear recruitment of different MVB components to the PV, although most strongly the protein CHMP4b, core of the ESCRT III complex. In addition, upon DC infection by *T. gondii*, we evidenced a complete redistribution of CHMP4b around the PV and an over-expression of this protein, as compared to uninfected DC. Indeed, by Western blot analysis, we confirmed a gradual increase in CHMP4b expression along with the progression of *T. gondii* infection, suggesting a critical demand for CHMP4b by the parasite. To test this possibility, we silenced CHMP4b expression in DCs and analyzed the proliferation of a fluorescent *T. gondii* strain. Accordingly to our hypothesis, we found that the parasite is not able to replicate correctly in DCs after CHMP4b depletion, especially at later time points post-infection. Given the major role of CHMP4b in the formation of intraluminal vesicles, we think that it might be necessary for the adequate development of the *T. gondii* intravacuolar tubulo-vesicular network. Ongoing electron microscopy-based experiments in this direction will allow as to determine the relevance of CHMP4b during the formation of these intravacuolar structures.

CB-C07
IDENTIFICATION OF A MINIMAL SEQUENCE OF P21 THAT SENSITIZES TUMOR CELLS TO DNA-DAMAGING AGENTS

De la Vega MB, Mansilla SF, Bertolin A, Gottifredi V.
Fundación Instituto Leloir- Instituto de Investigaciones Bioquímicas de Buenos Aires (IBBA). E-mail: beldlv89@gmail.com

DNA-damaging agents used in traditional chemotherapy are cytotoxic by interfering with DNA replication. These agents target cells with a high proliferation rate by generating lesions in the DNA that impair replication and cause cell death. The efficacy of anticancer treatments is, however, highly influenced by the cellular capacity to respond to DNA damage. One central mechanism that enables cancer cells to survive is Translesion DNA Synthesis (TLS). This process involves specialized DNA polymerases that synthesize a short patch of DNA across the lesion, a situation where replicative DNA polymerases would normally stall. Therefore, inhibiting TLS would be deleterious to these cells when used in combination with DNA-damaging agents. Our group has previously identified the cyclin-CDK inhibitor p21 as the first global inhibitor of TLS. A stabilized version of p21 (sp21) can inhibit the recruitment of TLS polymerases to replication factories after DNA damage without interfering with normal DNA replication, and this is dependent on its PCNA-binding domain. In this work, we have found that a smaller version of p21, which contains only its PCNA-Interacting Region (sPIR), is sufficient to robustly inhibit the recruitment of TLS polymerases to replication factories post ultraviolet (UV) radiation. By using a non-replicative lentivirus system as an overexpression tool, we have found that the sPIR increases cell death in the context of many DNA-damage inductors such as UV, cisplatin, and hydroxyurea. Lately, we have also found that, in many tumor cell lines, co-inhibition of TLS and Chk1, a protein with a pivotal role in the intra S-phase checkpoint, efficiently synergize to enhance replicative stress and cell death. Our data suggest that the PIR domain of p21 is a versatile tool with potential therapeutic utility.

CB-C08
GENETIC POLYMORPHISMS ON G-QUADRUPLEXES AS A CAUSE OF ONCOGENES TRANSCRIPTIONAL AND TRANSLATIONAL EXPRESSION VARIATIONS

Piga E, Bezzi G, Lorenzatti A, Binolfi A, Calcaterra NB, Armas P.
Instituto de Biología Molecular y Celular de Rosario (IBR) – CONICET-UNR. E-mail: armas@ibr-conicet.gov.ar

G-quadruplexes (G4) are nucleic acid secondary structures that can be transiently folded within proximal promoter regions (PPRs) in G-rich single-stranded DNA regions exposed during transcription and in G-rich RNA sequences within 5' untranslated regions (5’ UTRs) or other mRNA regions involved in translational control. G4s have been proposed as novel transcriptional and translational regulatory elements originally and mainly described in oncogenes. On the other hand, genomic scale association studies by massive DNA sequencing revealed that single nucleotide polymorphisms (SPNs) associated with human diseases are present mainly near transcription start sites, within PPRs and 5' UTRs. The goal of this work was to identify SNPs overlapped with putative G4 forming sequences (PG4) described as transcriptional or translational regulators (located within PPRs or 5' UTRs, respectively) of oncogenes, that may affect G4 folding, hereafter called SNP-PG4. First, we performed a bioinformatic analysis using Ensembl database to identify the SNPs (reported in COSMIC, ClinVar, dbSNP and HGMD genetic variation databases) overlapped with the PG4s (and their +/− 5 bp flanking sequences), described as transcriptional regulators for 10 oncogenes and as translational regulators for 15 oncogenes. For each reference sequence, we generated a collection of variable sequences containing each polymorphism and a mutant sequence with no PG4 (unable to form G4). Then we used several DNA and RNA G4 folding predictors to identify those SNP-PG4 that may affect G4 folding or stability. Based on the results of this analysis, from 88 DNA and 256 RNA sequences corresponding to the SNP-PG4 of the analyzed oncogenes, we chose 41 and 15, respectively, for further analysis. The selected sequences correspond to the *c-MYC*, *BCL2*, *cKIT*, *RET*, and *VEGF* oncogenes for G4s in PPRs and the *CCND3*, *NRAS*, *HSAFY*, *ESR1*, *FGF2*, *ZIC1*, and *TRF2* oncogenes for G4s in 5’ UTRs. Spectroscopic analyses by Circular Dichroism (CD) demonstrated that some SNPs cause quantitative or qualitative spectral changes. Moreover, qPCR stop assays and

CD melting assays indicate that the same SNPs induce G4 stability changes. In agreement, 1D ^{1}H NMR spectroscopy confirmed that SNPs induce quantitative and qualitative changes for the SNP-PG4s identified for *c-MYC* and *NRAS*. Finally, SNP-PG4s that produced significant structural variations *in vitro* were cloned into a pGL3 promoter vector (for PG4s controlling transcription) or into a psiCHECK-2 vector (for RNA PG4 controlling translation) and were transfected into HEK293 cells, revealing that SNPs altered luciferase reporter activity. Results gathered in this work suggest that SNP-PG4s that alter G4 folding may be the cause of differential expression of oncogenes leading to tumor predisposition, establishment, progression or metastasis and should be considered as a novel molecular etiology mechanism for the predisposition or establishment of diseases.

CB-C09
CONVERGENT APPROACH TO THE STUDY OF LONGEVITY OF *CERATITIS CAPITATA* AND *DROSOPHILA MELANOGASTER* MALES

Rabossi A , Bochicchio PA , Quesada-Allué LA , Pérez , MM.
IIBBA-Conicet, Depto. Química Biológica-FCEyN-UBA and Fundación Instituto Leloir. E-mail: arabossi@leloir.org.ar

Our main objective is to correlate the physiological and gene expression profiles from fly males showing unusual longevity. We studied two classical models for senescence profiles: the Medfly *Ceratitis capitata* and the vinegar fly *Drosophila melanogaster*. We developed laboratory studies of behavioral parameters of male medflies in a novel arena containing lek-like arrangements of three flies. We were able to determine that young males showing none or very low rate of spontaneous supine falls (VLS males) and showing significant higher adult mean life expectancy (46.9 days of VLS males instead 27.1 days of supine males:), were significantly more longevous. Thus, we considered the supine behavior as a longevity predictor. We then studied the longevous and non-longevous male gene expression. In a completely different but convergent approach, we studied for the first time, the physiological characteristics and adult senescence profiles of the *D. melanogaster* mutant *tan-1,* unable to hydrolize N-β-alanyl-dopamine; that were compared to those of wild type Canton-S and mutant *ebony-1* (unable to synthesize N-β-alanyl-dopamine). We then demonstrated that *tan-1* was significantly longevous (up to 75.7 days-old instead of WT 60.8 days and 58.8 days of *ebony-1*). When performing qPCR studies in these peculiar longevous flies, the results showed that, as somehow expected, the expression of "antioxidant" genes in *tan-1* was higher than the one in wt (a 1.5-fold increase in SOD-2 mRNA and a 2-fold increase in Catalase expression). Strikingly, we found that in VLS male Medflies the expression of these enzymes was normal but was enhanced in "Supine" males. When the expression from VLS and normal Medfly male brains was compared, by RNASeq studies, the results revealed that other key genes significantly changed more than 2.5 times in longevous flies, thus pointing to a polygenic contribution to longevity, as also expected. Most significant were xenobiotic detoxification and ROS detoxification systems as well as genes involved in neural system connectivity and innate immune response.

CB-C10
EVIDENCE OF ALTERED ENDOMEMBRANES IN FISSION YEASTS LACKING GLUCOSIDASE I, A MODEL FOR HUMAN CONGENITAL DISORDER OF GLYCOSYLATION CDG IIb

Valko A[1,2], Etchegaray E[2], Aramburu SI[1], González AC[2], Gallo GL[1], D'Alessio, C[1,2].
[1]Fundación Instituto Leloir, IIBBA-CONICET and [2]Facultad de Ciencias Exactas y Naturales, UBA. E-mail: cdalessio@leloir.org.ar

Glucosidase I (GI) is an endoplasmic reticulum (ER) membrane protein that removes the outermost glucose from the glycan $Glc_3Man_9GlcNAc_2$ (G3M9) immediately after protein *N*-glycosylation. Mutations in GI-encoding gene (*gls1*$^{+}$*)* result in human congenital disorders of glycosylation (CDG) IIb, also called MOGS-CDG. Using the fission yeast *Schizosaccharomyces pombe* lacking GI as a model organism we demonstrated that the main cause of the morphological and growth defects observed in mutant cells was the persistence of G3M9 structures in glycoproteins, as a second mutation in *alg10+* gene (which is responsible for the addition of the last Glc during the lipid-linked G3M9 synthesis) substantially suppressed the observed defects. The sick phenotype of *Δgls1* mutant cells could not be ascribed to a product inhibition of oligosaccharyltransferase transfer reaction, to the inability of glycoproteins to enter into calnexin-folding cycles, or to a potentially reduced ER-associated degradation. Glycan elongation of glycoproteins in the Golgi and the overall cell wall (CW) monosaccharide composition of *Δgls1* mutants were indistinguishable from those observed in cells lacking glucosidase II (*Δgls2α*), which display a wild type phenotype. However, transmission electron microscopy (TEM) showed that the CW of *Δgls1* mutants was thicker than *WT* and *Δgls2α* ones, presenting a feathered appearance, and a disorganized arrangement without its characteristic three-layered structure. Endomembrane system was also altered in cells lacking GI as: 1) subcortical ER structures localized below the plasma membrane were apparently absent or mislocalized in mutant cells observed by TEM, 2) CW glycoproteins region was wider in *Δgls1* cells than in WT ones as revealed by staining with fluorescent-labeled lectins *Griffonia (bandeiraea) simplicifolia* (recognizes Galactose terminal residues) and Concanavalin A (recognizes high-mannose glycans) , and 3) the lack of GI produces cells with highly fragmented vacuoles in hypotonic conditions (revealed by FM4-64 staining) which possibly cannot undergo homotypic fusion. Collectively, these results suggest the occurrence of alterations in the secretory/endocytic pathway in cells lacking GI and shed light on the underlying molecular and cellular mechanisms of CDG IIb disease.

CB-C11
THE FLIGHT RESPONSE INDUCES THE RELEASE OF AN ILP FROM THE INTESTINE TO INHIBIT CYTOPROTECTIVE MECHANISMS IN *CAENORHABDITIS ELEGANS*

Veuthey T, Giunti S, De Rosa MJ, Rayes D.
Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB) (CONICET–UNS)/ DBByF-UNS. E-mail: tveuthey@uns.edu.ar

The perpetuation of the flight response inhibits defensive cytoprotective mechanisms, leading to reduced resistance to environmental stressors, early onset of age-related disorders and shorter lifespan from invertebrates to mammals. We have recently shown that, in *Caenorhabditis elegans*, the flight response induces the neuronal release of Tyramine (TA, the invertebrate analog of adrenaline), which stimulates the adrenergic-like receptor TYRA-3 in the intestine. This leads to the activation of the DAF-2/Insulin/IGF-1 pathway and the inhibition of cytoprotective mechanisms, such as translocation of DAF-16/FOXO or HSF-1, not only in the intestine but also in other tissues. However, the signals that bridge the stimulation of TYRA-3 in the intestine with the activation of the DAF-2 insulin receptor in other tissues remain unknown. *C. elegans* genome encodes 40 Insulin-like peptides (ILPs), which in principle could bind to DAF-2, and many of them are expressed in the intestine. We, therefore, used RNAi to individually silence intestinal ILPs and test the resistance to environmental stressors such as oxidative and thermal stress. We found that the silencing of one of those ILPS, *ins-3*, improves the resistance to environmental stressors. In contrast to control, the addition of exogenous TA does not impair the oxidative or thermal stress resistance in *ins-3*-silenced animals. Moreover, we generated double null mutants of *ins-3* and TA-deficient mutants and found that this double mutant is as resistant to environmental stress as single mutants. This suggests that tyramine and INS-3 act in the same pathway to control stress resistance. Since *ins-3* is also expressed in neurons, we injected *ins-3* cDNA driven by intestinal and neuronal promoters to *ins-3* null mutant animals, to assess the tissue where the expression of *ins-3* is relevant for controlling stress resistance. We found that only intestinal expression of *ins-3* restores the resistance to wild-type levels. Moreover, we found that the stress resistance of *ins-3 null mutants* is mediated, at least partially, by DAF-16/FOXO. We, therefore, propose that the activation of the intestinal GPCR TYRA-3 by the escape neurohormone TA leads to the release of INS-3 which acts as endocrine, autocrine and/or paracrine signal to activate the insulin receptor DAF-2 not only in the intestine but also in distal tissues. Given the high degree of conservation of fundamental mechanisms among species, this study can contribute to understanding molecular pathways and cellular communication involved in neural regulation of stress response in multicellular organisms.

LIPIDS

LI-C01
α-SYNUCLEIN AND LIPID METABOLISM: INTERSECTING PATHWAYS

Alza, NP[1,2], Conde, MA[1,3], Scodelaro Bilbao PG[3,4], González Pardo V[2], Salvador GA[1,3].
[1]INIBIBB-CONICET, [2]DQ-UNS, [3]DBByF -UNS, [4]CERZOS-CONICET, Bahía Blanca, Argentina. E-mail: natalia.alza@uns.edu.ar

α-synuclein (α-syn) aggregation and fibrillation is a hallmark of a class of neurodegenerative disorders known as synucleinopathies. An intriguing and not completely clarified feature of α-syn is the many ways in which it interacts with lipids. In the present study, we aimed to investigate the effect of α-syn overexpression on neuronal lipid metabolism. For this purpose, human IMR-32 neuroblastoma cells stably transfected with either pcDNA3 vector (control) or pcDNA3-WT-α-syn (WT α-syn) were used. We observed that α-syn overexpression induced the accumulation of cytosolic lipid droplets (LD) and cholesterol (Chol) in lysosomes. LD increase was coincident with a rise in triacylglycerol (TAG) and Chol esters content. To ascertain the mechanism involved in LD accumulation, pharmacological inhibitors of proteasomal degradation and autophagy were used. Whereas autophagy inhibition did not affect neutral lipids content, the blockage of proteasomal degradation was able to increase LD accumulation in WT α-syn cells. *In silico* analysis performed with MyProteinNet server (Yeger-Lotem lab) postulates a positive correlation between α-syn and sterol regulatory element-binding gen (SREBF-2). To corroborate these data in our experimental model, we evaluated the status of the transcription factors SREBP-1 and SREBP-2. SREBP-1 nuclear localization was slightly diminished by α-syn overexpression with decreased levels of fatty acid synthase protein expression. In contrast, α-syn overexpression promoted SREBP-2 nuclear translocation, with no increment in the expression levels of the downstream genes related to Chol synthesis. Intriguingly, fatty acid Coenzyme A esterification and acylation into Chol and diacylglycerides were increased in WT α-syn cells. To elucidate the source of fatty acids availability, we measured phospholipid content and TAG hydrolysis. WT α-syn cells displayed diminished levels of cardiolipin and phosphatidic acid with no changes in TAG hydrolysis. Our results allow us to conclude that: α-syn overexpression induces a metabolic switch that triggers the neuronal accumulation of neutral lipids by activating several mechanisms: (i) increased phospholipid hydrolysis, (ii) a rise in fatty acids esterification into Chol and diacylglycerols, and (iii) Chol accumulation in lysosomes probably due to an increment in its uptake. *Funding: ANPCyT, CONICET, and UNS.*

LI-C02
LIPID DROPLETS POPULATIONS IN THE INSECT VECTOR OF CHAGAS DISEASE (*TRIATOMA INFESTANS*)

Girotti JR[1], Borús DL[1], Scelsio NS[1], Favale NO[2,3], Ves-Losada A[1,4].
[1]INIBIOLP-CCT-La Plata-CONICET-UNLP, [2]Cat Biol Cel Mol, FFB, UBA, [3]IQUIFIB-CONICET, [4]Dep. Cs Biol. FCE, UNLP, Argentina.
E-mail: avlosada@biol.unlp.edu.ar

The objective of the work was to characterize populations of Lipid Droplets (LD) in *Triatoma infestans* ('vinchuca'). The insect is one the main vector of the parasite *Trypanosoma cruzi*, the causative agent of Chagas disease in Argentina and the Americas. The cuticle (C) is the insect's 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 identify possible populations of LD in these cells as organoids involved in the genesis of C. 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 to identify and characterize LD populations in FB samples, whole cuticle (C) and scraping of the epidermis (E) of insects fast or feed. Light field microscopy and fluorescence (epifluorescence and confocal) and hematoxylin / Oil Red and DAPY / BODYPY stains were used, respectively. In all the samples studied, populations of LD were observed in the cytosol (cLD). The FB has an important population of cLD characterized by large LD; while, the cuticle (C and E), and in particular, in OE cells, the population of cLD that is large, is made up of LD of a smaller size than those of FB. In OE, the main LD population is located in the cytosol and a small population within the cell nucleus (nLD). These results would confirm the role assigned to OE to actively participate in the anabolism of the cuticle components, moreover, small LDs are metabolically more active than larger LD. In conclusion, *Triatoma infestans* cuticular oenocytes were characterized as cells that have a very varied morphology, depending on the developmental stage of the insect, and are larger than the surrounding epithelial cells. The OEs have two LD populations, a main cytosolic and a nuclear one. These are the first results where nLDs are described in insects.

LI-C03
THE REGULATION OF PROTEINS 14-3-3 AND THE HIPPO VIA AFFECT THE ADIPOGENESIS OF 3T3-L1

Del Veliz S[1,3], Uhart M[1], Lim Gareth E[3], Bustos Diego M[1,2].
IHEM[1] (CONICET-UNCuyo), FECEN[2], UNCuyo, Argentina. CRCHUM[3], Canada. E-mail: delvelizsamanta@gmail.com

Many transcription factors act sequentially to activate adipocyte differentiation. Multiple signal transduction pathways govern the adipocyte physiology. The Hippo kinase pathway is the main regulator of proliferation and differentiation of stem cells and adipocyte precursor cells. The 14-3-3 protein family, comprised of 7 paralogs in mammals, interacts with components of the Hippo kinase pathway regulating the adipogenic differentiation, although the specificity of these proteins in the process remains elusive. Deciphering the subcellular dynamics of 14-3-3 proteins will be instrumental in the discovery of new targets for the manipulation of stem cell fate decisions or treatment of chronic diseases, such as obesity and type 2 diabetes. *In vitro* adipocyte differentiation occurs by the addition of an Adipogenic Differentiation Medium (ADM) including Dulbecco's Modified Eagle Medium, 10% Fetal Bovine Serum, synthetic drugs (dexamethasone, IBMX, rosiglitazone) and peptide hormones (insulin). Since these mentioned chemical drugs are not present in physiological environments, it would be important to achieve a correct activated adipogenic program using fewer drugs and with natural origin. We have decided to evaluate the adipogenic potential of glucagon-like peptide 1 (GLP-1) analogs. In contrast to Native GLP-1 action, which is rapidly degraded by circulating Dipeptidyl Peptidase-4, GLP-1 analogs have shown positive effects on glycemic control and body weight due to their greater stability and longer half-life. In this project, experiments were performed on 3T3-L1 preadipocytes. First, the effect of different combinations of drugs for 7 days in adipogenesis was evaluated. Then, we carried out qPCR experiments to measure the gene expression of 14-3-3 and the most important proteins of the Hippo pathway, on days 3 and 7 of differentiation. We have determined that conditions resulting in greater adipogenic differentiation showed higher levels of Hippo pathway proteins and 14-3-3 gamma and beta isoforms on day 7. These effects were especially evident when IBMX was replaced by GLP-1 in the ADM. These results suggest that different inducers (glucocorticoids, thiazolidinediones, incretins), have different abilities for regulating the expression of 14-3-3 and hippo pathway proteins, thus affecting adipogenic differentiation. We are currently focused on elucidating the mechanisms of action of these drugs to have a greater understanding of the events that are necessary for adipogenesis to occur.

LI-C05
SPHINGOSINE KINASE 2 AS REGULATOR OF LIPID DROPLETS BIOGENESIS

Santacreu BJ, Romero, DJ, Tarallo E, Otero D, Sterin de Speziale NB, Favale NO.
Facultad de Farmacia y Bioquímica, Cátedra de Biología Celular y Molecular, UBA, Argentina. IQUIFIB-CONICET Buenos Aires, Argentina.
Email: bsantacreu@ffyb.uba.ar

Sphingolipids (SLP) participate in several different, even opposite, cellular processes. We have described their participation in the cellular differentiation of renal collecting tubule. We have observed that the levels of sphingosine 1 phosphate (S1P) regulate the levels of the other SLP by inhibition of enzymes involved in *de novo* SLP synthesis. In this way, S1P emerges as a general regulator of the level of sphingolipids, which adapts the SLP levels to the cellular fate. However, less it is known about the interrelation of S1P with phospholipids, triglycerides or other lipids. In this work, we study the importance of S1P in relation to the dynamics of lipid droplets. We found that the activity of sphingosine kinase 2 (SphK2), one isoform of the enzyme that synthetizes S1P, is necessary for the formation of lipid droplet (LD). SphK2 activity modification by pharmacological, siRNA and CRISPR/cas9 strategies produced a decrease in LD size and number. This observation was accompanied by a decrease in triglycerides and was not reversed by S1P receptor antagonists or inhibitors of the *de novo* SLP synthesis. Additionally, by using

bioinformatic analysis of the RNA-seq database, we found that SphK2 knockout mice present an alteration in the mRNA levels of the key enzymes of triglyceride synthesis. On the other hand, we found that SphK2 is located on the surface of lipid droplets by fluorescence microscopy. These results show that the SphK2/S1P pathway could play a central role in the regulation of the complex lipid metabolism, regulating, in this case, the biogenesis of lipid droplets.

MICROBIOLOGY

MI-C01
RETHINKING THE MECHANISM OF ACTION OF CLASS II BACTERIOCINS: A COMPARATIVE STUDY THROUGH THE USE OF SUICIDE PROBES

Rios Colombo NS, Chalón MC, Dupuy FG, Bellomio A.
Instituto Superior de Investigaciones Biológicas (INSIBIO), CONICET-UNT and Instituto de Química Biológica "Dr. Bernabé Bloj", Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán. Argentina. E-mail: rioscnatalia@gmail.com

Class II bacteriocins are membrane-active peptides that act over a narrow spectrum of target bacteria. It is known that the antimicrobial activity of these compounds is carried out via pore formation in the membrane after recognition of a specific receptor. Nonetheless, little is known about the molecular events after the bacteriocin-receptor interaction. It is not clear yet whether the receptor is involved in the pore structure or if it only acts as a docking molecule for the bacteriocins. In this work, we grasped the previous design of "suicide probes" that were first designed to study the insertion and topology of membrane proteins. We repurposed this tool to investigate the mode of action of different class II bacteriocins, namely microcin V (active against Gram-negative bacteria), pediocin PA-1, and enterocin CRL35 (active against Gram-positive bacteria). Suicide probes are hybrid peptides containing the different bacteriocins fused to the membrane protein EtpM. Arabinose-induced expression of these fusions attaches the bacteriocins to the periplasmic side of the *Escherichia coli* inner membrane. The constructs showed to be toxic for *E. coli* MC4100 cells lacking the specific receptor, thus supporting the role of the receptor as a binding site for bacteriocin anchoring to the membrane and not as a structural piece of the pore. In addition, microcin-based suicide probes are much more toxic than enterocin and pediocin-based fusions. Therefore, we discuss how the membrane composition, different in Gram-positive and Gram-negative bacteria, might be significant for bacteriocins activity. The developed probes were also applied to investigate and compare the effects of bacteriocins on membrane fluidity and transmembrane potential using fluorescence spectroscopy. The membrane-attached bacteriocins were proven to increase phospholipid order and depolarize the membranes of the receptor-free bacterial cells, upon arabinose induction. Since this system allows us to evaluate the interaction of bacteriocins *in vivo* with real bacterial membranes, we consider that it could be exploited to complement *in vitro* studies performed in model membranes, gaining molecular information of these and other antimicrobial peptides.

MI-C02
BVGR REGULATES THE TRANSCRIPTION OF A WIDE VARIETY OF GENES IN *BORDETELLA BRONCHISEPTICA*, INCLUDING VIRULENCE FACTORS

Gutiérrez MP[1], Wong TY[2], Sen-Kilic E[2], Damron FH[2], Fernández J[1], Sisti J[1].
[1]IBBM-CCT-CONICET-La Plata, Dto Cs Biológicas, FCE, UNLP. [2]West Virginia University, USA. E-mail:mariadelapaz.gutierrez@gmail.com

Bordetella bronchiseptica (*Bb*) is a pathogenic bacterium that causes respiratory infections in animals. *Bb* virulence factors are regulated by several mechanisms, being the most well-characterized the *Bordetella* three-component system, BvgASR. When this system is active, the response regulator BvgA activates the expression of virulence-activated genes. On the contrary, BvgR represses the transcription of some virulence-repressed genes, such as *Bb* flagellin. No DNA binding domain has been described for BvgR. To date, microarray analyses have been used to identify some genes controlled by BvgR but the totality of the genes regulated by BvgR is unknown. In order to characterize more deeply the BvgR regulon, we performed RNA seq on RNA samples from a wild type Bb (*Bb WT*) and a mutant in *bvgR* (*Bb bvgR-*). Comparing the *bvgR* mutant to the wild type revealed differential expression of 319 genes, of which 221 and 98 were upregulated or downregulated, respectively. Among the upregulated genes, we could detect the entire flagellar and chemotaxis genes regulons; the flagellar master regulator encoded by *flhD* was the most highly upregulated gene. In addition, several transcriptional regulators, chaperons, heat shock proteins, and proteins related to the second messenger c-di-GMP metabolism were found differentially expressed between the strains. The transcriptomic analysis also revealed 98 genes with higher expression in *Bb WT* than in *Bb BvgR-*, some of which encoded for virulence factors such as *bipA* and Type Three Secretion System proteins. qRT-PCR and western blots confirmed these data. In order to evaluate the impact of the downregulation of the TTSS on Bb bvgR-, cytotoxicity assays on J774 macrophages were performed. A decrease in the percent LDH release induced by *Bb bvgR-* confirmed that this strain is less cytotoxic than *Bb WT*. Altogether, these data indicate that BvgR regulates a complex network of genes and different phenotypes such as motility and cytotoxicity. In future studies, we will further characterize the downstream regulatory networks controlled by BvgR.

MI-C03
CHARACTERIZATION OF *BORDETELLA BRONCHISEPTICA* DIGUANILATE CYCLASE BdcB

Belhart K, Sisti F, Fernández J.

IBBM-CCT-CONICET-La Plata, Dto de Cs Biológicas, FCE, UNLP, La Plata, Argentina. E-mail: keibelhart@hotmail.com

Bordetella bronchiseptica (*Bb*) is a Gram-negative respiratory pathogen that forms biofilm-like structures *in vivo*. According to the signals it receives from the environment presents at least three phenotypically different phases: an avirulent phase, an intermediate phase, and a virulent phase. We previously showed that c-di-GMP regulates biofilm formation in *B. bronchiseptica*, like in other bacteria. However, c-di-GMP may be involved in other stages required for effective infection and transmission. *Bb* can survive inside immune cells like macrophages. In order to determine if c-di-GMP is involved in this process, we evaluated *Bb* survival with deletions in multiple diguanylate cyclases. Deletion of *bdcB* (*Bordetella* diguanylate cyclase B) impaired bacterial survival inside macrophages. Four hours post-infection, bacterial numbers in macrophages were below detection limits. Intracellular bacteria can resist macrophage bactericidal activity through different mechanisms. We evaluated *B. bronchiseptica* Δ*bdcB* (*Bb*Δ*bdcB*) resistance to acidic pH or oxidative burden (H_2O_2). While the deletion of *bdcB* was not detrimental to H_2O_2 resistance, survival at pH lower than 5.0 was significantly affected. To further characterize BdcB, we overexpressed *bdcB* in *Bb*. We observed enhanced biofilm formation and inhibition of motility, as expected for an active diguanylate cyclase. These phenotypes were dependent on diguanylate cyclase (DGC) activity and the N-terminal domain, because an inactive version of BdcB (GGDEFxGGAAF) and an N-term-truncated BdcB did not inhibit motility, neither enhanced biofilm formation. Finally, we analyzed the expression of *bdcB* by a transcriptional fusion of the promoter to *gfp* in the three phenotypic phases of *Bb*. The *bdcB* expression was significantly higher in the intermediate phase. We also determined that *bdcB* expression is under the repression of a response regulator required for bacterial resistance to oxidative stress and *in vivo* persistence, RisA. The present work represents another step on the role of c-di-GMP comprehension in *Bordetella* pathogenesis and particularly the function of one of the ten diguanylate cyclases present in *B. bronchiseptica* genome in intracellular survival.

MI-C04
A PUTATIVE PAUSE ENCODED IN THE *ureA* mRNA OF *ASPERGILLUS NIDULANS*, MIGHT PLAY A ROLE IN THE CORRECT RECOGNITION BY FACTORS AT EARLY BIOGENESIS

Sanguinetti M[1], Iriarte A[1], Amillis S[2], Musto H[1], Marin M[1], Ramón A[1].
1-Faculty of Sciences, UdelaR, Montevideo, Uruguay; 2-Faculty of Biology, University of Athens, Greece. E-mail: anacramonp@gmail.com

Codon usage has been proposed to assist in the establishment of a pause necessary for the correct targeting of the nascent membrane proteins to the translocon in the membrane of the ER. In the case of UreA, the *Aspergillus nidulans* urea transporter, we found that a pair of nonoptimal codons (codons 24 and 25) encoding amino acids situated at the boundary between the N-terminus and the first transmembrane segment is necessary for proper biogenesis of the protein. When these two non-optimal codons are changed into synonymous, optimal ones, the resulting mutant strain shows impaired ability to grow on urea at 37°C. ^{14}C-urea transport assays support these results, whereas Western blot and epifluorescence microscopy show a lower amount of protein in the membrane of the mutant strain, apparently due to a decrease in UreA synthesis or translocation to the membrane. No significant differences could be determined in *ureA* mRNA levels or predicted mRNA structures between the wild type and the strain carrying the synonymous mutations. We propose that nonoptimal codons 24 and 25 might regulate translation rate, contributing to the correct interaction of *ureA*-translating ribosome nascent-chain complexes with the signal recognition particle (SRP) and/or other factors, while the polypeptide has not yet emerged from the ribosomal tunnel. Our results suggest that the presence of the pair of nonoptimal codons would not be functionally important in all cellular conditions. Whether this mechanism would affect other proteins remains to be determined.

MI-C05
REPURPOSING TRICLABENDAZOLE AND CLOFAZIMINE AS PUTATIVE ANTI-TOXOPLASMIC COMPOUNDS

Ganuza A[1], Alberca LN[2], Dietrich RC[2], Gavernet L[2], Talevi A[2], Corvi MM[1].
[1]INTECH, CONICET-UNSAM.[2]LIDeB, Universidad Nacional de La Plata, E-mail: mcorvi@intech.gov.ar

Toxoplasmosis is an infection caused by the parasite *Toxoplasma gondii*. Although healthy individuals present few symptoms, the disease could have a high impact on immunocompromised individuals and congenital infection, leading to serious health problems. Although the combination of pyrimethamine and sulphonamides is still very effective for the treatment of toxoplasmosis, the use of these two drugs in immunocompromised individuals for long periods frequently leads to adverse reactions. As such, there is a need for alternative therapeutic options. Recently, by application of *in silico* drug repurposing, it was reported that cisapride (gastroprokinetic agent), cinnarizine (antihistamine used to treat travel sickness), clofazimine (antimycobacterial compound), triclabendazole (antihelminthic drug) and paroxetine (antidepressant) inhibit putrescine uptake in *Trypanosoma cruzi*. Given that *T. gondii* is auxotroph for polyamines, here we evaluated these compounds on *T. gondii* growth *in vitro*. All the tested compounds presented an anti-toxoplasmic effect. The calculated IC_{50} for paroxetine, cinnarizine, and cisapride were 2.42 µM, 3.12 µM, and 4.72 µM, respectively. However, triclabendazole and clofazimine presented a higher selectivity towards *T. gondii* inhibition growth, with IC_{50} 0.61 µM and 0.3 µM, and selectivity indexes of 15.67 and 10.3 respectively. Our results suggest that target and drug repurposing are valid approaches for the study of putative antiparasitic compounds, especially for neglected diseases.

MI-C06
IDENTIFICATION OF A *SALMONELLA* PhoP/PhoQ SYSTEM INHIBITOR FROM A DYNAMIC COMBINATORIAL LIBRARY

Lobertti CA[1], Cabezudo I[2], Furlán RLE[2], García Véscovi E[1].

[1]Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET),[2]Área de Farmacognosia, Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR). E-mail: lobertti@ibr-conicet.gov.ar

Salmonella is an enteropathogen that causes a wide range of diseases in humans and animals. PhoP/PhoQ is a two-component system (TCS) distributed amongst several Gramnegative bacteria, consisting of the histidine kinase PhoQ, and the transcriptional regulator PhoP. In *Salmonella* Typhimurium, the PhoP/PhoQ system regulates the adaptation to Mg^{2+}-limiting environments and controls key virulence phenotypes such as the invasion and proliferation within host cells. As signal transduction in mammals does not involve TCS, the PhoP/PhoQ system is an attractive target to develop new antimicrobial agents. We have previously reported a methodology based on a TLC-overlay as a new strategy for the search and identification of antimicrobial agents targeting the PhoP/PhoQ system. We applied this bio-guided strategy using a strain carrying a PhoP-controlled reporter gene, to the screening of a dynamic combinatorial library of hydrazones in the search for inhibitors. As a result, two libraries of hydrazones and three libraries of thiocarbazones totaling over 370 members were screened for their inhibitory activity through a rapid inexpensive TLC strategy. Satisfactorily, a complex library of hydrazones that can repress the PhoP/PhoQ system was selected from the initial screening, to further study its members. Through iterative deconvolution of over 100 library members, we identified a potential inhibitor, A25B4. This compound could be synthesized in its pure form, characterized, and it was confirmed that it does not affect the growth of Salmonella. By quantitative β-galactosidase assays, we confirmed its inhibitory activity and it was found that the response was dose-dependent and selective as well. Once the mechanism of action of A25B4 in the system is known, a target protein domain of the TCS will be used to template a library of hydrazones, biasing the composition of the dynamic library towards A25B4. This step will further confirm its affinity and mechanism of action. This strategy allows us to establish a novel methodology for the discovery of the PhoP/PhoQ system inhibitors to fight against *Salmonella*-borne diseases.

PLANTS

PL-C01
CONTRIBUTION OF FLAVODOXIN EXPRESSION IN POTATO PLANTS TO IMPROVED TOLERANCE AGAINST DROUGHT

Arce RC, Pierella Karlusich JJ, Zurbriggen MD, Hajirezaei M, Carrillo N.
Instituto de Biología Molecular y Celular de Rosario, CONICET. Ocampo y Esmeralda s/n. Argentina. E-mail: arce@ibr-conicet.gov.ar

Environmental stress represents the most important factor limiting the yield of crops worldwide, a situation that will surely get worse in the near future as a consequence of the deterioration of the global climate. For that reason, numerous strategies have been developed to increase stress tolerance in plants. Within this context, it has been demonstrated that the introduction of a plastid-targeted cyanobacterial flavodoxin (Fld) in transgenic plants resulted in increased tolerance to multiple sources of biotic and abiotic stress. Taking into account these observations and as potato is regarded as the third most important food crop in the world, we generated transgenic potato plants that express Fld in chloroplasts (*Stpfld*) to evaluate their performance under water deficit, which has the highest impact in quantitative terms. We characterized these plants and their wild-type (WT) siblings under hydric stress by interrupting irrigation. We found that negative consequences of water deprivation such as impairment of photosynthesis and increased propagation of reactive oxygen species were detected in WT leaves long before visual symptoms of leaf wilting became apparent, and that these adverse effects were prevented by expression of a plastid-located Fld. These results prompted us to gain further knowledge on the mechanism of tolerance conferred by Fld, therefore a transcriptomic analysis of these plants subjected to drought and control conditions was carried out in potatoes. Water deprivation induced 2529 genes in WT plants against 1697 in *Stpfld*252 siblings and repressed 3172 genes in the wild type versus 2400 in the transformant. Then, the overall effect of Fld presence was to mitigate the changes in gene expression driven by the drought treatment; either induction or repression, suggesting that plants accumulating chloroplast Fld sensed less stress than their WT counterparts. The results showed partial or complete protection of primary metabolisms affected by drought, including the photosynthetic electron transport chain and the Calvin Cycle, indicating that this genetic intervention increases stress tolerance in this species. In addition, these results provide a detailed snapshot of how chloroplast redox biochemistry affects gene expression and metabolism during drought.

PL-C02
NEW ROLES FOR OLD FRIENDS: A MICROTUBULE-LOCALIZED COP1-INTERACTING PROTEIN PROMOTES HYPOCOTYL ELONGATION IN THE DARK

Arico DS[1], Wengier DL[1], Castro LM[1],Muschietti JP[1,2],Mazzella MA[1].
[1]Instituto de Investigaciones en Ingeniería Genética y Biología Molecular "Dr. Héctor Torres" (INGEBI-CONICET) C.A.B.A., Argentina. [2]DBBE, FCEN, UBA, Argentina. E-mail: denise.s.arico@gmail.com

Plant irritability for light stimuli becomes crucial to cope with ambient fluctuations in order to keep up homeostasis and accomplish the life cycle successfully. Light environment governs plant development. Perception of light is carried out by photoreceptors, such as phytochromes (phyA to phyE) that absorb primarily in red and far-red; and cryptochromes (cry1 to cry3) that are predominantly blue-light receptors. Once perceived, plants are able to integrate light signals into biochemical networks that conduce to proper response. Transducing these light signals involve changes in the phosphorylation state of proteins. In an early light-induced phosphoproteome study in *Arabidopsis thaliana*, we identified a protein that presents light-responsive dephosphorylation in the presence of photoactivated photoreceptors. This protein was particularly interesting because it was reported to interact with the key repressor of photomorphogenesis COP1 and thus, it is potentially involved in early photomorphogenesis

events. *In vivo* assays with a transcriptional reporter revealed it is expressed in cotyledons and elongation zones of hypocotyl and root. Its expression is regulated negatively by light. CRISPR-CAS9 mutated lines exhibit shorter hypocotyls in darkness. Confocal microscopy assays with stably transgenic lines expressing translational reporters revealed localization to cortical microtubules. We are currently studying the biological implications of its microtubule association and its regulation by COP1 through changes in phosphorylation patterns. All these results suggest this protein promotes growth in darkness by affecting microtubules dynamics.

PL-C03
DYNAMIC REGULATION OF CHROMATIN TOPOLOGY BY INVERTED REPEAT-DERIVED SMALL RNAS IN SUNFLOWER

Gagliardi D, Cambiagno DA, Arce AL, Tomassi AH, Giacomelli JI, Ariel FD, Manavella PA.
Instituto de Agrobiotecnología del Litoral (IAL CONICET-UNL). E-mail: dgagliardi@santafe-conicet.gov.ar

Transposable elements (TEs) are extremely abundant in complex plant genomes. Small RNAs (siRNAs) of 24 nucleotides in length control its activity in a process that involves *de novo* methylation of targeted loci known as RNA-Dependent DNA Methylation (RdDM). Usually, the epigenetic modifications induced by RdDM trigger nucleosome condensation and a permanent silencing of the affected loci. Here, we show that a TE-derived inverted repeat element (IR), inserted near the sunflower *HaWRKY6* locus dynamically regulates the expression of the encoded gene by altering chromatin topology in different ways. The transcripts of this IR element are processed into 24-nt siRNAs, triggering its DNA methylation together with another two regions of the locus. These epigenetic marks then stabilize the formation of different tissue-specific loops in the chromatin that affect the gene expression in specific ways. In leaves, an intragenic loop is formed, blocking *HaWRKY6* transcription by disrupting the progress of RNA Polymerase II (RNAPII) along the gene. While in cotyledons, the formation of an alternative loop, encompassing the whole *HaWRKY6* gene, enhances transcription of the gene in a phenomenon known as "gene looping" where the RNAPII recycles along the gene. The formation of the latter loop also changes promoter directionality, reducing IR transcription, ultimately releasing the loop. Our results provide evidence that TEs can act as active and dynamic regulatory elements within coding loci in a mechanism that combines RNA silencing, epigenetic modification, and chromatin remodeling machinery.

PL-C04
CONTRIBUTION OF THE DNA GLYCOSYLASE MBD4L TO DNA REPAIR DURING SEED GERMINATION

Lescano I, Nota MF, Torres JR, Cecchini NM, Álvarez ME.
CIQUIBIC-CONICET, Dpto de Química Biológica Ranwel Caputto - Facultad de Ciencias Químicas – UNC.
E-mail: ignaciolescano@gmail.com

DNA repair is crucial to maintain genome integrity and ensure cell survival and accurate transmission of genetic information. Plants experience high levels of DNA damage at different stages of their life, which is often caused by stressful conditions. Cycles of dehydration and rehydration during seed development are associated with high levels of reactive oxygen species, resulting in oxidation of DNA bases and DNA strand breaks. Genome damage is exacerbated during seed aging, decreasing seed vigor and viability. Consequently, DNA must be repaired prior to germination to prevent genomic damage from being fixed after cell division. The base excision repair (BER) contributes to this end by using DNA glycosylases to excise damaged bases from the genome. Here, we studied the expression pattern of the *Arabidopsis* DNA glycosylase MBD4L (methyl-binding domain protein 4 like) during seed development, germination, and seedling establishment. We further analyzed germination phenotypes associated with the deficiency/overexpression of MBD4L. Interestingly, mbd4l mutants showed late germination under basal conditions. This phenotype was not caused by enhanced sensitivity to abscisic acid (ABA). Interestingly, late germination was exacerbated by exposure to genotoxic agents. Therefore, MBD4L may contribute to DNA repair, and consequently to proper plant development, by activating the BER system before seed germination. To test this hypothesis, the expression pattern of MBD4L and genes responding to DNA damaged (LIG1, RAD51, PARP2) has been analyzed along with germination.

PL-C05
EXPRESSION OF NOS ENZYME FROM PHOTOSYNTHETIC MICROORGANISMS IN HIGHER PLANTS: A TOOL TO IMPROVE NITROGEN USE EFFICIENCY

Del Castello F, Nejamkin A, Foresi N, Lamattina L, Correa-Aragunde N.
Instituto de Investigaciones Biológicas, Universidad Nacional de Mar del Plata. E-mail: fioredc@hotmail.com

Nitrogen (N) is one of the major macronutrients for plants. The massive use of N fertilizers in agricultural production has a negative impact on the environment, biodiversity, and human health. The development of strategies to improve the nitrogen use efficiency (NUE) in plants is of relevance in plant biology. Some studies showed that nitric oxide (NO) is a signal for N deficiency in plants as well as a potential source of N since it can be oxidized to $NO3^-$ by phytoglobin. In animals, the enzyme NO synthase (NOS) catalyzes the biosynthesis of NO from the arginine substrate. Some evidence suggests the existence of a putative NOS activity in plants; however, NOS sequences were not found in land plant genomes. In recent years, NOS enzymes were identified in photosynthetic microorganisms such as green algae, diatoms, and cyanobacteria. In our lab, the functionality of the NOS from the cyanobacteria *Synechococcus* PCC 7335 (SyNOS) was characterized. SyNOS has a similar structure to animal NOS with both oxygenase and reductase domains and contains an additional domain in the N terminus that encodes to a globin. It has been demonstrated that the globin domain of SyNOS acts as a NO dioxygenase, oxidizing NO to $NO3^-$. As a result, SyNOS is able not only to produce

NO from arginine but also to catalyze NO to NO_3^-. Since arginine is a N storage amino acid in plants, it was hypothesized that the expression of SyNOS in plants may remobilize N from arginine making it more available. We have generated Arabidopsis transgenic lines that express SyNOS under the constitutive ubiquitin promoter. As expected, the transgenic lines display increased NOS activity and less arginine content. Our results show that SyNOS-transgenic plants growing in N deficiency have greater shoot height, shoot branching and seed production compared to the Wt plants. Nitrate content in seeds/plants is higher in the SyNOS-lines than in wt. No statistical differences were detected in the fresh weight and area of rosette leaves. Bioassays for cytokinin (CK) showed a high CK content in SyNOS-plants respect to wt. Our results suggest that the SyNOS expression would increase $NO3^-$ availability and CK levels, generating a positive effect on growth and seed production in *Arabidopsis* plants growing under limiting N conditions.

PL-C06
MITOCHONDRIAL SMALL HEAT SHOCK PROTEIN AND CHILLING TOLERANCE IN TOMATO FRUIT

Escobar M[1], Herrfurth C[2], Boggio S[1], Feussner I[2], Valle EM[1].
[1]Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET-UNR), Ocampo y Esmeralda, Predio CCT, Rosario. [2]Georg-August-University Göttingen, Albrecht-von-Haller-Institute for Plant Sciences. Ernst-Caspari-Building, Dept. of Plant Biochemistry, Justus-von-Liebig-Weg 11, Göttingen, Germany. E-mail: escobar@ibr-conicet.gov.ar

Chilling injury (CI) is a physiological disorder that appears when plants and plant organs are exposed to low, but non-freezing temperatures. In tomato fruit, low temperature is used as a strategy to extend the commercialization period after harvesting. But this procedure can lead to CI affecting the production yield and quality. A comparative study showed contrasting postharvest chilling tolerance between two tomato varieties, cv. Micro-Tom was more tolerant while cv. Minitomato showed susceptibility to CI. It was previously reported that these two varieties exhibited different expression of small heat shock proteins (sHSPs) after chilling, with an increase of sHSPs in Micro-Tom while the more susceptible Minitomato fruit showed down-regulation of all sHSPs analyzed during cold storage. Among them, one mitochondrial sHSPs (sHSP23.8) showed higher expression in Micro-Tom green mature fruit after cold storage, indicating a potential correlation between the accumulation of sHSP23.8 and the amelioration of chilling symptoms in tomato fruit. In this work, the functional consequences of the down-regulation of sHSP23.8 in tomato fruit using artificial microRNA technology were investigated. The *amiR23.8* mutants were analyzed in their phenotype and susceptibility to CI shortly after and during several days after chilling treatments. Symptoms of CI were especially evident in *amiR23.8* fruit after 15 days of recovery. Compared to WT, *amiR23.8* fruit showed partial discoloration, wilting and wrinkles at the surface. In addition, pre-chilled fruit of *amiR23.8* mutant showed higher loss of water and increased ion leakage of pericarp tissue compared to WT fruit. The *amiR23.8* fruit deterioration indicates that it is highly susceptible to cold stress and developed chilling injury symptoms. The lipidome of *amiR23.8* fruit after chilling showed altered amounts of glycerolipids and saturated lipids, compared to WT. The relative percentage of unsaturated lipids were significantly lower in the fruit of *amiR23.8* compared to WT in normal conditions and after chilling. The results indicate a differential degradation of extraplastidic and plastidic lipids in *amiR23.8* fruit, and alterations in the remodeling of the lipidome after cold stress, which may lead to higher sensitivity to CI. The results presented here suggest that sHSP23.8 may be directly involved in the protection mechanisms against chilling stress in tomato fruit.

PL-C07
A TALE OF TWO PROTEINS: GAINING INSIGHT INTO GRF/GIF REGULATORY NETWORK

Hedin N, Perrone AP, Ferella A, Ercoli MF, Rodriguez R, Palatnik J.
[1]Instituto de Biología Molecular y Celular de Rosario (IBR) – CONICET – UNR. E-mail: hedin@ibr-conicet.gov.ar

While plant leaves grow in a defined fashion, its roots grow indeterminately through the meristem. This micro-environment that has active cell proliferation capabilities but limited cell differentiation is present both in plant roots and stem. Several regulatory networks have been associated with the maintenance of the meristems; among them, we focused our studies on the transcription factors that belong to the GROWTH-REGULATING FACTOR (GRF) family. *Arabidopsis thaliana* GRF family is composed of nine different members, among which seven of them are post-transcriptionally repressed by microRNA miR396. GRFs have two highly conserved domains: on the one hand the QLQ domain is responsible for its interaction with other proteins, and on the other hand, the WRC domain, which has a nuclear localization signal, is necessary for DNA binding. According to previous results from our and other groups, GRF interacts *in vivo* with a transcriptional co-regulator called GRF-INTERACTING FACTOR 1 (GIF1). GIF1, in turn, can also interact with BRAHMA, a central component of chromatin remodeling complexes. In this work, we studied the role of GRF/GIF interaction in cell proliferation and root meristem identity. First, we generated inducible lines for both genes that could complement each mutation. These lines were used for RNAseq transcriptomic analysis that allowed us to study genes regulated by GRF3 and GIF1. Common genes regulated by both proteins included *PLETHORA1* and *2*, which encode transcription factors that function as master regulators of the root. Then, we explored the interaction at a protein level on the stable inducible lines and also on transient infiltrated *Nicotiana benthamiana* leaves. The results obtained allowed us to propose a model for the function of the GRF/GIF network that might be relevant for varieties of agronomical interest.

PL-C08
UNDERSTANDING THE EXPANSION OF microRNA NETWORKS IN PLANTS

Larran AS[1], Hedin N[1], Rodriguez RE[1], Debernardi J[1], Palatnik JF[1].
[1]Instituto de Biología Molecular y Celular de Rosario (IBR) – CONICET – UNR. E-mail: larran@ibr-conicet.gov.ar

MicroRNAs (miRNAs) are a type of small RNAs that play a key role in the developmental programs of higher organisms. Several studies suggest that, among all the miRNAs conserved in plants, miR396 is the one that more target genes has gained throughout the evolution. While the miR396-GRF node is present in all vascular plants, different lineages have independently incorporated other target genes into the miR396 regulatory network, which is revealed by the emergence of binding sites with substantial differences in their sequences. In this context, some interesting questions arise. For example, how the *cis*-context of miR396 binding site affects its efficiency, and also which is the biological relevance of these new nodes that evolution has incorporated. In the first instance, we built sensors carrying different miR396 binding sites. We generated sensors with target sites either within the GFP coding region (CDS) or within the 3' UTR. Transient expression analysis in *Nicotiana benthamiana* showed that fluorescence levels were considerably different for the vectors used, and quantitative real-time PCR assays confirmed these observations. These results indicate that there may be a difference in the performance of miR396 in a context-dependent manner. In addition, it is well known that overexpression of miR396 causes a significant reduction of leaf size in *Arabidopsis thaliana* and other plant species, mainly due to the inactivation of *GRF* transcription factors. However, *in silico* studies predict that miR396 could regulate genes with a sensitive response to photoperiod. Therefore, we analyzed miR396a and miR396b overexpressing plants grown at differential light conditions, in contrast to *grf* mutants. Interestingly, we observed that the phenotype of plants overexpressing these miRNAs was significantly affected by the different environmental conditions tested. Consequently, our studies open new avenues for the empirical exploration of new target genes of the evolutionary-conserved miR396.

PL-C09
PSI ACCEPTOR-SIDE STATUS CONTROLS CHLOROPLAST DEVELOPMENT AND PLANT GROWTH

Lobais C, Blanco NE.
Centre of Photosynthetic and Biochemical Studies (CEFOBI/UNR-CONICET). Rosario, Argentina. E-mail: blanco@cefobi-conicet.gov.ar

Transplastomic tobacco plants overexpressing the minor pea Ferredoxin (Fd) isoform (*Oe*Fd1) were originally generated to increase the availability of soluble electron transporters at the acceptor-side of Photosystem I (PSI). Besides having an augmented amount of soluble acceptor carriers in the stroma, these plants exhibited unexpectedly lower linear electron flow (LEF) than control plants, a variegated phenotype, reduced growth as well as increased non-photochemical quenching (NPQ) under normal light intensities (150 µmol m^{-2} s^{-1}). The patchy chlorophyll distribution in leaves was linked to cells with abnormally developed chloroplasts and suggested an effect of the redox status of Photosystem I (PSI) in plant cell development. To gain insight into the underlying mechanism that provokes this phenotype, we evaluated the response of one-month-old variegated *Oe*Fd1 plants to changes in the growth light intensity and studied the performance of their photosynthetic electron transport chain (PETC). Four-week-old *Oe*Fd1 plants shifted to lower growth-light intensity did not exhibit an improvement in growth rate either in PSII or PSI functionality. Moreover, a physiological chlorophyll response to a decrease in light intensity was not observed in *Oe*Fd1 plants, and leaf phenotype remained variegated. In contrast, transgenic plants shifted to higher light intensities increased their growth rate (number of leaves generated after the light intensity change), LEF, and PSII functionality. When compared with wild-type siblings, *Oe*Fd1 plants had a better photosynthetic performance, especially at PSII. Altogether, these results confirm the importance of the regulation of the PSI redox status for plant growth and chloroplast development and present a novel strategy to improve photoprotective mechanisms based on the manipulation of electron partitioning at the PSI acceptor-side.

PL-C10
IDENTIFICATION AND FUNCTIONAL STUDIES OF THE REQUIREMENTS FOR EMBRYONIC NADP-MALIC ENZYME GENE TRANSCRIPTION

Gismondi M[1], Souza D[2], Permingeat H[2], Borras, L[2], Andreo CS[1], Drincovich, MF[1], Grotewold E[3], Saigo M[1].
[1]Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI-CONICET), Rosario, Santa Fe, Argentina. [2]Instituto de Investigaciones en Ciencias Agrarias de Rosario (IICAR-CONICET), Zavalla, Santa Fe, Argentina. [3] Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI, USA. E-mail: gismondi@cefobi-conicet.gov.ar

The NADP-dependent malic enzyme (NADP-ME) is widespread in nature and involved in different metabolic pathways due to the relevant physiological functions of its substrates and products (malate, and pyruvate and NADPH, respectively). In plants, this gene family contains several members although the biological roles of each of them remain still unknown. Phylogenetic analysis of plant NADP-ME protein sequences revealed the existence of different clades, being embryo-specific *ME1*lineage the only one that contains monocot and dicot members. Although the members of each clade have common subcellular localization, biochemical and structural features, and abundance in particular organs and during development, little is known about the mechanisms that govern their gene expression. Here, we present the *in silico* analysis of *ME1* lineage-specific features and performed experimental approaches on *ZmME1* promoter, to discover new players that could control ME1 particular abundance in plants. We verified the functionality of *promZmME1::GFP* constructs in transient expression experiments consisting of isolated maize embryo gene bombardments. Moreover, we performed a Yeast One Hybrid (Y1H) screen with a TFome library (containing only transcription regulators) and using a histidine auxotrophy-based selection of putative positive interactions. Different members of particular TF families (GNAT, MADS, bZIP, MYB, bHLH, HB) and also different combinations of TFs were identified as capable of binding to a transcriptional start proximal region of *ZmME1* gene, suggesting their importance of these protein-DNA interactions in its transcription regulation. Validations were carried out by direct transformation of identified TFs in yeast bait strain cells. Finally, the integration of obtained results with reported gene expression data in maize and other monocot species increased the confidence about the participation of the identified TFs in *ME1* clade-specific transcription. These findings provide new insights and trigger new hypotheses to better understand the *ME1* lineage function and the importance of their substrates and products for embryo development in species of agronomical interest.

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

THE *ARABIDOPSIS* TRANSCRIPTION FACTOR ATHB40 INHIBITS ROOT ELONGATION AND THE RESPONSE TO GRAVITROPISM

Mora CC, Ribone PA, Ariel F, Chan RL[1].
[1]IAL, UNL, CONICET, FBCB, Santa Fe, Argentina. E-mail: cmora@santafe-conicet.gov.ar

Homeodomain-leucine zipper I (HD-Zip I) transcription factors (TFs) are unique to plants and have been mainly associated with developmental processes and also related to abiotic stress responses in several species. It was previously reported that *AtHB40,* an *Arabidopsis* member of this TF subfamily, is expressed in roots, particularly in the tip of the main and lateral roots. Such expression circumscribed to the quiescent center, columella cells, and the vascular system. The obtaining and characterization of *AtHB40* mutants (*athb40*) and overexpressors (*OE40*) as well as plants transformed with *AtHB40* promoter driving *GUS* expression (*PrAtHB40:GUS*), allowed us to determine that AtHB40 is a repressor of main root elongation in an ABA-dependent manner. Regarding the inhibition of root elongation produced by AtHB40, we stated several hypotheses: (a) AtHB40 regulates cyclins, (b) AtHB40 regulates auxin transporters, (c) AtHB40 inhibits root elongation when seedlings are subjected to abiotic stress such as high salinity, (d) the gravitropic response is altered in *athb40* and *OE40* plants. To investigate our hypotheses, we obtained crossed plants in which the promoters of the putative target genes fused to the reporter *GUS* were expressed in *AtHB40* mutant or overexpressor backgrounds. The analyses of these crossed lines indicated that *LAX2* is downregulated by AtHB40 in 3-day-old seedlings. Moreover, *CYCB1* (cyclin) was repressed by AtHB40 in the root tip of 7-day-old plants. *AtHB40* mutant plants exhibited longer main roots than controls in the presence of ABA, Fluridone (an inhibitor of ABA synthesis), auxin (IAA) or NaCl indicating certain insensitivity to the growing media. Moreover, *athb40* mutants showed a larger survival percentage when seedlings were grown in high salinity medium. The response to gravitropism was also investigated indicating that *athb40* lines exhibited enhanced positive gravitropism whereas *OE40* a reduced response compared to WT plants. Altogether, our results suggest that *AtHB40* is a repressor of main root elongation and has a functional role in the gravitropic response as well as in cell division and auxin transport in the root tip.

PL-C12
PROLYL HYDROXYLATION IS NECESSARY FOR PROPER LOCALIZATION OF CELL WALL PROTEINS AND POLLEN GERMINATION IN *ARABIDOPSIS THALIANA*

Sede AR[1], Wengier D[1], Estevez JM[2,3], Muschietti JP[1,4].
[1] INGEBI–CONICET, Buenos aires, Argentina. [2]FIL-IIBBA-CONICET, Buenos Aires, Argentina. [3] Centro de Biotecnología Vegetal (CBV), Facultad de Ciencias Biológicas, Universidad Andrés Bello, Santiago, Chile. [4]DBBE, FCEYN-UBA, Buenos Aires, Argentina.
E-mail: ana.rocio@live.com.ar

To produce fertilization, pollen tubes have to travel along the pistil and then deliver sperm cells upon reaching the ovules. To sustain the polarized growth of pollen tubes, the role of the cell wall, which is constantly being remodeled in the apical region, is crucial. Different polysaccharides such as callose, pectin, and cellulose together with structural proteins that belong to the family of hydroxyprolyl-rich glycoproteins (HRGP) are involved in cell wall organization. Members of HRGPs family are the Leucine-rich repeat extensins (LRXs), hybrids proteins that contain an N-terminal domain involved in protein-ligand interactions and a C-terminal extensin-like domain with Ser-$Pro_{(3-5)}$ repetitions plausible to be glycosylated. We have previously demonstrated that *Arabidopsis* pollen-specific LRXs (LRX8-11) are necessary to maintain cell wall integrity since polarized growth of pollen tubes in loss of function *lrx9-2 lrx10-1 lrx11-1* triple mutant is altered both *in vitro* and *in vivo*. The lack of LRXs caused severe abnormalities in pollen tube morphology, a decrease in pollen germination rate and a skewed pollen segregation ratio. Moreover, microscopy analysis showed an altered deposition of polysaccharides, such as callose and pectin, in the cell wall of triple mutant pollen tubes. To determine whether post-translational modifications are required for the functionality of LRXs, we aim to study the importance of proline hydroxylation, catalyzed by prolyl-4-hydroxylases (P4H), necessary to define future *O*-glycosylation sites. We hypothesize that pollen-specific P4H4 and P4H6 catalyze the hydroxylation of prolines at the extensin domain of LRXs. Simple loss of function *p4h4* and *p4h6* mutants and *p4h4p4h6* double mutant showed a reduction in pollen germination rates; similar results were obtained by applying specific P4Hs inhibitors to the pollen germination medium. Transgenic plants expressing the construction pP4H4::P4H4-YFP showed that P4H4 is localized in the Golgi apparatus and/or endoplasmic reticulum. In addition, pollen tubes from transgenic plants expressing pLRX11::LRX11-GFP in the *p4h4p4h6* background showed a re-localization of LRX11-GFP from the tip to the cytoplasm. Together these results suggest that LRXs are putative targets of the P4H4 and P4H6 enzymes since the lack of hydroxylation and subsequent glycosylation in the *p4h4ph6* double mutant, prevents LRX11 from proper cross-linking at the pollen tube cell wall.

PL-C13
STUDY OF RALF4/19 PEPTIDES ROLE DURING POLLEN TUBE GROWTH IN *ARABIDOPSIS THALIANA*

Somoza SC[1], Boccardo NA[1], Sede AR[1], Wengier DL[1], Muschietti JP[1,2].
[1]Instituto de Investigaciones en Ingeniería Genética y Biología Molecular (INGEBI) [2]DBBE, FCEyN, UBA. E-mail: sofiacsomoza@gmail.com

Polarized cell growth involves the expansion of one of its ends, making a cell elongate in a single direction. In plants, this process occurs in growing pollen tubes, root hairs, and cotton fibers and is regulated by cytoskeletal reorganization, vesicular movement, Ca^{2+}, and reactive oxygen species (ROS) signaling. Polarized cell growth requires the synthesis and deposition of an extracellular layer rigid enough to withstand substantial internal turgor pressure, but at the same time flexible enough to permit the cell to grow. RAPID ALKALINIZATION FACTORs (RALFs) are secreted peptides relevant for transducing extracellular signals to the inside of the cell. With 36 members in *Arabidopsis thaliana*, RALFs regulate diverse developmental and physiological processes. In particular, RALFs are known to inhibit cell elongation. Eight *Arabidopsis* RALFs are expressed in pollen and we focused our study on two of them, RALF4 and RALF19, closely related to each other. RALF4 and RALF19 are required to maintain proper pollen tube growth, through the interaction with LEUCINE-REICH EXTENSIN (LRXs) and *Catharanthus roseus* receptor-like kinases (CrRLK1Ls) proteins. This interaction regulates changes in the integrity of the pollen tube cell wall transmitting this signal to the interior of the pollen tube. At the end of the journey, a pistil RALF (RALF34) triggers pollen tubes burst in order to release the sperm cells for fertilization, suggesting that regulation must be different at this point. Understanding how these complexes regulate pollen tube growth will shed some light on how plant reproduction works.

PL-C14
THE TRANSCRIPTION FACTOR AtPHL1 MODULATES SUCROSE TRANSPORT AFFECTING LIPID CONTENT IN *ARABIDOPSIS* SEEDS

Spies FP[1], Raineri J, Chan RL[1].
[1]Laboratorio de Biotecnología Vegetal - Instituto de Agrobiotecnología del Litoral – UNL – CONICET. E-mail: fspies@santafe-conicet.gov.ar

Plants are continuously subjected to stressful situations and they have evolved molecular and physiological responses allowing them to deal with such conditions. Molecular responses mainly occur at the transcriptional level, mediated by transcription factors (TFs). Plant TFs are classified in different families and among them, the MYB-CC has been associated with development in response to environmental or nutritional changes. AtPHL1 is a poorly characterized member of the MYB-CC family. It was previously informed that this TF can interact with the homeodomain-leucine zipper I TF AtHB23. Aiming at understanding the function of AtPHL1 and the interaction with AtHB23, *Arabidopsis* plants were transformed with a construct in which the expression of the *GUS* reporter gene is driven by the promoter of *AtPHL1* (*prPHL1:GUS*). Moreover, we obtained overexpressor (*35S:AtPHL1*) and mutant homozygous lines (*phl1*). The analysis of *prPHL1:G*US plants indicated that this gene is expressed in roots, in the pedicel-silique node, and the silique funiculus. Phenotyping of *phl1* mutant and *35S:AtPHL1* plants showed that the lack of PHL1 (*phl1)* provoked an early silique opening and a lesser lipid content in the seeds compared to Col-0 ones. No differences were detected in the glucose, fructose, sucrose, and protein seed contents. *Phl1* mutant plants also exhibited decreased aerial biomass both in stems and siliques. In spite of these differential characteristics, we stated the hypothesis that *phl1* mutants have difficulties to transport carbohydrates. To test this hypothesis, we performed a study with the reagent CFDA tracer (5(6)-carboxyfluorescein diacetate, a phloem-mobile probe analog to sucrose), which was transported slower to *phl1* siliques compared to control ones. Histological transversal cuts were carried out on stems to investigate xylem morphology. Surprisingly, *phl1* plants exhibited a higher number of vascular bundles in the first internode than the WT control. Altogether, the results suggest that PHL1 is a positive regulator of sucrose transport from roots to shoot, particularly to seeds. The decreased lipid content could be the consequence of slower transport capacity of *phl1* plants and could be partially compensated by an increase in the number of vascular bundles in *phl1* plants. Further studies will be necessary to corroborate this last hypothesis.

PL-C15
FUNCTIONAL CHARACTERIZATION OF AN ERF TRANSCRIPTION FACTOR WHICH MODULATES ABA SENSITIVITY AND JA-RELATED RESPONSES IN *ARABIDOPSIS*

Mengarelli DA, Osella AV, Valle EM, Zanor MI.
Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET-UNR). E-mail: mengarelli@ibr-conicet.gov.ar

Stress signal transduction pathways are still a matter of debate. In *Arabidopsis*, several members of the APETALA2/ETHYLENE RESPONSE FACTOR transcription factor (TF) superfamily were found to be involved in plant development and environmental stress responses. Here, we report the functional analysis of an uncharacterized member of this family in *Arabidopsis* that we called *ERF-SR* (for *ERF-stress related*). This TF belongs to the ERF subfamily and possesses only one AP2 domain and does not have any additional conserved motif. We have found it as superoxide specific induced gene and lines with altered levels of *ERF-SR* showed differential accumulation of ROS. Constitutive overexpression (o*x)* lines accumulate lower levels of ROS and in contrast, artificial micro RNA-(*ami*) lines show increased ROS levels in leaves when compared to wild type plants. Other contrasting phenotypes were related to ABA responses. *Ox* lines were hypersensitive to ABA in seed germination and root growth assays and also were hypersensitive to osmotic stress conditions, (both in the presence of high salt and PEG). On the contrary, *ami* lines showed enhanced tolerance to salt stress during the early stages of growth. To dissect the altered stress-related processes observed in these lines, proteomic analysis of the lines grown in the presence of 0.5 μM ABA was performed. The results revealed mainly changes in metabolic processes, photosynthesis, redox processes, responses to stress and responses to hormones. Changes in processes like glutamate and chlorophyll metabolism are in agreement with the observation that chlorophyll content of *ami* lines are higher compared to wild type and *ox* lines levels of plants grown in the presence of ABA. Additionally, several proteins related to jasmonic acid (JA) were found differentially accumulated in *ami* lines, such as VSP1 and JR1. By analysis of transcripts levels of genes involved in JA responses by qPCR of plants with altered levels of this TF, we confirmed this observation. Transcripts of *ERF1* and *ORA59* which are an upstream component of JA-signaling as well as the JA-marker *PDF1.2* were found significantly induced in *ami* lines reinforcing the idea that JA processes are altered in these lines. There is increasing evidence that ABA and other hormonal signaling pathways are interwoven with each other into an intricate network to regulate plant responses to osmotic

and salt stresses. Altogether, these data suggest that ERF-SR regulates the expression of genes involved in ABA- and JA- signal-transduction pathways.

SIGNAL TRANSDUCTION

ST-C01
ROLE OF THE P53 TARGET ICMT IN METASTASIS: POST-PRENYLATION PROCESSING AT THE CENTER OF THERAPEUTIC STRATEGIES IN CANCER

Borini Etichetti C[1,4], Cerri A [2], Arel Zalazar E[2], Larocca MC[1,4], Menacho-Márquez M[2], Porta E [3,4], Labadie, G[3,4] Girardini J[2,4].
[1] IFISE-CONICET. [2] IDICER-CONICET. [3] IQUIR-CONICET. [4] FCByF, Universidad Nacional de Rosario.
E-mail: girardini@idicer-conicet.gob.ar

The mevalonate pathway catalyzes the *de novo* synthesis of cholesterol, but also provides isoprenoids that may be used for protein modification through a complex process known as prenylation. Some proteins involved in oncogenic processes, such as Ras and Rho GTPases are among the targets of this modification. Pioneering studies with statins have suggested that mevalonate pathway alteration cooperates with tumor aggressiveness. More recently, other evidence has confirmed that alteration of this pathway may promote aggressive phenotypes and pointed out at enhanced protein prenylation as a possible mechanism underlying this effect. ICMT plays a central role in this posttranslational modification process by catalyzing the last step, carboxymethylation of the prenylated C-terminus in target proteins. We have recently unveiled a link between post-prenylation processing and the p53 pathway by showing that *ICMT* expression is repressed by wt p53, but enhanced by cancer-associated p53 point mutants. Moreover, our analysis of Breast and Lung cancer databases showed a negative correlation between *ICMT* expression and wt p53 status. Moreover, we found a significantly decreased metastasis-free survival frequency in patients with high *ICMT* expression. Basing on these results, we wondered if alteration of ICMT levels enhances metastasis development. To answer this question, we studied the effect of ICMT overexpression on metastasis *in vivo*, using Triple Negative Breast Cancer cells in an immunocompetent mouse model. We extended our previous analysis on breast cancer patients and we found that p53 status affects the impact of ICMT overexpression on clinical outcomes. Besides, our studies on the regulation of *ICMT* expression showed that other p53 family members affect its transcription. Our results suggest that ICMT levels are affected by alterations in the functional equilibrium between different members of this family during tumor progression. To explore the potential of pharmacological manipulation of ICMT function, we analyzed the impact of the ICMT inhibitor Cysmethynil to affect tumor-associated phenotypes. We found that ICMT inhibition affects clonogenic potential, as well as phenotypes associated with metastatic cells, such as migration and invasion *in vitro*. In an effort to develop novel ICMT inhibitors and inspired on Salirasib (*S*-trans-trans-farnesylthiosalicylic acid) we synthesized novel thiosalicylic acid derivatives. As a preliminary characterization, we analyzed the antiproliferative activity of our compounds *in vitro* on MDA-MB-231 cells. Our results suggest that ICMT overexpression affects tumor progression and that molecules interfering with the function of prenylated proteins are potentially useful in therapeutic strategies.

ST-C02
NITRIC OXIDE AND AUXIN REGULATE ROOT MERISTEM DURING GRAVITROPISM IN *ARABIDOPSIS THALIANA*

Vazquez MM[1], Goldy C[2], Rodríguez R[2,3], Casalongué C[1], París R[1].
[1] IIB-CONICET-UNMdP, Funes 3250, 4to nivel, Mar del Plata, Argentina, [2] Instituto de Biología Molecular y Celular de Rosario (CONICET-UNR), [3] Centro de Estudios Interdisciplinarios (UNR). E-mail: vazquezmaguie@gmail.com

Nitric oxide (NO) is a gaseous redox-active molecule with a role in different physiological auxin-mediated processes including gravitropism. Roots bend in response to gravity by the formation of an asymmetric distribution auxin pattern between the upper and the lower sides of elongation and meristematic zones of the root. However, the mechanisms by which auxin and NO interplay during the gravitropic response are not still fully understood. In this work, we focus on deciphering the spatio-temporal pattern of auxin and the functional contribution of NO in the meristematic cells during early events of gravitropism in *Arabidopsis* roots. In *Arabidopsis thaliana,* the meristematic root zone comprises all the cells that undergo mitotic divisions and stretches longitudinally up to 350 µm from the quiescent center (QC). By using the auxin sensor DII-VENUS we demonstrated that within the first 30 min of a 90º gravity stimulus, the hormone was distributed asymmetrically between the upper and lower sides in lateral root cap, epidermal and cortical cells extending up to 120 µm from the QC in the meristematic region of the root. In addition, we demonstrated that NO is accumulated asymmetrically between the lower and upper sides of the root meristem. Next, we measured the length of individual epidermal cells along the meristematic zone. Scavenging of endogenous NO affects the characteristic epidermal cell length observed during the gravitropic response. Therefore, we hypothesize that the disturbance of the interaction between auxin and NO signals could affect the meristematic cell size pattern which leads to an agravitropic response in *Arabidopsis* roots. Since cyclins play a vital role in controlling cell cycle progress, one of our current challenges is to investigate how auxin- and NO-mediated regulation affects the dynamic and functionality of root meristem during gravitropism. *Supported by UNMdP, CONICET, ANPCyT.*

ST-C03

HISTONE POST-TRANSLATIONAL MODIFICATIONS IN THE PROTOZOAN PARASITE *GIARDIA LAMBLIA*

Salusso A[1], Jaquet S[2], Diaz Perez L[1], Ciborowski P[2], Rópolo AS[1].
[1]INIMEC-CONICET-UNC, Córdoba, Argentina. [2]Dept. of Pharmacology and Exp. Neuroscience, University of Nebraska Medical Center. Omaha, USA. Email: asalusso@immf.uncor.edu

Histones are very low molecular weight proteins that together with DNA build chromatin in eukaryotic cells. The amino-terminal ends of histones are susceptible to post-translational modifications, which have an impact on chromatin structure and therefore on the regulation of gene expression. At present, it is proposed that all of these modifications on histone tails form an undercover language known as the "histone code", which is read by different proteins, and govern chromatin structure and regulate gene expression. In the protozoan parasite *Giardia lamblia*, epigenetic modifications have begun to be studied in recent years, analyzing histone-modifying enzymes, but so far, the particular modifications of each histone are unknown. The main objective of this work was to obtain the complete map of the post-translational modifications in the histones of *Giardia lamblia*. For that, we isolated the histones of growing trophozoites and performed mass spectrometry tests using two liquid chromatography and mass spectrometry platforms. We identified different peptides of H2A, H2B, H3, and H4 included in the *Giardia* histone code. We found modifications preserved in other organisms, such as lysine acetylation, lysine and arginine methylation, threonine and tyrosine phosphorylation and lysine ubiquitination. In turn, we described for the first time the propionylation of amino acids in *Giardia* trophozoites. Moreover, in the general analysis, we observed that the same amino acid might suffer different types of modifications and depending on the type of that modification, different modifications may appear in close residues, which would indicate that there is an exchange of information (cross talk) in neighboring amino acids. Interestingly, we found arginine methylation although in the *Giardia* DataBase there are no enzymes described as HRMT (histone-arginine methyltransferase). However, three enzymes were described as possible histone-lysine methyltransferases (HMT1, HMT2, and SET2), therefore the presence of methylated arginines indicates that possibly some of the HMTs that modify lysines, could also be modifying arginines. Finally, our work provides the first large-scale characterization of the *Giardia* histone code, which constitutes an essential initial platform for the development of future research in the field of epigenetics in this parasite.

WORKSHOP DRUG DISCOVERY

WS-C01
DRUG DISCOVERY OF TLS INHIBITORS TO SELECTIVELY TARGET CANCER CELLS WITH HOMOLOGOUS RECOMBINATION REPAIR DEFICIENCIES

García IA[1], Villafañez F[1]*, Quiroga R[2]*, Bocco JL[1], Villareal M[2], Soria G[1].*
**These authors contributed equally to this work.*
[1]CIBICI-CONICET, [2]INFIQC-CONICET. Facultad de Ciencias Químicas. Universidad Nacional de Córdoba.
E-mail: garcia.iris.alejandra@gmail.com

Translesion DNA Synthesis (TLS) and homologous recombination (HR) cooperate during S-phase to ensure replication forks integrity and cell survival. Consequently, TLS inhibition emerges as a promising strategy for the therapeutic intervention of HR-deficient tumors by synthetic lethality (SL) induction. Given the current lack of selective TLS pharmacological inhibitors to evaluate this hypothesis, we developed different approaches to identify small molecules able to impair PCNA mono-ubiquitination, a key post-translational modification required for the efficient activation of TLS. Initially, we developed a miniaturized WB assay using complementary antibodies that simultaneously detect ubi-PCNA and total PCNA. Using this assay, we screened a library of 627 kinase inhibitors. We found that targeting the pro-survival kinase AKT leads to a strong impairment of PCNA ubiquitination. Remarkably, such inhibition triggered the induction of SL in BRCA-deficient cells submitted to replication stress. The follow-up strategy was to focus on the identification of PCNA ubiquitination inhibitors with more selective mechanisms of action. To tackle this challenge, we designed a virtual screening approach to identify direct blockers of PCNA-ubiquitination through molecular modeling from a 10K collection of structurally diverse small molecules. We found several putative compounds that block PCNA-ubiquitination *in silico*, which after experimental validation led to the identification of a small group of strong PCNA ubiquitination inhibitors. Collectively, this work shows for the first time that TLS inhibition can be achieved by the pharmacological impairment of PCNA ubiquitination and provides the proof-of-concept of TLS inhibition as a therapeutic strategy to selectively kill HR-deficient cells.

WS-C02
ROCK INHIBITION INDUCES SYNTHETIC LETHALITY IN BRCA2-DEFICIENT CELL LINES

Martino J[1], Paviolo NS[1], Siri S[1], Pansa MF[2], Carbajosa S[2], Garro CA[2], Soria G[2], Gottifredi V[1].
[1]Fundación Instituto Leloir, Buenos Aires, Argentina, [2] CIBICI-Universidad Nacional de Córdoba, Córdoba, Argentina.
E-mail: jmartino@leloir.org.ar

BRCA2 (Breast cancer susceptibility protein 2) is involved in homologous recombination repair, a pathway that repairs DNA double-strand breaks, one of the most lethal DNA lesions. Hereditary and somatic loss of function mutations in BRCA2 are correlated with breast and ovarian cancers. These tumors are highly invasive, do not respond well to conventional chemotherapy and have a poor prognosis. In recent years, it was shown that BRCA2 deficient cells can be selectively killed using PARP (Poly-ADP Ribose Polymerase) inhibition and PARP inhibitors were quickly approved to be used in the clinic. Unfortunately, these treatments often develop resistance and there is an urgent need to find alternative therapies for BRCA2 patients. To find novel alternatives, we screened a library of kinase inhibitors and identified that inhibition of ROCK kinases kills cells deficient in BRCA2. ROCK kinases are key regulators of cytoskeleton functions and few data exist regarding crosstalk with HR. Using Fasudil, a commercially available ROCK inhibitor, we validated our screen hit in multiple BRCA2 deficient cell lines. Additionally, we found that Fasudil treatment in BRCA2 cells induces abnormal cell cycle distributions characterized by a population of polyploid cells. Consistent with this, we observed an increase in multinucleated cells. The observed changes were not due to an abnormal S phase since both the percent of EdU^+ cells and EdU intensity were similar in wild-type vs. BRCA2 cells. The replication stress marker γH2AX was also unaffected. We also did not observe increased genome instability, measured by micronuclei and chromosome aberrations. Additionally, mitotic entry, measured by phosphorylation of H3, was also normal. This suggests the observed phenotypes could be due to an abnormal transition through the M phase. Interestingly, we found that BRCA2 cells treated with Fasudil display aberrant metaphases, chromosome bridges and have a decreased percentage of cells in late mitotic stages such as anaphase and telophase. Moreover, abnormal mitotic figures were often accompanied by multipolar spindle poles and supernumerary centrosomes. Altogether, our data suggest that ROCK inhibition induces mitotic abnormalities and polyploidy in BRCA2 cells which are the likely cause of cell death. Intriguingly, these phenotypes are different than what is observed with PARP inhibition suggesting a new Achilles heel for BRCA2 cells. Future experiments aim at connecting cell death with the observed mitotic defects, as well as using a pre-clinical model to test if Fasudil can affect BRCA2 tumors.

WS-C03
EXPLORATION OF VITAMIN D RECEPTOR PHARMACODYNAMIC FEATURES UNDER TUMORAL AND NORMAL CELLS MICROENVIROMENTS: A MOLECULAR MODELING STUDY

Ribone SR[1] , Cerutti JP[1], Vitale C[2], Fall F[3], Ferronato MJ[4] , Curino AC [4], Facchinetti MM[4], Quevedo MA[1].
[1]UNITEFA-CONICET and Departamento de Ciencias Farmacéuticas, Facultad de Ciencias Químicas, UNC, [2]Laboratorio de Química Orgánica, INQUISUR, UNS, CONICET, Departamento de Química, UNS, Bahía Blanca, Argentina.[3]Departamento de Química Orgánica, Facultad de Química e IBI, Universidad de Vigo, Campus Lagoas de Marcosende, 36310 Vigo, Spain.[4]Laboratorio de Biología del Cáncer, INIBIBB, UNS, CONICET, Departamento de Biología, Bioquímica y Farmacia, Bahía Blanca, Argentina. E-mail: sribone@fcq.unc.edu.ar

The vitamin D receptor (VDR) is a member of the nuclear receptor (NR) superfamily that binds different isotypes of retinoid X coregulators. The involvement of VDR in antiproliferative and antitumoral cellular pathways has attracted much scientific attention, mostly as a therapeutic target to treat cancer. Unfortunately, Calcitriol (CTR), a recognized agonist of VDR, cannot be used as an antiproliferative agent due to its hypercalcemic effects. Among the pharmacophoric contacts established by CTR, the interactions with residues His305 and His397 of VDR are particularly relevant, forming a network of hydrogen bond interactions through the hydroxyl group present in the ligand sidechain. We have already reported the synthesis and evaluation of several CTR analogues, obtained by modification of its sidechain, with some compounds exhibiting interesting antiproliferative and/or selectivity properties, with associated hypercalcemic effects. In this context, the possibility of obtaining CTR analogs with selective antiproliferative effect towards tumoral cells constitutes a particularly interesting aspect. We have previously reported that differences in the pH environment between tumoral and normal cells may represent a key physicochemical property driving this selectivity. In this work, we present molecular modeling studies (i.e. constant pH molecular dynamics, molecular docking, classical molecular dynamics and free energy of binding analysis) performed in order to elucidate at an atomistic level the molecular basis behind the potential of CTR analogs to reach tumoral cells selectivity. In this way, we found that His305 and His397 residues exhibited a specific tautomeric configuration, which is required for the genomic effects of VDR. This feature is closely related to a hydrogen bond network connecting the ligand-binding site and the activating factor 2 (AF-2) domain, which in turn produces an allosteric control of VDR agonism. Also, differential acid-base properties were observed for His305 and His397 as evidenced by constant pH molecular dynamic simulations (CpHMD). In this way, His305 exhibited an increased acidity (pKa 5.5) compared to His397(pKa 6.6) and typical histidine residues. This feature favors the pharmacodynamic interaction of CTR analogs possessing electron-rich groups as part of the sidechain, thus conferring them a higher affinity for VDR at pH 5.5 (tumoral environment) compared to the VDR receptor modeled at pH 7.4 (normal cells environment). On the other hand, non-selective compounds (such as CTR), exhibits homologous interaction patterns with VDR at both pH conditions. To the best of our knowledge, the results presented constitutes the first evidence related to physicochemical features of VDR that may represent a key molecular feature for the design of new CTR analogs exhibiting not only improved potency and lowered hypercalcemic effects, but also high selectivity indexes.

WS-C04
NOVEL FUNCTIONS OF THE UPS IN THE CONTROL OF TUMOR CELLS INVASIVENESS

Rossi FA[1], Enriqué Steinberg JH[1], Calvo Roitberg EH[1], Espinosa JM[2], Rossi M[1].
[1] Instituto de Investigaciones en Medicina Traslacional (IIMT), Universidad Austral, [2] Linda Crnic Institute for Down Syndrome and the Department of Pharmacology, University of Colorado, USA. E-mail: mrossi-conicet@austral.edu.ar

Despite formidable advances in the prevention, early detection and treatment of a great number of cancers, the development of metastasis foci in patients suffering from this disease still represents a significant reduction in their survival and life quality. Although metastatic cells have partially been characterized, there is yet not an effective treatment for this pathological condition. The Ubiquitin-Proteasome System (UPS) plays a fundamental role in the maintenance of protein homeostasis both in normal and stressed conditions. This enzymatic cascade represents one of the most important metabolic protein degradation pathways and plays a fundamental role in the control of almost every single cellular process. Since alterations in the ubiquitylation cascade have been shown to be associated with malignant transformation, invasive potential of cells and metastasis, we sought to investigate the role of the UPS in the regulation of tumor-cell migration and invasion. To this end, we performed a genetic screen using an shRNA library against UPS genes, and Boyden chambers to analyze the migrating/invasive potential of breast cancer cells infected with this library. After the selection process, we characterized the non-migrating cell population and obtained a list of 30 candidate positive migration regulator genes, half of which had already been associated with the regulation of migration, invasion, tumorigenic processes or metastasis. Among the candidates, we focused on a specific DUB and demonstrated that its silencing reduces the migratory/invasive potential of different tumor-cell lines. Since silenced cells proliferation was impaired using *in vitro* three-dimensional setups, we furthered our investigation with *in vivo* studies. We demonstrated that NOD/SCID mice inoculated with silenced cells present Kaplan Meier curves for tumor-free survival with a clear separation within the control group, as well as a delay in the onset of the tumor formation compared to the tumors generated by control cells. In addition, our results also show a significant impairment in the generation of metastatic foci, indicating that tumor cell niche colonization might be impaired. Finally, we performed a small clinical study that demonstrated that this DUB's protein expression is a prognostic predictor of distant relapse-free survival in patients with breast cancer. Altogether, these findings demonstrate that shRNA screens using Boyden chambers are useful for finding novel genes that regulate migration and invasion, which might represent novel therapeutic targets for the development or improvement of cancer treatments.

WS-C05
USE OF *IN VIVO* IMAGING SYSTEM FOR PRECLINICAL EVALUATION: EXAMPLES OF ITS APPLICATION IN DRUG DEVELOPMENT

Salinas FJ, Berengeno AL, Santiago G, Marelli BE, Baravalle ME, Salvetti NR, Ortega HH.
Centro de Medicina Comparada, ICiVet-Litoral (UNL-CONICET), Esperanza, Santa Fe, Argentina. Facultad de Ciencias Veterinarias – Universidad Nacional del Litoral, Esperanza, Santa Fe, Argentina. E-mail: hhortega@fcv.unl.edu.ar

The most recent techniques available for real-time *in vivo* imaging of the distribution of drugs and their metabolism in the body provide a unique, early opportunity to identify which drugs will fail in the later stages of drug development, thereby improving the quality of the molecules ultimately selected to move forward. This cutting-edge research area is so important that new equipment and technologies are presented day by day. This technology allows the visualization, characterization, and measurement of biological processes in living systems. In the past decade, it has been increasingly recognized as an important preclinical and clinical research tool that can be used to speed up the long-term engagement of the drug development process. The use of *in vivo* images during preclinical development is a fundamental tool to improve the efficiency of the development process of new drugs and allow reducing the number of animals required in longitudinal studies as well as increasing the data obtained from each animal. Here we present two examples of the use of this technology in preclinical trials, developed under an ISO 9001 certified Quality Management System in accordance with the principles of Good Laboratory Practices (BPL-OECD recognized facility). Trials were designed and executed using a Small Animals Imaging System (Pearl Trilogy, Licor) that allowed analyzing in real-time the distribution of the drug in BALB/cCmedc mice. The biodistribution of nanoparticles administered intravenously was observed by loading with specific fluorophores in the NIR range of 700 nm and organs involved in their metabolism, accumulation, and elimination could be identified (liver, gallbladder, and kidneys). It was also possible to analyze the real-time distribution of heterologous immunoglobulins, administered intravenously, allowing to identify the target organs, elimination times and exception route. For this, the immunoglobulins were labeled with fluorophores with fluorescence at 800 nm. At 0.5, 1, 2, 4, 6, 8, 12, and 24 h after inoculation images were acquired. After *in vivo* imaging, animals were sacrificed. Ex vivo imaging after removal of the brain, heart, lungs, liver, spleen, kidneys, stomach, intestines, adrenal glands, eyes, seminal vesicles, prostate, and bladder was performed. The intensity and location of the signal were determined by digital analysis of the images. We can conclude that *in vivo* imaging techniques are extremely useful for longitudinal evaluation in pharmacodynamic studies. Also, the intensity and location of the bioluminescent signal can provide information about the distribution of specific molecules in animals reducing the number of animals to be used in pharmacokinetic and pharmacodynamic tests, added to the fact that their high sensitivity facilitates the adequate identification of end-points and white organs in development of new molecules.

WS-C06
VINARDO2: A NEW SCORING FUNCTION FOR MOLECULAR DOCKING WITH IMPROVED VIRTUAL SCREENING

Quiroga R, Villarreal MA.
INFIQC-CONICET, Departamento de Química Teórica y Computacional - Facultad de Ciencias Químicas – UNC

E-mail: rquiroga@unc.edu.ar

Molecular Docking is a computational method that aims to predict the optimal position and orientation of a ligand binding to a protein, as well as the strength of this interaction. Docking is a key tool in structure-based drug design. In essence, the method consists of the search for the global maximum of a mathematical function which represents the predicted affinity of the interaction between a ligand and a protein. This mathematical function, termed scoring function, is developed based on different approaches. Among them, there are semi-empirical, fully empirical, knowledge and artificial-intelligence-based methods. For the past few years, we have been developing an empirical scoring function based on the well-known Vina scoring function. Autodock Vina was the most cited and used docking software worldwide in 2018 (more than 1700 citations in 2018 and 8800 since its publication in 2010). In this work, we present the newest version of our scoring function which includes a completely reworked hydrogen bond function, improved atom-typing, improved exploration of ligand conformational space, and a novel solvation term. The philosophy behind our development was to come up with coarse grained interactions, where the atomic parameters that define the interactions are picked from a set of predefined and discrete values. The performance of the Vinardo2 scoring function was evaluated in rescoring, redocking and virtual screening tasks. The results show that the new function performs significantly better at all three tasks when compared with our previous version (Vinardo) and also with the original Vina scoring function. This improved performance of Vinardo2 comes with no additional computational cost or complicated ligand preparation procedures. This makes Vinardo2 an all-around improved scoring function which can be used in direct replacement of the Vina scoring function with improved performance, especially in regards to virtual screening and computational drug discovery.

WS-C07
HYDROXYLAMINE CHEMICALLY ENGINEERED EXTRACTS AS SOURCE OF ANTIMYCOBACTERIAL COMPOUNDS

Ramallo IA[1], Parés VS[1], Gago G[2], Gramajo H[2], Furlan RLE[1].
[1]Fac. de Ciencias. Bioquímicas y Farmacéuticas, UNR-CONICET. [2]IBR-CONICET, Rosario. E-mail:aramallo@fbioyf.unr.edu.ar

Tuberculosis (TB) is one of the top ten causes of death and the leading cause, from a single infectious agent, exceeding both malaria and HIV. The emergence of multidrug-resistant TB (MDR-TB) presents an increasingly difficult therapeutic challenge; so much that MDR-TB is now the main cause of death due to antimicrobial resistance. Unfortunately, the lethality of TB combined with its multidrug-resistant capacity has now transformed this long-neglected disease into a global health priority. Therefore, there is an urgent need for the development of new drugs that could reduce the duration and complexity of current therapies. Biological selection makes natural products (NPs) an excellent source of bioactive substructures. The chemical diversification of natural extracts uses natural substructures as scaffolds to produce new bioactive semi-synthetic molecules. This approach involves the chemical transformation of reactive fragments commonly found in NPs (like carbonyl groups) to introduce functionalities that are relevant for bioactivity (like N-O fragment). We report here the chemical diversification of a set of 18 essential oils (EOs) with $NH_2OH.HCl$ in refluxing ethanol for 7 h to produce chemically engineered EOs with different chemical compositions, according to the GC-MS/NMR analysis. The chemically engineered EOs were used to study *Mycobacterium smegmatis* growth inhibition using a microplate assay at 200 µg/mL final concentration. Of the complete set, the chemically engineered *Cuminum cyminum* L. essential oil (CCY-M) was the only mixture that showed inhibitory properties against this microorganism. Quantitative biological analysis of CCY-M and the starting essential oil *C. cyminum* (CCY) was performed giving a MIC = 50 µg/mL and a MIC > 200 µg/mL, respectively. A *M. smegmatis* TLC-bioautography assay was developed *ad hoc* and applied to the CCY-M showing an intense inhibition halo that was absent in CCY. With this evidence, the reaction was scaled up and, the bioassay-guided fractionation of CCY-M led to the purification of one compound that was identified by NMR and HRMS as (*Z*)-4-isopropyl benzaldehyde oxime (**1**). This isolated structure contains the N-OH moiety confirming that it is a product of the reaction. This compound was probably generated by the reaction of cuminaldhyde (the major constituent of CCY) with hydroxylamine. Interestingly, the oxime **1** MIC was 9.06 µM, two and a half times better than isoniazid (first-line antibiotic in the treatment of TB, MIC = 36 µM). The potency of the oxime **1** against *M. tuberculosis* H37Ra was tested, but the results showed a MIC = 72.45 µM, much higher than the expected according to the previous *M. smegmatis* results. Future chemical modifications of oxime **1** could be introduced to increase its activity against M. *tuberculosis* H37Ra. However, the most relevant point reported here is the chemical diversification of natural mixtures itself: it is a simple and low-cost strategy to produce new antimycobacterial molecules.

WS-C08
CHEMICAL BIOLOGY TO UNDERSTAND MOLECULAR MECHANISMS OF REGULATION AND AS POTENTIAL STARTING POINTS FOR DEVELOPMENT OF INNOVATIVE DRUGS

Sacerdoti M[1], Gross LZF[1], Suess E[2], Bollini M[3], Taylor M[1], Leroux AE[1], Biondi RM[1,2].
[1] IBioBA, CONICET, Partner Institute of the Max Planck Society. [2] Medizinische Klinik 1, Universitätsklinikum Frankfurt. [3]CIBION - CONICET.
E-mail: rbiondi@ibioba-mpsp-conicet.gov.ar

The object of the poster is to introduce our group, our know-how and capabilities at the 1st Workshop in Drug Discovery (SAIB 2019). Our laboratory uses interdisciplinary approaches in the field of chemical biology and early drug discovery to investigate the mechanism of regulation of proteins of biomedical interest, most notably protein kinases. Protein kinases are tightly regulated enzymes that mediate multiple cellular responses and their miss-regulation can lead to diseases, like diabetes and cancer. There is a large interest in the development of drugs directed to protein kinases. Most drugs are directed to the ATP-binding site. Our strongest focus over the years has been to understand the mechanism of regulation of protein kinase PDK1 and a group of protein kinases, termed AGC kinases. We described a key ON-OFF switch on the kinase domain, which we termed "PIF-pocket", which participates in the activation of AGC kinases by phosphorylation. We then developed small molecules that bind to the PIF-pocket regulatory site and can "activate" the kinase *in vitro* mimicking the mechanism of activation by phosphorylation, and compounds that bind to the PIF-pocket and "inactivate" an AGC kinase. Throughout the years we integrated structural biology, rational design,

and synthesis of small compounds – medicinal chemistry – and used structural, biochemical, biophysical and in silico methods to investigate the allosteric mechanism: how compounds binding at the PIF-pocket enhance or inhibit the activity of AGC kinases and the reverse path, i.e. how compounds binding at the ATP-binding site can enhance or inhibit interactions at the PIF-pocket. Modern allosteric drugs in development to the AGC kinase Akt/PKB (i.e. MK-2206) target the inactive structure of the PIF-pocket, the site we originally described as a regulatory site and as a potential drug target site. As part of collaborative research projects, we have set-up innovative protein-protein, DNA-protein or peptide–protein interaction assays (AlphaScreen homogenous assays) and performed wet-screenings of libraries of small compounds (15000 small compounds in a "hit-finder" approach). Using our screening approach, we identify "hit" compounds and employ different methods to "validate" the compound "hits" that have new mechanisms of action. With our approach, we identify new small molecules that can serve as tools to study the mechanisms of regulation of the target proteins and to validate those small compounds can indeed modulate the desired interactions. The finding that small compounds can modulate important biomedical interactions opens the possibility to follow-up the discoveries into early drug discovery and drug development programs. In 2016 we established our laboratory at IBioBA-CONICET while we keep our Frankfurt laboratory active. Structural biology studies are performed with the crystallography node of the Argentinian PLABEM platform (Sebastián Klinke, Fernando Goldbaum et al. at Leloir Institute).

POSTERS

BIOTECHNOLOGY

BT-P01
EXPRESSION, PURIFICATION AND CHARACTERIZATION OF RECOMBINANT HUMAN ALPHA-GALACTOSIDASE A MUTANTS

Amaranto M[1], Godino A[1], Carreño A[2], Mendoza R[2,3], Barra JL[1], Corchero J L[2,3].
[1]CIQUIBIC-DQBRC, FCQ, UNC, Córdoba, Argentina, [2]CIBER-BBN, Madrid, Spain, [3]IBB, UAB, Bellaterra, Spain.
E-mail: marilla.amaranto@gmail.com

Fabry disease is a recessive X-linked lysosomal storage disorder caused by a deficiency of the alpha-galactosidase A (GLA). This disorder can be treated using enzyme replacement therapy with recombinant GLA. However, there are several limitations in this type of therapy such as the high amounts of enzyme required and the associated cost, the possible unwanted immune reactions, and the stability of the enzyme, among others. To overcome some of these limitations, an alternative could be the use of mutant enzymes with a higher specific activity or with a higher half-life. In this work we produced and analyzed the enzymatic activity of three-point mutations (C90T, N139S and R252T), combinations thereof [C90T plus N139S (C-N), C90T plus R252T (C-R) and N139S plus R252T (N-R)] and two deletion mutants of GLA (Δ7 and Δ10, having 7 or 10 amino acids deletions at the C-terminal end, respectively). All these recombinant proteins contain a C-terminal His-tag fusion. The wild type GLA and mutant derivatives were expressed by transient gene expression in a human cell line (HEK 293F cells) and then purified by affinity chromatography from culture supernatants. The analysis of the culture supernatant and purified proteins showed that C90T, N139S, C-N, and C-R mutants displayed a lower specific enzyme activity compared with the WT enzyme. However, R252T, R-N, Δ7, and Δ10 mutants showed increased specific activity compared with WT GLA. More interesting, the last four mutants also showed increased stability, compared with the WT enzyme, after storage in a non-optimal buffer at 4°C, being the Δ7 derivative the most stable. WT enzyme maintained less than 1% of its enzyme activity while Δ7 derivative maintained more than 80% of its enzyme activity after 8 days under this storage condition. These results suggest that some point or deletion mutants of the GLA enzyme could be considered as potentially more efficient drugs for the Fabry enzyme replacement therapy.

BT-P02
OPTIMIZATION OF *PICHIA PASTORIS* FERMENTATION PROCESS FOR PC-PLC PRODUCTION

Cerminati S, Marchisio F, Val DS, Cansino Quispe CE, Paoletti L, Aguirre A, Peirú S, Menzella H, Castelli ME.
Laboratorio de Desarrollo de enzimas industriales, IPROBYQ-CONICET, Universidad Nacional de Rosario, Rosario, Santa Fe, Argentina.
E-mail: marchisio@iprobyq-conicet.gob.ar

Crude vegetable oils are a complex mix of triglycerides, phospholipids, sterols, glycolipids, free fatty acids, metallic traces, and other minor compounds. Oil degumming (or phospholipid removal) is the first step in the industrial process of oil refining and is the one having the major associated losses. Traditionally, physical and chemical methods have been used. More recently, enzymatic degumming has been adopted by many industries, with several advantages over the formers. Enzymatic degumming reduces the gums' volume and generates products that are more easily removed by centrifugation, increasing the overall yield, with a significant economic benefit for the industry. Our group has developed an enzymatic mixture of two phospholipases C (PC-PLC, PI-PLC) and a lisoacyltransferase GCAT (L-GCAT) that can be employed in the conditions currently used in industry for the treatment of crude oil, efficiently hydrolyzing phospholipids in oil. Each of these enzymes is obtained by heterologous production in fed-batch fermentation processes using different microbial hosts, e.g., *Escherichia coli* for PI-PLC and L-GCAT, and *Pichia pastoris* for PC-PLC. In this work, the optimization of the *Pichia pastoris* fermentation process to obtain PC-PLC is described. We optimized the culture medium composition, the adaptation phase length, and the feeding strategy. We finally established that a continuous strategy using a protease

deficient strain is the best option, with remarkably high productivity. This optimized fermentation strategy, together with efficient downstream processing, provides a manufacturing alternative for the cost-effective production of the enzyme.

BT-P03
SUSTAINABLE PRODUCTION OF ACTINOBACTERIA FOR USE IN BIOREMEDIATION

Costa-Gutierrez SB[1], Aparicio JD[1,2], Sineli PE[1], Benimeli CS[1], Polti MA[1].
1-PROIMI-CONICET, Tucumán, Argentina; 2-FBQF, Tucumán, Argentina. E-mail: stefanie.costa91@gmail.com

Pollution by pesticides and heavy metals is an environmental problem that strongly has concerned the scientific community in the last decades. Although great advances have been made in this area, there are relatively few successful treatments for the restoration of polluted environments nowadays. One promising solution is bioremediation by bacteria, especially actinobacteria because they are ubiquitous and have a wide spectrum of activities. It is estimated that 40 kg of microbial biomass is required to decontaminate one hectare of soil *in situ*. Therefore, it is necessary to develop a sustainable process that allows the generation of a large amount of biomass using low-cost substrates. Biofuels, such as biodiesel and bioethanol, are renewable and environmentally friendly alternatives to fossil fuels. Biodiesel is produced by the transesterification of vegetable oils, animal fats or microbial oils with alcohol, being glycerol the main product, which represents 10% of its weight. Although glycerol is used for different applications, the increasing production of biodiesel in the world causes an excess of this material. Hence, glycerol can be used as a substrate to obtain products with high added value. In the present work, four actinobacteria with known bioremediation activity (*Streptomyces* sp. M7, *Streptomyces* sp. MC1, *Streptomyces* sp. A5, and *Amycolatopsis tucumanensis* AB0) were evaluated for the production of biomass at laboratory scale, using glycerol as a source of carbon and energy in liquid minimal medium (30 mL). Biomass production was measured by dry weight, and residual glycerol concentration was determined using a commercial kit. *Streptomyces* sp. A5 was able to produce the greatest amount of biomass and it was selected to evaluate the influence of culture parameters through a two-level fractional factorial design which allowed studying the main effects of the involved factors. From this design, the variables with the greatest effect on the response (biomass g/L) were selected. The factors evaluated were: inoculum concentration, glycerol concentration, agitation, temperature, and pH. Subsequently, in order to optimize the biomass production of the actinobacteria, a complete factorial design was carried out by selecting the most important factors previously determined. The simple effects and cross effects were studied, and a response surface design was applied to adjust the model and optimize the microbial biomass production. All factorial designs were made and analyzed using the statistical software Minitab® 17.2.1. Finally, the selected conditions will be used for biomass production of *Streptomyces* sp. A5 in a greater scale instrumented bioreactor of 2 L capacity.

BT-P04
USE OF A MINI-INTEIN FOR THE AFFINITY PURIFICATION OF RECOMBINANT THERAPEUTIC PROTEINS FROM *ESCHERICHIA COLI* PERIPLASM

Godino A; Amaranto M, Barra JL.
CIQUIBIC–CONICET, DQBRC, FCQ, UNC, Córdoba, Argentina. E-mail: agustinagodino@gmail.com

The aim of this work was to develop a new expression and affinity purification methodology for the production of recombinant therapeutic proteins from *Escherichia coli* periplasm. This development combines the delivery of proteins to the periplasmic space, together with a self-cleavable affinity tag for the purification process. The expression of recombinant proteins directed by a periplasmic signal peptide allows the expression of proteins with any N-terminal amino acid (except Pro), and the periplasmic environment facilitates disulfide bridge formation. We use the recombinant human growth hormone (rhGH) as a model protein. The therapeutic rhGH starts with a Phe residue, it has two disulfide bridges and cannot have affinity tags. We designed an expression vector to produce the rhGH N-terminal fused to a periplasmic signal peptide and the C-terminal fused to a mini-intein / Chitin Binding Domain (CBD) chimeric affinity tag. The CBD allows purification of recombinant proteins by chitin affinity chromatography, while the mini-intein undergoes a self-cleavage reaction enabling the elution of rhGH without any extra amino acid. Optimization of the upstream conditions allowed us the expression of six times more recombinant protein in the periplasm than that obtained under non-optimized conditions. The mini-intein/CBD polypeptide allowed the purification of the recombinant protein by affinity chromatography using a chitin column. Elution of the rhGH without any extra amino acid was achieved after induction of the self-cleavage of the mini-intein by decreasing the pH of the buffer and increasing the temperature of the affinity column. The rhGH without any tag was efficiently recovered with high purity under these experimental conditions. These results suggest that this expression and purification system could be used for the production of therapeutic and research proteins with any N-terminal amino acid and without affinity tag.

BT-P05
POTENTIAL USE OF BACTERIAL FLAVING-CONTAINING MONOOXYGENASES FOR INDIGO AND INDIRUBIN PRODUCTION IN *ESCHERICHIA COLI*

Guendulain TV, Barra JL.
CIQUIBIC–CONICET, DQBRC, FCQ, UNC, Córdoba, Argentina. E-mail: tatianaguendulain@gmail.com

Indole alkaloids are a large group of secondary metabolites. Indigoid compounds such as indigo and indirubin have been employed as natural dyes and drugs since ancient times. Indigo is a commercially important blue pigment that is used to dye both cotton and wool, while indirubin is currently used as a drug to treat chronic granulocytic leukemia and is a potential drug against cancer and Alzheimer's disease as well. In this work we analyzed the potential use of flaving-containing monooxygenases (FMO) from *Roseovarius* sp. and/or *Methylocella silvestris* for indigo and indirubin production in *Escherichia coli*. The FMO coding sequences were *E. coli* codon optimized and synthesized having a C-terminal his-tag coding sequence. Overexpression of FMOs in *E. coli* produced a large amount of the recombinant proteins. The analysis of the purified proteins showed that his-tagged FMOs are fully active. However, production of indigo and indirubin was only observed under specific culture conditions. Optimization of the upstream conditions (culture media composition, proteins overexpression time, addition of tryptophan, temperature of growth, etc) allowed the production of a large amount of indigo and indirubin in *E. coli*. The potential use of these enzymes for the commercial production of indigo, indirubin and other indole derivatives of biotechnological interest will be discussed.

BT-P06
RECOVERY OF TOXIC PROTEIN FROM INCLUSION BODIES

Hails G, Lacava F, *All JD, Cerchi MN, Anselmi PA, Castelli ME Peirú S, Menzella HG, Aguirre A.*
Laboratorio de Fermentaciones, IPROBYQ-CONICET, Universidad Nacional de Rosario, Rosario, Santa Fe, Argentina.
E-mail: hails@iprobyq-conicet.gob.ar

In the last decades, the world demand for oils to be used as food and for the production of fuels has been in constant increase. In order to produce edible oils, crude oils need to be refined in a process that involves several steps. The first of these steps is phospholipid removal or oil degumming. Several methods such as water degumming and acid degumming have been used for this purpose. More recently, enzymatic degumming has been implemented showing several advantages over traditional methods, including the reduction of gum volume and an extra-yield of the oil produced. For enzymatic degumming, phospholipases are employed. The most commonly used are phospholipases C (PLC) and phospholipases A (PLA). Also, glycerophospholipid:cholesterol acyltranferase (GCAT) enzymes were described to be suitable for this purpose. GCAT enzymes attack acyl groups from phospholipids just like PLA enzymes, but they transfer the acyl group to a free sterol instead of water, reducing the amount of free fatty acids in treated oil. Our group has recently developed an enzymatic oil degumming process using a GCAT from *Aeromonas enteropelogenes* (GCATae). We have demonstrated that this enzyme remains active even in the harsh conditions such as the currently used in industry and that it hydrolyzes all the phospholipids present both in crude oils and water degummed oils. However, the development of a manufacturing process for the efficient production of this enzyme was not simple. GCATae has shown to be toxic to microbial hosts like *Escherichia coli* and *Pichia pastoris* since it recognizes phospholipids present in the host membrane, leading to cell lysis and foam production. As a consequence, the heterologous production of soluble GCATae in a fed-batch fermentation process is poor and makes its application non-viable. In order to increase the amount of GCAT produced, we used *E. coli* thioredoxin (TRX) as a fusion partner. This strategy has been widely used to increase the expression level and the solubility of heterologous proteins synthesized in the *E. coli* cytoplasm. Surprisingly, the TRX-GCATae fusion protein constructed was expressed as inclusion bodies (IB). This partially folded and inactive form of the protein was not toxic and allowed the host cells to produce up to 17 g of IB per liter of culture. Refolded fusion protein TRX-GCATae showed good activity and was suitable for enzymatic degumming. After several optimizations of the IB refolding protocol, GCAT activity per liter of culture obtained was 2.3 folds higher than the activity reached in the soluble GCATae expression system. Such an increase turns TRX-GCATae a cost-effective enzyme for its use in industrial enzymatic degumming.

BT-P07
SIMPLE AND COST-EFFECTIVE PROTOCOL FOR BACTERIAL DNA EXTRACTION FROM STOOL SAMPLES, SUITABLE FOR PCR AND qPCR

Ousset MJ, Venturino A, Jaureguiberry MS.
Centro de Investigación en Toxicología Ambiental y Agrobiotecnología del Comahue (CITAAC) CONICET-UNCo.
E-mail: juousset@gmail.com

In recent years we have witnessed a paradigm shift regarding the microbiome and its role in health and disease. Our distal gastrointestinal tract hosts for over a trillion bacteria of several species and dysbiosis (imbalance of microbial community composition and functionality) has been shown to underlie a wide range of diseases, including not only gastroenterological issues, but also immune, respiratory, metabolic, hepatic, cardiovascular, and even neurologic disorders such autism, Parkinson's disease, anxiety and depression. Thus, microbiome characterization studies have become a topic of great interest in current research, many of which require bacterial DNA extraction from stool samples. Because of the highly complex composition of feces, consistent extraction of high-quality DNA from fecal samples is challenging due to the presence of inhibitors (i.e., bile salts, lipids, urates, and complex polysaccharides) that might be co-extracted with DNA, and could affect downstream techniques such as PCR, dramatically reducing sensitivity and amplification efficiency. Several methods have been proposed in order to extract DNA from feces that are, in some cases, laborious, and combine multiple purification steps. DNA extraction commercial kits are a very popular choice. Although efficient and convenient, they are comparatively expensive and sometimes, like other methods, fail with samples from herbivorous species. They also include reagents of unknown composition (e.g., lysis buffer, inhibitors absorber), and silica gel membrane to further remove inhibitors from the DNA solution. Considering complex polysaccharides from the diet are major PCR inhibitors, we adapted and modified a CTAB-PVP based procedure for plant RNA extraction, and developed a cost-effective, simple workflow that renders quality, non-fragmented DNA suitable for PCR and RT-PCR from feces. Moreover, our method replaces expensive enzymes (for breaking down bacterial cell wall) such as lysozyme or proteinase-K with liquid nitrogen rupture, and the optional use of silica columns for further purification.

BT-P08
IS IT POSSIBLE TO USE A CELL-PENETRATING PEPTIDE AS A VECTOR TO THE INTRACELLULAR DELIVERY OF MOLECULES INTO THE OOCYTE?

Klinsky Lahoz OG, Wetten PA, Berberian MV, Michaut MA.
Instituto de Histología y Embriología de Mendoza (IHEM, UNCUYO-CONICET), Mendoza, Argentina. E-mail: omargklinsky@gmail.com

The oocyte possesses a cell membrane named oolema that, in addition to the zona pellucida, protects the cytosol from the extracellular space and is essential in the sperm-egg interaction during fertilization. Nevertheless, it can be bypassed by intracytoplasmic microinjection, a physical technique used to introduce sperm or a number of different molecules into the oocyte cytosol. However, it is an invasive process because it implies the penetration of the plasma membrane and the zona pellucida. Cell-penetrating peptides (CPPs) have been introduced as novel biocarriers since they are able to translocate the cellular membranes without damaging it. These CPPs are small molecules composed of positive-charged amino acids that can be attached to fluorophores, proteins or nanoparticles. CPPs are used as carriers or vectors to introduce these molecules into the cell. The mechanisms by which these permeable peptides manage to enter the cell depend on the concentration of CPP and the incubated cellular type. However, the capacity of the oocyte to allow or deny the entrance of CPPs into the cytoplasm remains unknown. Therefore, the aim of this work was to study if a CPP is capable of penetrating the oocyte oolema, in order to determine if it constitutes an alternative of intracytoplasmic microinjection for the intracellular delivery of different molecules. Thus, CF-1 mouse oocytes of two maturation stages, immature (Germinal Vesicle, GV) and mature (Metaphase II; MII) oocytes were incubated in medium with increasing concentrations of an arginine-rich CPP attached to a fluorophore. The incubation was carried out at different times and different temperatures (4°C and 37°C). The cells were analyzed with confocal microscopy and the fluorescence intensity was used to graph concentration and time curves. Apparently, the MII oocytes incorporated CPP in a concentration and time-independent manner, at 4°C and 37°C. On the other hand, the penetration of peptide into GV oocytes was concentration and time-dependent, only at 37°C. The comparison of GV and MII oocyte showed that the first one incorporated more CPP than the second one, in all concentrations used during the incubation. These results show that CPP penetrates the oolema in both maturation stages and that the penetration was higher in GV oocytes.

BT-P09
TOPICAL SYSTEM OF CONTROLLED RELEASE OF NANOPARTICLES LOADED WITH ANTITUMORAL DRUGS

Stagnoli AS[1], Garro C[2], Ertekin O[3], Niebylski A[4], Leal A[3], Soria G[2], Boccaccini, A[3], Correa NM[1].
[1]IDAS, UNCR-CONICET; [2]CIBICI, UNC-CONICET; [3] Institute of Biomaterials, FAU,[4]INBIAS, UNRC-CONICET.
E-mail: asstagnoli@gmail.com

Two of the main setbacks of many chemotherapeutical drugs are their widespread systemic distribution and limited permeability across the cell membrane. Indeed, to reach effective concentrations of the drugs at tumor sites, it is usually necessary to administer relatively high doses of drugs which ultimately lead to severe side effects. In this work, our goal was to develop a new system capable of stably encapsulate drugs that target tumor cells with controlled release of chemotherapeutic drugs. Moreover, we aimed to design a system that can be used on patients topically. Topical drug delivery eliminates the need for systemic drug administration and offers a less invasive alternative than conventional therapy. Nanocarriers could be used to improve solubility, cellular delivery, distribution *in vivo*, control release and decrease the toxicity of antitumoral drugs. In the present study, we used the biocompatible materials DOPC (D) and BHD-AOT (B) as nanocarriers capable of stably encapsulating antitumor drugs, such as Curcumin (C). C is a polyphenolic natural compound with well-known antitumoral, antioxidant and anti-inflammatory properties. However, the therapeutic efficacy of C is limited due to its poor aqueous solubility and its difficulty to cross the cell membrane. In this study, the *in vitro* biocompatibility of vesicles and the ability to encapsulate C of B (BC) and D (DC) was evaluated. The cellular interaction and antitumor activity of BC and DC were studied by nano-zetasizer and flow cytometry. Finally, to perform a local and controlled release of BC and DC we use a new type of hydrogel (oxidized alginate co-polymerized with gelatin) as a support matrix. This assay was carried out using rat skin by Franz diffusion cell and fluorescence spectroscopy. Our results showed that B and D are highly efficient to encapsulate C and interact with tumor cells. D was harmless in a wider range of concentrations compared to B (concentrations lower than 0.05 mg/mL). DC was more efficient than BC because it was able to deliver the high concentrations of C necessary to reach its antitumoral activity. Another important finding was that the incorporation of DC in tumor cells was greater ($\geq$ 70%) than in non-tumoral cell lines ($\leq$ 10%). The hydrogel incorporated and protected BC and DC efficiently, after lyophilization and in humid conditions both at low and physiological temperatures. Finally, after the controlled degradation of the hydrogel, both BC and DC vesicles crossed the stratum corneum without morphological alterations and without losing the encapsulated drug. Together, our results suggest that this type of vesicles can become ideal systems for the delivery of antitumor drugs in minimally invasive topical applications.

BT-P10
HUMAN RECOMBINANT GALECTIN 1 PRODUCED BY TRANSPLASTOMIC TOBACCO PLANTS

Vater CF[1], Stupirski JC[2], Bravo-Almonacid FF[1], Rabinovich GA[2], Pérez Sáez JM[2], Morgenfeld MM[1*]*
**These authors contributed equally to this work.*
[1]Laboratorio de Biotecnología Vegetal (INGEBI-CONICET), [2] Laboratorio de Inmunopatología y Oncoinmunología (IBYME-CONICET)

E-mail: mmorgen@dna.uba.ar

Transplastomic plants stand out from other molecular farming platforms because of the highly efficient production of recombinant proteins (>50% of the total soluble protein). In some cases, however, low and even undetectable levels of heterologous protein expression have been reported in this system. This fact makes it necessary to evaluate and study the expression system whenever a new protein emerges as a candidate to be produced using this platform. The aim of this work, therefore, was to assess the feasibility of producing transplastomic plants that express a human protein with immunomodulatory activity and potential therapeutic use. For this purpose, we expressed human Galectin 1 (hGal-1), a carbohydrate-binding protein with proven immunomodulatory and anti-inflammatory activities. This protein has broad therapeutic potential and the appropriate biochemical characteristics to be expressed in the plastid system. Initially, we cloned the *LGALS1* gene in a plastid transformation vector (pBSW5'UTR). This vector was used to transform *Nicotiana tabacum* leaves using biolistics. Transplastomic lines, obtained from three independent recombination events (verified by PCR), were characterized at the molecular level. In order to confirm the homoplasty of the lines, we performed a Southern blot. We corroborated the transgene transcriptional activity by Northern blot. The expression of hGal-1 in transplastomic plants was analyzed by Western blot and quantified by ELISA. In both cases, we used a specific anti-Gal1 polyclonal antibody for detection and recombinant hGal-1 expressed in *Escherichia coli* as the positive control. The standard protocol of protein extraction was adapted in order to increase the recovery of active Gal-1 by adding a reducing agent. This recombinant protein, accumulated in the soluble protein fraction of the transplastomic plants, was finally purified by affinity chromatography with a lactosyl-Sepharose column. In conclusion, homoplastic tobacco plants capable of producing hGal-1 were obtained. Human Galectin 1 produced by tobacco chloroplasts was electrophoretically indistinguishable from bacterial hGal-1. Purification by affinity chromatography demonstrated an intact carbohydrate recognition domain, suggesting preservation of the biochemical activity of the recombinant hGal-1. Further *in vitro* and *in vivo* experiments are currently in process in order to corroborate the biological activity of the recombinant protein. Given the therapeutic potential of this protein in the treatment of autoimmune and chronic inflammatory disorders, this new expression system may serve to produce endotoxin-free, hGal-1 for pre-clinical and clinical studies.

BT-P11
CONSTRUCTION OF A *SACCHAROMYCES CEREVISIAE* STRAIN FOR THE BIOREMEDIATION OF DAIRY INDUSTRY WASTE COUPLED WITH ETHANOL PRODUCTION

Zubak TA[1], Cassain SJ*[1], D'Alessio C[1,2].*
**These authors contributed equally to this work.*
[1]Departamento de Fisiología y Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, [2]Fundación Instituto Leloir-IIBBA-CONICET. E-mail: tomaszubak@hotmail.com

The dairy industry is an integral part of the food industry, being one of the largest, most important and dynamic agro-food complexes within the national economy. This industrial segment produces considerable amounts of liquid waste with a high content of organic matter. Whey is the remaining liquid from the precipitation and removal of casein from milk during the cheese manufacturing process. It is made up of several components, with lactose being the most abundant (4.5–5 % w/v). This sugar contributes to the high chemical oxygen demand and the biochemical oxygen demand of whey. Bioethanol may be obtained from various compounds by microorganisms as yeasts, which have the ability to ferment a wide variety of sugars to alcohol. Yeasts are used in industrial plants because of their great fermentation yield, ethanol tolerance, productivity and their efficient growth in simple and economical media. Thus, the use of whey for the production of ethanol from the fermentation of lactose would be a beneficial process due to the reuse and bioremediation of this highly polluting by-product. By integrating the genes LAC4 and LAC12 from *Kluyveromyces marxinau*s to the genome of the *Saccharomyces cerevisiae* laboratory strain BY4742, we have developed several transgenic strains capable of using lactose as a sole carbon source. Using a spectroscopy technique, we determined the ability of these strains to produce bioethanol both from sucrose and lactose and compared it to a wild type *S. cerevisiae* strain. Our results show that while wild type *S. cerevisiae* strain is not capable of growing even in rich mediums if they contain lactose as the sole carbon source, the engineered strains are able to efficiently catabolize lactose into bioethanol in anaerobic conditions. Two of the strains obtained, BY4742-11F and BY4742-51, were able to produce a final concentration of 1.1% and 0.74% ethanol from a 2% lactose rich medium, values close to the maximum theoretical yield. These promising results justify further studies leading to an optimization in the production of bioethanol from this food processing waste using the strains obtained.

BT-P12
GLYPHOSATE REMOVAL BY RIPARIAN VEGETABLE SPECIES AND ISOLATION OF ASSOCIATED BACTERIA

Giaccio G[1], Aparicio V[2,3], Estévez C[4,5], De Gerónimo E [2,3], Corral R [1,3], Dávila Costa JS [4], Álvarez A[4,5]
[1]INTA-Barrow, [2]INTA-Balcarce, [3]CONICET, [4]PROIMI-CONICET, [5]UNT. E-mail: alvanalia@gmail.com

Glyphosate (Gly) is the most used herbicide in Argentina. Consequently, a higher occurrence of Gly and its major metabolite (AMPA) in different environmental compartments are currently found. Plants growing in such environments can reduce pollutant loads. Contaminated soils and vegetation represent a source of potentially beneficial plant associated-bacteria that could be used within microbial-assisted remediation strategies. The objectives of this study were (1) to isolate bacterial strains from Gly contaminated soil and rhizosphere of *Salix fragilis* (*Sf*) and *Festuca arundinacea* (*Fa*) spontaneously grown on soils contaminated, and (2) to evaluate Gly and AMPA content in soil and plant tissue of *Sf* and *Fa* grown in a greenhouse experiment. Samples of top soils contaminated with Gly and samples of *Sf* and *Fa* growing in the surroundings of the Claromecó stream (Tres Arroyos) were collected. For the bacterial isolation, one g of bulk soil (S) and rhizosphere soil (R) was suspended in a sterile solution. Soil suspensions were diluted and plated. After 7 days of incubation, distinct colony morphotypes isolated were screened according to use Gly (0.5 g L^{-1}) as only carbon source (minimal medium+Gly) and to use Gly as only P source (mineral salt medium+sodium glutamate+Gly).

For the greenhouse experiment, pots were filled with 2 kg of contaminated soil and one *Sf* cutting and 6 *Fa* seedlings were planted per pot, for 3 months. Four pot replicates were prepared for each treatment, including control pots. At the end of the experiment, plants were harvested, and soil samples were taken for Gly and AMPA analysis by UPLC-MS/MS. Sixty-nine different colonies morphotypes, 23 from S and 46 from R (26 from *Fa* and 20 from *Sf*) were isolated. Seventeen of the isolates were able to grow on Gly as source of P and 14 were able to grow using Gly as source of C. Five of different bacterial morphotypes were able to grow using Gly as source of P and C. In the greenhouse experiment, Gly and AMPA initial content in soils were 5512 ± 1369 μg kg^{-1} and 2353 ± 181 μg kg^{-1}, (respectively). At the end of the assay, Gly final content was 325 ± 23 μg kg^{-1} (*Sf*) and 25 ± 2 μg kg^{-1} (*Fa*) showing both a noticeable decrease in planted soils. AMPA final content was also decreased in *Fa* (822 ± 104 μg kg^{-1}) while for *Sf* AMPA was enhanced (3853 ± 207 μg kg^{-1}). Gly detected in plant biomass was 513 ± 97 μg kg^{-1} (*Sf*) and 164 ± 50 μg kg^{-1} (*Fa)*. AMPA content in plants was 2385 ± 726 μg kg^{-1} (*Sf*) and 575 ± 87 μg kg^{-1} (*Fa*). In control pots, differences in contaminant content were not significant during the assay. Since *Fa* treatment showed decreased values of Gly and AMPA both in plant and soil, and five of different bacterial morphotypes were able to grow using Gly as a source of P and C, bioassays combining both bacterial inoculant and *Fa* are currently in course. The microbial-plant system could be considered a promising tool for the phytoremediation of Gly and AMPA.

BT-P13
GOAT MILK CHEESE ENRICHED WITH *SMALLANTHUS SONCHIFOLIUS* (YACON) ATTENUATES REDOX STATUS IN AN ANIMAL MODEL OF OBESITY

Díaz Miranda EN[1], Fabersani E[2], Gomez Rojas JR[1], Sánchez SS[1], Grau A[3,4], Oliszewski R[2], Honoré SM[1].
[1]INSIBIO (CONICET-UNT). [2]Fac. de Agronomía y Zootecnia, UNT. [3]Fac. Cs. Naturales- Instituto Miguel Lillo, UNT. [4]IER (CONICET-UNT). E-mail: smhonore@gmail.com

Oxidative stress is a critical factor linking obesity with its associated complications such as diabetes, cardiovascular and hepatic dysfunctions. Excessive visceral fat increases oxidative stress in several organs leading to insulin resistance. Nowadays, the focus has been geared towards new functional foods to avoid the progression of metabolic complications. Cheese provides a valuable option as a food vehicle for prebiotic delivery. Also, phenolic compounds have been proposed as nutritional ingredients to improve the functional properties of milk and dairy products. This work investigated the effects of the addition *Smallanthus sonchifolius* (yacon) roots, a natural source of fructooligosaccharides (FOS) and phenolic compounds, to goat milk cheese on the antioxidant properties *in vitro* and *in vivo*. Cheese was elaborated from goat milk and *Lactobacillus bulgaricus, Streptococcus termophillus* (Chr. Hansen, Denmark) as starters. Yacon flour was added in a concentration of 20% (w/v). The centesimal composition of the product was determined. Wistar male rats (n = 30) were fed a standard diet (CD) or high-fat diet (HFD) for 12 weeks. Then HFD divided into four groups: HFD; HFD plus goat cheese (HFD-GC); HFD plus yacon flour (HFD-Y); HFD plus goat cheese + yacon (HFD-GCY). After 8 weeks of treatment, anthropometric, feeding, biochemical and oxidative stress parameters were measured. The formulation containing yacon had higher nutritional values (fats 21.6%, proteins 16%, and carbohydrates 18.54%), increased prebiotic FOS (4.55%), fibers (1.8%), and total phenolic content. The product had acceptable sensory attributes, high count (10^7 CFU/g) of viable probiotic microorganisms and high antioxidant activity determined as DPPH-free-radical scavenging activity ($p < 0.05$). Regular ingestion of the GCY provided substantial protection against oxidative stress, increasing serum levels of reduced glutation in HFD-fed rats. Moreover, GCY consumption increased superoxide dismutase, catalase and glutathione peroxidase antioxidant activities in the liver. Non change in body weight gain, fat pads weight or lipid profile was observed after supplementation with GCY to a HFD-animals ($p > 0.05$), however a tendency to improve has been observed. In addition, an improvement in fasting glucose levels and insulin sensitivity was detected ($p < 0.05$). Our results showed conclusive evidence indicating that GCY is an excellent functional food that avoids the oxidative impact of high fat feeding. Overall, yacon flour showed good potential as an antioxidant supplement for dairy products.

BT-P14
METABOLIC EFFECTS OF GOAT MILK YOGURT SUPPLEMENTED WITH *SMALLANTHUS SONCHIFOLIUS* (YACON) ROOT FLOUR IN RATS ON A HIGH-FAT DIET

Grande MV[1], Fabersani E[2], Coll Aráoz MV[3,4], Zannier ML[4], Sánchez SS[1], Grau A[4,5], Oliszewski R[2], Honoré SM[1].
[1]INSIBIO (CONICET-UNT). [2]Fac. de Agronomía y Zootecnia, UNT. [3] PROIMI (CONICET). [4] Fac. Ciencias Naturales e Instituto Miguel Lillo, UNT. [5]IER (CONICET-UNT). E-mail: rubenoliszewski311@yahoo.com

Overweight and obesity have increased dramatically in the world during recent decades, reaching epidemic levels. Currently, functional foods represent one of the most intensively investigated and widely promoted areas in the food and nutrition sciences in order to collaborate in obesity management. Yogurt is the most popular of fermented milk, rich in calcium and milk proteins with higher biological value and is an excellent delivery vehicle for functional ingredients such as fructooligosaccharides (FOS). *Smallanthus sonchifolius* (yacon) roots are considered the best natural source of FOS, and their consumption is associated with several health benefits. This study aimed to evaluate the effects of the addition of yacon flour on the quality parameters of goat milk yogurt and investigate the metabolic effects of its regular consumption on a high-fat diet-fed Wistar rats. Yogurt was elaborated from goat milk and *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* as starters and analyzed microbiologically weekly for 30 days (shelf life of commercial yogurts). Yacon flour was added at a concentration of 7% (w/v). The centesimal composition of the product was determined. For the experimental model Wistar male rats (n = 30) were divided into five groups (n = 6 animals per group) receiving the specified diet: standard diet (Control), high-fat diet (HFD), high-fat diet plus yogurt (HFD-yogurt), high-fat diet plus yacon flour (HFD-yacon), high-fat diet plus yogurt + yacon (HFD-yogurt + yacon), for 30 days. The formulation containing yacon flour had higher nutritional values (fats 4%, proteins 4% and carbohydrates 6.74%) and improved sugar profile (reduced lactose 0.94% and increased prebiotic FOS 4.55%) content. The final product had acceptable sensory attributes and a higher count (10^7 CFU/g) of viable probiotic microorganisms, with a shelf life of at least 30 days. Supplementation of goat yogurt + yacon to an HFD resulted in a marked attenuation of weight gain and a decrease in visceral fat pad weight ($p < 0.05$). Moreover, goat yogurt + yacon restored serum lipid profile, reduced fasting glucose

levels, HOMA-IR, and atherogenic indices of rats, improving the effects of goat yogurt or yacon flour alone ($p < 0.05$). Our results showed conclusive evidence indicating that goat yogurt + yacon is an excellent functional food that avoids the metabolic impact of high-fat feeding, representing a novel food product for the management of obesity.

BT-P15
EVALUATION OF ENZYMATIC ACTIVITIES IN LINDANE-CONTAMINATED SOILS DURING THEIR RESTORATION BY BIOREMEDIATION TECHNIQUES

Raimondo EE[1,2], Costa Gutierrez [SB1], Sineli PE[1], Fuentes M S[1], Benimeli CS[1,3]
1PROIMI-CONICET. 2Fac. de Bioquímica, Química y Farmacia. UNT. 3Fac. de Ciencias Exactas y Naturales. UNCA.
E-mail: enzo_er_25@hotmail.com

Lindane is an organochlorine pesticide that, due to its persistence in the environment, is still detected in different matrices. Bioremediation using actinobacteria consortia and agriculture residues proved to be successful for the restoration of lindane-contaminated soils. Furthermore, soil enzymatic activities, including oxidoreductases and hydrolases, are used as sensitive indicators to evaluate the soil quality, due to their participation in a range of biochemical reactions that take place in the environment. The aim of this work was to select soil enzymatic activities in order to be used as indicators of efficiency during the bioremediation of lindane-contaminated soils. Bioremediation tests were carried out in microcosms formulated with different soil types, contaminated with 2 mg kg^{-1} of lindane, bioaugmented with 2 g kg^{-1} of an actinobacteria consortium, and biostimulated with sugarcane bagasse or filter cake in the following soil:amendment proportions (100:0, 98:2, 90:10), under previously optimized conditions. The microcosms were incubated at 30°C for 14 days, and periodic samples were taken to determine residual lindane by gas chromatography and enzymatic activities using the traditional techniques reported in the literature with slight variations. All appropriated controls were performed. At the end of the assay, the pesticide removal percentages were different among the treatments and soil types, and the enzymatic activities were greater at day 14 than at day 0. In bioaugmented soils, the enzymatic activities were greater than in non-bioaugmented controls. In addition, biostimulation of bioaugmented and non-bioaugmented microcosms increased the values of these biological parameters. However, it was observed that lindane had an inhibitory effect on dehydrogenase, fluorescein diacetate hydrolysis, acid and alkaline phosphatases activities, while catalase was stimulated by the pesticide. Urease was slightly inhibited or not affected by the presence of the pesticide, depending on the evaluated condition. Based on their sensitivity, catalase, fluorescein diacetate hydrolysis, and acid phosphatase were selected as appropriate indicators to assess the effectiveness of the bioremediation process in subsequent studies. The obtained results demonstrated that the simultaneous use of the actinobacteria consortium and the agro-industrial residues was suitable for the treatment of soils of different textural classes contaminated with lindane, which led to an increase in the enzymatic activities values, with a consequent improvement in the quality of bioremediated soils.

BT-P16
IRON AS A MULTIFUNCTIONAL FACTOR IN *ASPERGILLUS NIGER* MYA 135: FUNGAL MORPHOLOGY, LIPASE PRODUCTION AND LIPASE ENHANCER

Salvatierra HN[1], Vázquez SC[1], Baigori MD[2], Pera LM[2]
NANOBIOTEC UBA-Conicet[1]; PROIMI-Conicet[2] E-mail: liciapera@gmail.com

Filamentous fungi have been broadly used in biotechnological processes as cell factories due to their metabolic versatility. They are able to secrete high levels of enzymes, antibiotics, vitamins, polysaccharides, and organic acids. However, one particular obstacle with these kinds of microorganisms focuses on their morphological form. They can show linear filaments to highly branched structures, and in submerged culture, growth morphologies varying from compact pellets to dispersed mycelia. In turn, several fungal processes can be directly or indirectly affected. Those growth morphological patterns are generally induced by extracellular factors and accomplished by genetic and biochemical factors. In this connection, we previously reported that $FeCl_3$ decreases the mycelium-bound β-N-Acetyl-D-glucosaminidase activity (a relative marker of the wall lytic potential) from *Aspergillus niger* ATCC MYA 135 and yields a dispersed mycelium in its presence. Here, both the fungal morphology and the lipase activity obtained in the presence of an optimized culture medium supplemented with $FeCl_3$ were analyzed. The role of this salt as a lipase enhancer was assessed as well. Firstly, the extracellular lipase production was conducted in an orbital shaker at 30°C during 192 h by using a mineral medium supplemented with 1 g/L $FeCl_3$ and a final conidial concentration of about 10^5 conidia per mL. After 24 h of fermentation, 2 % (v/v) of olive oil was added as an inducer. Thus, the highest specific activity (15.51 ± 0.78 U/mg) was obtained at 96 h of cultivation. This activity value was 10-fold compared with its control without $FeCl_3$ supplementation. Secondly, a new fermentation of 96 h was conducted. The mycelium was examined by scanning electron microscopy displaying clumps structures with scarce ramified hyphae. The supernatant, collected by filtration, was also evaluated as a biocatalyst in hydrolytic and synthetic reactions as follows. The role of iron as a lipase enhancer was studied in native PAGE by using 1.3 mM of α-naphthyl acetate as a substrate. Released naphthol was bound with 1 mM Fast Blue to give a colored product. Preincubation of lipase bands during 30 min in the presence of 0.1 g/L $FeCl_3$ resulted in a significant increase of the activity signal. Additionally, the extracellular lipase activity was immobilized in silica gel by adsorption. The elemental analysis performed under SEM-EDX (Energy-dispersive X-ray spectroscopy) evidenced the presence of iron. This biocatalyst was assayed to produce biodiesel compounds in a solvent-free system using soybean oil and butanol (1:4) as substrates. After a three-stepwise addition of butanol, a biodiesel conversion of 93.36 % was reached. Therefore, it can be concluded that $FeCl_3$ acted by altering fungal morphology, increasing lipase production, and improving the performance of the enzymatic activity. This research was supported by the following funding sources: FONCYT (PICT 2015-2596) CONICET (P-UE 2016-0012) and UNT (PIUNT D606).

BT-P17
BIOAUGMENTATION OF A BIOMIXTURE WITH ACTINOBACTERIA FOR ATRAZINE REMOVAL

Ocante TAL[1], Bigliardo AL[1], Sineli PE[1], Saez JM[1,2], Benimeli CS[1,3]
[1]PROIMI-CONICET. Tucumán, [2]Fac. de Cs. Naturales e Instituto Miguel Lillo, UNT. Tucumán, [3]Fac. de Cs. Exactas y Naturales, UNCA. Catamarca, Argentina. E-mail: any.ocante@gmail.com

Biopurification systems (BPS) are simple and economical constructions designed to retain and degrade pesticides, in order to reduce or avoid point-source contamination. An organic biomixture (BM) constitutes the most abundant and important component of a BPS. The bioaugmentation of the BM with pesticide-degrading microorganisms is a very interesting approach to optimize its efficiency. Atrazine (ATZ) is a selective herbicide commonly applied to control the appearance of weeds in crops, mainly corn, sorghum, and sugarcane. The aims of this study were to select actinobacteria capable of removing ATZ and to evaluate the effect of the bioaugmentation of a BM with the selected actinobacteria on ATZ dissipation. A qualitative determination of tolerance to ATZ was performed by streaking 14 actinobacteria strains on Petri dishes containing starch casein agar medium with a central channel containing the ATZ solution (1000 mg L^{-1} and 50000 mg L^{-1}). The tolerant strains were used to perform a quantitative determination of their ability to grow in the presence of ATZ (50 mg L^{-1}) and to remove it from liquid minimal medium (MM). Controls were carried out in MM supplemented with glucose (1 g L^{-1}). Microbial growth (dry weight) and residual concentration of ATZ (gas chromatography, GC) were determined after 96 h. The actinobacterium which presented the highest removal of ATZ and did not show growth inhibition in the presence of ATZ was used to inoculate a BM composed of soil, straw, and agricultural sugarcane crop residue (25:25:50). Periodic samples were collected to determine residual ATZ, total heterotrophic microorganisms (CFU g^{-1} BM), fluorescein diacetate hydrolysis (FDA) and acid phosphatase (AP) activities. All actinobacteria were highly tolerant to 1000 mg L^{-1} of ATZ and moderately tolerant to 50000 mg L^{-1} of ATZ. All of them were able to grow in MM supplemented with ATZ, however, most of them reached statistically lower biomass than the obtained with glucose as a carbon source, with the exception of *Streptomyces* sp. A2, A11, and M7. These strains were able to use ATZ as carbon source and to remove it from MM, presenting removal values ranging between 10% and 75%. Based on the microbial growth and ATZ removal, *Streptomyces* sp. M7 was selected to bioaugment the BM. The inoculation of the BM with *Streptomyces* sp. M7 improved significantly the ATZ removal (58%) respect to the non-bioaugmented BM (38%) after 28 days of incubation. The total heterotrophic population in the bioaugmented and contaminated BM (4.55×10^7 CFU g^{-1}) did not present statistical variation respect to non-inoculated control, nor non-contaminated control. Enzymatic activities obtained in these systems ranged between 44.9 ± 0.03 and 87.2 ± 20.3 µg fluorescein g^{-1} h^{-1} for FDA and 91.82 ± 5.7 and 159.0 ± 9.0 µg p-nitrophenol g^{-1} h^{-1} for AP. These results suggest that the bioaugmentation of BM with *Streptomyces* sp. M7 represents a promising tool to reduce ATZ concentration in BPS.

BT-P18
EXPRESSION AND CHARACTERIZATION OF AN ASPARTIC PROTEASE FROM *SOLANUM TUBEROSUM* IN *KLUYVEROMYCES LACTIS*

Tito FR[1], Pepe A[2], Tonón CV[1], Daleo GR[1], Guevara MG[1]
[1] Instituto de Investigaciones Biológicas (IIB-UNMDP-CONICET), [2] Instituto de Investigaciones en Ciencia y Tecnología de Materiales
E-mail: ftito@mdp.edu.ar

In the last years, the gradual decrease of cow population, the increasing global demands for cheese, the ethical problems associated with the isolation of the animal rennet, and the incidence of bovine spongiform encephalopathy (BSE) have reduced both supply and demand for bovine rennet. Therefore, much research has been focused on discovering new natural milk-clotting enzymes from microorganisms and plants that would successfully replace the calf rennet in cheese manufacture. Previously, we have isolated and characterized an aspartic protease from *Solanum tuberosum* named *St*AP3. *St*AP3 is able to clot bovine milk and to degrade bovine casein subunits. In this work, we report the development of a new *St*AP3-derived produced in the generally regarded as safe (GRAS) yeast *Kluyveromyces lactis*. Using a stepwise optimization strategy – consisting of culture media screening, complemented with chromatography in a pepstatin A–agarose column step – we successfully improve *St*AP3 production in *K. lactis* (r*St*AP3). As *St*AP3, r*St*AP3 has caseinolytic and milk clotting activity with an optimum MCA value in accordance with those used in the industrial manufacture of cheeses (pH 6–6.2 and temperature 40–42 ºC). These results, in terms of milk-clotting activity, suggest the suitability of *rSt*AP3 to producing milk clots and the possibility of using these proteases in the artisanal and industrial cheese production.

BT-P19
HIGH CELL DENSITY *ESCHERICHIA COLI* COCULTURE SYSTEM FOR *DE NOVO* PRODUCTION OF MULTI-METHYL-BRANCHED ESTERS

Bracalente F, Sabatini M, Galván V, Arabolaza, A, Gramajo H.
IBR-CONICET. Facultad de Ciencias Bioquímicas y Farmacéuticas. UNR. E-mail: gramajo@ibr-conicet.gov.ar

Microbial lipid production represents a potential alternative feedstock for oleochemical industries. In our laboratory, we managed to turn *Escherichia coli* into a biofactory, by the heterologous expression of a polyketide synthase (PKS)-based biosynthetic pathway from *Mycobacterium tuberculosis* and redefinition of its biological role towards the production of a variety of multi-methyl-branched esters (MBE). The final step of this pathway involves the transesterification of a multi-methyl-branched fatty acid, synthetized by the PKS Mas, to an acceptor alcohol that was supplemented into the culture media. With the aim to develop a whole *de novo* bioprocess (i.e. without adding exogenous alcohol), an *E. coli* co-culture system was designed, where the MBE producing strain (RQ5 *pMB23*) is grown with another *E. coli* strain engineered to produce the branched alcohol isobutanol (CB1 *pIAA11*). Previous results indicated that the consortium CB1/RQ5, at intermediate inoculation ratios (~1:1) in batch cocultures of M9 medium, had a very closed productivity to the one exhibited by a monoculture based on the single-cell RQ5 *pMB23* with the external addition of isobutanol to the growth media. Based on this evidence, in this work, we decided to scale-up this coculture system for the *de novo* production of MBE of isobutanol, so we undertook the optimization of a high cell density fed-batch fermentation process. To set the

cultivation conditions, 1 L fed-batch fermentations (BIOFlo115 Bioreactor) were carried out using the consortium CB1/ RQ5. Variables to evaluate were: (1) inoculation ratio between the two cells; (2) time course progression of each constituent strain; (3) alcohol accumulation; and both (4) final cell density and (5) quantity of MBE produced per DCW. Interestingly, we found out that, due to different growth rate of each strain, co-cultures with dominant CB1 ratios (above 4:1) were necessary to reach amounts of MBE comparable to the fermentation of RQ5 *pMB23* monoculture. In conclusion, we demonstrate the feasibility and scability of the production of industrially relevant bio-esters by the coculture of two *E. coli* strain in a minimal medium.

CELL BIOLOGY

CB-P01
MODULATING TRANSCRIPTIONAL NOISE THROUGH GENETIC MODIFICATION OF EUKARYOTIC PROMOTERS

Iungman M, Schor IE.
IFIBYNE, UBA-CONICET; DFBMC, FCEN-UBA. E-mail: martin.iungman@gmail.com

The promoter regions of eukaryotic genes control both the average expression levels and their cell-to-cell variability, a quality known as intrinsic transcriptional noise. We are interested in understanding how the transcriptional noise affects other cellular processes, such as the coupling between transcription and RNA processing or the robustness of regulatory networks directing cellular processes like differentiation. Here we present the development of tools attempting to modulate transcriptional noise independently from transcript levels, the first step for our long-term aim. To measure intrinsic transcriptional noise, we use a dual fluorescent reporter containing the genes sfGFP under the control of the test promoter and mCherry under the regulation of a constitutive mammalian promoter. This plasmid was obtained from TIPR-cherry, a similar vector developed for usage in *Drosophila* cells, by replacing the fly constitutive actin promoter with the hPGK human promoter from the Tet-ONE vector (Clontech). For this purpose, we amplified by PCR a fragment containing the hPGK promoter from Tet-ONE and cloned it using Gibson cloning into a HindIII-KpnI fragment from TIPR-cherry. This vector, which we termed hTIPR-cherry, has an inducible promoter controlling sfGFP expression, with the MMTV minimal promoter downstream seven Tet-responsive elements (TRE). At this time, we are testing the transcriptional properties of this initial reporter through analytic flow cytometry, using the single-cell sfGFP/mCherry fluorescence ratio as readout. We have also engineered different variants of this promoter region in order to uncouple variations in transcriptional noise from those in average transcriptional strength. The hypothesis is that these two parameters are controlled by the number and affinity of transcription factor binding sites, which are expected to increase both, and the presence of nucleosome positioning elements, which have been reported to increase strength but not noise. In the first place, we are obtaining promoters with a different number of TREs, which we will subsequently combine with single nucleotide substitutions on these elements to modify their affinity. In parallel, we have obtained two different nucleosome positioning elements: a 20bp-long dA:dT stretch, which has been used as a nucleosome exclusion sequence in yeast, and a nucleosome positioning region already tested in human IFN-beta gene. We are currently performing overlap extension PCR to amplify these two parts of the test regulatory region and combine them, and Gibson cloning to introduce these new variants onto hTIPR-cherry. We expect that these synthetic promoters allow us to scan different combinations of transcriptional noise vs. strength, becoming useful tools for testing the separate effects of these two parameters of transcription.

CB-P02
INFLUENCE OF TARGET RNA TOPOLOGY ON miRNA STABILITY

Wightman FF[1], Lukin J[2], Giusti S[2], Refojo D[2], De la Mata M[1].
[1]Instituto de Fisiología, Biología Molecular y Neurociencias, UBA-CONICET, Argentina, [2]IBIOBA, CONICET, Argentina.
E-mail: fuchsf@fbmc.fcen.uba.ar

MicroRNAs function by fine-tuning gene expression throughout different tissues and cell types, conferring an important layer of post-transcriptional control. Consequently, regulation of miRNAs levels themselves is of crucial importance. Accordingly, the mechanisms of microRNA biogenesis and function have been studied extensively, while their degradation mechanisms remain less explored. Nevertheless, several cases of specific miRNAs that decay quickly under certain conditions such as viral infections or particular stages of the cell cycle, suggested that active miRNA degradation takes place. Indeed, in the past few years, endogenous instances of miRNA active degradation have been described. One of the pathways responsible for this phenomenon is known as TDMD, which stands for Target Directed MicroRNA Degradation. TDMD was first described in *Drosophila* and in mammalian cells, where it was shown that target RNAs can themselves trigger specific miRNA degradation. Unlike the canonical miRNA silencing pathway, TDMD is characterized by a different target-miRNA architecture. While target repression is achieved by a complementarity limited to the 5' seed region of the miRNA, a more extensive base pairing of a target RNA to both the 5' seed and 3' regions can lead to miRNA destabilization. In the past few years, circular RNA molecules (circRNAs) have attracted a lot of interest. CircRNAs are produced through backsplicing of pre-mRNAs by the spliceosome, they are highly stable molecules and tend to accumulate in the cytoplasm. Some circRNAs can be translated and more recently they have been associated with the innate immune response during viral infections, yet the functions for most circRNAs remain largely unknown. CircRNAs were initially proposed as "sponges" that can titrate and block miRNA function and since then a plethora of publications seems to support this phenomenon. Nevertheless, a systematic approach exploring which impact, if any, circRNAs have on miRNA stability is still lacking. To shed light on this matter, we are studying an endogenous instance of this kind of interaction, namely the miR-7/CDR1as/Cyrano network, where a circRNA (CDR1as) prevents degradation of a specific miRNA (miR-7). Furthermore, we

are developing tools capable of expressing artificial circRNAs in an exclusive manner, by selectively degrading the linear form "leaked" by the construct. We are currently studying the effects of the artificial circRNAs on their cognate miRNAs.

CB-P03
ACTIVATED ALPHA-2 MACROGLOBULIN INDUCES THE GLUT4 TRAFFIC TO CELL SURFACE THROUGH LRP1 IN CARDIOMYOCYTES

Actis Dato V, Chiabrando GA.
Universidad Nacional de Córdoba-Faculta de Ciencias Químicas-Departamento de Bioquímica Clínica (CIBICI-CONICET).
E-mail: vactisdato@fcq.unc.edu.ar

Diabetes mellitus (DM) is a highly-incidence chronic disease that affects global health. The major cause of death in diabetic patients is produced by cardiovascular complications, which include myocardial infarction. The heart requires an enormous amount of energy for its daily function and the insulin-induced glucose uptake represents approximately 30% of the energy source available for this tissue. In this way, insulin resistance has a marked influence on cardiac metabolism because the action of insulin in the myocardium is affected, the contribution of substrates is altered and there are drawbacks in the metabolic adaptations in this organ. In addition, activated α2-macroglobulin (α2M*) is involved in cardiac hypertrophy of diabetic patients. α2M* is a proteinase-inhibitor complex that is specifically recognized and internalized by endocytosis through low-density lipoprotein receptor-related protein 1 (LRP1), which is an endocytic and signaling receptor expressed in cardiomyocytes. This receptor is stored in vesicles and sorted to the plasma membrane (PM) by insulin and α2M* through a regulated exocytic route. These vesicles also contain glucose transporter type 4 (GLUT4), which is the main insulin-sensitive glucose transporter in the myocardium. In the present work, we investigate whether α2M* may promote the GLUT4 intracellular traffic to PM in cardiomyocytes mimicking the insulin function. The experiments were performed in cell cultures of mouse cardiomyocyte cell line, HL-1. Several cellular and molecular tools were applied in order to evaluate intracellular signaling activation and membrane protein trafficking in HL-1 cells stimulated for different times with α2M* (60 nM). Our results demonstrate that α2M* promoted a rapid and significant activation of the PI_3K/Akt pathway as well as increased expression of GLUT4 and LRP1 on the cell surface in HL-1 cells. This intracellular signaling activation and GLUT4 sorting to the PM was comparable to those induced by insulin (100 nM for 30 min). In conclusion, these results taken together may have pathophysiological implications on the extracellular regulation of glucose in cardiomyocytes.

CB-P04
EFFECT OF RESVERATROL ON RENAL EPITHELIUM DAMAGED BY OXALATE

Artuch A[1], Fernández Tome M[1,2], Casali CI[1,2]
[1]Cátedra de Biología Celular y Molecular, Facultad de Farmacia y Bioquímica, UBA. [2]IQUIFIB-UBA-CONICET.
E-mail: ayelenartuch@gmail.com

One of the kidneys functions consists of filtering the total circulating blood to remove metabolic waste and other toxic substances. For that reason, tubular epithelial cells, which constitute renal parenchyma, are constantly exposed to high concentrations of these substances that can be toxic for them. Such is the case of oxalate (Oxa), coming from diet or hepatic synthesis, which can generate calcium oxalate crystals in renal tubular lumen causing injuries in the epithelium and tubular structures, leading to the development of the chronic renal disease. Resveratrol (trans-3,4′,5-trihydroxystlbene, Rsv) is a polyphenolic compound whose main intake sources in humans are grapes, peanuts, and wine. It has numerous beneficial health effects due to its anti-oxidative and anti-inflammatory actions. One of the Rsv mechanisms is through the modulation of cyclooxygenase 2 (COX2) expression and activity. In our lab, we had demonstrated that COX2 is a survival gene in renal cells subjected to an abrupt osmolarity change. Thus, the aim of the present work is to assess the possible protective effect of this polyphenol on a model of renal tissue damage induced by oxalate in renal collecting duct derived cells and to evaluate the role of COX2 in that process. To do this, MDCK cells were subjected to high-NaCl media (512 mOsm/kg H_2O) for 72 h to obtain a differentiated epithelium, then were treated with different concentrations of Rsv (0.1, 1, 5, and 10 μM), and, 30 min later, with oxalate (1.5 μM). Two controls were used: treatment only with NaCl and treatment with NaCl and oxalate, both without Rsv. After treatment, cells were collected, counted, the morphology was analyzed by phase-contrast microscopy and the COX2 expression was evaluated by western blot. The treatment with oxalate to the differentiated epithelia induced cell damage with changes in cell morphology and decreased the cell number, compared to NaCl control. The treatment with Rsv decreased cell number and altered cell morphology (form and size) at 5 and 10 μM, compared to NaCl control. The concentration of 0.1 and 1 μM Rsv did not change the cell number and morphology compared to NaCl control. COX2 expression was decreased by oxalate respect to NaCl control and it was increased by Rsv in a concentration-dependent manner. According to these results, Rsv would have a protective effect at 0.1 and 1 μM concentrations, in contrast with Rsv at 5 and 10 μM concentrations, where the protective capacity is not evidenced. The maintenance of a differentiated epithelial morphology with Rsv 0.1 and 1 μM could explain the Rsv protective effect. However, Rsv 5 and 10 μM did not preserve the morphology and did not protect from oxalate damage. This fact infers the possibility that Rsv acts by different molecular mechanisms depending on its concentration.

CB-P05
FOLIC ACID SUPPLEMENTATION INDUCES GENE EXPRESSION CHANGES IN BOVINE OVIDUCT EPITHELIAL CELLS CULTURED *IN VITRO*

Mansilla MJ[1], García EV[1,2], Barrera AD[1,2]
[1]Inst. de Biología, FBQF-UNT. [2]INSIBIO, CONICET-UNT. Tucumán. Argentina. E-mail: abarrera@fbqf.unt.edu.ar

Folate is a well-known epi-nutrient that influences genomic methylation marks, which in turn, are crucial to orchestrate a tightly regulated pattern of gene expression through epigenetic mechanisms. In a previous study, we determined the presence and intra-oviductal concentration of folate, as well as the existence of a fine-tuned regulation of the gene expression of its receptors and transporters in bovine oviduct epithelial cells (BOECs). Considering this, we hypothesized that folate could have a functional impact on BOECs. The present study aimed to assess the effect of different concentrations of folic acid (FA, the synthetic form of folate) on cell health and gene expression levels in BOECs, by using a cell suspension culture system. BOEC explants from the ampulla and isthmus regions were separately cultured in TCM.199 medium with FA in either low (20 nM, usual FA content in culture medium, control group), physiological (1 µM, intra-oviductal concentration), elevated (10 µM) or supra-physiological (100 µM) concentration at 38.5°C, 5% CO_2, and 100% humidity, for 24 h. Measurement of cytotoxicity was performed by evaluating LDH activity in the culture medium. Moreover, treated BOECs were processed for gene expression analysis using RT-qPCR. The genes examined were linked to important cellular processes including folate transport (*FOLR1, FOLR2, FOLR3, SLC19A1, SLC46A1*), DNA methylation (*DNMT1, DNMT3A, DNMT3B*), cell-cell interaction (*CDH1*), antioxidant activity (*SOD2*) and signaling pathways (*TGFB1, MTOR)*. Cytotoxicity analysis showed a low LDH activity into the culture media of the experimental groups, indicating that FA did not affect epithelial cell integrity in the concentrations tested. Additionally, supplementation of culture medium with an oviductal-like concentration of FA (1 µM) induced only the increase of mRNA levels for *FOLR1* and *FOLR3* in the ampulla and isthmus explants, respectively, compared to the control group. In contrast, BOEC explants cultured in the presence of an elevated FA concentration (10 µM) showed increased mRNA expression levels of genes associated with folate transport (*FOLR1*, *FOLR2*, *FOLR3*, *SLC19A1*), DNA methylation (*DNMT1*, *DNMT3A*) and antioxidant activity (*SOD2*). Finally, it is worth noting that at a supra-physiological concentration of FA (100 µM), the transcriptional response in BOEC explants resulted in decreased mRNA levels for most analyzed genes with respect to the control group in a region-dependent manner. These data allow us to suggest that fluctuations in extracellular folate levels can promote changes at the molecular level in BOECs, providing new insights about the impact of maternal folate in the oviductal context.

CB-P06
ROL OF TRANSMEMBRANE DOMAIN GEOMETRY IN PROTEIN LOCALIZATION TO MEMBRANE SUB-DOMAINS *IN VIVO*

Bigliani G, Valdez Taubas J
[1]CIQUIBIC, CONICET y [2]Dpto. de Química Biológica Ranwel Caputto, FCQ, UNC, Córdoba, Argentina. E-mail: gbigliani@fcq.unc.edu.ar

The geometric features of protein transmembrane domains (TMDs), including the length of the TMD and the volume of the amino acids that constitute their exoplasmic halves, can determine traffic and localization of single spanning membrane proteins. Using transmembrane SNAREs as models, we previously showed that short TMDs with high-volume exoplasmic halves are retained in the Golgi, while short TMDs with low-volume exoplasmic halves are transported to the Plasma Membrane (PM). Proteins with long TMDs are transported to the PM regardless of the volume of their exoplasmic halves. Increasing the volume of the residues that constitute the exoplasmic hemi-TMD, however, results in a polarized distribution at the PM through endocytic cycling. An interesting possibility is that the length and volume of the exoplasmic hemi-TMD may induce clustering and/or segregation of proteins to membrane subdomains and in turn, this would determine traffic and localization. In the yeast *Saccharomyces cerevisiae,* coexisting subdomains have been observed at the plasma membrane. Also, stable micrometer-scale membrane domains are formed in the yeast vacuolar membrane in response to nutrient deprivation and other stresses. Here, we use a set of constructs bearing endogenous and chimeric TMDs of different geometry to analyze whether this is sufficient to confer a differential partition in membrane subdomains *in vivo*. To compare these proteins, which are normally localized to different organelles, it was necessary to express them in the same membrane. To this end, we changed their cytoplasmic (Sso1) domains, for that of the endosomal/vacuolar SNARE Pep12, which has a signal that could override the sorting information present in the TMDs. Long TMDs were effectively localized to the vacuolar membrane as expected. The chimeric proteins with short TMDs however, localize to the vacuole lumen because they are recognized by a quality control mechanism that involves the ubiquitin ligase Tul1 and the Bsd2 protein. The expression of the chimeras in a *bsd2*Δ*tul1*Δ mutant strain results in the localization of the proteins at the vacuolar membrane. Finally, we induce micro-domains formation in the vacuolar membrane. These domains were labeled by the liquid disordered (*Ld*) domain markers Vph1 and FM4-64. Preliminary evidence indicates that the volume of the exoplasmic halves in long TMDs do indeed drive to partition to different membrane subdomains *in vivo,* suggesting that this phenomenon may underlie their different trafficking behavior in cells.

CB-P07
GAMMA CARBONIC ANHIDRASE LIKE PROTEINS ARE PRESENT IN MITOCHONDRIAL COMPLEX I OF THE DIATOM *PHAEODACTYLUM TRICORNUTUM*

Cainzos M, Marchetti F, Pagnussat G, Zabaleta E

Instituto de Investigaciones Biológicas, Universidad Nacional de Mar del Plata, CONICET. E-mail: cainzos.unmdp@gmail.com

Diatoms are a major group of algae responsible for 20% of CO_2 global fixation. *Phaeodactylum tricornutum* is commonly used in molecular biology as a model organism for understanding the biology of diatoms. Diatoms are the result of a symbiosis between an ancestral heterotrophic organism (called the exosymbiont) with a red alga (known as the endosymbiont) from which has only the chloroplast left. Diatom mitochondria are then believed to derive from the exosymbiont, i.e., the "animal" part. However, these organisms seem to contain an ancestral OXPHOS (Mitochondrial Oxidative Phosphorylation System) different from that of Opisthokonta (animals and fungi). In support of this, genes encoding gamma type carbonic anhydrases that were shown to be intrinsic complex I subunits in plants and amoeba but not in animals and fungi were found in all other eukaryote lineages. Here, we show that the diatom complex I is a large complex containing gamma type carbonic anhydrase subunits, supporting an ancestral origin.

CB-P08
CHECKPOINT KINASE1 TRIGGERS CELL DEATH AND GENOMIC INSTABILITY BY INDEPENDENT PATHWAYS

Calzetta NL[1], González Besteiro[1], Marina A[1], Gottifredi V[1].
[1]Fundación Instituto Leloir (IIBBA-CONICET). E-mail: ncalzetta@leloir.org.ar

The DNA damage response (DDR) is a complex network of inter-dependent signaling pathways that are activated upon DNA insults to assist the completion and fidelity of DNA replication. Checkpoint Kinase 1 (Chk1) is a key mediator factor of the DDR whose inhibitors are undergoing clinical evaluation across a variety of cancers. As different lines of evidence indicate that Chk1 inhibition or loss increases replication stress, genomic instability, and cell death, it is currently accepted that such events happen in a lineal and interdependent fashion. The link between replicative stress and cell death has been unequivocally proven in Chk1-deficient cells, but the link between genomic instability and cell death has not been unambiguously established. Such a link is important because genomic instability can also contribute to the augmentation of intratumoral genetic heterogeneity, which implies the risk of developing resistance to the treatment. Thus, elucidating the mechanisms that trigger genomic instability and how genomic instability impacts on cell survival are of utmost importance to cancer research. In this respect, we show here that genomic instability in Chk1-deficient cells emerges from DNA damage in mitosis. In particular, we uncover an unprecedented mechanism of genomic instability that involves the cleavage of under-replicated by the structure-specific endonuclease Mus81-Eme1 during mitosis. In sharp contrast, we show that cell death results from altered replication dynamics and Mus81-Eme2-dependent cleavage of DNA in the S phase. Together these results demonstrate that, in the context of Chk1 inhibition/loss, cell death is not the result of genomic instability and is not ruled by the same molecular effectors. As a consequence, cancer treatment with Chk1i leads to the enrichment of a genomically unstable subpopulation that could be prevented by inhibiting the pathway identified by us. We suggest that Chk1-directed therapies could be improved by further exploring the specific signals of genomic instability discovered by us.

CB-P09
PLATELET RICH PLASMA (PRP) STIMULATES OSTEOBLAST PRECURSORS THROUGH AUTOPHAGY INDUCTION

Carminati S[1,2], Bertoldi MV[3], Fader CM[1,2].
[1] Universidad Nacional de Cuyo, Facultad de Odontología, Mendoza, Argentina, [2] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Instituto de Histología y Embriología (IHEM), Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina, [3] Universidad Nacional de Cuyo, Facultad de Ciencias Agrarias, Mendoza, Argentina.
E-mail: sacarminati@mendoza-onicet.gob.ar

Platelet-rich plasma (PRP) is a preparation containing a higher concentration of platelets, isolated from autologous blood, which contains numerous different growth factors and cytokines that activates several cellular signaling cascades. Some of these signalings are wound healing and osteogenesis promotion by stimulation of homeostatic responses to injury. Autophagy is an essential cellular homeostatic mechanism by which intracellular components are delivered into the lysosomes for degradation and recycling. Autophagy has been related to a diversity of pathological or physiological dentary processes such as bone remodeling, skeletal aging, osteoclastogenesis, osteoblastogenesis, and different types of oral cancer. Bone homeostasis is a tightly controlled mechanism in which osteoblasts (OB, the cells responsible for bone formation), osteoclasts (OC, the cells specialized for bone resorption) and osteocytes (the multifunctional mechanosensing cells embedded in the bone matrix), are the main actors in bone remodeling. Osteoblast and adipocytes originate from common mesenchymal stem cells (MSCs), and several transcription factors control the differentiation of the two lineages. It is known that 3T3-L1 cells, an immortalized preadipocyte cell line, are able to differentiate into bone-forming osteoblasts by transdifferentiation. This differentiation produces an increase of alkaline phosphatase (ALP) activity and expression of osteocalcin (OC), some known osteoblast factors. These data indicate that 3T3-L1 cells are a good model to study the molecular mechanisms of osteoblast function and differentiation. This report aimed to show whether PRP was able to induce autophagy in osteoblast precursors 3T3-L1 cells. Our results showed that PRP can increase the number of autophagic structures in 3T3-L1 and HeLa (cervical cancer cells) cells. Moreover, we have determined by Western blot a rise in the lipidated form of the autophagic protein LC3 (i.e. LC3-II) upon PRP treatment. Taken together, our results suggest that PRP is able to induce a strong autophagy response in osteoblast precursor and, to a lesser extent, in non-related osteoblastic cells, suggesting that PRP could be a potential therapeutic tool for some autophagy-related diseases associated with bone homeostasis.

CB-P10

MYO1C, MYO6 AND MYO18A ARE NECESSARY FOR *CHLAMYDIA TRACHOMATIS* DEVELOPMENT

Cuervo Bustamante ME[1], Del Balzo D[1,2], Damiani MT[1,2], Capmany A[1].
[1] Instituto de Instituto de Bioquímica y Biotecnología, Facultad de Ciencias Médicas, UNCUYO. [2] Laboratorio de Bioquímica e Inmunidad, IMBECU-CONICET. E-mail: anahicapmany@hotmail.com

Chlamydia trachomatis (CT) is an obligate intracellular bacterium and the most frequent bacterial agent of sexually transmitted infections. The latest research has placed CT as a risk factor of cellular transformation, which could lead to cervical or ovarian cancer development. This bacterium induces several alterations in the host cell such as inhibition of apoptosis and cytokinesis, a decrease in molecules involved in cell adhesion, and loss of front–rear polarity in migrating cells. The cytoskeleton and the associated proteins are the main factors that ensure polarized trafficking and a correct cell division. Particularly, myosins stand out, not only because of their role in actin cytoskeleton arrangement but also because of their implication in vesicular transport. By confocal microscopy, we observed that MYO1C is recruited to the chlamydial inclusion at 24 h post-infection. We recently published that MYO1C stabilizes actin at the Golgi apparatus facilitating the arrival of incoming transport carriers at this organelle. Strikingly, CT establishes a close relationship with the Golgi apparatus, receiving from this organelle a continuous supply of vesicles loaded with essential nutrients. Thus, CT could recruit this myosin as a strategy to ensure the arrival of post-Golgi vesicles. Interestingly, the knockdown of MYO1C impairs the CT development, assessed by flow cytometry and confocal microscopy. Moreover, this function could be shared with MYO6 and MYO18A that are also necessary for the normal chlamydial development and function of the Golgi apparatus. Our results suggest that MYO1C, MYO6, and MYO18A are manipulated by CT to ensure its development.

CB-P11
CHLAMYDIA TRACHOMATIS CAUSES THE INTRACELLULAR REDISTRIBUTION OF MHC-I AND IMPAIRS ANTIGEN PRESENTATION IN DENDRITIC CELLS

Del Balzo D[1]; Capmany A[1]; Cebrián I[2], Damiani MT[1].
[1]Laboratorio de Bioquímica e Inmunidad. Instituto de Bioquímica y Biotecnología. FCM-UNCuyo. IMBECU-CONICET. [2]IHEM-CONICET, FCEN- UNCUYO. Mendoza. E-mail: ddelbalzo@mendoza-conicet.gob.ar

Chlamydia trachomatis (CT) is the most frequent bacterial cause of sexually transmitted infections worldwide. This highly adapted intracellular bacterium has evolved multiple strategies to hide inside cells. However, little is known about the molecular mechanisms underlying CT evasion of the immune response. Dendritic cells (DCs) are the most efficient antigen-presenting cells of the immune system and an essential link between innate and adaptive immunity. Therefore, DCs could play a key role in CT´s clearance. In this study, we analyzed in CT-infected DCs the process of cross-presentation, in which exogenous antigens are associated with MHC-I molecules to activate CD8+ T lymphocytes. By confocal microscopy and flow cytometry-based approaches, we observed, after chlamydial infection, a decrease in MHC-I molecules exposed at the plasma membrane while they are redistributed intracellularly. However, the total amount of MHC-I molecules did not change after infection, as assessed by western blot analysis. Finally, we found that CT-infected DCs were less efficient than non-infected ones to cross-present the model antigen Ovalbumin, as measured colorimetrically by the activation of the antigen-specific CD8+ T cell hybrid called B3Z. Altogether, these findings indicate that CT infection impairs antigen-cross presentation in DCs through the disturbance of MHC-I transport.

CB-P12
NOVEL CAFFEINE ANALOGS AS POTENTIAL LEADERS ON THE CHOLINERGIC SYSTEM

Fabiani C[1], Biscussi B[2], Munafó JP[1], Salvador G[1], Corradi J[1], Murray AP[2], Antollini SS[1].
[1]INIBIBB, Dpto. Biol. Bioq. Farmacia; [2]INQUISUR, Dpto. Qca. Univ. Nac. del Sur – CONICET, Bahía Blanca, Argentina.
E-mail: cfabiani@inibibb-conicet.gob.ar

Cholinergic deficit is regarded as an important factor responsible for Alzheimer's disease symptoms. Two molecular targets for the treatment of this disease are acetylcholinesterase (AChE) and nicotinic receptor (nAChR). We previously demonstrated that caffeine has a dual effect on muscle and a7 nAChRs, behaving as a weak agonist at low concentrations and as a negative modulator at high concentrations. Furthermore, it is well-known that caffeine also acts as an inhibitor of AChE. The aim of this work was to synthesize more potent caffeine analogs with a dual effect on the cholinergic system by inhibiting AChE and potentiating nAChRs. With this objective, a theophylline fragment, resembling the caffeine chemical structure, was connected with a pyrrole fragment, which is present in the nicotinic chemical structure, through homologation from 3 to 6 carbon atoms (C_n). We first tested the capacity of the different compounds to inhibit the AChE. We found that whether theophylline alone inhibited the enzyme, pyrrolidine did not. With respect to C_n, they all can inhibit the AChE at concentrations of 100, 200, and 400 µM, having C6 the strongest effect. We then explored if theophylline, pyrrolidine, and C_n influence the nAChR conformational state. To this end, we used the AChR conformational-sensitive fluorescence probe crystal violet (CrV) and AChR-rich membranes from *Torpedo californica*. We found that whether pyrrolidine induced changes in the K_D values of CrV taking the nAChR to a state close to the desensitized one at concentrations of 200 and 400 µM, theophylline did not show a significant change in the K_D value. The combined analogs also produced changes in the K_D values of CrV. This effect was dependent on the length of homologation, being C5 and C6 the most potent analogs with effect at concentrations lower than 50 nM. To understand the molecular mechanism underlying the conformational changes of the nAChR, we expressed adult muscle nAChR in HEK293 cells and performed single-channel recordings with different C_n concentrations. We found that C5 activated muscle nAChR at very low concentrations (from 0.01 pM). At the highest tested concentration (30 µM), we observed a decrease in the mean open duration, which suggests that C5 also acts as an open channel blocker. As a partial conclusion, we can say that we have synthesized more potent caffeine analogs through the combination of caffeine and nicotinic structures. The effect of theophylline on AChE, the effect of pyrrole on AChR, and the effect of C_n on

both proteins suggest different pharmacophore profiles for these target molecules. Our results bring new information about the mechanism of modulation of pharmacologic targets for the design of new therapies for the intervention in neurological diseases.

CB-P13
DISSECTING THE INTRACELLULAR CHOLESTEROL TRANSPORT IN *TETRAHYMENA THERMOPHILA*

Gabrielli M, Costa J, Hernández J, Uttaro AD.
Instituto de Biología Molecular y Celular de Rosario, UNR-CONICET. E-mail: gabrielli@ibr-conicet.gov.ar

The ciliated protozoa *Tetrahymena thermophila* has not sterol requirements, but when available, they are incorporated and converted to 7,22-*bis* dehydrocholesterol, replacing the endogenous sterol surrogate, tetrahymanol, in the cell membranes. In this process, genes involved in sterol bioconversion, such as sterol C22 desaturase (*Des22*), are induced whereas tetrahymanol synthesis genes, like squalene synthase (*SqS*), are repressed. Although phagocytosis through the oral apparatus is the main route for sterol uptake, we have previously found that there is at least a second internalization pathway, and furthermore, some genes responded differently depending on the entry mechanism. However, the details of the intracellular traffic of sterols in *T. thermophila* and the associated signaling pathways involved in the regulation of gene expression have not yet been established. Using radiolabeled cholesterol and following its incorporation and esterification by thin-layer chromatography, we have previously observed that U18666A, an inhibitor of the export of sterols from lysosomes to the endoplasmic reticulum, impaired cholesterol esterification. This effect was not due to inhibition of ACAT activity since no differences in cholesteryl esters levels were detected in assays with cell-free extracts, therefore indicating that sterol transport to endoplasmic reticulum was indeed affected. In addition, the time-course analysis of gene expression upon treatment with U18666A revealed a delay in the cholesterol-induced upregulation of *Des22* and an absence of *SqS* repression. When cells were treated with the Golgi disrupting agent Brefeldin A neither cholesterol uptake nor its esterification were affected, but, interestingly, RT-qPCR data showed higher levels of *Des22* mRNA and a further downregulation of *SqS*. Together, these results suggest that cholesterol reaches the endoplasmic reticulum where it is modified and from where it triggers signaling pathways leading to changes in gene expression.

CB-P14
PALMITOYLATION OF THE CYSTM FAMILY OF PROTEINS IN YEAST

Giolito ML , Bigliani G , Valdez Taubas J.
CIQUIBIC, CONICET y Dpto. de Química Biológica Ranwel Caputto, FCQ, UNC, Córdoba, Argentina. E-mail: lgiolito@fcq.unc.edu.ar

A superfamily of proteins called CYSTM proteins was identified using a bioinformatics approach and found to be widely distributed among eukaryotes. These proteins are in general small, ranging from 60 to 120 aminoacids. The family is characterized by the presence of a conserved motif at the C-terminal region, which is rich in cysteine and that has been annotated as a transmembrane helix. High-throughput studies suggest that members of this family are localized to the plasma membrane. Orthologues of these proteins are involved in resistance to pathogens and they might be involved in resistance to different kinds of stress, including that caused by heavy metals. However, no thorough experimental analysis of this family of proteins has been carried out. In yeast, the family comprises the genes YDL012C, YBR016W, YDR034W-B, YDR210W and the recently characterized Mnc1 (YBR056W-A) Manganese-chelating protein 1. We became interested in these proteins because the CYSTM module could be the substrate of palmitoylation and if so, might not be able to form a TMD as predicted. Moreover, YBR016W was suggested to be palmitoylated in a high-throughput study. Protein S-acylation, commonly known as palmitoylation, is a post-translational modification (PTM) that consists of the addition of long-chain lipids on cysteine residues. This modification plays some critical roles in the regulation of many cellular processes. Palmitoylation is mediated by a family of transmembrane palmitoyltransferases (PATs), which are defined by the presence of a conserved Asp-His-His-Cys (DHHC) catalytic domain. *Saccharomyces cerevisiae* has seven members of this family in its genome. YBR016W, YDR034W-B, YDR210W were fused to GFP and we confirmed that they are indeed localized to the plasma membrane, although in polarized fashion. This polarity is achieved by endocytic cycling since it is lost in the endocytosis mutant *sla1Δ* and the proteins are retained in inner structures in a recycling mutant *ric1Δ*. Acyl-biotin exchange (ABE) experiments indicate that these proteins are indeed palmitoylated. Expression of YBR016W in strains lacking each of the yeast PATs showed that the plasma membrane localization, and most of the fluorescence, is lost in the strain that lacks the PAT Akr1. This degradation was confirmed by Western blot. ABE-PEG indicates that although the protein is degraded, palmitoylation is not completely lost suggesting that other PAT must be modifying it. Finally, treatment with the palmitoylation inhibitor 2-bromopalmitate results in loss of fluorescence from the plasma membrane, suggesting that these proteins are indeed bound to the membrane via palmitates and that the CYSTM module is, in fact, a palmitoylation motif.

CB-P15

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

PLK 1 INHIBITION AS A THERAPEUTIC APPROACH TO TARGET BRCA1-DEFICIENT CANCER CELLS BY SYNTHETIC LETHALITY

*Pansa MF[*1] , Carbajosa S[*1], Guantay L[*1] , Paviolo N[2] , Castellaro A[3] , Andino D[4] , Nigra A[3] , García IA[1] , Gil G[3] , Bocco JL[1] , Gottifredi V[2] , Soria G[1].*
[1]CIBICI-CONICET. FCQ, UNC. [2] Fundación Instituto Leloir. [3] CIQUIBIC-CONICET. [4] CIDIE-CONICET. E-mail: lguantay@fcq.unc.edu.ar

BRCA-deficiencies are widespread drivers of human cancers that await the development of targeted therapies. In this work, we aimed to identify novel synthetic–lethal interactions with therapeutic potential using BRCA-deficient isogenic backgrounds. To reach this goal, we developed a phenotypic screening technology to simultaneously search for synthetic-lethal (SL) interactions in BRCA1 and BRCA2-deficient contexts. The screening of a kinase inhibitors library revealed that Polo-like Kinase 1 (PLK1) inhibition triggers strong SL-induction in BRCA1-deficient cells. We uncovered that BRCA1 down-regulation and PLK1 inhibition lead to aberrant mitotic phenotypes with altered centrosomal duplication and cytokinesis, which severely reduced the clonogenic potential of these cells. The penetrance of PLK1/BRCA1 SL-interaction was validated using several isogenic and non-isogenic cellular models, chimeric spheroids, and mice xenografts. Moreover, bioinformatics analysis revealed high-PLK1 expression in BRCA1-deficient tumors, a phenotype that was consistently recapitulated by inducing BRCA1 deficiency in multiple cell lines as well as in BRCA1-mutant cells. Collectively, we uncovered an unforeseen addiction of BRCA1-deficient cancer cells to PLK1 expression, which provides a new means to exploit the therapeutic potential of PLK1 inhibitors in clinical trials, by generating stratification schemes that consider this molecular trait in patients' cohorts.

CB-P16
THE QUINONE FORM OF RIFAMPICIN IMPROVES NEURON SURVIVAL BY SUPPRESSION OF α-SYNUCLEIN-INDUCED MICROGLIAL ACTIVATION

Acuña L[1,2] , Hamadat S[1] , Corbalán N[3] , González F[4] , Rocca J[1] , Sepúlveda J[1] , Chehín R[4] , Papy D[3], Michel P[1] , Raisman R[1].
[1]Institut du Cerveau et de la Moelle Epinière (ICM), Inserm U 1127, CNRS UMR 7225, Sorbonne Université, Hôpital de la Pitié-Salpêtrière. Paris France, [2]Instituto de Patología Experimental (IPE) CONICET-UNSa. Salta, Argentina, [3]Laboratoire Croissance Cellulaire, Réparation et Régénération Tissulaires, Université Paris Est Créteil, Université Paris Est, Créteil, France, [4]Instituto de Medicina Molecular y Celular Aplicada (IMMCA) CONICET/UNT and SIPROSA. Tucumán, Argentina. E-mail: leonardo.a@conicet.gov.ar

Microglial cells are the resident immune cells of the brain parenchyma. Sustained activation of microglia is known to play a role in the progression of neurodegenerative diseases such as Parkinson's disease (PD). It has been suggested that the modulation of microglial activation could prevent neuronal demise, and thus the progression of neurodegeneration. Based on clinical studies in the context of infectious diseases where Rifampicin seems to protect patients from neurodegeneration, we hypothesize that rifampicin quinone (RifQ), an autoxidation product of Rifampicin, is responsible for a neuroprotective effect by suppressing microglial activation induced by endogenous pro-inflammatory mediators, such as α-synuclein fibrils (αS_f). Primary microglial cells purified from post-natal day 1 C57BL/6J mouse pup brains were pre-treated or not with RifQ, then challenged with αS_f and incubated for 24 h. Conditioned media were collected to measure cytokine levels (TNF-α, IL-1b, IL-6) by ELISA assays. Adherent cells were either fixed for immunostaining procedures or lysed for western blot assays. The modulatory effect of drugs on cell proliferation was also followed by thymidine incorporation. Cortical neurons purified from C57BL/6J mouse embryos were challenged to microglial induced conditioned media. The viability was measured using CCK-8 and LDH release. RifQ readily reduced prototypical markers of inflammation induced by αS_f such as (i) Iba-1 expression, (ii) TNF-α and IL-6 release, (iii) morphological changes, and (iv) cell proliferation, by blocking PI3K/pAKT signaling pathway, leading to neuron survival. Globally, our results suggest that RifQ inhibits microglial activation induced by αS_f. We thus suggest that RifQ should be studied in depth to propose it as a novel treatment for neurodegenerative diseases such as PD.

CB-P17
INTRACELLULAR AGGREGATION OF A PDK1 SUBSTRATE BY BLOCKING THE CONFORMATIONAL SENSOR POCKET ON PDK1

Leroux AE[1] , Biondi RM[1,2]
[1] Instituto de Investigación en Biomedicina de Buenos Aires (IBioBA) - CONICET - Partner Institute of the Max Planck Society. [2] Medizinische Klinik 1, Universitätsklinikum Frankfurt. E-mail: aleroux@ibioba-mpsp-conicet.gov.ar

Conformational disorders are a group of pathologies that share aggregation of specific proteins as a common feature. They include the prion disease, Alzheimer's disease, diverse tauopathies, and Parkinson's disease. There is no successful disease-modifying treatment yet available for any of the conformational disorders, possibly because we lack a detailed molecular understanding of the mechanisms that cause them. The seeding hypothesis is considered to be the general mechanism explaining the aggregation. Nevertheless, even after 20 years of being accepted, aggregation by seeding remains vastly inefficient in cell-free assays and cannot explain the existence of "strains" of proteins that aggregate. The phosphoinositide-dependent protein kinase 1 (PDK1) is a master kinase that phosphorylates the activation loop site and is required for the activity of at least 23 other AGC kinases. Some of the substrates, like PKCs, are constitutively phosphorylated while others, like S6K and Akt/PKB, are dynamically phosphorylated by PDK1. Protein kinase C zeta (PKCzeta), and its brain-specific splice variant PKMzeta have important roles in late long-term potentiation and long-term memory. Additionally, PKCzeta/ PKMzeta aggregates are present in the neurofibrillary tangles that affect the brain function of Alzheimer's disease patients. We have previously described the mechanisms used by PDK1 to sense the conformation of its substrates during cell signaling. We now show through a variety of techniques that blocking the "conformational sensor" pocket on PDK1 can trigger the cellular aggregation of PKCzeta. Based on our results, we elaborated a novel hypothesis that can explain how proteins aggregate in conformational disorders and the existence of "strains". The main goal of the follow-up project is to identify specific conformational sensors that are responsible

for the cellular aggregation of Tau, alpha-synuclein, and p62/SQSTM1, all proteins that aggregate in conformational disorders. We envisage that the identification of the "conformational sensors" and the actual mechanisms involved in protein aggregation will enable the development of innovative drugs that target the original cause of major global diseases that have highly unmet medical needs.

CB-P18

PROTEOLYTIC FRAGMENTS OF GHRELIN N-TERMINUS SHOW DIFFERENTIAL OREXIGENIC EFFECTS THAN THE PARENTAL HORMONE

Lufrano D[1,2], Fittipaldi A[1], Fernández G[1], Castrogiovanni D[1], De Francesco PN[1], Valacco P[3], Trejo S[4], Perello M[1].
[1] IMBICE-CIC-CONICET-UNLP; [2] Fac. Cs. Exactas-UNLP; [3] CEQUIBIEM-UBA-CONICET; [4] YTEC-YPF-CONICET.
E-mail: dlufrano@biol.unlp.edu.ar

The stomach-derived hormone ghrelin was first reported as a growth hormone secretagogue and a "hunger" hormone that stimulates food intake and the preference for energy-dense foods. However, since its discovery in 1999, the number and the kind of activities reported for ghrelin has been increasing over time, including gut motility and gastric secretion/emptying, regulation of adiposity, blood glucose levels, bone metabolism, sleep, stress and hedonic feeding, enhancement of contractility and vasodilatation. Ghrelin is a peptide of 28 residues acylated with an octanoic acid at Ser3. The N-terminal sequence of ghrelin along with the octanoyl group is essential to act on the ghrelin receptor (GHSR1a) through which the hormone triggers its effects. As many peptide hormones undergo proteolytic processing as a regulatory mechanism–producing fragments that may differ not only in the magnitude of the effects but also the type of bioactivity they exert–here, we explored the hypothesis that circulating ghrelin can be cleaved in order to generate ghrelin-derived peptides with differential bioactivities. Initially, by *in vitro* digestion of ghrelin with human plasma followed by MALDI-TOF MS detection, we found that the bonds of ghrelin sequence extended from residue 11 to 16, are hydrolyzed by proteases present in plasma. Then, we also incubated ghrelin with a human hepatocarcinoma cell line (HepG2) or with the extracellular medium of a 48h-culture of these cells. The MALDI-TOF MS analysis of these digests showed the same "hot cleavage zone" in the ghrelin sequence previously found with plasma digestion samples. In addition, using a fluorescent analogue of ghrelin, extracellular medium as a source of proteases and different proteases inhibitors, we have been able to elucidate that HepG2 cells secrete, at least, two different proteases able to cleave ghrelin peptide bonds. In order to evaluate the impact of proteolysis on the orexigenic effects of ghrelin, we tested one of the ghrelin-derived fragments detected in MALDI-TOF MS analysis of *in vitro* digests (ghrelin(1-14)). Despite having the active core of the hormone, we found that ghrelin(1-14) failed to induce neuronal activation, assessed by the marker of neuronal activation c-Fos, nor to increase food intake in mice. Additionally, this ghrelin-derived peptide was unable to impair the orexigenic effect of full-length ghrelin in competition assays. Together, these data support the existence of a proteolytic extracellular mechanism that generates ghrelin-derived peptides with different bioactivity than full-length ghrelin. Moreover, the liver may be involved in this mechanism during the passage of ghrelin through the hepatic portal circulation.

CB-P19

EXTRACELLULAR VESICLE SECRETION: INVOLVEMENT OF THE SNARE VAMP7 AND THE GTPASE Rab39a

Lujan AL[1], Wojnacki J[2] Rabinovich GA[3], Galli T[2], Damiani MT[1].
[1]Lab. de Bioquímica e Inmunidad, IMBECU-CONICET. Inst. Bioquímica y Biotecnología, FCM- UNCUYO, Mendoza. [2]Membrane Traffic in Healthy & Diseased Brain, IPNP, INSERM, Paris. [3]Lab. de Inmunopatología, IBYME-CONICET, UBA, CABA.
E-mail: agustinleonardolujan@gmail.com

The secretion of extracellular vesicles (EVs) exerts fundamental roles in almost every tissue, in both physiological and pathological conditions. EVs emerge as important structures involved in cellular communication, particularly in cancer, autoimmunity, infectious diseases, and neurodegenerative disorders. These small membranous formations contain a vast number of different molecules like nucleic acids, lipids, proteins, and sugars. Hence, EVs could be useful as novel biomarkers and therapies for the treatment of numerous pathologies. Understanding the role played by proteins involved in EVs exocytosis is crucial to control their burden. Rab proteins are the master controllers of vesicular transport. Our laboratory has demonstrated that Rab39a co-localizes with CD63 at multivesicular bodies (MVBs) and regulates the transport of these vesicles. We have shown the role of VAMP7 protein in EVs fusion with the plasma membrane. In this collaborative study, we found that Rab39a co-localized with VAMP7 in small vesicles. Furthermore, these proteins modulated EVs secretion in HeLa cells. Interestingly, in VAMP7-knockout cells, the amount of EVs released to the extracellular medium significantly decreased; therefore, EVs cargoes, including molecules with important functions in the immune system, were retained within cells. We found that Galectin-1, a protein of the lectin family, is released through VAMP7-dependent exocytosis. Consequently, impairing the VAMP7 function might distort communication and signal transmission among cells, overall affecting immune response.

CB-P20

ANGIOTENSIN II, K AND CHEMOTHERAPEUTIC DRUGS INCREASE CELL RESISTANCE VIA ACSL4 AND ABCG2 IN ADRENOCORTICAL CARCINOMA

Rios Medrano M , Prada J , Maloberti P , Castillo AF , Podestá E , Orlando U.
INBIOMED UBA-CONICET, School of Medicine, UBA, Argentina. Email: mayra.riosmedrano@gmail.com

Acyl CoA synthetase 4 (ACSL4) is an enzyme that regulates steroidogenesis in 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 that use energy through ATP hydrolysis to translocate low-weight molecules. Whereas in physiological conditions these transporters are in charge of maintaining homeostasis in different tissues, for example through steroid efflux, their participation in pathological events is associated with drug resistance, for example through chemotherapeutic drug efflux. Our group has previously reported the involvement of ACSL4 in steroidogenesis and breast cancer chemotherapy resistance, partly mediated by the regulation of the ABCG2 transporter. In this context and given that adrenocortical cells generate both steroids and highly aggressive tumors such as adrenocortical carcinoma, we used NCI-H295R, a cell line developed from a human adrenocortical tumor, as a steroidogenic and carcinogenic model to study the participation of ACSL4 and ABCG2 in chemotherapeutic drug resistance. Short-term treatment with angiotensin II or K increased ACSL4 expression and ABCG2 mRNA expression and protein levels. This treatment increased fluorescent compound efflux from cells. Inhibition assays further proved that compound efflux was mediated by ABCG2. Moreover, the continuous chemotherapeutic treatment also rendered an increase in ACSL4 and ABCG2 expression and drug efflux from cells. In addition, both pre-treatment with angiotensin II or K followed by high-concentration chemotherapeutic treatment and continuously increasing chemotherapeutic treatment induced an increase in cell survival rates as compared to non-stimulated non-treated cells. Altogether, these results suggest the participation of ACSL4 and ABCG2 in the mechanisms underlying adrenocortical carcinoma cell resistance to chemotherapy and hint at ACSL4 as a potential therapeutic target in this type of tumor to inhibit steroid synthesis, resistance to treatment and tumor growth.

CB-P21
EXTRACELLULAR VESICLES OF PATHOGENICALLY DIFFERENT *GIARDIA* STRAINS REVEAL DIFFERENTIAL PROTEOMIC CONTENT

Moyano S[1] , Musso J[1] , Camerini S[2] , Casella M[2] , Touz MC[1] , Lalle M[2].
[1]INIMEC-CONICET-UNC, Córdoba, Argentina. [2]Istituto Superiore di Sanità, Rome, Italy. *Email: smoyano@immf.uncor.edu*

Extracellular vesicles (EVs) are small vesicles that operate as cargoes from cell to cell, carrying proteins and nucleic acids, and are involved in physiological and pathological processes. EVs are also emerging as a potential therapeutic tool for the treatment of infectious diseases. The anaerobic parasitic protozoan *Giardia lamblia* is the etiological agent of human giardiasis, a diarrheal disease with a worldwide distribution, higher prevalence in developing countries, and mainly affecting children. Human infection results in a wide range of clinical outcomes and is almost due to two *Giardia* genetic groups, namely assemblages A and B. Differences at a genomic level occur between and within assemblages, which account for variations in growth rate, infectivity, and pathogenicity. Since *Giardia* releases EVs, to elucidate if variation in EVs content can associate with differences in assemblage pathogenicity, we analyzed the protein content of EVs released by *G. lamblia* WB (assemblage A) and GS (assemblage B) isolates. EVs released by trophozoites in well-defined culture conditions were purified by differential ultracentrifugation (microvesicles and exosome fractions). EVs were morphologically and biochemically characterized by electron microscopy, dynamic light scattering (DLS), proteomic assays, and immunoblotting. Our results indicate that EVs with different sizes and shapes are produced by both *Giardia* isolates, although in different amounts. EVs proteome was characterized by high-resolution mass spectrometry, showing the presence of typical EVs markers as well as proteins unique to *Giardia.* Proteins exclusively associated with exosomes (endosomal origin) or microvesicles (plasma membrane origin) were also identified. Virulence factor candidates and assemblage-specific proteins were also present. Our results provide evidence that differences in the amount and content of EVs occur between assemblages A and B, pointing on their role in determining the difference in Giardia isolate pathogenicity and virulence.

CB-P22
TRANSCRIPTION ELONGATION RATE MODULATES RESPONSE TO DNA DAMAGE OF ALTERNATIVE EXONS WITH SHORT UPSTREAM INTRONS

Prego AF, Muñoz MJ, Schor IE.
DFBMC, FCEN-UBA, IFIBYNE, UBA-CONICET. E-mail: aprego@fbmc.fcen.uba.ar

DNA damage affects gene expression patterns by different mechanisms, one of these being the regulation of alternative splicing (AS). In previous reports, a pathway that modulates the AS patterns in human keratinocytes exposed to UV light was characterized, which includes the generation of pyrimidine dimers, the ATR kinase and the phosphorylation of the C-terminal domain of RNA polymerase II (RNAPII), an important mediator of the response. From an RNA-seq experiment assessing the effect of DNA damage and its repair on global AS regulation, we found that the response of the alternative exons to the damage is correlated with the length of the flanking introns. In particular, shorter upstream introns favor higher inclusion of alternative exons, which can be related to changes in the RNAPII elongation rate. We selected exons with short upstream introns from those that showed regulation by UV light on the RNA-seq and performed a validation of this effect by semi-quantitative RT-PCR, showing higher reproducibility for those cases where the UV-effect was reverted after damage repair. To corroborate whether DNA damage causes changes in the transcriptional kinetics for these genes, the elongation properties of RNAPII were analyzed in one of them using a biochemical approach. We also tested the influence of elongation changes on both the AS patterns of these endogenous genes and the UV-exerted regulation,

through the use of drugs that affect RNAPII elongation. This work proposes regulatory mechanisms within the response to genotoxic damage, through a combination of genomic analysis with experimental validation.

CB-P23
TEMPORAL CONTROL OF TUMOR GROWTH IN NOCTURNAL MAMMALS: IMPACT OF THE CIRCADIAN SYSTEM

Prucca CG, Wagner PM, Suarez T, Velázquez F, Caputto BL, Guido ME.
CIQUIBIC-CONICET, Departamento de Química Biológica Ranwel Caputto. FCQ-UNC, Córdoba, Argentina. E-mail: cprucca@fcq.unc.edu.ar

Perturbations of the circadian clock function have a profound impact on numerous cellular pathways and thereby likely to contribute to many of the hallmarks of cancer. It has been proposed that the misalignment between the endogenous circadian clock and external time correlates with increased cancer risk. However, little is known about the circadian clock function during tumorigenesis. Here we investigate the day/night differences in the growth rate of peripheral nervous system tumors generated after the injection of A530 cells isolated from NPcis ($Trp53^{+/-}$; $Nf1^{+/-}$) heterozygous mice. First, synchronized A530 cultures exhibited temporal fluctuations in PER1 protein and ROS (Reactive Oxygen Species) levels as well as in their susceptibility to Bortezomib chemotherapy (a well-known proteasome inhibitor). Secondly, A530 or B16 melanoma cells injected on C57BL/6 mice at the beginning of the day or the night showed differences in the tumor growth rate being higher when mice were injected at night as compared with those injected at the beginning of the day in animals maintained in a 12:12 LD regular cycle. This phenomenon was also observed in mice released to constant dark after LD synchronization and injected at the beginning of subjective day or night. Lastly, when we examined the role of the molecular clock activator *Bmal*1, a higher tumor growth rate was observed in mice injected with cells (A530 A5) in which *Bmal*1 expression was diminished by CRISPR/Cas9 compared with controls. Our observations strongly suggest an important circadian regulation on tumor growth, mainly dependent on the host state.

CB-P24
HUMAN PROGESTERONE AND GLUCOCORTICOID NUCLEAR RECEPTORS ARE CO-REPRESSED BY THE HOMEOBOX PROTEIN DUX4 IN HEK293 CELLS

Quintero J[1], Saad N[2], Harper SQ[2], Rosa AL[1].
[1]IRNASUS-CONICET, Facultad de Ciencias Químicas, Universidad Católica de Córdoba. Argentina. [2]Center for Gene Therapy, The Research Institute at Nationwide Children's Hospital, Columbus, OH 43205, USA. Email: jquintero@ucc.edu.ar

DUX4 is a transcription factor that regulates the expression of zygote activated genes in placental mammals. We have demonstrated that DUX4 is a toxic pro-apoptotic protein associated with the pathogenesis of facioscapulohumeral muscular dystrophy (FSHD), the second most common form of inherited myopathy in humans. We also showed that DUX4 co-repress the activity of the progesterone and glucocorticoid nuclear receptor (NR) in T47D and HepG2 (reconstituted system) cells. Here, we explored if DUX4 also co-regulates the activity of the glucocorticoid (GC) and progesterone (PR) nuclear receptors. The activity of DUX4 on these NRs was studied in a reconstituted system of cultured HEK293 cells, which do not express the GC or PR nuclear receptors. Cells were co-transfected with a plasmid expressing either GC or PR nuclear receptors and the reporter system MMTV-Luc/*Renilla*. The potential co-repressor activity of DUX4 was monitored using a plasmid expressing either wild-type or tagged versions of DUX4. The results of these studies indicate that DUX4 dramatically inhibits the transcriptional activity of the GC and PR nuclear receptors in HEK293 cells. Taken together, our data show that DUX4 is also a strong co-repressor of the GC and PR NRs in the reconstituted system of cultured HEK293 cells. Although DUX4 is mostly considered a transcriptional activator, our results show that this protein could indirectly modulate gene expression by repressing the activity of hormone NRs.

CB-P25
OXIDATIVE STRESS INDUCED BY ORGANOPHOPHORUS PESTICIDES DEPENDS ON MEMBRANE LIPID COMPOSITION AND DOMAIN ORGANIZATION.

Belver Gottau MB, Ousset MJ, Venturino A, Jaureguiberry MS.
Centro de Investigación de Toxicología Ambiental y Agrobiotecnología del Comahue (CITAAC) CONICET-UNCO.
E-mail: belen.belver.g@gmail.com

Chlorpyrifos (CP) is an organophosphorus pesticide (OP) widely used in the Alto Valle region (Rio Negro) since the fruit growing market is a major economic activity. Over the last decades, urban areas have overlapped with producing lands, leaving periurban inhabitants chronically exposed to environmental hazards. Although acetylcholinesterase (AChE) is the primary target of OPs, secondary targets such as oxidative stress enzymes become relevant at sub-lethal concentrations. Plasma membrane (PM) cholesterol (Chol) content and domain organization influence cell function. OPs have been suggested to compete with Chol for partition domains at the PM and to cause structure perturbations. We aimed to analyze the effect of PM lipid composition on the expression of enzymes involved in oxidative stress response, upon CP exposure *in vitro*. Our cell line model consists of three different PM lipid composition achieved by stable transfection of CHO-K1 cells with rat fatty acid desaturases. CHO-K1 cells transfected with stearoyl Co-A desaturase (SCD cells) show higher Chol content while cells co-transfected with fatty acid desaturases 1 and 2 (FADS cells), have lower Chol level at the PM due to higher polyunsaturated fatty acids content. First, we analyzed AChE expression by RT-PCR and found no constitutive expression compared to two housekeeping genes (beta-actin and GAPDH), validating this cell model as ideal for studying CP secondary targets. Then we analyzed catalase (CAT) and glutathione S-transferase (GST) expression after 1 µM or 500 µM CP chronic (24 h) exposure, relative to three housekeeping genes (beta-actin, GAPDH, and Eif3-i). Regarding GST, the expression level was not significantly

altered at 1 µM CP for either cell line. However, CHO-K1 and SCD showed a moderate increase at 500 µM CP, while expression in FADS cells raised over 2-fold after exposure to the highest concentration. Additionally, CAT results exhibited higher expression only at 500 µM CP both in SCD and FADS cells, whereas CHO-K1 CAT expression was not significantly altered by CP exposure. We also analyzed enzymatic activity by spectrophotometry (for CAT and GST) and found differential response upon CP exposure, depending on membrane lipid composition. These preliminary results suggest that lipid composition and domain organization at the PM affect not only CP entry into target cells but also their response to oxidative stress induced by this pesticide, probably through toxicant absorption kinetics.

CB-P26
CORTICAL GRANULES ANALYSIS IN POSTOVULATORY AGED OOCYTES

Wetten PA, Klinsky Lahoz OG, Michaut MA.
Instituto de Histología y Embriología de Mendoza (IHEM, UNCUYO-CONICET), Mendoza, Argentina. Email: paulawetten@gmail.com

Once oocytes are ovulated, there is a time window in which they are normally fertilized. After this period of time, those non-fertilized oocytes will decrease their quality leading to postovulatory aging. Postovulatory oocyte aging can occur at any age of a fertile female´s life and during oocyte manipulation in assisted fertilization treatments. Cortical granule exocytosis (CGE), also referred to as "cortical reaction", is a secretory process triggered by sperm-oocyte fusion during fertilization and is involved in blocking polyspermy. Although it is known that aging produces a decrease in fertilization rate and an increase in the polyspermy, there is no evidence about cortical granule density in postovulatory aged oocytes. Therefore, the aim of this research was to analyze cortical granules density in postovulatory aged oocytes. Two models of postovulatory aging were tested – *in vitro* and *in vivo* aged oocytes– and compared with a control condition. To obtain *in vitro* aged oocytes, mature oocytes were collected from hormonally stimulated female mice 16 h post hCG injection (control) and were *in vitro* incubated for 4 and 8 h. To obtain *in vivo* aged oocytes, mature oocytes were collected from hormonally stimulated female mice 20 and 24 h post hCG. Results showed that cortical granules density was decreased in both postovulatory aged oocytes when compared to control cells. The secretion of cortical granules was confirmed by staining and quantification of exudate dots. Next, because secretion of cortical granules is a calcium-dependent process, we determined calcium cytoplasmic level using Fura2-AM. We found that in both conditions of aging, the basal calcium level diminished compared to the control oocytes. Previous findings have reported that cortical granules are immersed in an actin network in the cortical region, so we explored actin localization in aged and control oocytes. Control oocytes presented cortical actin localization whereas aged oocytes, in both conditions, showed cortical and cytoplasmic actin localization. Altogether, our results suggest that postovulatory aging affects the physiology of cortical granules in mouse oocytes.

CB-P27
DEEP DESCRIPTION OF MITOCHONDRIAL GUIDE RNA REPERTOIRES IN THE SIX MAIN LINEAGES OF *TRYPANOSOMA CRUZI*

Rusman F, Tomasini N, Floridia Yapur N, Ragone PG, Acuña L, Diosque P.
Instituto de Patología Experimental, UNSA-CONICET, Argentina. E-mail: fannyrusman@gmail.com

The kinetoplast (mitochondrial) DNA of *Trypanosoma cruzi* consists of two types of molecules: maxicircles and minicircles. Maxicircles encode typical mitochondrial products (rRNA and respiratory chain subunits), although some of the protein-coding genes are encrypted. To generate functional mRNAs, it is required an intricate process of RNA editing that involves the insertion and elimination of uridine residues at specific messenger sites. Genetic information for editing is provided by guide RNAs (gRNAs) encoded by minicircles. Unfortunately, mRNA edition and gRNA repertoires are mostly unknown in *T. cruzi*. In the present work, we have inferred gRNAs on deep-sequenced minicircle hypervariable regions (mHVRs) and rebuilt edition cascades for nine *T. cruzi* strains belonging to the six main lineages (TcI-TcVI). A total of 6743029 gRNAs were detected. Inferred gRNAs were clustered according to sequence similarity to constitute gRNA classes. Results showed an extreme diversity of gRNA classes, highly frequent minicircles that presumably do not code gRNAs, and very divergent gRNA repertoires, among lineages and within some lineages. In addition, we observed variable gRNA class redundancy (different gRNA classes that edit the same mRNA region) among strains. It was also observed that some strains have upon four times more gRNA classes than the others. In addition, we failed to rebuild complete cascades for components of the respiratory complex I in several strains. Finally, we observed that gRNA classes from different strains may potentially edit mRNAs from other lineages in the same way as they edit their self mRNAs. This is a requisite for the suitability of biparental inheritance of minicircles in hybrids, a recently proposed hypothesis in *T. cruzi*. Different evolutionary mechanisms and biological processes are discussed in order to explain differences among strains and lineages.

CB-P28
ISOLATION AND CHARACTERIZATION OF EXTRACELLULAR VESICLES COMING FROM BREAST CANCER CELLS AND MACROPHAGES.

Rodriguez-Baili MC[1], Dhooge SL[1], Théry C[2], Gil GA[1].
[1]CIQUIBIC-CONICET.Química Biológica Ranwel Caputto, FCQ, UNC. [2]Institut Curie, France. E-mail: mrodriguez@fcq.unc.edu.ar

Extracellular vesicles (EVs) are lipid bilayer-enclosed extracellular structures secreted by almost all cell lines with the capacity to transfer their cargo, playing an important role in cellular communication, growth of microenvironment and tumor progression, generating substantial interest in the scientific community. Worldwide, breast cancer is the most frequently diagnosed malignant cancer and the main cause of mortality in women. It is known that tumor-associated macrophages (TAMs) promote tumor growth; however, it is unknown how this communication occurs, and we

proposed that extracellular vesicles coming from both cellular lines are involved. We performed two different isolation techniques of EVs, coming from breast cancer cells and macrophages cell lines. EVs were isolated by ultracentrifugation and ultrafiltration following ultracentrifugation; in both cases, five million cells were seeded per plate and cultured for 24 h, after that, complete medium was removed, and changed for fetal bovine serum (FBS) free medium for 24 h. Following the protocols of both methods, the medium was collected and intact EVs recovered and resuspended in PBS. For the EVs characterization, we revealed by Western blotting anti-tetraspanins antibodies, (proteins widely used in literature) and with other proteins of our interest. Moreover, we observed the absence of Golgi markers, assuming that there is no contamination with other vesicles. The presence of total proteins and DNA in EVs was detected using bicinchoninic acid assay (BCA assay) and qPCR, respectively. We also performed Nanotracker analysis (analysis of particles) to estimate the total amount of vesicles in each fraction. Isolation and characterization of EVs is the first step to analyze the content of the vesicles in our cells of interest for further functional assays that will allow us to determinate if they are involved in the communication between TAMs and tumoral cells, and if EVs are responsible for the endocrine resistance that some estrogen receptor-positive cancers acquire with endocrine treatments.

CB-P29
ROLE OF STARD7 IN MITOCHONDRIAL DYNAMICS

Rojas ML, Cruz Del Puerto MM, Miranda AL, Kourdova LT, Panzetta-Dutari G, Genti-Raimondi S.
Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI)-CONICET, Facultad de Ciencias Químicas, UNC, X5000HUA, Córdoba, Argentina. Email: mlrojas@fcq.unc.edu.ar

Mitochondria are dynamic organelles crucial for cell function and survival implicated in oxidative energy production. Mitochondrial lipids affect several important functions such as respiratory metabolism, membrane architecture, protein import, mitophagy, and mitochondrial dynamics. StarD7.I is a lipid transport protein isoform that has a mitochondrial targeting signal involved in phosphatidylcholine (PC) delivery to the mitochondria. Previous studies have shown that StarD7 knockdown induces alterations in mitochondria and endoplasmic reticulum morphology with a reduction in mitochondrial PC content, however, how different StarD7 levels affect the mitochondrial dynamics has been not explored yet. Here, we generated two HTR8/SVneo stable cell lines expressing the mitochondrial StarD7.I or the cytosolic StarD7.II isoforms. We demonstrated that StarD7.I overexpression promotes altered mitochondrial morphology with a significant increase in mitochondrial fragmentation, whereas no changes were observed in stable StarD7.II cells compared to control cells. These data were confirmed by immunofluorescence analysis in HTR8/SVneo wild type cells transiently transfected with the bicistronic p-Lenti-StarD7.I-IRES-EGFP or p-Lenti-StarD7.II-IRES-EGFP plasmids. Mitochondrial targeting photoactivatable (PA-GFP) protein assays indicated that mitochondria are able to yield fusions with higher motility in StarD7.I-overexpressing cells than those in control cells. Stable StarD7.I cells maintain the mitochondrial membrane potential and produce lower ROS generation than control cells. Additionally, an increase in the expression of Drp1 and Mfn2 proteins was established in StarD7.I cells. In contrast, the amount of Mfn1 was decreased and no changes were observed in PGC1α protein levels. Moreover, the overexpression of Drp1K38A, used to induce Drp1 loss-of-function, promotes mitochondrial network invariably to collapse into large perinuclear aggregates, inhibiting StarD7 overexpression-induced mitochondrial fragmentation. These results indicate that StarD7 overexpression-mediated mitochondrial fragmentation occurs in a fission-dependent manner via Drp1. Nocodazole treatment separates the aggregates showing how mutant Drp1 affects the mitochondria morphology. Finally, StarD7 silencing leads to mitochondrial fragmentation with a donut phenotype, without motility and an increase in ROS generation. In this condition, the Mfn2 level was decreased without modifications in Drp1, Mfn1, and PGC1α proteins. Altogether these findings indicate that alterations in StarD7.I expression produce significant changes in the proteins that control the mitochondrial morphology impacting mitochondrial dynamics. *Granted by FONCyT, SECyT-UNC.*

CB-P30
KLF6 IS REQUIRED FOR OXIDATIVE AND ONCOGENE-INDUCED CELLULAR SENESCENCE

Sabatino ME, Castellaro A, Racca A, Carbajosa Gonzalez S, Pansa MF, Soria G, Bocco JL.
Centro de Investigaciones en Bioquímica Clínica e Inmunología, CIBICI-CONICET, Facultad de Ciencias Químicas, UNC
E-mail: mesabatino@fcq.unc.edu.ar

The KLF6 protein is a transcription factor involved in the regulation of several cellular processes during the cell cycle. Regarding its role in tumorigenesis, KLF6 is reputed as a tumor suppressor since numerous reports demonstrate its frequent genomic loss or downregulation, implying a functional inactivation, in a broad range of human cancers. Previous work from our laboratory showed that the downregulation of KLF6 expression in normal fibroblasts led to cellular transformation, whereas its ectopic expression interfered with oncogenic transformation triggered by activated Ras, through a cell cycle arrest. We hypothesize that the growth suppressor activity of KLF6 may involve the induction of cellular senescence, thereby preventing the proliferation of cells at risk of neoplastic transformation. Here, we determined the association of KLF6 upregulation in two different cellular senescence scenarios. We also observed that KLF6 silencing bypassed either oxidative or oncogene-induced senescence. Moreover, KLF6 expression *per se* was capable to trigger cellular senescence in both normal and tumoral contexts. Results presented in this work provide insights into a potential mechanism by which KLF6 may play a suppressing role of uncontrolled or damaged cell proliferation.

CB-P31
MECHANISMS INVOLVED IN RENAL CELL ADAPTATION TO STRESS BY THALLIUM

Morel Gomez E, Verstraeten SV, Fernández MC.*
BCM; IQUIFIB (UBA-CONICET), School of Pharmacy and Biochemistry, UBA. E-mail: emorelgomez@ffyb.uba.ar

Thallium (Tl) is a non-essential toxic heavy metal that contaminates the environment (water, air, and soils). Human intoxication with Tl affects several organs and tissues through still poorly understood mechanisms, in which the kidney is the main target of Tl toxicity. Tl has two oxidation states, the monovalent [Tl(I)] and trivalent [Tl(III)] cations, the latter being a strong oxidant. We previously demonstrated that Tl decreased the number of proliferating renal epithelial (MDCK) cells, altered their lipid metabolism, and induced changes in cellular complexity and morphology. Other non-essential metals (cadmium, lead, and mercury) give way to trigger diverse cellular processes in response to stress, such as endoplasmic reticulum (ER) stress, autophagy, and paraptosis. Hence, the present study seeks to investigate if some of these molecular mechanisms are likely to mediate Tl effects on MDCK cells. Confluent cell cultures were incubated for 3, 6, 24, or 48 h in the absence or presence of Tl(I) or Tl(III) (100 μM). Expression levels of proteins involved in autophagy and ER stress were analyzed by Western blot. Tl(I) and Tl(III) increased XBP-1(s) expression at 6 and 24 h of cell exposure and increased those of IRE-1, ATF-6, beclin-1, and sequestrin-1 at 24 and 48 h of treatment. The involvement of MAPK expression in ER stress and paraptosis was next analyzed. Tl increased the p-JNK/JNK ratio without changing in the p-ERK/ERK ratio after 3 h of treatment. RT-PCR analysis indicated that both Tl(I) and Tl(III) increased prohibitin (a positive paraptosis modulator) expression after 24 h of treatment without affecting the expression of phosphatidylethanolamine-binding protein (a negative paraptosis modulator) expression. Phase-contrast and transmission electron microscopy evidenced cytoplasmic vacuolation compatible with paraptosis-like death. Tl also induced changes in LC3 intracellular distribution, as evidenced by fluorescence microscopy. To sum up, current results provide evidence that Tl might induce two cellular responses: in the early stages of exposure, cells activate ER stress and autophagy as adaptative and pro-survival responses. In advanced stages of exposure, severe ER stress leads to cytoplasm vacuolation and paraptosis. Considering that Tl impairs human health at different levels, including alterations in the kidneys, our findings contribute to further understand the biochemical mechanisms underlying Tl toxicity in humans. However, additional studies are required to investigate further the adaptation and survival mechanisms that are involved in Tl intoxication.

CB-P32
ROLE OF ACYL-COA SYNTHETASE 4 IN PROSTATE CANCER CELLS

Prada JG, Orlando UD, Solano AR, Podesta EJ, Maloberti PM, Castillo AF.
INBIOMED (UBA-CONICET), School of Medicine, UBA, Buenos Aires, Argentina. E-mail: jesicaprada@hotmail.com

Acyl-CoA synthetase 4 (ACSL4) is an enzyme taking part in arachidonic acid metabolism. The expression of ACSL4 has been shown to be associated with the aggressiveness of several types of cancer such as colon and hepatocellular carcinoma. ACSL4 is part of the mechanism responsible for the aggressiveness in breast and prostate cancer cells and is increased in malignant cells, particularly in castration-resistant prostate cancer (CRPC). It plays a key role in the regulation of cell growth by controlling upstream effectors of the mTOR pathway in breast cancer cells. In addition, ACSL4 participates in steroid synthesis through arachidonic acid release and induction of Steroidogenic Acute Regulatory protein (StAR). The aim of this work was to analyze the effect of ACSL4 inhibition in prostate cancer cells on steroidogenesis and signaling pathways regulated by this enzyme. Also, we characterized the effect of PRGL493 in prostate cells, the specific ACSL4 inhibitor recently developed in our lab. PC3 prostate cancer cells treated with PRGL493 produced a significant cell proliferation inhibition and migration measured by BrdU incorporation assay and wound healing assay. Moreover, the combination of sub-maximal doses of PGRL493 (5 μM) and docetaxel (1 nM), a chemotherapeutic drug, generated a synergistic effect on inhibiting cell proliferation. It is known that androgen receptor (AR) expression and ACSL4 levels are inversely correlated in human prostate tumors, being associated with a high ACSL4 expression with loss of steroid hormones sensitivity. After ACSL4 inhibition with PRGL493, we could detect an increase in AR mRNA levels in the PC3 cell line through real-time PCR. Aggressive prostate tumors are able to synthesize steroids, and even in low amounts are very important in tumor biology and its malignant behavior. ACSL4 inhibition in PC3 produced a decrease in steroid levels detected by radioimmunoassay. Also, a decrease in StAR protein expression, the key steroidogenesis protein involved in the limiting step of steroid production, was detected. Then, we evaluated the role of ACSL4 on mTOR pathway regulation in prostate cancer cells. We analyzed the expression of different proteins such as pS6, pGSK3αβ, pRICTOR and pAKT by Western Blot, finding a decrease in their expression after treatment with PRGL493. These results all together contribute to show the effect of ACSL4 and its new developed inhibitor on steroidogenesis, mTOR pathway, migration and proliferation in prostate cancer cells, supporting the relevance of targeting ACSL4 in prostate cancer treatments, especially in CRPC, aggressive hormone-independent tumor for which there is no fully effective treatment.

CB-P33
COMPOSITON AND DYNAMICS OF RIBOPROTEOME IN *SACCHAROMYCES CEREVISIAE* DURING QUIESCENCE AND TRANSLATIONAL RECOVERY

Solari C[1], Valacco MP[2], Moreno S[2], Portela P[1].
[1]Departamento de Química Biológica FCEN UBA IQUIBICE, [2]CEBIQUIEM-IQUIBICEN. E-mail: pportela@qb.fcen.uba.ar

Ribosomal heterogeneity describes the existence of different populations of ribosomes, which provides translational selectivity for mRNAs. Ribosomal heterogeneity is evident in ribosomopathies, in which specific-tissue diseases are associated with mutations in ribosomal proteins. To study ribosomal heterogeneity, we used label-free quantitative proteomics and characterized the relative abundance and composition of proteins

that belong to monosomal and polysomal ribosomes during the transition from quiescence to yeast cell proliferation. We identified 315 proteins belonging to different functional groups as analyzed by Gene Ontology: translation, protein folding, α-amino acid metabolism, cellular response to oxidative stress, and glycolytic process. We characterized a specific repertoire of protein complexes that were fractionated with polysomes or monosomes. In addition, we identified 69 of the 79 ribosomal proteins of the 80S: 18 ribosomal proteins encoded by a single copy gene and 51 ribosomal proteins that are encoded by paralogous genes. Our results show that there is an exchange between protein paralogs and differential distribution of relative abundance resulting, in some cases, in undetectable levels of some ribosomal proteins either in the monosome or the polysome. In addition, 28 pairs of ribosomal protein paralogous show differences in translational fitness. Our results suggest that ribosomal heterogeneity is achieved by changes in the ribosomal core proteins and accessory proteins that could modify ribosomal function depending on growth conditions and the number of ribosomes associated with an mRNA.

CB-P34
HUMAN ERYTHROPOIESIS: MOLECULAR AND CELLULAR STUDY OF LRP1 DURING ERYTHROID MATURATION

Vieyra C[1], Martina Moras M[2], Sánchez MC[3] , Chiabrando GA[3] , Lefevre S[2] , Ostuni M[2] , Fader CM[1,4].
[1]Laboratorio de Biología Celular y Molecular, Instituto de Histología y Embriología (IHEM-CONICET), UNCUYO, Mendoza, Argentina. [2]Centro de investigaciones en Bioquímica Clínica Inmunología, CIBICI-CONICET, Facultad de Ciencias Químicas, UNC.[3]Integrated Biology of Red Blood Cell, INTS, Paris Diderot University, Paris, France. [4]Facultad de Odontología, UNCUYO, Mendoza, Argentina.
E-mail: cfader@fcm.uncu.edu.ar

Erythroid maturation is a highly regulated process where immature cells from bone marrow go through a series of differentiation stages to become mature red blood cells. During this, essential intracellular modifications take place such as degradation of entire organelles that are non-necessary for erythrocyte functionality. Autophagy is a lysosomal degradative pathway where macromolecules and organelles are surrounded by double-membrane vesicles called autophagosomes and then targeted to lysosomes for its degradation. Autophagy participates actively in erythropoiesis being responsible for engulfment and elimination of mitochondria (mitophagy) and ribosomes once all hemoglobin has been synthesized. Low-density lipoprotein receptor-related protein 1 (LRP1) is a transmembrane receptor involved in a wide range of cellular processes such as proliferation, metabolism, and differentiation. LRP1 participates as a scavenger receptor for the hemin–hemopexin complex, leading to its endocytic internalization for metabolism. Previous results from our group demonstrated that hemin, an erythropoiesis inductor, is able to generate an autophagic response (mitophagy) in erythroleukemia cell lines (K562 cells), inducing the expression of LRP1 and some autophagic genes such as LC3, Atg5, and Beclin1. Moreover, we have demonstrated that LRP1is in part responsible for hemin autophagy activation. In this work, we show that LRP1 has an expression peak at day 10 of ex vivo erythropoiesis, being practically undetectable at final maturation stages (reticulocytes and erythrocytes). Similar results were observed by confocal microscopy where there was an increased number of cells labeled with this receptor at day 10 compared with cells at day 0, 7, or 17 of ex vivo maturation. These results suggest that LRP1 has an important role in a specific stage during erythropoiesis. Taken together, our results suggest that LRP1 is favoring erythroid maturation, maybe by inducing an autophagic response, being a possible therapeutic candidate that helps in hematopoietic disorders as well as the chronic myelogenous leukemia (CML) treatment.

CB-P35
STUDIES ON MECHANISMS UNDERLYING REPLICATIVE LIFESPAN EXTENTION IN YEAST WITH DISASSEMBLED EISOSOMES MEMBRANE DOMAINS

Salzman V, Nievas M, Correa F, Aguilar PS.
IFIBYNE (Instituto de Fisiología, Biología Molecular y Neurociencias)-UBA-CONICET, CABA, Argentina.
E-mail: salzmanvalentin @gmail.com

Eisosomes are plasma membrane (PM) domains concentrating lipids and proteins in the budding yeast *Saccharomyces cerevisiae*. These are domains shaped as 200–400-nm long and 50-nm-deep invaginated furrows structured by scaffolds composed mainly by two cytoplasmic proteins Pil1 and Lsp1. Deletion of *PIL1* leads to the disappearance of invaginations, very few and large clusters persist at the PM concentrating a fraction of the original eisosomal proteins. We study eisosomes' role in aging measuring the number of daughters produced by yeast mother cells of a dividing culture (replicative aging model, RLS). Performing RLS assays we found that knockout strains for *PIL1* have significantly enhanced longevity. We are interested in determining the mechanism underlying this phenotype. Eisosomes have been associated with the regulation of endocytosis of certain eisosomal proteins as well as the functionality of some of them. These domains concentrate at least 25 different proteins including signaling proteins and nutrient transporters such as Tat2, a permease that mediates high-affinity tryptophan (Trp) import. Knowing that *TAT2* gene deletion causes nutrient limitation and RLS extension in *S. cerevisiae*, we challenged a specific hypothesis: eisosomes´ disassembly decreases Tat2 permease activity, increasing lifespan. We set up an assay to measure ^{3}HTrp import *in vivo* in order to compare Trp uptake between the pil1 mutant and WT strain. In addition to Tat2, there is a second protein (Tat1) that transports Trp with lesser efficiency. Using tat2tat1 and tat2 mutants as control strains, we found that at [Trp] lower than 1uM, most of the amino acid is imported through Tat2 in the WT strain. Under these conditions, PIL1 deletion does not affect Trp uptake. Next, we estimated the kinetic parameters for Trp uptake in the WT strain and found that the apparent Km (Kmap) value is 30 μM. Taking into account that Tat2 activity could be lower in a strain with disassembled eisosomes not only because Kmap is affected but also because Vmax could be reduced, we decided to extend the range of [Trp] tested when comparing Tat2 import activity. As the kinetic parameters of both Trp permeases individually are unknown, it was not possible to infer the contribution of each of them at high substrate concentrations. Therefore, the Tat1 low-affinity permease gene was eliminated and Tat2 activity was measured in a wide range of substrate concentrations in tat1 and tat1pil1 strains. Additionally, to determine if eisosomes disassemble decreases Tat2 protein levels; we imaged cells expressing Tat2–GFP by fluorescence microscopy and quantified the protein fraction localized at the PM. The results contribute

to understanding the Tat2 role in the pil1 mutant longevity phenotype in order to further describing complex *S. cerevisiae* aging process and nanoscale PM domains function.

CB-P36
A NOVEL PROTEIN INVOLVED IN GOLGI HOMEOSTASIS

Zarelli VEP[1] , Lopez de Armentia MM[2] , Ortega M[1] , Colombo MI[1].
[1]Instituto de Histología y Embriología de Mendoza, CONICET. FCM-UNCuyo. [2]FFB-UMaza, Mendoza. E-mail: valezarelli@gmail.com

Kctd15 belongs to the potassium channel tetramerization domain (KCTD) proteins that contain a Broad-Complex, Tramtrack, and Bric-a-brac (BTB) domain. The BTB domain is a protein–protein interaction motif present among eukaryotes. Kctd15 homologs are found in different vertebrates, representing a well-conserved family. It has been demonstrated that Kctd15 affects Wnt signaling and AP-2 transcription factor function during embryonic development in zebrafish. The precise molecular mechanism of these processes remains unknown. To better understand the role of Kctd15, we transfected CHO, MEF, and HeLa cells with Kctd15-FOS plasmid and analyzed the protein localization by immunofluorescence using a confocal microscope. We observed the formation of giant vacuoles when Kctd15 levels increased in mammalian cells. These structures did not acquire Rab5, Rab7, or CD63. In addition, Lysotracker was not observed inside the vacuoles. Taken together, these results suggest that the vacuoles induced by Kctd15 are not endosomal compartments. In addition, we analyzed the recruitment of other Rab proteins to characterize these vacuoles and did observe Rab29, Rab32, and Rab38 in the membrane of these structures. To further characterize this compartment, we were able to trace the origin of these vacuoles to the Golgi complex. Furthermore, Kctd15 colocalized with TGN38/46 suggesting a link with a most posterior Golgi region. Interestingly, Kctd15 overexpression mimics the Brefeldin A effects disrupting the *cis*-Golgi cisternae. We consider that the particular phenomenon induced by Kctd15 overexpression might be part of the regulation of some of the pathways it modulates by affecting cell homeostasis.

CB-P37
TARGETING GLUCOSE METABOLISM FOR THE TREATMENT OF GLIOBLASTOMA

Arbe MF[1], Pan MD[1]*, Belgorosky D[1], Eiján AM, Zaloff Dakoff JM[2], Moughty Cueto C[2], Mazzon AJ[2], Gómez-Escalante JI[2], Prost D[2], Glikin GC, Finocchiaro LME[1], Villaverde MS[1]. *These authors contributed equally to this work.*
[1]Área de Investigación, Instituto de Oncología Ángel H. Roffo (IOHAR), Facultad de Medicina, UBA, [2]Unidad Funcional de Neurocirugía, Instituto de Oncología Ángel H. Roffo, Facultad de Medicina, UBA. E-mail: marcelavillaverde@hotmail.com

Glioblastoma (GBM) has the highest incidence among primary brain cancers and is also associated with poor prognosis. As in many other types of cancers, glycolysis and pentose phosphate pathway (PPP) support GBM proliferation and contribute to cancer development. Metformin (MET) is used as a first-line oral drug for the treatment of Type 2 Diabetes Mellitus (T2MD). Interestingly, MET can efficiently inhibit the progression of oral premalignant lesions and diminish tumor growth in different types of xenografts cancer models, including GBM. Here, we combined MET with 2DG, which is a glucose analog that inhibits Hexokinase (HK, the first and limiting enzyme of glycolysis), or with 6-aminonicotinamide (6AN), which is a Glucose-6 phosphate dehydrogenase (G6PD, the first and limiting enzyme of PPP) in 3 well-characterized human GBM cell lines, U87, U373, and U251. We found that 2DG decreased the viability (APH assay, $p < 0.05$) of U87 and U373, whereas U251 was highly resistant to HK inhibition. GBM cells resulted mildly (U87) or highly (U373 and U251) sensitive to metformin ($p < 0.05$), while all cell lines resulted highly sensitive to PPP inhibition by 6AN ($p < 0.01$). In addition, combinatory therapies showed even better results than monotherapies, especially in the case of MET/6AN. Among the mechanisms involved in the effectivity of MET and MET/6AN, we found a decreased in the mitochondrial membrane potential (TMRM) and an increase in apoptotic events (AO/EtBr). Interestingly, G6PD inhibition induced astroglia differentiated-like phenotype. Next, we were able to establish a novel GBM cell line, derived from a tumoral sample of a patient of our Institution (IOHAR), characterized as small cell GBM with EGFR amplification (FISH). These cells, particularly resistant to Temozolomide (TMZ, standard treatment) were sensitive to MET and 6AN, and highly sensitive to MET/6AN. Moreover, we also found an astroglia differentiated-like phenotype after the inhibition of G6PD by 6AN. These promising results support further studies to uncover the mechanism behind the synergistic effect of MET alone or in a combination of glucose metabolism inhibitors as a potential treatment of GBM.

CB-P38
RAB24, A NOVEL REGULATOR OF THE INITIAL STEPS OF THE AUTOPHAGIC PATHWAY

Germ Gomez E, Amaya C, Colombo MI.
IHEM, CONICET, FCM, UNCuyo, Mendoza Argentina. E-mail: emilianogerm@gmail.com

Autophagy is an intracellular degradation/recycling process that has fundamental roles to preserve cell homeostasis. The process must be tightly regulated since the deregulation of this pathway contributes to several human diseases, including cancer, neurodegeneration, and infections. This dynamic process requires the formation of double-membrane vesicles, termed autophagosomes, which engulf several cytoplasmic components such as molecules and organelles to finally fuse with lysosomes to digest the incorporated material. Rab proteins, a family of small GTPases, are essential for vesicle trafficking events, including autophagy, since they control vesicle budding, targeting, and fusion through the recruitment of effector proteins. In our laboratory, we have shown that Rab24 participates in vesicle trafficking events such as endosomal degradation and autophagy, but beyond its already established role in endosome-lysosome fusion, its specific role in autophagy remains elusive. For this reason, we have studied, in cellular models, the participation of Rab24 in autophagy events. To do this, through the use of various techniques, such as protein transfection, RNAi knockdown, microscopy, and Western blot assays, we have evaluated how Rab24 modulates the distribution and level

of proteins involved in the initial stages of the autophagic process. Our findings reveal Rab24 as a novel positive regulator of autophagosome formation and elucidate relevant insights about the role of Rab24 in autophagy.

CB-P39
SMALLANTHUS SONCHIFOLIUS (YACON) ROOTS IMPROVE THE DAMAGE INDUCED BY OXIDATIVE STRESS IN THE DIABETIC INTESTINE

Gomez Rojas JR[1] , Sánchez SS[1] , Honoré SM[1,2].
[1]INSIBIO (CONICET-UNT). [2]Fac. de Bioquímica, Química y Farmacia-UNT. E-mail: smhonore@gmail.com

Diabetes mellitus is becoming an increasingly prevalent disease with acute and chronic complications. Gastrointestinal symptoms associated with diabetes, known as diabetic enteropathy, are dyspepsia, constipation, diarrhea, and fecal incontinence. Previous findings indicate that hyperglycemia and the resulting oxidative stress on neural networks, including interstitial cells of Cajal, play a central role in the development of diabetic enteropathy. Nowadays, non-pharmacologic treatment options to manage these complications are becoming relevant. *Smallanthus sonchifolius* (yacon) is an Andean crop tuber characterized by its low caloric value and high fiber content, with a low glycemic response that makes it appropriate for consumption by diabetic individuals. In the present work, we investigate the effect of the yacon flour, rich in fructooligosaccharides (FOS) and phenolic compounds, in the intestine of diabetic rats. Diabetes was induced in male Wistar rats, using a single intraperitoneal dose (50 mg/kg bw) of streptozotocin (STZ). The rats were randomly assigned in the following groups (n = 6 animals/group): control, control supplemented with yacon flour (340 mg FOS/kg bw, Control+Y), diabetic (STZ), and STZ supplemented with yacon (340 mg FOS/kg bw, STZ+Y). Clinical, biochemical, molecular parameters were evaluated, and histological studies of intestinal tissue were performed. The results were analyzed statistically (*t*-test and ANOVA/post hoc test) considering a value of $p < 0.05$. Administration of yacon for 4 weeks did not change the levels of glucose, insulin, total cholesterol, and HDLc in STZ+Y animals. However, triglyceride and VLDLc levels were significantly reduced in this group. Clinically, the STZ+Y animals maintained their body and intestine weight, while the cecum presented marked hypertrophy. From a functional point of view, gastrointestinal transit increased significantly, accompanied by variations in the expression of intestinal peptides, with an increase in substance P and a decrease in the vasoactive intestinal polypeptide. Histological analysis revealed that the consumption of yacon prevents structural disorders of the intestine, particularly at the level of nerve plexuses. The inflammatory state decreased both systemically and in intestinal tissue in animals that received yacon. The redox balance was restored in the STZ+Y group showing a decreased lipid peroxidation and increased antioxidant enzymes activity. These results lead us to conclude that the consumption of yacon roots prevents the development of structural and functional alterations in the intestine of diabetic rodents, improving the inflammatory state and restoring redox balance, showing the potentiality of these roots in diabetes management.

CB-P40
ROLE OF EZRIN IN CELL SPHEROIDS FORMATION

Lujea NC, Masner M, Kunda P.
Centro de Investigación en Medicina Traslacional Severo Amuchástegui (CIMETSA - IUCBC). Córdoba, Argentina. E-mail: noelujea@gmail.com

ERM proteins (Ezrin, Radixin, and Moesin) link the actin cytoskeleton to the cell membrane forming the cell cortex. Thus, the actin cortex plays an important function in cell survival and proliferation. We suggest that the integrity of cortical actin and ezrin could play a role in spheroid formation in soft environments such as fluid. Understanding the mechanism of spheroids formation in fluids is crucial to understand tumor growth in ascitic fluid during ovarian cancer intraperitoneal metastasis. The epithelial ovarian cancer cell line SKOV3 and CRISPR-CAS9 modified cell line against ezrin (Ez6148) were used. Spheroids were generated by two methods: soft agar method, in which cells were cultured in 0.5% agar up to 10 DIV, and the aggregation method, in which cells were allowed to aggregate over an agar base for 24, 48, 72, and 96 h after seeding. Cytochalasin D (CD) was used to disrupt the actin cytoskeleton. Spheroid formation was analyzed by IF and confocal microscopy imaging. The experiments showed that, while SKOV3 in soft agar proliferated and formed spheroids from 3 DIV, Ez6148 did not proliferate at any time of the assay. Furthermore, when cells were allowed to aggregate over an agar base, Ez6148 failed to form a compact spheroid as the control cells did. Moreover, disruption of the actin cytoskeleton with CD did not alter the formation but changed the level of cell compaction at different times after cells seeding. These results show that the anchorage of the actin cytoskeleton to the plasma membrane is essential for the spheroid formation in soft environments, while actin dynamic and organization is not necessary. This reveals the importance of ezrin in favoring the formation of cell spheroids and could have an implication in the development of ovarian cancer intraperitoneal metastasis.

CB-P41
OLEIC ACID PROMOTES THE MIGRATION OF OVARIAN CANCER CELLS, AFFECTING THE POLARIZATION AND CYTOSKELETON DYNAMICS

Masner M[1], Lujea N[1] , Bisbal M[2] , Acosta C[3] , Kunda P[1].
[1]Centro de Investigación en Medicina Traslacional Severo Amuchástegui (CIMETSA). Instituto Universitario de Ciencias Biomédicas de Córdoba (IUCBC), Córdoba. [2]Instituto de Investigación Médica Mercedes y Martín Ferreyra - INIMEC-CONICET-UN. [3]Instituto de Histologia y Embriologia de Mendoza IHEM, FCM, UNCUYO. E-mail: mmasner@gmail.com

Ovarian cancer develops as a disease of high metastatic potential and mortality, with few effective treatment choices. The morphological transitions of the cell, cell-environment interactions, cell-cell interactions, and migrations determine the dispersion of tumoral cells, which generate secondary

growth foci. Within the hallmark alterations of the tumor environment, the differential accumulation of unsaturated fatty acids such as Oleic Acid (OL) in the ascitic fluid is associated with a phenotype of higher metastatic potential. In our laboratory, we investigate the effects of OL on ovarian tumoral cells, regarding its influence on the cytoskeleton, at the level of structure, dynamics, and polarization. Using the human ovarian tumoral cell line SKOV-3, we take an in-vitro approach to structures and dynamics of the actin and tubulin cytoskeleton, combining cell culture, molecular biology, epifluorescence and confocal microscopy with live and fixed specimens. Our results have shown that the addition of OL to the culture media enhances the migration and orientation capabilities of cells in the wound assay. Time-lapse microscopy enabled us to detect the combined effect on trajectory alignment and orientation to the front. Video microscopy experiments using the SKOV-3 LifeAct-GFP revealed that there are more abundant and mobile actin structures at the cell front. In parallel, FRAP experiments using SKOV-3 LifeAct-GFP showed that OL treatment accentuates the difference between the front and back of the cell during migration. Cells transfected with the FRET probes for the Rho-GTPases Rho/Rac/CDC42 exhibited an increase in Rac induced by OL at the cell front. Analyzing the tubulin cytoskeleton during migration, we found that OL favors an earlier repositioning of the MTOC to the cell front. EB3-Cherry comet experiments resulted in an increase in microtubule speed at the cell front induced by OL. In conclusion, OL enhances cell migration, by incrementing the actin cytoskeleton dynamics, through Rac activation, and the increment in microtubules at the cell front. Our findings support the incipient role of these fatty acids in the pathogenesis of ovary cancer and give way to speculations about its possible prognostic and therapeutic use.

CB-P42
GLYCOGEN SYNTHASE KINASE 3 PLAYS A CENTRAL ROLE IN THE TRANSCRIPTIONAL RESPONSE TO UV-INDUCED DNA DAMAGE

Nieto Moreno N[1], Villafáñez F[2] Soria G[2], Muñoz MJ[1], Kornblihtt AR[1].
[1]IFIBYNE-UBA-CONICET, Argentina. [2]CIBICI-UNC-CONICET, Argentina. E-mail: nnietomoreno@fbmc.fcen.uba.ar

DNA integrity is a major requisite for life and, therefore, cells develop a concerted response upon DNA damage. At the gene expression level, this response consists of a genome-wide downregulation of transcription initiation and elongation rates, as well as the modulation of alternative splicing (AS) patterns. In particular, we have described that UV-induced DNA lesions trigger an *in trans* signaling cascade that promotes hyperphosphorylation of the carboxyl-terminal domain (CTD) of RNA polymerase II (RNAPII), a decrease in transcription elongation rates and changes in AS patterns in the context of the kinetic coupling model between transcription and splicing. In order to identify kinases mediating this response, we performed a screening with the Public Kinase Inhibitors Library (PKIS2) from GlaxoSmithKline and identified glycogen synthase kinase 3 (GSK-3) as a central player: GSK-3 inhibition prevents UV-induced RNAPII hyperphosphorylation, as well as the changes in AS patterns and the decrease in transcription elongation rates. Using RNAPII ChIP analysis we detected that while intragenic RNAPII occupancy is increased upon UV irradiation, consistent with a decrease in RNAPII elongation rates, GSK-3 inhibition prevents this increase. In addition, GSK-3 inhibition prevents UV-induced apoptosis. Finally, since RNAPII CTD adjusts to the GSK-3 consensus site, and it was reported that GSK-3 translocates to the nucleus in stress conditions, we are currently testing the hypothesis that GSK-3 may be phosphorylating RNAPII in a direct manner. Altogether, our results set GSK-3 as a central kinase in the transcriptional response to DNA damage.

CB-P43
ADAPTATION OF THE SECRETORY PATHWAY IN NEURONAL DIFFERENTIATION MODELS

Sampieri L, Di Giusto P, Álvarez C.
CIBICI-CONICET, Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, UNC. E-mail: lsampieri@fcq.unc.edu.ar

Intracellular protein trafficking is essential for all eukaryotic cells. The secretory pathway targets proteins to the extracellular space and is responsible for protein and lipid transport among different cellular compartments. To allow membrane flow and maintenance of organelle size and cellular homeostasis a high degree of coordination in every step of intracellular trafficking is required. The role of the secretory pathway during cell differentiation is crucial and particularly special for neurons because they need to develop an extremely polarized phenotype which is accompanied by a large cell surface expansion, implying that an increase in plasma membrane protein and lipid synthesis and transport is required. Therefore, the secretory pathway must adapt to respond to this greater demand of its function. The mechanisms that regulate the expression of secretory pathway proteins in this type of cells have been poorly described. The CREB3 family of bZIP transcription factors, which comprises CREB3, CREB3L1, CREB3L2, CREB3L3, and CREB3L4, has been described to participate in cell differentiation in various tissues by regulating the secretory machinery. However, very little is known about their role in the nervous system. Our goal is to analyze the regulation of the secretory pathway and the participation of CREB3 transcription factors in neuronal differentiation models. To accomplish this, we used two cellular models: rat pheochromocytoma cells from a PC12 cell line, which respond to nerve growth factor (NGF) and *in vitro* cultured primary rat hippocampal neurons. Both models have been extensively characterized and widely used to study neuron development. We demonstrated, in PC12 cells, that NGF treatment induces an increase in the levels of transport proteins and Golgi complex size. Also, we found that CREB3L2 increases during NGF-induced differentiation and that MAPK and PKA signaling pathways, both regulators of neurite outgrowth, control CREB3L2 response to NGF. We observed that partial inhibition of CREB3L2 expression alters normal neurite outgrowth in response to NGF. Interestingly, we found that, during the early development of hippocampal neurons *in vitro*, levels of transport proteins and CREB3L1 increase. In this context, blocking CREB3L1 transcriptional activity impairs normal neurite development and Golgi morphology. Altogether, these data indicate that during neuronal differentiation an adaptative response of the secretory pathway occurs, presumably mediated by CREB3 factors and that this response is required for proper development, highlighting a novel role for these transcription factors in neuronal cells.

CB-P44
ROLE OF THE TRANSCRIPTION FACTOR CREB3L1 IN THYROID CELLS

Di Giusto P, Sampieri L , Alvarez C.
CIBICI-CONICET / Depto. de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina.
E-mail: pdigiusto@fcq.unc.edu.ar

CREB3L1 is a transcription factor member of the CREB3 subfamily, expressed in a wide variety of tissues, including cartilage, pancreas, and bone. It has been reported that, in bone cells, this transcription factor regulates the expression of proteins involved in the secretory pathway (transport factors) as well as the expression of bone-specific genes. We have previously shown that, in thyroid cells, CREB3L1 acts as a downstream effector of the Thyroid Stimulating Hormone (TSH) and promotes the expression of transport factors as well as the expansion of the Golgi volume. However, the CREB3L1 function in the regulation of thyroid-specific genes has not been analyzed. The aim of this work is to analyze the role of CREB3L1 in the regulation of NIS, a thyroid-specific protein that is also expressed in extra-thyroidal tissues including lactating mice mammary gland and human breast cancers. NIS is responsible for the uptake of iodine, which is then incorporated into the metabolic regulators, triiodothyronine (T3) and tetraiodothyronine (T4). Moreover, NIS expression is also fundamental for the success of the radioactive iodine therapy, standard and main practice for some types of differentiated thyroid carcinomas. Here we show that inhibition of CREB3L1 with a specific siRNA reduces NIS protein and mRNA levels in FRTL5 cells (a well-established cell model for the study of thyroid function). Moreover, we assessed the effect of CREB3L1 on the activity of the rat *NIS* promoter using luciferase-reporter assays in HeLa cells. Results indicated that CREB3L1 enhanced NIS promoter transcriptional activity when compared to mock cells. Taken all together, our results indicate that CREB3L1 regulates NIS expression, and suggest that it acts as a transcriptional regulator of the *NIS* gene. Further analysis will be required to confirm direct CREB3L1-NIS promoter interaction.

CB-P45
ANALYSES OF ALELIC VARIANTS OF A SUBTELOMERIC pLAM HAPLOTYPE LINKED TO THE HUMAN D4Z4 TANDEM REPEAT AT 4Q35

Pagnoni SM[1,2,3], Bidinost C[1,2] , Rosa AL[1,3].
[1]Laboratorio de Biología Celular y Molecular, Fundación Allende; [2]Laboratorio de Genética y Biología Molecular, Sanatorio Allende; [3]IRNASUS-CONICET, Facultad de Ciencias Químicas, Universidad Católica de Córdoba. E-mail: sabri.pagnoni@gmail.com

A polymorphic tandem repeat of 3.3 kb (D4Z4), consisting of 8–100 units, is present at the subtelomeric region of human chromosome 4q. Contractions in this tandem are associated with facioscapulohumeral muscular dystrophy (FSHD), an inherited progressive autosomal dominant neurodegenerative muscular disease. A D4Z4-linked polymorphic sequence named pLAM, distal to the tandem D4Z4, shows three alternative haplotypes, named 4qA, 4qB, and 4qC. In previous reports, we used a molecular strategy to characterize the molecular structure of complex 4q35-linked D4Z4 alleles using pulsed-field gel electrophoresis (PFGE), followed by Southern blot hybridization and a chemiluminescence detection method. Alternative specific probes from the 4q35 region, as well as single and double digestions with alternative restriction enzymes, allowed us to establish the size of multiple D4Z4 alleles as well as to differentiate D4Z4 alleles from chromosome 4q versus chromosome 10q. In this report, we characterized a large cohort of anonymous genomic DNA samples from individuals originally diagnosed as FSHD. Optimum conditions of PFGE-Southern blot, using specific p13E11, 4qA, and 4qB probes, allowed us to define the size and haplotype of complex D4Z4 alleles.

CB-P46
ENDOGENOUS ANTIOXIDANT DEFENSES IN YOUNG ADULTS OF THE REPUBLIC OF PANAMA

Fernández MO, Díaz CV.
Departamento de Bioquímica y Nutrición, Facultad de Medicina, Universidad de Panamá. E-mail: proyectoeon@gmail.com

The cells need oxygen to produce energy and some metabolic reactions, but cellular respiration and other metabolic reactions produce reactive oxygen species (ROS) that cause oxidative damage to the cells. The damages to ADN, membrane phospholipids and proteins are related to the origin and the development of non-communicable diseases such as cardiovascular disease, diabetes, and cancer that are listed among the main causes of morbidity and mortality in Panama. The organism has endogenous antioxidant defenses, metabolites, and enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR). The unbalance between the production of oxidants and the capacity of the organism to fight them produces oxidative stress. The hydroperoxides, the carbonyl groups, the conjugated dienes, and malondialdehyde are biomarkers of this stress and can be analyzed in plasma. In order to study the complex relation between oxidative stress and non-communicable diseases, we are developing a research project to analyze endogenous defenses, exogenous defenses (antioxidant consumption), and the plasma oxidative biomarkers. The results will be used to build a national database for a prevention program of non-communicable diseases based on scientific evidence that will benefit the country. In this work we present the results of the antioxidant enzyme activities of a population of 783 Panamanians, 57% males and 43% females, a mean age of 22 ± 2.4 years from all provinces and indigenous regions of the country and a comparison of cut-off points obtained for the population with other countries population results, since they are influenced by lifestyles and environmental factors.

CB-P47
THE INITIAL PICTURE OF PREADIPOCYTE RESPONSE TO A NITRO-OXIDATIVE ENVIRONMENT

Funk MI[1], Bruzzone A[1,2], Salvador GA[1,2], Uranga RM[1,2].
[1]INIBIBB-CONICET, [2]DBByF -UNS, Bahía Blanca, Argentina. E-mail: ruranga@criba.edu.ar

Cumulative evidence indicates that obesity-related metabolic dysfunction is more associated with the existence of hypertrophic dysfunctional adipocytes rather than to numerous small adipocytes. For this reason, the study of adipocyte development as a major cause of obesity has become a relevant area of research. We have previously shown that menadione-induced oxidative stress inhibits adipogenesis and increases triglyceride content in the mature adipocyte, with the PI3K/Akt pathway playing a crucial role. Here, we tested the effect of nitro-oxidative stress (NS) induced by sodium nitroprusside (SNP) on 3T3-L1 preadipocytes. For this purpose, we exposed 3T3-L1 preadipocytes to 0.0–1.0 mM SNP for 24 h, and we characterized the level of cellular injury triggered by NS. Cell mortality was close to 100% after 24-h incubation in the presence of concentrations of SNP over 0.2 mM. Low SNP concentrations (0.1–0.2 mM) altered neither mitochondrial functionality (MF) nor increased reactive oxygen species (ROS). However, lipid peroxides were found to be increased, and the permeability of the cell membrane (measured as LDH leakage) was significantly altered. These findings indicate that SNP triggers the generation of ROS that are early scavenged, and only the remaining lipid peroxidation products and damage to cell membrane are detected after 24 h. We also evaluated the short-term effect of the exposure to SNP by incubating 3T3-L1 cells with 0.0–10.0 mM SNP for 2 h. It was found that MF was not affected by 0.5 mM SNP, but significantly diminished by concentrations of SNP over that (20%, 40%, and 60% decrease by 1 mM, 5 mM, and 10 mM, respectively). Moreover, 0.5 mM tripled ROS levels, doubled the levels of lipid peroxides and significantly altered membrane permeability (4-fold increased LDH leakage). We then evaluated the phosphorylation levels of Akt, ERK1/2, and the stress-activated protein kinase/Jun-amino-terminal kinase (SAPK/JNK), four well known kinases involved in cellular redox signaling, after the incubation with 0.5 mM SNP (increased markers of NS without affecting MF) and 5 mM SNP (increased markers of NS with decreased MF). The four kinases were found to increase their phosphorylation level, thus, to be activated by both concentrations of SNP. Immunocytochemistry analysis revealed a different subcellular distribution of the kinases upon SNP treatment: the active form of Akt showed a preferential nuclear localization, regardless of the concentration of SNP; p-ERK1/2 increased their nuclear localization only in the condition where MF was not affected; p-SAPK/JNK increased all over the cell. Together, our findings show the response-time distribution that arises from the SNP treatment of preadipocytes. Our next goal is to evaluate the differentiation capacity of these preadipocytes acutely exposed to SNP.

ENZYMOLOGY

EN-P01
A CO-PURIFYING CONTAMINANT LOWERS THE PHOSPHOENZYME LEVEL OF THE SPF1

Mazzitelli LR, Corradi GC, Petrovich GD, Grenon P, de Tezanos Pinto F, Adamo HP.
Instituto de Química y Fisicoquímica Biológicas (IQUIFIB), CONICET-UBA, Argentina. E-mail: lrmazzitelli@qb.ffyb.uba.ar

P5-ATPases are eukaryotic proteins believed to actively transport a yet not identified substrate. They are necessary for cellular functions associated with the ER and the endo-lysosomal membranes, and in humans, mutations in the P5-ATPases genes are associated with neurological disorders. At the molecular level, the best-characterized P5-ATPase is the Spf1 from *Saccharomyces cerevisiae*. We have previously shown that purified micellar preparations of recombinant Spf1 can hydrolyze ATP and produce the phosphoenzyme intermediate (EP) characteristic of the transport reaction cycle of P-ATPases. Moreover, Spf1 was proposed to be modulated by Ca^{2+} by decreasing the level of EP. Here we present results suggesting that at least part of the effect of Ca^{2+} is mediated by traces of contaminant proteins that co-purify with Spf1. When the reaction media contained EGTA, the preparation exhibited a low ATPase activity that was either increased or inhibited by the addition of $CaCl_2$ depending on each particular preparation. The addition of 1 mM of the phosphatase inhibitor ammonium molybdate abolished the stimulation of the ATPase. The catalytic death mutant Spf1-D487N showed a marginal ATPase activity in EGTA but was highly stimulated by Ca^{2+}. These results suggest that the increase of ATPase did not result from a direct effect of Ca^{2+} on Spf1. Moreover, the stimulation of the ATPase activity was also produced by other metals like Mn and Zn, suggesting that the increase in ATPase was not a specific effect of Ca^{2+} but the result of decreasing the free EGTA. The level of EP formed by Spf1 was higher in the presence of EGTA and decreased with the increase in the Ca^{2+} of the media. The magnitude of this Ca^{2+} effect on the EP level showed a positive correlation with the stimulation of the ATPase activity in each preparation of Spf1 tested. Altogether these results suggest that the catalytic function of Spf1 may be regulated by a protein phosphatase. Analysis by mass spectrometry of the Spf1 preparation after SDS-PAGE detected the presence of several kinases and phosphatases proteins.

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

EN-P02
ANALYSIS OF SUBSTRATE SPECIFICITY OF GALACTINOL SYNTHASE FROM *BRACHYPODIUM DISTACHYON*

Martinez MP, Minen RI, Iglesias AA, Figueroa CM.
Instituto de Agrobiotecnología del Litoral (UNL-CONICET). E-mail: pilarmartinez678@gmail.com

Raffinose (Raf) is an α-1,6-galactosyl extension of sucrose that plays a key role in the stabilization of membranes during seed desiccation. Plants from the family Cucurbitaceae also use Raf for transporting carbon from photosynthetic to heterotrophic tissues. Additionally, Raf accumulates in tissues exposed to several abiotic stress conditions, such as heat, cold, salinity, and drought. Considering the importance of Raf for plant physiology and biochemistry, the number of studies dealing with enzymes involved in Raf biosynthesis are relatively scarce. To better understand the kinetic and regulatory properties of the enzymes involved in Raf metabolism, we cloned the genes encoding UDP-sugar pyrophosphorylase (USPPase, EC 2.7.7.64), galactinol synthase (EC 2.4.1.123), and Raf synthase (EC 2.4.1.82) from *Brachypodium distachyon*, a model grass evolutionary related to several economically important species, including rice and wheat. USPPase catalyzes the production of activated sugars, mainly UDP-galactose (UDP-Gal), the natural substrate of galactinol synthase, which in turn produces galactinol from UDP-Gal and *myo*-inositol. Afterward, Raf synthase transfers the Gal moiety from galactinol to a preformed sucrose molecule to produce Raf. The genes encoding USPPase, galactinol synthase, and Raf synthase from *B. distachyon* were synthesized *de novo*, and the recombinant proteins were expressed with an N-term His-tag in *Escherichia coli* cells and purified in a single step by IMAC. The activity of USPPase with different hexoses-1P was as follows: Gal-1P ≅ glucose-1P > glucuronic acid-1P > mannose-1P > glucosamine-1P. We found that galactinol synthase uses UDP-glucose as an alternative substrate, although with a significantly lower catalytic efficiency (196 M^{-1} s^{-1}) than with UDP-Gal (2.6 x 10^5 M^{-1} s^{-1}). Our results suggest that galactinol synthase might use other UDP-sugars *in vitro*, thus leading to novel *myo*-inositol derivatives different from galactinol. These molecules could then be used by Raf synthase to produce original oligosaccharides with multiple applications in biorefineries.

EN-P03
A FIRST EVIDENCE OF A GLUTAREDOXIN-LIKE PROTEIN IN *ENTAMOEBA HISTOLYTICA*

Birocco F, Guerrero SA, Iglesias AA, Arias DG.
Laboratorio de Enzimología Molecular, IAL (CONICET- UNL), Santa Fe - Argentina. E-mail: francobirocco@gmail.com

Entamoeba histolytica, an intestinal parasitic protozoan, is the causative agent of amoebiasis. The parasite usually lives and multiplies within the human gut, an environment of reduced oxygen pressure. During tissue invasion, *E. histolytica* is exposed to elevated amounts of exogenous reactive oxygen species (ROS), which are highly toxic for the parasite. The metabolic pathway for ROS detoxification in this organism is a matter of controversy. Because neither glutathione nor its associated enzymes were found to occur, it has been proposed the cysteine as a main intracellular thiol and one of the compounds responsible for maintaining the intracellular redox balance. In this work, we present the functional characterization of a glutaredoxin-like protein from *E. histolytica* (*Eh*Grx1). Biochemical assays showed that *Eh*Grx1 was able to catalyze the *in vitro* reduction of GSH-derivate low molecular mass disulfides and cystine. The protein obtained by recombinant expression in *Escherichia coli* presented an apo-monomeric structure; however, a holo-protein form was obtained from supplemented culture media with ferric citrate and cysteine. The ability to ligate iron-sulfur centers (ISCs) was evaluated by UV-Vis spectroscopy and gel filtration chromatography, showing evidence that *Eh*Grx1 could bind ISCs. The Grx activity was not detected in holo-*Eh*Grx1, suggesting that its catalytic cysteine residue would be linked to ISC. We also evaluated by western blot the relative abundance of *Eh*Grx1 in *E. histolytica* cells exposed to exogenous oxidative species and metronidazole (the preferred drug for amoebiasis treatment). The results showed that the protein level increases respect to no-treated cells. Similar behavior was observed in the subcellular localization analysis, carried out for different oxidative conditions by confocal immunofluorescence microscopy. Altogether, the results suggest that *Eh*Grx1 could be involved in oxidative and nitrosative stress protection in the parasite. To the best of our knowledge, this is the first characterization of this type of protein in *E. histolytica*. Granted by ANPCyT (PICT2016-1778 and PICT2017-2268).

EN-P04
KINETIC AND STRUCTURAL CHARACTERIZATION OF A GLYCOSYL PHOSPHORYLASE FROM *EUGLENA GRACILIS*

Calloni RD, Muchut JM, Arias DG, Iglesias A, Guerrero SA.
Instituto de Agrobiotecnología del Litoral (Universidad Nacional del Litoral-CONICET). E-mail: rcalloni@santafe-conicet.gov.ar

Euglena gracilis is a freshwater protist with a large metabolic capacity because it is able to grow photosynthetically or heterotrophically. *E. gracilis* is a microorganism of interest in biotechnology and biomedicine due to its ability to generate bioproducts such as polysaccharides, polyunsaturated fatty acids, vitamins, wax esters, and other metabolites. Paramylon is the main reserve polymer of *E. gracilis*. It is a water-insoluble β-1,3-glucan with a high degree of polymerization. There is little information about how *E. gracilis* is able to metabolize this polymer. Recently, the presence of a protein in *E. gracilis* belonging to the family 149 of glycosyl hydrolases (*Eg*GH149) was reported. GH149 is a new family of "Carbohydrate-Active Enzyme" (CAZyme) and is thought to group glycosyl phosphorylase. Glycosyl phosphorylases can catalyze the degradation/synthesis of β-1,3-glucan. The kinetic parameters of the enzyme in both senses (and for several substrates) were determined. We have studied the partition of a disaccharide of glucose with β-1,3 bond (Lamianribiose or Lam2) with inorganic phosphate and we found that the enzyme had its maximum activity at pH 7.5 and 40°C (*Kcat* of 9.1 s^{-1} and a *Km* values of 1.57 mM for inorganic phosphate and 1.24 mM for the Lam2). We observed that the enzyme had no activity when testing other types of disaccharides. The influence of polymerization degree (PD) was evaluated, being the efficiency of the enzyme 10-fold lower for both laminaritetrose (PD = 4) and laminarihexose (PD = 6). No activity using laminarin (PD = 30) as a substrate was detected. On the other hand, *Eg*GH149 catalyzed the condensation of glucose with glucose-1-Phosphate (*Kcat* 1.32 s^{-1} and *Km*

1.81 for the glucose). We also carry out promiscuity tests for sugar phosphate and free sugar, finding that it is specific for the use of glucose-1-Phosphate without reaction with other sugar-1-phosphate. We also show that it is capable of using Lam2 and 2-deoxy-glucose with lower affinity than glucose. We do not detect enzymatic activity when evaluating other acceptors. In order to know the quaternary structure of the enzyme, we performed a gel filtration chromatography, showing that the *Eg*GH149 forms homodimers in agreement with previous reports for the same enzyme from other sources. With this information and the crystallized structure of another protein belonging to GH149, we made a 3D model of the protein in which we detected a laminarihexose binding surface away from the active site. In this regard, we decided to study the ability of the enzyme to bind paramylon and laminarin. We did not observe the binding of the *Eg*GH149 to the substrates tested. We recently obtained rabbit serum antibodies immunized against the protein of interest that will help to determine its cellular location. This result contributes to the right assignation structure–function for this polypeptide, being necessary further works for in-depth analyses of the functionality of *Eg*GH149 in *E. gracilis*.

EN-P05
A NOVEL SCREENING SYSTEM TO IDENTIFY SMALL-MOLECULE INHIBITORS OF S-ACYLTRANSFERASES

Coronel C[1], Giolito M L[1], García I A[2], Soria G[2], Valdez Taubas, J[1].
[1]CIQUIBIC-CONICET, Fac. Cs. Químicas, UNC, Córdoba, Argentina, [2]CIBICI-CONICET, Fac. Cs. Químicas, UNC, Córdoba, Argentina
E-mail: ccoronel@fcq.unc.edu.ar

Protein S-acylation or palmitoylation is a post-translational modification (PTM) that consists of the addition of long-chain fatty acids on cysteine residues through a thioester bond. The labile nature of this bond makes this PTM the only lipid modification that is reversible and, therefore, with regulatory capabilities. Palmitoylation has multiple roles in many cellular processes, including signal transduction, protein traffic, and even gene expression. This modification is highly prevalent and more than 10% of the human proteome is thought to be palmitoylated. Additionally, there is growing evidence that palmitoylation is closely linked to human health. For example, its misregulation has been associated with many types of cancers and disorders of the nervous system, and many viral and bacterial pathogens require palmitoylation by the host machinery to thrive, making the enzymes responsible for this modification attractive drug targets. Palmitoylation is catalyzed by a family of palmitoyltransferases (PATs) or zDHHC proteins, which are polytopic membrane proteins characterized by the presence of a conserved DHHC-Cysteine Rich domain. There are 7 members of this family in yeast and 23 in humans. Despite the importance of palmitoylation, no specific inhibitors of mammalian DHHC proteins are currently available. There is a great need to develop such inhibitors to aid in the study of this modification and to test their possible therapeutic implications. The aim of this work is to develop an *in vivo* screening system to identify putative small-molecule inhibitors for PATs. We describe the creation of yeast strains that enable us to positively select the cells in which palmitoylation by an individual PAT is inhibited. This system is based on a reporter gene (HIS3) that responds to a transcription factor fused to a palmitoylation substrate. We show that the system works well for the endogenous yeast PAT Akr1 and it can be adapted for the isolation of inhibitors of heterologous PATs, both from mammalian and parasite origins, such as human DHHC20, mouse DHHC21, and *Giardia lamblia* GL50803_6733. We are currently screening a 10000 compound library (3D Biodiversity by ChemDiv) and have several candidate molecules for further validation.

EN-P06
FUNCTIONAL CHARACTERIZATION OF A HYBRID FMSR PROTEIN FROM *TRYPANOSOMA CRUZI DM*28C

Gonzalez LN, Birocco F, Sasoni N, Guerrero SA, Iglesias AA, Arias DG.
Laboratorio de Enzimología Molecular, Inst. de Agrobiotecnología del Litoral (UNL-CONICET), (3000) Santa Fe, Argentina
E-mail: nadia.lihue@gmail.com

Methionine is an amino acid susceptible to being oxidized to methionine sulfoxide (MetSO). The reduction of MetSO to methionine is catalyzed by methionine sulfoxide reductase (MSR), an enzyme present in almost all organisms. In trypanosomatids, the study of antioxidant systems has been mainly focused on the involvement of trypanothione, a specific redox component in these organisms. *Trypanosoma cruzi*, the etiological agent of Chagas disease, is auxotroph by methionine. Through an analysis of its genome project, we identified a coding sequence of free methionine sulfoxide reductase (fMSR), a protein only present in unicellular organisms. In *T. cruzi*, this protein is constituted by a GAF-like domain fused to the TIP41 domain (homologous to yeast TIP41 protein involved in the TOR pathway negative regulation). The encoding sequence for the GAF domain was expressed in *Escherichia coli*, and the corresponding recombinant protein was purified and functionally characterized. The recombinant protein exhibited MSR activity with L-Met(*R*)SO and *T. cruzi* tryparedoxins and thioredoxin as the reducing substrates. Our results supported that this enzyme has non-saturation ping-pong kinetics. In addition, based on the fact that the GAF domain is present in proteins capable to bind nucleotides, we evaluated the effect of AMP, ADP or ATP on its MSR activity. We observed an inhibitory effect at low substrate concentration (in the presence of $MgCl_2$), mainly by ATP. On the other hand, we performed a yeast complementation assay using a Δ*tip41* mutant. The results showed that *Tc*fMSR (both isolated TIP41 domain as the full protein) could compensate the sensitive to rapamycin phenotype. This result indicates that the *Tc*fMSR is active acting in the yeast TOR pathway. These results suggest that the *Tc*fMSR protein is a possible link between the redox metabolism and the TOR pathway in *Trypanosoma cruzi. Granted by ANPCyT (PICT2016-1778 and PICT2017-2268).*

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

EN-P07
A CHEMICAL BIOLOGY APPROACH TO UNDERSTAND THE REGULATION OF FULL-LENGTH PHOSPHOINOSITIDE-DEPENDENT PROTEIN KINASE 1 (PDK1)

Gross LZF [1], Sacerdoti M [1], Leroux AE[1], Cappellari MV [4], Aramendía PF [4], Ghode A[5], Anand GS [5], Klinke S, Biondi RM [1, 2]
[1] IBioBA, CONICET; [2] Department of Internal Medicine I, Universitätsklinikum Frankfurt, Germany; [3] IIBBA-CONICET; [4] CIBION; [5] Department of Biological Sciences, National University of Singapore, Singapore. E-mail: lgross@ibioba-mpsp-conicet.gov.ar

Phosphoinositide-dependent protein kinase 1 (PDK1) is a master AGC kinase that phosphorylates at least other 23 AGC kinases, being PKB/Akt the most relevant substrate downstream of PI3-kinase, important for growth and cell survival, and a drug target for cancer treatment. Over the years, our laboratory studied and characterized in detail the catalytic domain of PDK1, as well as the selective activation of substrates such as SGK or S6K, which in order to be phosphorylated require a docking interaction with a hydrophobic site in PDK1 termed the *PIF-Pocket*. However, this is not the case of Akt/PKB, since it can be activated in a *PIF-Pocket* independent way. On the other hand, up to date little is known about the mechanistic and structural details of PDK1 full length. Therefore, we are using an interdisciplinary approach to understand how the full-length protein is regulated and how this regulation mechanism can be manipulated to specifically inhibit the activation of PKB/Akt. As a result of a medium-scale screening of small compounds we carried out using AlphaScreen technology, we validated a series of small compounds "hits" that modulate PDK1 structure by interaction at different sites on PDK1. We performed hydrogen/deuterium exchange (HDX) experiments and crystallization screenings to understand the structure of full-length PDK1 and how it can be modulated with small compounds. We here present a series of results obtained using HDX on full-length PDK1 and the crystal structure of the catalytic domain of PDK1 bound to a small compound identified in our screening. We also present our *in vitro* studies to understand the oligomerization of PDK1 visualizing single particles by using STORM fluorescence microscopy. Finally, we integrate our data to present an updated model on the molecular mechanism of regulation of PDK1.

EN-P08
KINETIC AND STRUCTURAL CHARACTERIZATION OF GALACTINOL SYNTHASE FROM *BRACHYPODIUM DISTACHYON*

Minen RI, Iglesias AA, Figueroa CM.
Instituto de Agrobiotecnología del Litoral (UNL-CONICET) E-mail: rminen@santafe-conicet.gov.ar

Raffinose (Raf) is an α-1,6-galactosyl extension of sucrose that is used for carbon export from source to sink tissues in many plants. Raf plays an important role in the stabilization of membranes during seed desiccation, being accumulated under certain abiotic stress conditions, including heat, cold, salinity, and drought. The first committed step in the pathway of Raf biosynthesis is the reaction catalyzed by galactinol synthase (EC 2.4.1.123), a member of the glycosyltransferase family 8, which produces galactinol from UDP-galactose (UDP-Gal) and *myo*-inositol. Then, raffinose synthase (EC 2.4.1.82) transfers the galactosyl moiety from galactinol to sucrose, thus producing Raf. Several works have shown the relationship of galactinol synthase transcripts with galactinol and Raf levels in response to various types of stressful conditions. However, little is known about the structural, kinetic, and regulatory properties of this enzyme. The present work focuses on galactinol synthase from *Brachypodium distachyon*, a model grass closely related to economically important crops, such as rice and wheat. The gene coding for this enzyme was synthesized *de novo* and the recombinant enzyme was expressed fused to an N-terminal His-tag in *Escherichia coli* cells and purified by IMAC. The activity of *B. distachyon* galactinol synthase was 2-fold higher in presence of Mn^{2+} than with Mg^{2+}, the enzyme exhibiting a 10-fold higher affinity for the former. Enzyme activity in the physiological direction of the reaction (synthesis of galactinol) was optimal at pH values from 7 to 9 and at 35°C. Under standard conditions, the $S_{0.5}$ for UDP-Gal and *myo*-inositol were 0.08 and 2.9 mM, respectively. Interestingly, the recombinant enzyme was inactivated by oxidation with diamide, and the activity was recovered by reduction with DTT, suggesting the existence of a redox regulatory mechanism. A structural model of the enzyme was obtained by protein threading (fold recognition), which was used to determine the residues putatively involved in substrate and metal binding. Overall, our work lays the ground to understand better the synthesis of Raf in plants.

EN-P09
DIFFERENTIAL $NADP^+$ BINDING MODE IN BACTERIAL AND PLASTIDIC FERREDOXIN-$NADP^+$ REDUCTASES

Monchietti P, Ceccarelli EA, Catalano Dupuy DL
Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET-UNR. E-mail: monchietti@ibr-conicet.gov.ar

Ferredoxin-$NADP^+$ reductases (FNRs) constitute a family of monomeric hydrophilic proteins that contain FAD as a prosthetic group. They are classified as either plant- or mitochondrial-type FNRs. Plant-type FNRs are divided into plastidic and bacterial classes. Bacterial FNRs participate in metabolic pathways that are especially appropriate for the development of microbicidal agents because they are not present in humans. Plastidic FNRs have a conserved tyrosine residue at the carboxyl terminus which is interacting with FAD isoalloxazine. This residue would be displaced to allow the entry of $NADP^+$. Plastidic FNRs show between 20- and 100-times greater exchange rates than bacterial enzymes. The latter, on the other hand, have a structured variable terminal carboxyl end that has not allowed to propose models justifying how the substrate reaches the active site. The crystallographic structure of bacterial *Escherichia coli* FNR (EcFPR) with the bound nucleotide shows that the $NADP^+$ molecule interacts with three arginines (R144, R174, and R184) that would generate a strongly structured site with high affinity for the $NADP^+$ substrate. These three amino acids are conserved in other bacterial FNR, but not in the highly efficient plastidic enzymes found in plant chloroplasts and cyanobacteria. The structural alignment of EcFPR with the plastidic *Pisum sativum* FNR (PeaFNR) shows that of these three arginines, only R174 in EcFPR is present in PeaFNR (R229); R144 corresponds to a proline (P199) and R184 to a tyrosine (Y240). We have found $NADP^+$ tightly bound to the EcFPR. The bound nucleotide and the structured carboxyl terminus in bacterial enzymes could be the cause of their slower exchange rate. We

propose a new model of catalysis for bacterial FNR in which $NADP^+$ would interact with two different affinity sites (N and P). The nucleotide tightly bound to the P site (the one of higher affinity) would be released from it only after the nicotinamide of the incoming substrate interacts at the N site. We have cross-substituted EcFPR arginines with proline and tyrosine residues and replaced both amino acids with arginines in PeaFNR. We analyzed all proteins by kinetic, thermodynamic, and stability studies. We found that the EcFPR mutants lost the ability to tightly bind $NADP^+$. Therefore the Arg mutations would be interfering with the $NADP^+$ binding site. In PeaFNR mutants, $NADP^+$ affinity was not affected, thus the substrate-binding mechanism could be different. The presence of $NADP^+$ in the reaction medium only decreased the catalytic efficiency of wild type EcFPR, indicating an inhibition by $NADP^+$. $NADP^+$ binding caused stabilization on wild type EcFPR but not on mutants or PeaFNR. Our results indicate that the high-affinity nucleotide binding is essential for the modulation of the catalytic activity of EcFPR. This phenomenon could be related to a general mechanism of activity regulation in bacterial enzymes.

EN-P10
CHARACTERIZATION OF AN ATYPICAL THIOREDOXIN FROM *ENTAMOEBA HISTOLYTICA* WITH SPECIFICITY FOR CYSTINE REDUCTION

Birocco F, Guerrero SA, Iglesias AA, Arias DG.
Laboratorio de Enzimología Molecular - IAL (UNL-CONICET), Santa Fe (3000) – Argentina. E-mail: darias@fbcb.unl.edu.ar

Entamoeba histolytica, a unicellular parasite, usually lives and multiplies within the human gut, under reduced oxygen pressure. During tissue invasion, it is exposed to increased amounts of reactive oxygen species, which are highly toxic for the parasite. The metabolic pathways used by this organism to cope with such environmental changes and redox homeostasis are a matter of our work. Recently, we characterized in *E. histolytica* its functional thioredoxin system, composed by thioredoxins (TRXs) and thioredoxin reductase (TRXR). In this work, we present the functional characterization of atypical thioredoxin (*Eh*TRX212) with specificity for cystine reduction from *E. histolytica*. By *in vitro* assays, we observed that *Eh*TRX212 was unable to accept reduction equivalents from *Eh*TRXR directly. However, the protein was able to catalyze the reduction of cystine, CySNO and cysteine-derivate low molecular mass disulfides via *Eh*TRXR in presence of *Eh*TRX8 (a canonical TRX). Interestingly, chemical substitutions (for example, N-acetylation) in cysteine moiety prevent the reduction by *Eh*TRX212 of derivative low molecular mass disulfides, indicating substrate specificity by cysteine-moiety in disulfide substrates. In addition, the protein catalyzed GSH-dependant cystine reduction, similar to classic glutaredoxin activity. In line with the above, *Eh*TRX212 was able to coordinate the iron-sulfur cluster (ISC) by an *in vitro* reconstitution assay. By gel filtration chromatography and UV-Vis spectroscopy experiments, we detected *Eh*TRX212-ISC complexes. Complementarily, by the biotin-switch technique, we evaluated the capacity of *Eh*TRX212 to reduce *S*-cysteinylated proteins from *E. histolytica* cells. Finally, we performed confocal microscopy experiments and *Eh*TRX212 has been immunolocalized in the cytosol of trophozoites. This work strongly supports the occurrence in *E. histolytica* of a new TRX, which was not previously described in the parasite. Our results extend the knowledge regarding *Eh*TRX function and suggest that these proteins have important functions in the redox metabolism of this pathogen parasite. Granted by ANPCyT (PICT2016-1778 and PICT2017-2268)

EN-P11
BIOCHEMICAL CHARACTERIZATION OF CATALASE, AN IMPORTANT ANTIOXIDANT ENZYME IN *LEPTOSPIRA INTERROGANS*

Sasoni N, Guerrero SA, Iglesias AA, Arias DG.
Laboratorio de Enzimología Molecular- IAL (UNL-CONICET), (3000) Santa Fe – Argentina. E-mail: sasoni.natalia@gmail.com

Catalase is an abundant protein and a virulence factor in *Leptospira interrogans*. Although the pathogenic and non-pathogenic strains of *Leptospira* have genes coding for catalase in their genomes, these are not homologous proteins and belong to different enzyme groups. Catalase is responsible for tolerance to exogenous oxidative species by *L. interrogans*. It was reported that pathogenic species (such as *L. interrogans*) have strong catalase activity and low peroxidase activity, while nonpathogenic species (such as *L. biflexa*) show only peroxidase activity, which suggests that catalase plays an important role during infection in the mammalian host. In this work, we studied biochemical properties of catalase of *L. interrogans.* The enzyme obtained by recombinant expression in *Escherichia coli* presented a homo-tetrameric structure. An amino acid sequence analysis indicated that the enzyme belongs to mono-functional catalases, however, its functional characteristics suggest that the protein could be a bifunctional enzyme. Both *tert*-butyl hydroperoxide (*t*-BuOOH, an organic hydroperoxide) and HClO exhibited an inhibitory effect on catalase activity. On the other hand, HClO inhibition brought changes in its oligomeric structure, with the consequent formation of high molecular mass oligomers. Alternatively, nitrosocysteine (CySNO) caused substrate-reversible nitrosylation on the protein heme group, without changes in its oligomeric structure. By western blot assays, we detected that catalase relative abundance was modified when *L. interrogans* is exposed to exogenous CySNO or HClO, but not with H_2O_2 or *t*-BuOOH. The results obtained indicate that the structure-function relationship of the protein is regulated in the presence of different types of redox agents. Granted by ANPCyT (PICT2014-2103, PICT2016-1778 and PICT2017-2268).

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

LIPIDS

LI-P01
MICROSOMAL TRIACYLGLYCEROL TRANSFER PROTEIN (MTP) INHIBITION, BY HYPOCHOLESTEROLEMIC DRUG LOMITAPIDE, FAVORS TUMOR GROWTH

Comanzo CG[1], Vera MC[1], Lucci A[1,2], Heit Barbini FJ[3], Ferretti AC[2], Ceballos MP[1], Carrillo MC[1,2], Quiroga AD[1,2,3]
[1]Instituto de Fisiología Experimental (IFISE-CONICET), [2]Área Morfología (FCByF – UNR), [3]CAESIHS (UAI).
E-mail: comanzo@ifise-conicet.gov.ar

Microsomal triacylglycerol transfer protein (MTP) locates in the lumen of the endoplasmic reticulum and participates in the secretion of lipids from the liver as very-low-density lipoproteins. There is evidence that MTP might be involved in other cellular processes, including the pathogenesis of different diseases; however, no studies were performed yet to evaluate whether MTP plays a role in cancer. The MTP inhibitor lomitapide binds directly to MTP, thereby inhibiting the synthesis of triglyceride-rich VLDL in the liver. Therefore, the objective of this work was to study the effect of MTP inhibition on tumor growth. Adult male Balb/c nude mice were subjected to a xenograft model where Huh7 cells (5 x 10^6 per mouse) were injected subcutaneously into the right flank of mice. Four days post-cell inoculation, mice were randomly divided into two groups (8 mice/group). One group (Control) received the vehicle (methylcellulose, gastric probe), and the other group received 5 mg/kg bw/day lomitapide (gastric probe) for 15 days. Tumors were monitored using a caliper, and volumes were estimated based on the formula "1/2 x length x width x height". At the end of the treatment, mice were sacrificed, and tumors were excised and weighed. After treatment, lomitapide-treated mice showed higher tumor volume and weight (2-fold) than control mice. Plasma levels of triacylglycerol and cholesterol were decreased (−30%, and −40%, respectively) in lomitapide-treated mice compared to control mice. Tumor histology analysis showed no differences between groups on tissue architecture and fibrosis; however, lomitapide-treated mice presented with an accumulation of cytosolic lipid droplets. Then, we evaluated proliferation by immunoblotting in total tumor homogenates. We found lomitapide-treated mice presented with increased protein expression of proliferation cell nuclear antigen (PCNA) (+58%) compared to control mice. In line, positive Ki-67-stained nuclei were increased in tumor sections from lomitapide-treated mice. In conclusion, these studies represent the first steps in the evaluation of the role of MTP in cancer development and demonstrate that MTP may be participating in tumor growth.

LI-P02
DOCOSAHEXAENOIC ACID EXERTS ANTIPROLIFERATIVE ACTIVITY ON PANCREATIC CELLS BY SHH AND IL-6 DOWNREGULATION

Garay MI[12], Barotto NN[2], Quiroga PL[2], Silva RA[2], Pasqualini ME[12].
[1] Inst. Invest. Cs. Salud (INICSA - UNC - CONICET), [2]Cat. Biol. Cel. Embr, Histol. FCM-UNC. E-mail: marisabel_1119@hotmail.com

Pancreatic cancer (PC) remains one of the deadliest malignancies worldwide. PC is characterized by activation of the Sonic Hedgehog (SHH) signaling pathway as well as by an increment on IL-6 levels, a pro-inflammatory cytokine. Tumor cells can produce the SHH ligand that functions either in an autocrine or paracrine manner to promote tumorigenesis and survival of the tumors. Studies on many carcinomas demonstrated a ligand-dependent activation of Hedgehog (HH) signaling. Recent evidence has identified the importance of IL-6 in the regulation of SHH secretion in the tumor microenvironment. IL-6 is released in the tumor microenvironment, and its activation results in SHH expression, which, in turn, promotes the expansion of progenitor populations and leads to the re-growth of tumors. This evidence strongly supports the notion that IL-6 may facilitate the production and distribution of SHH to metastatic sites. On the other hand, growing evidence implicates fatty acid-induced signals as contributing to pancreatic carcinogenesis. Dietary ω3 polyunsaturated fatty acids (PUFAs) may have a protective role whereas ω6 PUFAs are associated with greater incidence and growth of pancreatic cancer. Nevertheless, neither the mechanism by which ω3 PUFAs induce suppression of pancreatic tumorigenesis or their effects on the SHH/IL-6 pathway have not been elucidated clearly. In the present work, we studied the effects of two omega-3 PUFAs, the eicosapentaenoic acid (EPA) and the docosahexaenoic acid (DHA) and the omega-6 fatty acid, the arachidonic acid (AA) on SHH/IL-6 expression in a human pancreatic cancer cell line (PANC-1) in order to find new chemotherapeutical approaches. We evaluated gene expression by qRT-PCR, protein production by immunofluorescence and ELISA, cell viability by fluorometry using Resazurin, migration by wound-healing, lipid cell profile by gas chromatography (GC), and eicosanoids generation by high-pressure liquid chromatography (HPLC), respectively. Our results showed that SHH and IL-6 expression were significantly downregulated by DHA in correlation with a decrease in cell viability and migration ($p < 0.05$). Furthermore, DHA induced a significant reduction in proliferative eicosanoids release: 12(S) HETE and 13(S) HODE ($p < 0.05$). Changes in membrane lipids induced by ω3 PUFAs may affect eicosanoid release, modulating cell signaling pathways of IL-6 and SHH. In this sense, we found that DHA downregulated both SHH and IL-6 expression, probably due to the decrease in pro-tumorigenic 12(S) HETE and 13(S) HODE eicosanoids leading to a diminution of pancreatic cell viability and proliferation.

LI-P03
PHOSPHOLIPASES A2: DISTINCTIVE ROLES IN THE REGULATION OF α-SYNUCLEIN BIOLOGY AND NEURONAL REDOX RESPONSE

Iglesias González PA[1], Conde MA[1,2], Uranga RM[1,2], Salvador GA[1,2]
[1]INIBIBB-CONICET, [2]DBByF -UNS, Bahía Blanca, Argentina. E-mail: salvador@criba.edu.ar

Iron (Fe) accumulation and α-synuclein (α-syn) overexpression are hallmarks of several neurodegenerative disorders. We have previously reported that Fe-induced oxidative stress activates fatty acid release catalyzed by different phospholipase A2 (PLA2) isoforms in the nervous system. In

this work, our aim was to study the involvement of PLA2s in the regulation of α-syn biology and the neuronal redox response to Fe overload. We also investigated the role of glia-secreted factors in the neuronal outcome. For this purpose, we exposed human neuroblastoma cells (IMR-32) to different ferric ammonium citrate concentrations (300–1000 μM) or vehicle for different incubation times (24–72 h). Using these experimental conditions, redox status, α-syn expression and phosphorylation, and the participation of calcium-independent and calcium-dependent PLA2 isoforms (iPLA2 and cPLA2, respectively) in the regulation of these events were studied. IMR-32 neurons exposed to Fe overload showed increased expression levels of iPLA2, concomitantly with an increase in lipid peroxides and reactive oxygen species. The pharmacological blockage of iPLA2 activity increased, even more, the levels of lipid peroxides and the content of reactive oxygen species. On the contrary, the inhibition of cPLA2 showed the opposite effect by promoting a decrease in oxidative stress markers associated with increased neuronal viability. Fe-challenged neurons also displayed increased α-syn expression and phosphorylation. The phosphorylation of α-syn was blocked by the inhibition of iPLA2 activity. To study the role of glia in the neuronal response to Fe, C6 astroglioma cells were challenged with ferric ammonium citrate or vehicle, and the astrocyte-derived media were added to neuronal cultures. Astrocytes exposed to Fe showed an increase in the glial marker S100B and lipid peroxidation levels, thus indicating reactivity to oxidative stress. Neurons incubated with the mentioned astrocyte-derived media displayed lower levels of oxidative injury than neurons only exposed to Fe. Astrocytes were positive for the rate-limiting step enzyme for glutathione biosynthesis. Altogether, our results show specific roles for the different PLA2 isoforms in the neuronal response to Fe-induced injury: whereas iPLA2 showed to be neuroprotective and also to be involved in the regulation of α-syn phosphorylation, cPLA2 appeared to act as a damage promoter. To ascertain the nature of the effect exerted by astrocytes on the neuronal response to oxidative stress, we are currently studying glutathione synthesis and how the isoform-specific PLA2-inhibition could be involved. *Sponsored by FONCyT, CONICET, UNS.*

LI-P04
SUPPLEMENTATION OF *EX VIVO* MOUSE TESTES EXPLANTS WITH PUFA-RICH LIPIDS STIMULATES SPERMATOGENESIS

Luquez, JM[1], Santiago Valtierra FX[1], Isoler-Alcaraz J[2], Aveldaño MI[1], del Mazo J[2], Oresti GM[1]
[1]INIBIBB, CONICET y Depto. BByF, UNS, Bahía Blanca, Argentina. [2]CIB, CSIC, Madrid, España. E-mail: jluquez@criba.edu.ar

Using a gas-liquid interphase culture system from neonatal mouse testes, we previously observed in *ex vivo* explants a relationship between the progression of spermatogenesis at both cytological and histological levels and the gene expression of some of the enzymes involved in lipid metabolism. Here, we examined by RT-qPCR the expression of two PUFA elongases (*Elovl2* and *Elovl4*), Δ6-desaturase (*Fads2*), fatty acid 2-hydroxylase (*Fa2h*), two fatty-acid-binding proteins (*Fabp3* and *Fabp9*) and a diacylglycerol acyltransferase (*Dgat2).* Testis explants from 6 days old mice cultured for 22 days evidenced progress in spermatogenesis beyond the meiotic phase in some of the tubules. Although delayed *in vitro* in comparison with the *in vivo* development, in both cases the appearance of haploid germ cells occurred concomitantly with an increase in the expression of *Fabp9, Dgat2*, and *Fa2h*. Interestingly, the genes involved in PUFA synthesis (*Elovl2*, *Elovl4*, *Fads2*) and transport (*Fabp3*) were up-regulated in the testicular explants in comparison with the *in vivo* situation. This suggested, as a possible cause, partial insufficiency in the culture system of the C20-C22 PUFA required as substrates by these biosynthetic enzymes. This proved to be the case, as this medium contained low proportions (less than 4%) of these fatty acids. Supplementation of *ex vivo* explants with a PUFA-rich total lipid extract (TL) from adult mouse testis allowed progression into meiosis at the times in culture examined. Moreover, after 22 days in culture, the TL-supplemented explants contained more tubules with spermatogenic cells that had succeeded to reach the spermatid stage. Thus, in addition to growth factors and hormones, influences that promote the biosynthesis of PUFA-containing lipids are among the factors required to optimize spermatogenesis in *ex vivo* tissue explants. *Supported by FONCyT [PICT2017-2535] and PGI-UNS [24/B272] to GMO and by the MCIyU, Spain [BFU2017–87095-R] to JdM.*

LI-P05
IMPLICATION OF SPHINGOLIPIDS IN EPITHELIAL–MESENCHYMAL TRANSITION PROCESS IN RENAL COLLECTING DUCTS OF AGED RATS

Brandán YR[1], Guaytima EV[1], Pescio Lucila G[2], Favale NO[2], Carbajal Robledo ME[1], Sterin-Speziale NB[2], Márquez MG[1]
[1]Instituto de Investigaciones en Ciencias de la Salud Humana-UNLaR, [2]IQUIFIB-CONICET. E-mail: brandanyamila@gmail.com

The epithelial–mesenchymal transition (EMT) is a process in which the cells lose their epithelial phenotype and acquire the characteristics of mesenchymal cells, which includes loss of cell–cell binding. Renal function declines progressively with age, and the EMT process has been suggested as a mechanism that drives renal fibrosis, with the consequent loss of tissue functions, which occurs mainly in old age. In previous works, we demonstrated that the inhibition of sphingomyelin synthase 1—the enzyme responsible for the synthesis of sphingomyelin (SM) at the Golgi Ap level—induces an EMT process in CD cells from the renal papilla of young, 70-day-old rats. We also demonstrated that the EMT occurs spontaneously in renal papillary CD cells of middle-aged rats (6–8 months), denoted by an impairment of cell–cell adhesion, a higher number of CD cells expressing the mesenchymal protein vimentin, and the *de novo* synthesis of α-smooth muscle actin (α-SMA), another mesenchymal biomarker. These results motivated us to study the possible implication of sphingolipids, and in particular SM, in the occurrence of EMT in renal papilla CD cells during aging. Taking into account that the cells in culture behave as in intact tissue, primary cultures of CD cells isolated from the renal papilla of young and middle-aged rats were performed. Since the occurrence of the EMT process was observed in 6-month-old rats, we performed a recovery experiment using the exogenous addition of 10 μM C12-SM to primary cultured CD cells from middle-aged rats. For this purpose, we simultaneously evaluated the intercellular adhesions by α- catenin immunostaining, and the expression of the mesenchymal biomarker α-SMA. After the addition of exogenous SM, the intercellular spaces between the CD cells disappeared, and α- catenin lined the lateral cell membranes, reflecting the presence of mature adherens junctions. Moreover, the percentage of CD cells that express α-SMA decreased (young vs middle-aged, $p = 0.0006$). We also analyzed the total SM content in CD cells isolated from young, middle-aged and aged-rats (15 months old) by thin-layer chromatography (TLC) and densitometry. Surprisingly, although we observed an EMT in CD cells from middle-aged rats, the quantitative results showed a decrease in SM content only in CD cells isolated from the renal papilla of aged rats (young vs aged-rats, $p = 0.0030$). Taking into account our previous and present results, we conclude that the epithelial-mesenchymal phenotypic conversion that spontaneously

occurs in CD cells during aging is reversible, and can be reverted by the exogenous addition of SM. Altogether, we propose that sphingolipid metabolism plays a central role as a modulator of the fate of renal papilla CD cells during aging.

LI-P06
MOLECULAR MECHANISMS OF EPITHELIAL REGENERATION AFTER OXALATE DAMAGE: THE ROLE OF COX-2

Casali C.,[1-2]; Artuch A[1]; Erjavec L [1-2]; Fernández MC [1-2]
[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Biología Celular y Molecular, [2] IQUIFIB-UBA-CONICET
E-mail: ccasali@ffyb.uba.ar

The renal collecting ducts, which are involved in the urine concentration mechanism, are immersed in an extracellular matrix with the highest body osmolarity. This hyperosmolarity is a key signal for cell differentiation and maturation of the tubular structures and the establishment of the urine concentration mechanism. However, hyperosmolarity can induce cell death when there is a great osmolarity change. Renal cells activate adaptive and protective mechanisms to survive in the hyperosmolar environment. One important cell mechanism is the expression of osmoprotective genes such as cyclooxygenase 2 (COX-2). Moreover, renal ducts are exposed to wastes coming from blood filtration that include several nephrotoxic agents and kidney stones. Calcium oxalate stones are the most common type of kidney stone. The crystal aggregates are harmful to epithelial renal cells and tubular structures that could lead to renal kidney disease. It has been described that oxalate induces COX-2 mRNA and protein expression in renal epithelial cells, but the role of this protein is still unknown. The aim of the present work is to evaluate the role of COX-2 in the regeneration mechanism in differentiated renal epithelial cells treated with oxalate. To do that, the renal epithelial cells MDCK were grown in a hyperosmolar environment (NaCl 125 mM, 512 mOsm/kg H_2O) for 72 h in order to get a differentiated epithelium and then subjected to oxalate (Ox) 1.5 mM for 24, 48, and 72 h. After treatment, the cells were harvested, counted and the cell viability was determined by Trypan blue assay and MTT assay. Cell morphology was also evaluated. The expression of COX-2 was determined by western blot and PCR. The treatment with Ox decreased the number of cells at 24, 48, and 72 h compared to controls 24, 48, and 72 h, respectively. However, the number of cells recovered after Ox 72h was higher than Ox 48 h and Ox 24 h. Cell viability determined by Trypan blue did not change after Ox treatments but the MTT assay showed a decrease at 24, 48, and 72 h compared to controls 24, 48, and 72 h, respectively. The control conditions showed a typical epithelial cobblestone morphology, but the cells treated with Ox for 24 showed a spindle-shaped morphology characteristic of an epithelial–mesenchymal transition. After 48 h of Ox, the cells started to recover their morphology and, after 72 h of Ox, the epithelium is almost reestablished. Control conditions showed a high expression of COX-2 protein and the treatment with Ox decreased their expression compared to controls. However, the expression of COX-2 at Ox 72 h was higher than that at Ox 48 h. The COX-2 mRNA level was low in control conditions at 24 and 48 h but there was an increase at 72 h. The treatment with Ox increased the levels of COX-2 mRNA with a maximum level at 72 h. The results showed that COX-2 could be involved in the epithelial regeneration mechanisms after the damaged of differentiated renal epithelial cells caused by oxalate.

LI-P07
EFFECT OF ESSENTIAL OILS FROM INTRODUCED AND LOCAL PLANTS ON CHOLESTEROL METABOLISM IN HEPATIC AND FOAM CELLS. A SEARCH FOR NATURAL ANTIATHEROGENIC COMPOUNDS

Castro A[1]; Otero C[2], Dumrauf B[1], Rodenak-Kladniew B[1], Montero-Villegas S[1], Viña S[3], García de Bravo M[1], Crespo R[1]
[1]INIBIOLP "Prof. Dr. Rodolfo R. Brenner" (CONICET- CCT La Plata) Fac.de Cs Médicas-UNLP, [2] INIFTA (CONICET- CCT La Plata), [3]CIDCA (CONICET- CCT La Plata), Fac.de Cs Exactas- UNLP. E-mail: mgarcia@med.unlp.edu.ar

High blood cholesterol levels constitute one of the main risk factors for the development of cardiovascular diseases, including atherosclerosis. The aim of this study was to analyze the effects of essential oils (EOs) on cholesterol metabolism through its pathway of synthesis, the mevalonate pathway (MP), and its intracellular accumulation, looking for EOs of local production plants that show the greatest potential to prevent or decrease the atherogenic process. Human hepatic cell line (HepG2) and human THP-1 derived foam cells were treated with *Citrus reticulata*, *Lippia alba* (chemotypes tagetenone, linalool, and dihydrocarvone) and *Melissa calamintha* EOs and with D-limonene and 1,8-cineole (components of the EOs). MTT assays were used to determine working concentrations. Cholesterol synthesis was assessed by incorporation of [^{14}C]acetate in HepG2 cells, and Ro 48-8071 was used as a lanosterol synthase (LSS) inhibitor. Nonsaponifiable lipids were evaluated by radio-TLC. Foam cells were produced using 40 µg/mL oxidized low-density lipoproteins (oxLDL). Lipid droplets content was quantified spectrophotometrically by Oil Red O staining and cholesterol (total, free, and esterified) by commercial kits and TLC. A three-dimensional (3D) foam cell spheroid model was developed using the hanging droplet culture method. Results show that in hepatic cells EOs of *C. reticulata* and *L. alba* decrease the incorporation of [^{14}C]acetate in lanosterol and cholesterol, suggesting an inhibition of the enzyme lanosterol synthase (LSS) and or squalene synthase of MP. The incorporation of [^{14}C]acetate in squalene is up and down but always increases in cells incubated with its major components, D-limonene and 1,8-cineole, indicating LSS inhibition. In all cases, the levels of 2,3-oxidosqualene and / or ubiquinone are increased. Foam cells in 2D cultures show different IC50 values of cell viability in cells treated with the EOs, and the lowest values were obtained with the *M. calamintha* (IC50 =130 µL/L) treatment. Foam cells also show great variability on the content of lipids, mainly cholesterol, when they were incubated with the EOs. *Citrus reticulata* and *L. alba* significantly decreased intracellular lipid levels and cholesterol synthesis. Preliminary assays performed in the foam cells in a 3D culture model suggest similar results. We conclude that EOs from well adapted to local conditions plant species decrease cholesterol synthesis in hepatic cells by LSS inhibition, an enzyme of a post-squalene reaction without diminishing essential isoprenoids as ubiquinone and decrease cholesterol accumulation in foam cells. These results suggest that these EOs have great potential as natural drugs against atherosclerogenesis.

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

LI-P08
EFFECT OF PHOSPHOLIPIDS AND EXOSOMES ON NEURAL STEM CELLS DIFFERENTIATION UNDER OXIDATIVE STRESS

Delgado S, Pelusso C, Banchio C.
Instituto de Biología Molecular y Celular de Rosario IBR-CONICET Rosario, Argentina. E-mail: delgado@ibr-conicet.gov.ar

Neural Stem Cells (NSC) have the capacity to generate and regenerate the nervous system. In the central nervous system, there are niches of NSC that are located mainly in the subventricular zone and in the hippocampus. In case of injury, these cells migrate to the site of the lesion and repair the damaged tissue. However, their capacity is limited, due to their partial potential to replace dead neurons and rebuild the dendritic connections. For these reasons, it is important to evaluate the mechanisms of differentiation for therapies development. Preliminary studies in our laboratory have demonstrated that certain bioactive lipids have a regulatory effect on the fate of NSC. On the other hand, exosomes have been described as a new model of cell communication and have become a novel treatment for several pathologies. For the nervous system, several *in vitro* and *in vivo* studies have demonstrated the effects of exosomes derived from mesenchymal stem cells for the treatment of different injuries, such as stroke, traumatic brain injuries and neurodegenerative diseases such as Alzheimer and Parkinson. Using these two ideas, the aim is to evaluate the effect of certain lipids and exosomes on NSCs differentiation and proliferation under normal, oxidative stress or damage conditions. Mouse embryonic NSCs were exposed to different concentrations of hydrogen peroxide, ferrous sulfate, and cupric sulfate to generate oxidative damage. Moreover, NSCs were cultivated in the presence of the phospholipids after the damage was induced to evaluate their neuroprotective effect. Finally, by immunofluorescence analysis using specific markers, we studied the effect of PtdCho during NSCs differentiation under stress microenvironment. On the other hand, we demonstrated that treatment with exosomes isolated from neural stem cells promotes neuronal differentiation without affecting glial differentiation. In addition, exosomes produce a significative increasing in primary neurite length. According to these results in non-stressed conditions, both molecules can enhance neuronal differentiation, but in reactive condition future studies are needed. Here we provide evidence that reinforces the role of lipids and exosomes as signaling molecules, opening new avenues to restoratives therapies.

LI-P09
ROLE OF SPHINGOSINE KINASE ACTIVITY IN THE COLLECTING DUCT FORMATION DURING POSTNATAL RENAL DEVELOPMENT

Guaytima EV[1], Brandán YR[1], Saavedra Campillay JZ[1], Sterin-Speziale NB[2], Márquez MG[1]
[1]Instituto de Investigaciones en Ciencias de la Salud Humana, Universidad Nacional de La Rioja. [2]IQUIFIB-CONICET
E-mail: edithguaytima@hotmail.com

In mammals, nephrogenesis is completed postnatally. A characteristic of mammalian kidneys is the hypertonicity of the interstitial fluid in the renal papilla, which induces and maintains the differentiation of collecting duct (CD) cells. The osmolality of the renal papilla is much lower in neonatal rats than in adults and increases dramatically after weaning. Previously, we demonstrated that the sphingosine kinase (SK)/sphingosine 1 phosphate (S1P) pathway is developmentally regulated, leading sphingolipid metabolism to the formation of S1P in the neonatal period, which is consistent with the immature-proliferative stage of neonatal rat renal papilla. Thus, the aim of this work is to study the involvement of SK activity during postnatal CD development. Taking into account that the cells in culture behave as in intact tissue, primary cultures of CD cells isolated from the renal papilla of 10-day-old rats were performed. During the postnatal development, the renal papilla gradually acquires a hypertonic (HT) medium. To mimic this physiological condition, cultured CD cells were subjected to gradual increases of NaCl concentration until it reached the final concentration of 200 mM. D,L-threo-dihydrosphingosine (tDHS) was used as an SK activity inhibitor. CD cell morphology was analyzed in isotonic (ISO) and HT medium by phase-contrast microscopy and F-actin distribution, and the degree of differentiation was evaluated by immunofluorescence analyzing adherens junction (AJ) (E-cadherin, β- and α-catenin) and the presence of primary cilium. In ISO medium, cultured cells displayed an elongated morphology with an irregular distribution and intensity of AJ protein immunostaining, proper of immature epithelial cells. By contrast, HT medium induced a more mature cell phenotype, reflected by a morphological change from an elongated to a hexagonal shape, and a uniform and enhanced positive signal for AJ proteins. Likewise, a greater number of CD cells with primary cilium was observed. When cells were treated with tDHS 24 h before being subjected to HT, cell-cell adhesion was impaired, denoted by an irregular intensity and distribution of AJ protein immunostaining. To evaluate the effect of the inhibition of SK activity in already differentiated CD cells, tDHS was added to the culture after the cells were subjected to HT medium. In this condition, the epithelial morphology was not altered. Altogether, these results suggest that SK activity is necessary for the acquisition of the epithelial differentiated phenotype of CD cells in the physiological HT environment of the renal papilla, during postnatal development.

LI-P10
ANALYSIS OF SPHINGOLIPIDS FOR LIPIDOMICS USING MALDI AND LC-ESI MASS SPECTROMETRY

Pescio LG, Paván CH, Francisco MN, Sterin–Speziale NB.
Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. LANAIS PRO EM. IQUIFIB-CONICET. E- mail: lucilagpescio@ffyb.uba.ar

Sphingolipids are a complex family of bioactive lipids that are involved in many important processes such as cell signaling, cell differentiation, proliferation, cell survival and apoptosis. It is demonstrated that the different subspecies of sphingolipids containing different fatty acids have distinct functions. The most powerful and specific techniques for the identification and quantification of the molecular species of sphingolipids

are chromatography combined with mass spectrometry. In this work we analyzed sphingolipid standards and sphingolipid extracts from epithelial cell cultures by TLC - MALDI and LC – ESI MS, and compared both methodologies. The TLC was developed in two solvent systems for the separation of ceramide (Cer) and glucosylceramide (GlcCer) and stained with primuline. The bands were scraped off the TLC, extracted, mixed with the matrix (2,5-dihydroxylbenzoic acid, DHB) and analyzed using a 4800 MALDI TOF/TOF™ analyzer. We detected different subspecies of Cer, GlcCer, LacCer and sphingomyelin: d18:1/C16:0, d18:1/C18:0, d18:1/C20:0, d18:1/C22:0, d18:1/C24:1 and d18:1/C24:0. For ESI analysis, the sphingolipid extracts were separated by LC using different mobile phases and reverse phase columns. The equipment used was a LCQ-Duo ESI -IT. The comparison of both techniques showed that MALDI TOF TOF MS analyses required short times of fine tuning and detection, but need prior separation of the sphingolipids present in the lipid extract by TLC, since each revealed band was scraped, extracted from the silica and analyzed by MALDI TOF TOF to avoid signal suppression. Moreover, the spectra showed severe matrix background and the presence of multiple adducts that complicates quantitation. On the other hand, LC - ESI MS simplified the procedure for lipid analysis, since the complete lipid extract was injected directly into the column, allowing the simultaneous separation and detection in the same assay. However, the fine tuning of LC – ESI MS was more laboriously because of different variables must be adjusted: mobile phase composition and additives, type of column, flow rate, type and optimization of detection and fragmentation conditions. Furthermore, the results obtained from LC–ESI MS were more complex than MALDI MS results, complicating mass spectral interpretation. The selection of the technique for sphingolipid analyses depends on the complexity of the sample and the objective of the investigation.

LI-P11
PROINFLAMATORY MEDIATORS AFFECT NEURAL DIFFERENTIATION OF MURINE EMBRIONIC NEURAL STEM CELLS

Magaquian D, Perez C, Banchio C.
Instituto de Biología Molecular y Celular de Rosario - IBR-CONICET. E-mail: magaquian@ibr-conicet.gov.ar

Neuroinflammation is a common feature of acute neurological conditions such as stroke and spinal cord injury, as well as neurodegenerative conditions such as Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis. Previous studies have demonstrated that acute neuroinflammation can adversely affect the survival of neural stem cells (NSCs) and thereby limit the capacity for regeneration and repair. However, the mechanisms by which neuroinflammation induces NSCs death remain unclear. Macrophages are key mediators of neuroinflammation and when activated to induce a pro-inflammatory state secrete a number of factors that could affect NSCs survival. To investigate the effects of inflammatory cytokines on NSCs survival and differentiation, we utilized the RAW 264.7 mouse macrophages cell line as a homogeneous and renewable source of immune cells. We first examined whether RAW 264.7 cells activated by the bacterial endotoxin lipopolysaccharide (LPS) induced a pro-inflammatory phenotype that secrete soluble factors that could promote NSCs apoptosis. A RT-PCR analysis was performed in order to assess the presence of IL1β, IL6 and TNFα RNAm. In the present study we demonstrate that inflammatory mediators produced by LPS-activated RAW 264.7 macrophages affect NSCs survival after 72 and 96 hours of culture in a dose dependent manner. Furthermore, to determine whether the cytokines had any effect on neural stem cell differentiation, medium from LPS-stimulated macrophages was added to the dissociated stem cells at the time of plating for differentiation. By immunofluorescence analysis using neuronal and astroglial markers we demonstrated that the percentage of total βIII-tubulin positive cells increased by 3–4-fold within 72-hour treatment suggesting that the inflammatory microenvironment created during brain damage or neurodegenerative conditions could influence the ability of NSCs to repair damage. Additionally, we investigated the effects of medium from LPS-stimulated macrophages on the ability of NSCs to proliferate and generate new neurospheres. To determine the inflammatory factor involved in the effect, similar analysis was performed with pure IL1β or IL6. As phosphatidylcholine has a stimulatory effect on neuronal differentiation, we also evaluate this effect under inflammatory conditions. In conclusion, we demonstrated that inflammation induces neuronal differentiation, perhaps, as a mechanism to regenerate the damaged tissue.

LI-P12
EFFECT OF RESVERATROL ON RENAL COX-2-MEDIATED OSMOPROTECTIVE MECHANISMS

Erjavec LC, Casali CI, Fernández Tome MC.
UBA, Facultad de Farmacia y Bioquímica, Cátedra Biología Celular y Molecular- CONICET, IQUIFIB. E-mail: luerjavec@gmail.com

Resveratrol (*trans*-3,4′,5-trihydroxystilbene, RSV) is a stilbene polyphenol present in a large variety of plants such as mulberries, peanuts and grapes. In the last years RSV intake has increased, as it is sold as an over-the-counter dietary supplement due to its antioxidant, anti-inflammatory and antitumoral properties. However, it has also been reported that RSV may behave as a pro-oxidizing agent; thus, it may also have implication in pathology of diseases. Some studies suggest that RSV can modulate different pathways at a time, resulting in distinct biological effects depending on the cell type, its concentration or treatment time. In renal tissue, many studies describe the beneficial effects of RSV in kidney injury models; however, some studies detected nephrotoxicity in animal models subjected to chronic doses of RSV. Hence, RSV effects on renal tissue are still controversial. Renal medullary interstitium is characterized by an elevated osmolality due to the presence of high concentrations of sodium and urea. Depending on the hydric state of the body, renal interstitial osmolality can abruptly vary, reaching values up to 800-1200 mOsm/kg H_2O. To survive in such an adverse environment, protective pathways are activated. We have demonstrated that renal epithelial cells (MDCK) subjected to high osmolality undergo an adaptive process during the first 24 h, in which the transcription of several osmoprotective genes (sodium/myo-inositol transporter (SMIT), sodium/chloride/betaine transporter (BGT1), aldose reductase (AR), and cyclooxygenase 2 (COX-2), among others) is activated. After 48h, these cells are already adapted and acquire a polarized epithelium morphology. In the present work, we evaluate the effect of RSV on adaption and differentiation mechanisms, focusing particularly in COX-2 expression. To do this, MDCK cells were treated with different concentrations of RSV (1, 5, 10 and 25 μM) for 30 min and then cultured in hyperosmotic medium (NaCl 125 mM, 500 mOsm/kg H_2O) for 24 and 48h. Cells were then harvested to obtain cell number and viability and processed for protein or RNA extraction. Cell morphology was also

analyzed. Cell number recovered after RSV treatment was significantly lower in a concentration-dependent manner after 24 and 48h of hyperosmotic challenge. Cells treated with RSV did not reach typical epithelium morphology; moreover, cells treated with the highest concentrations showed a mesenchymal phenotype after 24 and 48h. mRNA expression of osmoprotective genes (COX2, BGT1, SMIT, AR) assessed by RT-PCR showed a decrease only after treatment with 25 µM of RSV, but no significant changes were found after treatment with 1, 5 or 10 µM. COX-2 protein expression was also evaluated by Western Blot and surprisingly, it was significantly upregulated by RSV treatment in a concentration-dependent manner at 24h. These results suggest that in renal cells RSV pretreatment did not affect osmoprotection but impeded monolayer differentiation.

LI-P13
THE IMPLICATION OF SPHINGOSINE-1-PHOSPHATE RECEPTOR 2 (S1PR2) IN EPITHELIAL CELL MIGRATION AND DIFFERENTIATION

Romero DJ, Santacreu, BJ, Tarallo E, Herrera P, Otero D, Favale NO.
Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, IQUIFIB-CONICET, Argentina. E-mail: danielaromero05@gmail.com

Epithelial cell differentiation is a process that involves the mesenchymal to epithelium transition (MET) and includes cell cycle arrest, cell-cell junction maturation in addition to changes in cell migration capacity. The epithelial-mesenchymal transition (EMT) is a dynamic process by which fully differentiated epithelial cells can acquire a mesenchymal phenotype. During EMT, cell adhesion and apical-basal polarity are lost, and the cytoskeleton is reorganized. Previous results from our laboratory shown that in Madin-Darby canine kidney cells (MDCK) under different culture conditions can achieve different stages of differentiation resembling MET. Sphingosine 1-phosphate (S1P) is a bioactive sphingolipid, produced by the phosphorylation of sphingosine by sphingosine kinases (SPHK), which is involved in different processes such as proliferation, cell growth, differentiation and migration. S1P can act both intracellularly as a second messenger and extracellularly as a ligand of 5 different G protein coupled receptors (S1PR1-5). In the present work we evaluated the importance of S1P acting on S1PR2 in the modulation of cell migration and its association with cell differentiation of MDCK cells. We found that there are differences in the migratory profile of MDCK cells that depends both on the differentiation stage and S1PR2 activity. In addition, we found that in differentiated cells, migration depends on EMT. Inhibition of S1PR2 triggers changes in EMT markers, such as rearrangements of the actin cytoskeleton, expression of vimentin and nuclear translocation of beta-catenin, as well as slug. The expression levels of S1PR2 in the different stages of differentiation of MDCK cells did not show significant differences. Instead, immunofluorescence studies showed that during cell differentiation, S1PR2 was progressively enriched at the plasma membrane. These results suggest that the location of S1PR2 depends on the stage of cell differentiation and this determines its role. S1PR2 can either reduce migration and this would be necessary for a successful MET and, on the other hand, this receptor can promote migration, through the stimulation of EMT.

LI-P14
TRIACYLGLYCERIDE (TAG) METABOLISM REGULATION BY HYPEROSMOTIC ENVIRONMENT IN RENAL EPITHELIAL CELLS.

Parra L, Weber K*, Casali C, Fernández Tome M, *These authors contributed equally to this work.*
Cátedra de Biología Celular y Molecular, Facultad de Farmacia y Bioquímica, UBA. IQUIFIB CONICET-UBA. E-mail: le.parra@live.com

Hyperosmolarity is a key controversial signal for renal cells. Under physiological conditions, it induces renal cell differentiation and maturation of urine concentrating system. However, abrupt changes in environmental osmolarity may also induce cell stress that can lead to death. Both conditions require lipid synthesis either for membrane expansion or for osmoprotection. In Madin-Darby canine kidney (MDCK) cells we showed that hyperosmolarity upregulates phospholipid (PL) as well as triglycerides (TAG) de novo synthesis. We also showed that hyperosmolarity activates SREBP-mediated transcriptional regulation of lipogenic genes such us lipin and diacylglycerol acyltransferase (DGAT) enzymes. In the present work we evaluated which signaling pathway mediates hyperosmolarity upregulation of lipid. MDCK were subjected to hyperosmolality (298-512 mOsm/kg H2O) for 48h, treated with different phospholipases (PLA2, PLD, PLC-PI and PLC-PC) or kinases (PI3K, PKC or MAPKs) inhibitors and labeled using [14C]-Glycerol. After treatments, lipids were extracted, separated by TLC and quantified. Neither MAPKs inhibitors nor PKC and PI3K were mediating TAG, but not PL, synthesis. PLC-PI and PLC-PC inhibitors increased PL and TAG synthesis; PLD activity was also involved in PL and TAG homeostasis. cPLA2 inhibitors prevented hyperosmotic-induced lipid synthesis. Such decrease seemed to be due to a down regulation of lipin2 and DGAT1 and DGAT2 expression which were evaluated by RT-PCR. As PLA2 activity generates arachidonic acid (AA) to form prostaglandins (PGs), we evaluated the effect of PGs synthesis inhibitor in lipid synthesis; either indomethacin or NS398 significantly increased lipid metabolism. Thus, hyperosmolar induced lipid metabolism is modulated by different signaling systems, specially by the axis PLA2 – COX2.

LI-P15
ROLE OF SPHINGOSINE-1-PHOSPHATE RECEPTOR 3 IN EPITHELIAL CELL DIFFERENTIATION

Tarallo E, Romero DJ, Santacreu BJ, Pescio LG, Favale NO
Cátedra de Biología Celular y Molecular, FFYB-UBA, IQUIFIB-CONICET, Argentina. E-mail: estefaniatarallo@gmail.com

Madin-Darby canine kidney cells (MDCK) acquired a differentiated phenotype when they are cultured in hypertonic medium. Previous works from our lab have demonstrated that sphingolipid metabolism is involved in such differentiation process. One of the most active sphingolipid is sphingosine-1-phosphate (S1P), that has been classically associated with the induction of a proliferative phenotype and anti-apoptotic activity. However, the participation of S1P in cell differentiation is not well understood. S1P can act both intracellularly as second messenger and

extracellularly as ligand for cell surface receptors (S1PRs). We have reported that S1P levels decreases during transition to the differentiated state and S1P acts by its receptors. In this work we evaluated whether changes in the expression and/or localization of S1PRs are involved in the differentiation process. Immunofluorescence studies showed that during cell differentiation S1PR3 was located in intracellular vesicles. However, S1PR3-vecicles changed their intracellular localization depending on cell differentiation state. In proliferative state S1PR3 was in all the cytoplasm, but was relocated to sub-apical distribution during differentiation process. At the final stage of cellular differentiation, where apical domain and primary cilium were present, S1PR3-vecicles signal decreased. Moreover, pharmacological inhibition of S1PR3 accelerated the apical membrane establishment. These results suggest that S1PR3 need to be inactivated/degraded for final epithelial cell differentiation, which propose a possible new function for the S1P/S1PR3 pathway in phenotypical epithelial cell differentiation.

MICROBIOLOGY

MI-P01
DEVELOPMENT OF A MULTIPLEX PCR SYSTEM FOR MULTI-PATHOGEN DETECTION IN ALFALFA PLANTS

Jaime CL[1,2], Gieco JO[1], Jáuregui JM[1], Dunger G[1,2]
[1]Facultad de Ciencias Agrarias, Universidad Nacional del Litoral. [2] CONICET. E-mail: caami_j@hotmail.com

Alfalfa (*Medicago sativa* L.) is a perennial legume plant, also known as queen of forages due to its high content of minerals and vitamins. In addition, it has 22% of its weight in proteins, which makes alfalfa an excellent forage for animal feed. Nevertheless, numerous diseases affect this plant. In Argentina, around 20 different diseases have been found in the last years. This leads not only to economic losses, also to a decrease in the soil's quality, more predisposition to pests, etc. Argentina is the second largest producer of alfalfa in the world, with a cultivated area of more than 3.2M hectares. Due to the importance of this crop in our country, an efficient mechanism of detection of pathogens is needed. This will lead to an improvement in the management of the crop and will facilitate the control of pests. Therefore, we developed a multiplex PCR procedure which will rapidly and simultaneously detect the presence of the most important fungal and bacterial pathogens in alfalfa plants. Fifteen samples of alfalfa plants were collected from different regions of the province of Santa Fe (Argentina) and were used to calibrate the multiplex PCR protocol. Moreover, Geographical Information System (GIS) was used to provide a representation and helps to the integration of the information collected from the PCRs results. Taken together, these results will facilitate the management of alfalfa crops by rural producers and the multiplex PCR technology could be used for multi-pathogen detection all over the world.

MI-P02
PACHYPODANTHINE INHIBITS VIRULENCE MARKERS OF *YERSINIA ENTEROCOLITICA* AT SUB-INHIBITORY CONCENTRATION

Di Marco N [1,3], Pungitore C [1,3], Lucero Estrada C [2,4]
[1]Área de Química Orgánica. [2]Área de Microbiología. FQBF. UNSL FQBF. UNSL, [3]INTEQUI-CONICET- San Luis, [4]IMIBIO-CONICET- San Luis. E-mail: nataliadimarc@gmail.com

Most bacteria live as heterogeneous congregations of surface associated microorganisms encapsulated within a self-produced polymer matrix called biofilms. This matrix protects cells in biofilms from the detrimental effects of chemical insults and harsh environmental conditions. An estimated 80% of all bacterial infections are biofilm related. Within biofilms, cells use a communication system known as quorum sensing (QS) to coordinate and cooperate for a joint behavior. QS in Gram-negative bacteria is coordinated by acil homoserin lactones (AHLs) using the LuxI/LuxR-type system, corresponding to AHLs synthase and the response regulator, respectively. Aporphinoid alkaloids are abundant secondary metabolites in plants and represent one of the most widespread classes of compounds endowed with multiple and varied pharmacological properties. Various aporphinoids were described as antimicrobials but no reports have been found on their inhibiting biofilm abilities. Recently, we have demonstrated the antimicrobial activity of an aporphinoid alkaloid named pachypodanthine against *Yersinia enterocolitica* (*Ye*) with MIC value of 100 µM. Inhibiting biofilm formations could be a novel therapeutic against antimicrobial resistance problems. Focusing on this "antipathogenesis" therapy and considering that plants provide unlimited opportunities for new drugs, the aim of this work was to examine a set of aporphinoid alkaloids as possible *Ye* biofilm inhibitors at sub-inhibitory concentrations. The strain used was *Ye* CLC001 bio-serotype 1A/O:7,8-8-8,19: isolated from pork sausage in our laboratory. Minimum biofilm inhibitory concentration (MBIC) was assayed by crystal violet technique. The QS technique was performed using the biosensor *Chromobacterium violaceum* CV026. Expression of QS *Ye* genes (*luxI: yenI* and *luxR: yenR* homologues) was analyzed by RT-PCR and scanning electron microscopy (SEM) was performed after coating biofilm with gold. Results revealed that pachypodanthine inhibited *Ye* biofilm formation at 12.5 µM, suggesting that it behaved as biofilm formation inhibitor at sub-inhibitory concentrations. In addition, QS was clearly affected, because AHLs production was inhibited by 55.17 ± 4.87 %, *yenI* gene was overexpressed and *yenR* gene expression was reduced. Biofilm morphology changes were observed by SEM, including monolayer cells added to the surface with a mixture of sparse palisade distribution, randomized cells with irregular edges, and heterogeneous bacillus length and thickness. We concluded that pachypodanthine behaves as biofilm formation and QS inhibitor at sub-inhibitory concentrations against *Ye*. The advantage of this alternative approach is that these new anti-microbials could be able to inhibit virulence rather than growth. Consequently, the problems of resistance associated with many bactericidal or bacteriostatic drugs could be avoided.

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

MI-P03
FUNCTIONAL CHARACTERIZATION OF PROBIOTIC SURFACE LAYER PROTEIN OF *LACTOBACILLUS ACIDOPHILUS* ATCC 4356

Fina Martin J, Ruzal SM, Barquero AA.
Dep. Química Biológica FCEN-UBA IQUIBICEN-CONICET. E-mail: sandra@qb.fcen.uba.ar

The surface layer protein (Slp) of *Lactobacillus acidophilus* ATCC 4356 is non-covalently bound to the outmost bacterial cell wall but its involvement in the adherence of bacteria to host cells remain unknown. We have previously described that the SlpA presents lectin-like activity located in the carboxyl-terminal portion of the protein, interacting with prokaryotic (peptidoglycan and lipoteichoic acids) and with eukaryotic (mucin) macromolecules, as well as with viruses, bacteria, yeast and blood cells. However, the amino-terminal portion is responsible for self-assembly and interaction with extracellular matrix (ECM) proteins, fibronectin and collagen. Here, we study the interaction of purified SlpA with intestinal epithelial cells and its ability to modulate the infection of herpes simplex type 1 virus (HSV-1). First, we observed that SlpA bound to the surface of Caco-2/TC7 and HT29 intestinal cells using indirect immunofluorescence (IFI) assay with Slp-specific antibodies. Then, we found that SlpA had no antiviral action against HSV-1 multiplication in HT29 cells. Moreover, we determined that the purified protein was able to protect HSV-1 from heat inactivation, probably through its carbohydrate recognition domains since this effect was inhibited by addition of D-mannose. In summary, our results suggested that SlpA mediates the adherence of bacteria to intestinal cells and interacts with viral glycosylated components.

MI-P04
BP1492 AN EXCLUSIVE *BORDETELLA PERTUSISS* DIGUANYLATE CYCLASE INVOLVED IN BIOFILM AND RESISTANCE TO ACIDIC STRESS

Zacca F, Fernández J, Sisti F
IBBM-CCT-CONICET-La Plata, Dto de Cs Biológicas, FCE, UNLP, La Plata, Argentina. E-mail: fedehzacca@gmail.com

Bordetella pertussis is a Gram-negative strictly human pathogen of the respiratory tract and the etiological agent of whooping cough. Despite the high vaccination coverage among children, pertussis is considered a re-emerging disease. Nearly all virulence factors in *B. pertussis* are activated by a master two-component system, BvgAS. However other regulators systems are present in B. pertussis and needs further research. In the present work we focused on the role of the second messenger c-di-GMP, a key signaling molecule that regulates many important physiological processes in bacteria. Particularly we described for the first time a unique diguanylate cyclase (DGC), BP1492. DGC synthesizes c-di-GMP from two GTP molecules and are present in most bacterial genomes. BP1492 is present in all *B. pertussis* isolates sequenced to date and there are not homologues in other Bordetella species. Tipically, overexpresion of DGC in heterologous or homologous bacteria augments c-di-GMP intracellular concentration and enhanced biofilm formation. However, after multiple efforts to express *bp1492* from plasmid in different bacteria, DGC activity has not been evident for bp1492. BP1492 present a GAF domain in the N term portion. We speculated that a signal sensed by this domain may trigger DGC activity. This hypothetical signal may be absent in the experiments we did. To study the role of this DGC in *B. pertussis* we interrupted *bp1492* gen and evaluate phenotypes previously described as c-di-GMP regulated in other bacteria. We evaluated biofilm formation over plastic surface with cristal violet technique. Wild type *B. pertussis* presented values similar to reported *B. pertussis* biofilms. However, the BP1492 mutant was able to produce as high values as the wild type strain. Resistance to acidic stress is a phenotype regulated by c-di-GMP in other bacteria. During infection *B. pertussis* is exposed to acidic conditions when phagocyte by host cells. We speculate that c-di-GMP may regulate response to acid. To evaluate this, we exposed wild type and mutant strains to pH = 4 and determinate viable bacteria after treatment. Interestingly, mutant strain presented a significant impaired survival at acidic pH. However, we did not observed differences in tolerance to oxidative stress induced by hydrogen peroxide. Overall, we described for the first time a *B. pertussis* exclusive DGC, BP1492. We showed that this DGC and thus the second messenger, c-di-GMP is involved in biofilm formation and resistance to acid in *B. pertussis*.

MI-P05
INVOLVEMENT OF TRANSCRIPTIONAL REGULATORS IN SUCROGLUCANS METABOLISM IN A HETEROCYST-FORMING CYANOBACTERIUM

Giarrocco LE[1,2], Marcozzi C[1], Cumino AC[1,3], Salerno GL[1,3]
[1]Centro de Investigaciones Biológicas, FIBA, Mar del Plata. [2]CIC, Prov. Bs.As. [3]FCEyN-UMMdP and CONICET.
E-mail: lgiarrocco@fiba.org.ar

The filamentous, nitrogen-fixing cyanobacterium *Nostoc* sp. strain PCC 7120 accumulates sucrose and sucroglucans as compatible solutes against salt stress. The expression of the enzymes involved in sucrose metabolism (SPS and SuS, encoded by *sps* and *susA* genes) are increased in the presence of sodium chloride. Additionally, we had shown that those genes exhibited an opposite expression regarding the nitrogen source (with combined nitrogen or under diazotrophic conditions). However, the mechanism underlying the regulation of sucroglucans metabolism remains unknown. The aim of this work was to investigate the participation of two transcriptional regulators in the expression of genes involved in the oligosaccharide metabolism, such as *susB* (a gene homologous to *susA*). We analyzed the expression of *susB* in two insertional mutants derived from *Nostoc* sp. PCC 7120 (SA6 and CSE). While the transcript levels were higher in salt-treated cells of *Nostoc* sp. PCC 7120 that accumulated sucroglucans, cells from the SA6 mutant did not synthesize them in the presence of sodium chloride and were more sensitive to the stress. On the other hand, after a nitrogen-step down, the transcript levels of *susB* were higher in cells of the CSE mutant (which has interrupted a gene encoding a transcription factor involved in the global nitrogen regulation and in diazotrophic growth), analyzed by primer extension experiments. We conclude that sucroglucans metabolism in *Nostoc* is

regulated at the transcriptional level when cells are exposed to salt and depends on the nitrogen source. Further studies are needed to understand the intricate regulation network that could be modulating the synthesis and degradation of the polymers. *Supported by UNMdP (EXA841/17), CIC, CONICET, and FIBA.*

MI-P06
BIOPHYSICAL AND ANTIBACTERIAL STUDIES OF ESSENTIAL OIL FROM LEAVES OF *SCHINUS AREIRA*

Cutro AC[1,2], Castelli MV[3], López SN[3], Rosales M[4], Hollmann A[1], Rodriguez S[4]
[1]CIBAAL -CONICET UNSE, [2] Fac. Cs Médicas-UNSE.,[3]Fac. Cs Bioq. y Farm.- CONICET UNR [4]Fac. Agronomía y Agroindustrias -UNSE – CONICET. E-mail: cutro.andrea@gmail.com

The genus *Schinus* (Anacardiaceae) is a native species from the north-west region of Argentina, which different traditional uses in medicine have been reported. However, only a few studies have been conducted screening the antimicrobial properties of *Schinus areira* essential oil (E.O.). In this context, *S. areira* E.O. isolated from leaves of plants located in Santiago del Estero was obtained by hydrodistillation; and its chemical composition and antibacterial action were determined. Bioautography assays have shown that *S. areira* E.O. presents antibacterial activity on *Staphylococcus aureus* but no effect was observed in *Escherichia coli.* The minimal inhibitory concentration and minimal bactericidal concentration were determined for *S. aureus* and the values obtained were 64 µg/mL and 256 µg/mL, respectively. In order to better describe the effect of *S. areira* E.O. on bacterial envelopes, changes of Zeta potential (ΔZ) and bacterial viability were determined at different E.O. concentrations. At concentrations higher than 200 µg/mL of E.O, a significant increase in ΔZ was noticed. This behavior was related to a significant decrease in the bacteria viability. Furthermore, significant ΔZ decrease of DMPC: DMPG(5:1) liposomes that mimic bacterial membranes, was observed after the addition of increasing E.O. concentrations. These results could indicate a direct and differential interaction of certain chemical components of *S. areira* E.O. with bacterial surface and membrane. Besides, further studies should be carried out. The fact that the E.O. exhibited a high antibacterial effect against *S. aureus* represents an important milestone for the future development of antimicrobial compounds.

MI-P07
MOLECULAR STUDIES AIMED AT THE IDENTIFICATION AND BIOCONTROL OF PLANT PATHOGENS

Príncipe A[1-5], Caligiore-Gei P[2], López JL[3], Pucci GN[4], Puche R[1], Medeot D[1-5], Jofré E[1-5].
[1]Departamento de Ciencias Naturales, FCEFco.Qcas y Naturales, UNRC. [2]INTA-EEA La Consulta. [3]IBBM-UNLP. [4]CEIMA-UNPSJB, [5]INBIAS-CONICET.
E-mail: aprincipe@exa.unrc.edu.ar

One of the main impacts of climate change on different crops is the outbreak of bacterial diseases that were previously of low incidence. The effects of bacterial diseases include yield loss and impairments of product quality in different plant species, particularly intensive crops like fruit trees and vegetables. The management of these emerging pathogens is mainly based on chemicals derived from copper, a heavy metal that may cause a negative impact on the environment. Hence, biological control emerges as an important alternative for integrated diseases management. This strategy is based on employing live microorganisms to reduce and/or maintain the population of a plant pathogen below the levels, which cause economic loss. Thus, the use of these eco-friendly formulations is an effective tool, useful to avoid the negative effects of the chemical treatments. The evaluation of the effectiveness of bioformulations is commonly performed against reference pathogenic strains, which usually do not respond in the same way as native phytopathogenic strains. For this reason, the main objective of this work was to characterize, at the molecular and genetic level, native pathogenic bacteria as well as to address biocontrol studies using different *Bacillus* formulations. Eleven bacterial strains were isolated in the Valle de Uco region, Argentina, where they cause emerging diseases in different crops, including tomato, onion, walnut and garlic. The strains were obtained from diseased plants and confirmed as pathogen following Koch's postulates. They were later characterized through their fatty acid profiles and bio-typed by MALDI-TOF. In addition, the 16S *rRNA* gene of each strain, amplified using universal primers rD1 and fD1, was sequenced. Biocontrol tests were performed under in-vitro conditions, challenging the bacterial pathogenic strains against different *Bacillus* formulations. The results showed that the strains belonged to different species grouped in the *Pantoea*, *Xanthomonas* and *Pseudomonas* genera. The in-vitro biocontrol tests demonstrated that both, the *Bacillus* strains (ARP2-3, MEP2-18, A6, and A7) and their metabolites, released to the culture medium, showed promising antagonistic activity against the evaluated phytopathogenic strains.

MI-P08
CATONIC PEPTIDE ENTEROCIN CRL35 IS A PROMISING ALTERNATIVE TO CLINICAL ANTIBIOTICS AGAINST CLINICAL ENTEROCOCCI

Masias E [1], Córdoba JM. [1], Aráoz EN, Farizano JV, Saavedra L [2], Minahk C [1]
[1](INSIBIO(CONICET/UNT) [2] CERELA (CONICET). E-mail: emilsemasias@gmail.com

Among the opportunistic pathogens that are isolated from nosocomial infections, *Enterococcus faecalis* and *E. faecium* are particularly tricky because they are often difficult to treat due to their intrinsic and acquired resistance to a number of conventional antibiotics. As a matter of fact, the emergence and unstopable spreading of antibiotic resistance not only in enterococci but in many other bacteria species is one of the most urgent concerns of the health systems worldwide. That is why new treatment options that allow to fight against these multidrug-resistant microorganisms

are critically needed. In this regard, the antimicrobial peptides surge as promising novel antibiotics that might help to control resistant isolates. Even though there are a myriad of cationic peptide displaying antibacterial activity, the bacteriocins from lactic acid bacteria constitute interesting candidates because the specificity and the particularly high specific activity. Among them, enterocin CRL35 has been studied in our group since a long time ago, and many features of this peptide have been characterized. This a pediocin-like bacteriocin produced by *Enterococcus mundtii* CRL35 that had been almost exclusively considered for the control of the foodborne pathogen *Listeria monocytogenes* so far. However, since bacteriocins are typically active against phylogenetically related bacteria, we hypothetized that enterocin CRL35 should be active against clinical isolates of *E. faecium* and *E. faecalis*. We focused on the strains isolated from patients with invasive infections in the Angel C. Padilla Hospital from San Miguel de Tucumán city (Tucumán, Argentina). Thirty-four strains were isolated and characterized. The resistance pattern of each isolate was determinated using qualitative (agar diffusion) and quantitative (minimum inhibitory concentration) techniques according to the Clinical and Laboratory Standards Institute recommendations. VITEK 2® Compact automated system was used as a second approach to confirm the previous characterization. Antimicrobial activity of enterocin CRL35 was initially evaluated by the cross-streak method. Subsequently, the MIC was determined with the agar diffusion method and then with serial dilutions in liquid medium. The results showed that 90% of all strains under study were sensitive to the cationic peptide. Most importantly, those isolates that were labeled as resistant to ampicillin, gentamicin and even vancomycin turned out to be sensitive to enterocin CRL35. The sensitivity was also checked by kill curve assays. We observed a rapid lose of viability of all the enterococci tested. Moreover, some bacteria proved to be hypersensitive to the peptide, undergoing even cell lysis with the highest concentrations tested. Overall, we can conclude that enterocin CRL35 represents a good candidate for the treatment of enterococcal infections.

MI-P09
THE NEGATIVE REGULATOR NfxB: A SHARED MECHANISM UNDERLYING BIOFILMS AND ANTIBIOTIC RESISTANCE IN *PSEUDOMONAS AERUGINOSA*

Martino RA, Tobares RA, Buffa ML, Colque CA, López VA, Moyano AJ, Smania AM
CIQUIBIC-CONICET; Dpto. Química Biológica Ranwel Caputto, Facultad de Ciencias Químicas, UNC. E-mail: romanmartino@fcq.unc.edu.ar

Pseudomonas aeruginosa grows as biofilm communities in the airways of cystic fibrosis patients, where small colony variants (SCVs) are frequently observed. These biofilm-adapted SCVs show a great instability when grown outside biofilms, thus revealing the ability to switch between phenotypes. We have previously performed an evolutionary assay to explore the adaptive potential of *P. aeruginosa* by subjecting clones to alternating SCV conversion and reversion cycles, from which we further isolated SCVs and revertant clones. Comparative genomics analysis showed that all the evolved lines accumulated 1 mutation/round in genes of the *wsp* and *yfi* systems, suggesting strong evolutionary parallelism. Both systems are key regulators of the second messenger c-di-GMP, which governs the transition between planktonic and biofilm mode of growth. By round 4, SCV conversion in all evolutionary lines was dramatically constrained. However, one of these lines evolved up to ten conversion/reversion cycles. Interestingly, subsequent mutations to those observed in the *wsp* and *yfi* systems were localized in *morA* and the repressor gene *nfxB*. MorA is a dual enzyme with diguanylate cyclase and phosphodiesterase domains and is associated with motility regulation. Gene *nfxB*, located 243 bp upstream of *morA*, is a self-regulating gene, which regulates the expression of the MexCD-OprJ pump, associated with resistance to fluoroquinolones. Interestingly, SCV conversion in round 7 (R7-SCV) showed a mutation (A600C) that removed the stop codon of *nfxB*. Importantly, this mutation was also able to provide resistance to the fluoroquinolone ciprofloxacin. We next wondered if this mutation was able to affect the expression of *morA*. RT-PCR analysis showed that transcripts of *morA* were significantly increased in R7-SCV compared to the parental wild type strain and to its immediate predecessor from round six. Heterologous expression of a wild type copy of *nfxB* in R7-SCV was able to revert the SCV phenotype, restore sensitivity to ciprofloxacin, decrease biofilm formation and, to fully eliminate transcripts of *morA*. By performing site directed mutagenesis we further generated a *nfxB-A600C* mutant under a parental wild type context. Surprisingly, although this mutation *per se* provided resistance to ciprofloxacin and increased the transcript levels of *morA*, this mutant did not show a SCV phenotype nor an increased ability to form biofilms. These results suggest that previous mutations in *wsp* and *yfi* systems provided the genetic context in which mutations in *nfxB* resulted adaptive for biofilm formation by providing a SCV phenotype. Most importantly, under this genetic context, resistance to fluoroquinolones can be co-selected in biofilm environments in association with the SCV phenotype. Our results constitute the first report describing the role of NfxB in biofilm formation and provides a new mechanism of association between biofilms and antibiotic resistance.

MI-P10
MICROBIAL SOURCE TRACKING TO IDENTIFY FECAL POLLUTION IN RECREATIONAL WATERS FROM THE WIERNA AND MOJOTORO RIVERS IN SALTA, ARGENTINA

Reyes SI[1] Said Adamo M M[1, 2], Poma H R [1], Rajal V B[1, 3], Cristóbal, H A [1, 2]
[1] INIQUI-CONICET-unas. [2]Facultad de Ciencias Naturales-unas. [3]Facultad de Ingeniería-UNSa, Salta, Argentina.
E-mail: hacristobal@gmail.com

The presence of fecal pollution in environmental waters, coming from wastewater treatment plant and illegal discharges, and runoff of animal feces, represents a threat to human health and an environmental concern all around the globe. The microbiological water quality is traditionally assessed by enumerating fecal indicator bacteria (FIB) (fecal coliforms, *Escherichia coli* and *Enterococcus*). Although these FIB are employed in the world, these indicators do not accurately predict the health risk of other human pathogens. Nevertheless, microbial source tracking (MST) methods, based in the determination of genetic markers from *Bacteroides* (bacteria found in the gastrointestinal tracts of warm-blooded organisms), can discriminate between human or animal fecal pollution sources. The main of this study was to identify the origin and magnitude of fecal pollution by detecting universal, human and bovine *Bacteroides* in water samples from the Wierna and Mojotoro rivers, at the Province of Salta, Argentina. Water samples were obtained from thee points (P): P1 at Wierna river, where recreational activities develop, and P2 and P3 from Mojotoro river, which receives the discharges of two wastewater treatment plants (one inactive and the other currently operating). A total of 30 20-l water samples were collected from each point and concentrated by ultrafiltration to a final volume of approximately 50 mL. DNA extractions

were performed from the concentrates and real-time PCR assays were carried out to detect 16S rDNA from *Bacteroides*. Universal (using BacUni oligonucleotides), human (with BacHum) and cow (with BacRum) markers were determined by duplicate. The universal marker was found in all the sites evaluated in a concentration range from 6.10 $x10^1$ to 7.71 $x10^3$ genomic copies per ml. The human marker was found in a concentration range from 5.44 $x10^2$ to 7.22 $x10^3$genomic copies per mL. however, the ruminant marker was found in less proportion in the samples analyzed. The outcome confirmed the existence of fecal contamination of human origin, which represents a health risk to people who use these rivers for recreational purposes. In conclusion the results highlight the importance of intensifying studies that contribute to improve the assessment of water quality using alternative strategies.

MI-P11
REGULATION OF SWIMMING MOTILITY AND BIOFILM FORMATION IN *MESORHIZOBIUM LOTI*: STUDY OF THE ROLE OF SECOND MESSENGERS

Basile LA, Escobar M, Lepek VC
Instituto de Investigaciones Biotecnológicas (IIBIO-CONICET-UNSAM). Buenos Aires, Argentina. E-mail: lbasile@iibintech.com.ar

Early steps of nodule development in rhizobia-legume symbiosis involve the induction of the bacterial Type III secretion system (T3SS) by specific flavonoids secreted by the leguminous plants. Flagella promote swimming and swarming motility in free-living bacteria being required for the bacterial movement to the roots proximity and attachment to the root hairs. We had previously described a negative regulation of swimming motility in soft-agar under T3SS induction conditions in *Mesorhizobium loti* MAFF303099. The transcriptional factor TtsI (which positively regulates T3SS genes expression) was involved in the inhibition of motility. Expression of *visN* gene (a positive regulator for flagellar genes) and flagella production were also affected under T3SS induction conditions. In this work we started to evaluate the role of the second messengers c-diGMP and cAMP in motility regulation, biofilm formation and interaction with the leguminous *Lotus* sp. A *M. loti* strain overexpressing a phosphodiesterase of c-diGMP (PDE, *mll2537*) did not show inhibition of swimming motility under T3SS induction conditions, suggesting a role of c-diGMP levels in the negative regulation of motility. Biofilm formation was increased in the wild type strain under T3SS induction conditions, but not in the strain overexpressing *mll2537* gene. This indicates that c-diGMP levels would be also implicated in the regulation of biofilm formation. The promoter region of the *mll9676* gene (a fosfoesterase of c-AMP) presents a *tts*-box like sequence. By fusion to the b-galactosidase gene, the activity of this promoter region was evaluated. Preliminary results showed activity at late-exponential growth phase, both in the wild type as in the *ttsI* mutant strain. Similar levels of promoter activity were detected for the wild type strain grown with or without T3SS induction conditions. These results suggest that *mll9676* gene is not under a direct regulation of the TtsI transcriptional factor. *M. loti* mutant strains for genes of c-diGMP and c-AMP metabolism are being constructed. In addition to biofilm and motility assays, their role in nodulation will be determined by inoculation of these strains in *Lotus tenuis* plants.

MI-P12
ASSESSMENT OF WATER QUALITY FROM WIERNA AND MOJOTORO RIVERS IN SALTA, ARGENTINA

Reyes SI[1], Jurado M A[3], Peñaloza LG[3], Rajal VB[1, 4], Celiz G[1, 3], Cristóbal HA[1, 2]
[1] INIQUI-CONICET-UNSa, [2] Facultad de Ciencias Naturales-UNSa, [3] Facultad de Ciencias Exactas-UNSa, [4] Facultad de Ingeniería-UNSa, Salta, Argentina. E-mail: hacristobal@gmail.com

The Wierna and Mojotoro rivers run from north to south crossing La Caldera, in the province of Salta. The Mojotoro River receives the discharge of two wastewater treatment plants. As both rivers are used for recreational purposes, it is important to assess the water quality. The objective of this work was to evaluate the influence of wastewater treatment plants (WWTP) discharges on the physicochemical and microbiological water quality. A total of 90 water samples were obtained from three points (P), one in the Wierna river (P1) and two in the Mojotoro river (P2, P3). Physico-chemical variables were measured *in situ*: temperature, pH, turbidity, conductivity, salinity, and dissolved oxygen, using a portable multiparameter analyzer. Density of total (TC) and fecal (FC) coliform bacteria was determined in the laboratory using the multiple tube method. The concentrations of Cu, Mn, Pb, Fe, Cr, Zn, and Cd were measured by atomic absorption spectrophotometry (AAS) and that of As by the Hydride Generation method (HG) coupled to AAS. Spearman correlation analysis was performed to search for correlation between variables analyzed and the potential influence of WWTP was evaluated using the Wilcoxon test. The minimum and maximum values of the physico-chemical variables were: 8.5-21 °C for temperature, pH 7.8-9.5, and 2 -> 999 NTU for turbidity, 0.19–0.32 mS/cm for conductivity, 0–0.02% salinity, and 2.3–7.7 mg/L of dissolved oxygen. In addition, the range for TC and FC were 0–1.09 x 10^6 and 0–1.19 x 10^5 NMP/100 mL, respectively. Iron and Mn at P1 and P2 exceeded the maximum permissible limits, established by international legislations (0.2 and 0.1 mg/L, respectively). Correlations between Zn and conductivity, TC and FC were statistically significant ($p > 0.05$). The Wilcoxon test showed significant differences ($p > 0.05$) for TC and FC between P1 and P2, and P2 and P3. The Mojotoro river showed deterioration in water quality after receiving the discharge of WWTP. High concentrations of bacterial indicators and heavy metals in these sites represents a risk to public health.

MI-P13

INCREASED PHOSPHATE SOLUBILIZATION BY RHIZOSPHERIC SUGARCANE INTERSPECIES INTERACTIONS

Rubio Molina AC, Bulacio Gil NM, Zenoff AM, Caram di Santo MC, de Cristobal RE, Pomares MF, Vincent PA, Adler C.
INSIBIO (CONICET-UNT), Tucumán, Argentina. E-mail: rubioc32@gmail.com.

Abstract: Plant-associated microbiota can enormously influence on plant traits. Manipulation of these microbial communities holds great potential as an effective way to improve crops, while contributing to a more environmentally benign agriculture. Revealing the chemical communication network in the rhizosphere will pave the way for the future production of bioinoculants using bacterial consortia. In this work, we isolated numerous microorganisms from the sugarcane rhizosphere, sequenced the 16S rDNA of a subset of isolates showing different cultural features and evaluated their ability to solubilize phosphate in NBRIP medium. Next, using only phylogenetically different isolates growing in solid medium containing salts, amino acids and sucrose, we tested interspecies interactions in pairwise combinations. Interactions were followed for 7 days and cultural traits such as growth inhibition, growth promotion, colony morphology changes and pigment production were registered. We focused in one peculiar interaction that involved isolate 258 promoting growth of isolate 214 while the latter inhibited growth of 258. In addition, 258 enhanced the ability of 214 to solubilize phosphate. To analyze if this phenomenon was due to a secreted metabolite, supernatants were tested. Interestingly, we observed that cell-free supernatants of isolate 258 were able to induce 214 phosphate solubilization. We are currently performing experiments to elucidate the chemical nature of the metabolites responsible for this phenotype.

MI-P14
STUDY OF GENOTOXICITY AND CITOTOXICITY OF MCCJ25 (G12Y)

Koltan M[1], Bulacio Gil NM[1], Rubio Molina AC[1], Zenoff AM[1], Caram di Santo MC[1], de Cristobal RE[1], Adler C[1], Vincent PA[1], Pomares MF[1].
1INSIBIO, CONICET-UNT), Tucumán, Argentina. E-mail: marianokoltan@gmail.com

MccJ25 (G12Y) is an antimicrobial peptide active against *Escherichia coli* O157 H: 7 and other diarrheagenic *E. coli* strains. MccJ25 (G12Y) presents advantageous properties as a food preservative: it resists to extreme temperatures, is stable in a wide range of pH and exerts antimicrobial activity in food samples (egg yolk and milk). Our long-term aim is to develop a food preservative based on MccJ25 (G12Y). Therefore, we were interested in studying MccJ25 (G12Y) safety for humans. For this, we evaluated the geno- and cytotoxicity of MccJ25 (G12Y) on *Salmonella* Typhimurium and *Artemia salina* respectively and MccJ25 (G12Y) acute toxicity in mice. In addition, we performed an Ames test to test the mutagenic potential of the compound. Results obtained confirmed the absence of genotoxic effects of MccJ25 (G12Y) on *S.* Typhimurium. Furthermore, the *Artemia salina* assay, which determined the Medium Lethal Concentration (LC_{50} value) of MccJ25 (G12Y), revealed that 0.31, 0.62, 1.25, 2.25 and 3 mg/mL of MccJ25 (G12Y) in the growing medium did not significantly affect the viability. In fact, the LD50 was not reached with any of the MccJ25 (G12Y) concentrations tested. When MccJ25 (G12Y) citotoxicity was evaluated with *Galleria mellonella,* a low toxicity was observed with the concentrations studied (1500 and 2500 mg/kg). Finally, acute toxicity was studied in female BALB-c mice. Neither death nor symptoms of toxicity in animals were observed. In addition, macroscopic pathological studies showed that the organs of MccJ25 (G12Y)-treated animals did not present any morphological alteration. Taken together, results indicate that MccJ25 (G12Y) would be safe for humans. This finding is relevant in view of its application as a natural preservative to extend the shelf-life of foods.

MI-P15
QUORUM SENSING MEDIATED TRADEOFF LIMITS MUTATORS FIXATION DURING POLYMICROBIAL INFECTIONS

Luján AM [1,2], Paterson S [4], Madsen Sommer L [5], Marvig RL [5,7], Sharma MD [1], Opsal E [1], Ciofu O [7], Smania AM [2,3], Molin S [5], Krogh Johansen H[5,6], Buckling A [1].
[1]ESI UoE UK. [2]CIQUIBIC/DQBRC-FCQ-UNC ARG. [4]IIB UoL UK. [5]DTU Biosustainability DK. [6]DCM CPH DK. [7]CGM CPH DK. [8]CBC II&M UCPH CPH DK. E-mail: adem.lujan@gmail.com

Bacteria with elevated mutations rates (mutators) can reach high frequencies in cystic fibrosis (CF) chronic infections and are associated with treatment failure in chronic respiratory infections. The causes and consequences of bacterial elevated mutation rates have been widely studied and results suggest that mutators might be selected because their higher probability of generating beneficial mutations. However, it is notable that there are large between-patient differences in mutator frequency; even where the age and treatment protocols of patients are similar. Understanding this variation may provide opportunities for interventions that could minimize mutator evolution and the severity of infections. Possible drivers of variation in mutator frequencies are interactions with other bacterial co-infecting bacterial species. Ecological and evolutionary changes in populations of co-occurring species may result in continually changing selection pressures, potentially selecting for mutators. Alternatively, mutators may be selected against if competitors constrain adaptation to other components of the environment. Here, we used a combination of correlational *in vivo* data and *in vitro* experiments to determine the role played by co-occurring pathogens in driving variation in *Pseudomonas aeruginosa* mutation rate in chronic infections. By performing metagenomic analysis of CF sputum samples we show that mutation frequency in *P. aeruginosa* is negatively correlated with the frequency and diversity of co-infecting bacteria in chronic lung infections of CF patients. By competing *in vitro P. aeruginosa* mutators against wild-type in the presence and in the absence of the bacterial community we demonstrate that mutators have a fitness advantage in the absence of other CF-associated species, and that this was in part because mutations in the main Quorum-Sensing (QS) regulators that were beneficial in the absence of competitors but deleterious in their presence. These QS genes were also more likely to be mutated in *P. aeruginosa* CF populations showing elevated mutations frequencies *in vivo*. Our results demonstrate that interspecific competition constrains the evolution of mutation rates, and more generally highlights the crucial role of the community context in microbial evolution and virulence.

MI-P16
SERRATIA MARCESCENS PRTA: A KEY FACTOR TO STRUCTURE A MATURE BIOFILM

Molino MV, Bruna RE, García Véscovi E.
Instituto de Biología Molecular y Celular de Rosario (UNR-CONICET). E-mail: molino@ibr-conicet.gov.ar

Serratia marcescens (Sma) is an environmentally ubiquitous bacterium also acting as an opportunistic pathogen. Moreover, *Sma* displays biofilm formation capacity that has been shown to be related to its ability to colonize, persist, and proliferate on either biological or inert surfaces. This capacity to adapt and survive in either hostile or changing environments can be related to the expression of a myriad of secreted hydrolytic enzymes, including proteases. Genomic analysis of *Sma* clinical strain RM66262 identified four zinc-metalloprotease-encoding genes. Amongst them, we previously showed that PrtA is prominently secreted and its expression depends on the bacterial growth temperature, being transcriptionally upregulated at 30°C in comparison with 37°C. We also found that, in *Sma*, the CpxAR signal transduction system, which responds to envelope stress and bacterial surface adhesion, is activated at 37°C and able to down-regulate PrtA expression by CpxR at the transcriptional level. Taking into account that, in other enterobacteria, the CpxAR pathway is involved in modulating the ability of biofilm generation, we examined whether PrtA could influence *Sma* biofilm formation capacity. To that aim, we performed *in vitro* biofilm assays in polystyrene microwell plates, followed by biofilm quantitation using crystal violet staining. When the strains were grown at 30°C or 37°C in SLB medium the lack of PrtA expression in the *prtA* strain reduced the capacity of the bacteria to form biofilm compared with that of the wild-type strain, being more attenuated at 37°C. Results of confocal microscopy also showed this deficiency in biofilm formation in the *prtA* strain. The defect in biofilm formation of the *prtA* strain could be complemented to wild-type levels by adding purified, catalytically active, PrtA. To further understand PrtA influence on biofilm formation, we built a single-aminoacid-mutant protein that annuls the protease hydrolytic capacity and performed biofilm assays. Our results show a defective biofilm phenotype, equivalent to a *prtA* strain. The addition of purified catalytically active PrtA to this mutant restored biofilm formation to wild-type levels, demonstrating that PrtA expression and activity contribute to the ability of *Sma* to structure a biofilm community.

MI-P17
SYNTHESIS OF STABILIZED SILVER NANOPARTICLES WITH EXOPOLYSSACCHARIDES OF LACTIC ACID BACTERIA AND EVALUATION OF ITS ANTIMICROBIAL ACTIVITY

Espeche Turbay MB[1], Rey V[1], Torino MI[2], Dorado RD[1], Borsarelli CD[1], Rodríguez MC[2]
[1]Instituto de Bionanotecnología del NOA (INBIONATEC), CONICET, UNSE, Santiago del Estero. [2]CERELA-CONICET, Tucumán.
E-mail: beaespeche@gmail.com

The development of new nanomaterials (NM) to be used in antimicrobial therapies, are currently an alternative to conventional antibiotics, which present multiresistance. Silver nanoparticles (AgNP) are used as antimicrobial agents because are able to release silver ion (Ag^+), damage the cell envelope or interact with intracellular essential components for microorganism functioning. AgNP synthesis requires stabilizing agents, and different types of molecules have been used with this porpoise, such as proteins, among others. NM are intended not only to exert a deleterious effect on bacteria, but also that these one be biocompatible. Lactic acid bacteria (BAL) are ubiquitous bacteria primarily used in food industry, because of an extra value added to food. Some of them are able to form exopolysaccharides (EPS) as a metabolism product, this being itself a bioactive compound. Considering these, EPS were used as stabilizing agents in AgNP synthesis. *Weissella cibaria* CRL 11 EPS (EPS_{CRL11}) produced from solid medium was isolated, purified and lyophilized. The photoreductive synthesis of AgNP was completed using EPS as a stabilizing agent (AgNP @ EPS_{CRL11}), obtaining stable nanoparticles, which were characterized by UV-Vis and infrared (FT-IR) spectroscopy, ligth dimanic scaterring (DLS), potential Z (pZ) and transmission electron microscopy (TEM). AgNP @ EPS_{CRL11} has a size of 12 ± 2 nM, and a concentration of 3.77 nM. Antinimicrobial (AA) activity was evaluated on Gram positive (*Staphylococcus aureus* ATCC 25923, *Bacillus sp*., *Micrococcus luteus*) and negative (*Escherichia coli* O157H7; *Serratia marcescens*, *Klebsiella pneumoniae*) strains. Agar diffusion technique was employed, demonstrating activity on gram-positive strains. The minimum inhibitory concentration (MIC) in the sensitive strains was determined, being possible to obtain it for *Micrococcus luteus* (MIC = 1.26nM). Subsequent studies carried out by increasing the concentration of the EPS, and consequently the concentration of the NP, demonstrated a higher AA. Our results show that is necessary not only to obtain new potentially active antimicrobial agents, but also their respective AA assessment on strains of interest.

MI-P18
USE OF *MYCOBACTERIUM BOVIS* ANTIGENS PRODUCED IN BACULOVIRUS IN AN IFN-Γ ELISA ASSAY FOR BOVINE TUBERCULOSIS DIAGNOSIS

Villafañe L, López MG, Garbaccio S, Ferrara X, Garro C, Campos E, Eirin ME, Forrellad MA, Taboga O, Bigi F.
Laboratorio de Tuberculosis – IABiMo – INTA Castelar. E-mail: villafanie.luciana@inta.gob.ar

The classical diagnostic method to detect *Mycobaterium bovis* infection, the causative agent of bovine tuberculosis (bTB), is the measure of the immune mediated response induced by the intradermal injection (IDR) of a protein purified derivative of *M. bovis* (PPDb). Another ancillary bTB test detects IFN-γ produced in whole blood upon stimulation with PPDb or with antigenic protein/peptide cocktails. Protein reagents for immune stimulation are generally obtained from *Escherichia coli*; however, *E. coli*-derived antigens may produce in livestock non-specific immune reactions derived from contamination with lipopolysaccharides (LPS). On the other hand, the use of baculovirus expression system to production of the antigenic protein cocktails would reduce the presence of LPS in these reagents. The aim of this study was to evaluate the performance of

two different antigenic cocktails of *M. bovis* proteins in the ancillary test; one of them produced in baculovirus (BvCK) and the other produced in *E.coli* (EcCk), to differentiate intradermal positive (IDR+) and intradermal negative (IDR-) reactor animals. The BvCk was composed by a mixture of the *M. bovis* antigens ESAT-6, CFP-10 and Rv3615c, fused to polyhedrine (PolH), produced by using the Bac-to-Bac system and the protein purification protocol described by O'Reilly et al. in 1994. The *E. coli* cocktail contains the same *M. bovis* antigens provided by Lionex. These cocktails were used to stimulate whole blood of 7 IDR- from an official bTB free herd from Santa Fé province, and 25 IDR+ bovines from herds located at Buenos Aires province. The plasmas were used for IFN-γ dosage by ELISA (Mabtech). Considering IDR+ animals, the IFN-γ response upon stimulation with BvCk was not statistically different from that observed upon stimulation with EcCk or even with the PPDb. Moreover, the IFN-γ response observed among IDR- animals stimulated with BvCk induced IFN-γ production at similar levels to that induced with PBS. Conversely, EcCk induced higher IFN-γ production than PBS stimulation, in 3 out of 7 samples. These values were statistically different to those obtained with BvCk. In conclusion, our results suggest that the PolH fusion proteins showed equivalent sensitivity but better specificity than the same *M. bovis* proteins produced in *E. coli*. These preliminary results suggest the use of BvCk as an alternative antigenic cocktail to the traditional recombinant antigenic cocktail produced in *E. coli* for using in the ancillary IFN-γ test.

MI-P19
KUP AND KIMA: TWO POTASSIUM UPTAKE SISTEMS REGULATED BY C-DI-AMP IN *ENTEROCOCCUS FAECALIS*

Acciarri G, Espariz M, Blancato V, Magni C.
IBR-CONICET, Rosario. E-mail: acciarri@ibr-conicet.gov.ar

Enterococcus faecalis is a natural commensal member of the human gut flora that belongs to the group of lactic acid bacteria (LAB). In recent years however, it has emerged as a clinical important opportunistic pathogen. A distinct trait in the physiology of these bacteria is the ability to persist and thrive in harsh environments, that include heat, acid, oxidative and hyperosmotic stress. Despite its controversial profile, *E. faecalis* are part of food products, either due to contamination or as part of starter, adjunct or non-starter cultures. Besides, ion homeostasis is a key factor for all living cells. Particularly, potassium (K^+) is the most abundant cation in the cytosol, and its uptake is tightly regulated. Intracellular K^+ is important for cellular metabolic processes such as gene expression, pH homeostasis, osmotic adaptation and enzymatic activity. A search for genes present in *E. faecalis* genome coding for proteins with high homology for K^+ transporters revealed the presence of a Kup as well as a KimA homologue. To study the functional properties of the proteins encoded by both genes we used *Escherichia coli* LB650 (ΔkdpABC5 ΔtrkH ΔtrkG). This strain is a triple mutant for the main K^+ transporter systems and, hence, it is unable to grow at low K^+ concentrations. *E. faecalis* JH2-2 *kup* and *kimA* genes were individually cloned in plasmid pWH844 using *E. coli* LB650 as host, to check if their expression could restore growth in minimal salt media when no KCl is added. Both evidence growth with or without K^+ supplementation. These results suggest that Kup and KimA are involved in the K^+ transport in. *E. faecalis.* On the other hand, Kup and KimA are regulated by c-di-AMP in *Lactococcus lactis* and *Bacillus subtilis*, respectively. c-di-AMP is a recently discovered second messenger molecule found in a wide range of bacteria and is the only that is essential for the bacteria that produce it. c-di-AMP plays important roles in the regulation of diverse cellular pathways, including K^+ homeostasis. Once Kup and KimA were identified as K^+ transporters, the next step was to analyze the impact of this metabolite on the activity of both proteins. A co-expression system was established in *E. coli* 2003. This strain is deficient in the three major K^+ uptake systems (ΔkdpABC5 kupD1 ΔtrkA) and is not able to grow in minimal salt media at low K^+ concentrations. Very importantly, *E. coli* lacks c-di-AMP synthesizing enzymes, for which the co-expression of a c-di-AMP synthesizing enzyme (CdaA) and Kup or KimA allows the analysis of the phenotypic effect of c-di-AMP on these transporters, without interference of host-synthesized c-di-AMP. Growth curves performed under low K^+ concentrations confirmed that c-di-AMP has an inhibitory effect on both Kup and KimA.

MI-P20
ROLE OF THE SIGMA FACTOR ALGT AND ITS ANTI-SIGMA MUCA IN *PSEUDOMONAS AERUGINOSA*

Hedemann LG, López VA, Moyano AJ, Smania AM.
Departamento de Química Biológica Ranwel Caputto, FCQ, UNC; CIQUIBIC-CONICET. E-mail: ghedemann@fcq.unc.edu.ar

Pseudomonas aeruginosa is an opportunistic pathogen that chronically infects the airways of cystic fibrosis patients. In *P. aeruginosa*, conversion to the mucoid (exopolysaccharide alginate-overproducing) phenotype marks the onset of chronic infection and constitutes a sign of poor prognosis. Mucoid conversion frequently involves the acquisition of mutations in the *mucA* gene. MucA is an anti-σ factor that negatively regulates alginate production by sequestration of AlgT, an alternative σ factor responsible for the transcription of the alginate biosynthetic operon. Mutations in *mucA* can also affect Quorum-Sensing (QS) signals, flagellum biosynthesis or survival under anaerobic and osmotic stress conditions, revealing the existence of an extensive regulon controlled by MucA and AlgT, which is not completely understood. The most frequent mutation responsible for mucoid conversion is a deletion of a G residue within a homopolymeric track of five Gs ($G5_{426}$), also known as *mucA22* allele, which causes the truncation of MucA C-terminal periplasmic domain. By engineering different *mucA* alleles, we previously showed that deletion of $G5_{426}$, although severely reduces mucoid conversion frequency and *mucA* mutations prevalence, the few remaining mutations that still occur in *mucA* keep targeting the gene periplasmic coding region, thus leading to truncated versions of MucA equivalent to *mucA22*. We advance in the knowledge of this regulatory network, by exploring whether MucA truncated proteins can still exert their regulatory function on AlgT. Moreover, we wondered whether MucA has biological roles independent of the known AlgT regulatory pathway. Here, we constructed and characterized a set of mutants containing different combinations of *mucA* and *algT* composition, namely, *ΔalgT, ΔalgTΔmucA, ΔalgTmucA22*, *mucA22* and partially deleted *mucA* mutants. Phenotypic characterizations included alginate overproduction, measurement of NO_2^- sensitivity under anaerobic conditions, osmotic stress tolerance, and production of acyl homoserine lactones (AHLs). Overexpression of the *mucA22* allele was able to suppress the mucoid phenotype, confirming that it partially maintains its anti-σ regulatory function. Whereas the *mucA22* strain showed a mucoid phenotype

and AHLs suppression, Δ*algT*, Δ*algT*Δ*mucA*, and *ΔalgTmucA22* remained non-mucoid and showed AHLs wild type levels. The *mucA22* strain was highly sensitive to NO_2^- under anaerobic conditions. Surprisingly, *ΔalgT* mutants were resistant to $NO2^-$like the wild-type strain. These results suggest that AlgT deregulation might be toxic under certain conditions. On the other hand, unlike the wild-type strain, all mutants failed to grow in high salt media, which suggests that tightly controlled AlgT levels are necessary to achieve tolerance to osmotic stress. Our results shed light on the regulatory pathways underlain mucoid conversion, providing potential targets for future therapeutic strategies to control chronic *P. aeruginosa* infections.

MI-P21
A BALANCE BETWEEN RESISTANCE AND VIRULENCE IN THE EVOLUTION OF *SALMONELLA* COPPER HOMEOSTASIS

Terán I, Mendoza JI, Giri GF, Checa S, Soncini FC.
Instituto de Biología Molecular y Celular de Rosario (IBR, CONICET-UNR). Email: soncini@ibr-conicet.gov.ar

Salmonellosis is among the most common foodborne diseases, with millions of human infections occurring worldwide every year. The ability of *Salmonella* to persist and proliferate both in contaminated food and water and within host's tissues reflects the versatility of its genetic repertoire that allows adaption to diverse and challenging environmental conditions. This also involves the participation of regulatory systems that perceive specific host's signals and respond to them by modulating the expression of factors essential for virulence. In particular, it has been shown that this pathogen detects the surge of copper (Cu) inside the *Salmonella*-containing vacuole in infected cells. In consequence, mutants affected in Cu-resistance have reduced survival in macrophages. Most known bacterial cuproproteins localize to the envelope, which makes this compartment the main target for Cu homeostasis. While in most enteric species periplasmic Cu homeostasis is maintained by the CusR/CusS-controlled CusCFBA efflux system that removes surplus cellular Cu ions, we noticed that this sensory and efflux system coding *cus* locus was lost in the core genome of most *Salmonella* subspecies. On the other hand, both *Salmonella* species acquired *cueP*, which codes for a periplasmic Cu-chaperone. We observed that mutants with a deletion in *cueP* have increased Cu sensitivity in anaerobic conditions, and that CueP can partially restore Cu-resistance of a Δ*cus Escherichia coli* mutant strain in the absence of oxygen, suggesting that the *Salmonella* periplasmic chaperone can substitute the CusCFBA system in the maintenance of the envelope Cu homeostasis. Reintroduction of the ancestral *cus* locus in the *Salmonella* chromosome did not affect Cu-resistance in aerobic conditions while its resistance to the metal ion in anaerobic conditions was even higher than that of the wild-type strain. Although the reasons for the loss of the ancestral locus are not clear, we speculated that virulence could be involved. In fact, we observed that regardless the presence or absence of the *cus* locus, the Δ*cueP* strain shows a deficiency in intracellular replication in macrophages compared to the wild type strain. Our results demonstrate that CueP and CusCFBA exert redundant functions for metal resistance, but not for macrophage survival and therefore for the virulence of this pathogen.

MI-P22
PHOSPHATE INFLUENCES AMMONIUM DEPENDENT-RNASEQ PROFILES IN *HERBASPIRILLUM SEROPEDICAE*

Farizano JV[1], Villegas, JM[1], Pankievicz VC[2], Tadra-Sfeir M[2], Brusamarello-Santos L[2], Rapisarda VA[1], Souza EM[2], Grillo-Puertas M[1]
[1]INSIBIO, CONICET-UNT, Tucumán, Argentina; [2]UFPR, Curitiba, Brazil. E-mail: juanvfarizano@gmail.com

Herbaspirillum seropedicae is a nitrogen-fixing β-proteobacterium capable of colonizing commercial crops to promote plant growth. The regulation of nitrogen fixation and assimilation in this bacterium has been the object of several studies. However, how the bacterium responds to different environmental nitrogen concentration was not yet been completely understood. It was previously described that *H. seropedicae* cells grown in high phosphate (Pi) medium reached higher cell densities than cultures grown in sufficient Pi media and maintained high polyP levels in stationary phase. In high Pi condition, genes involved in energy production process, two component regulatory systems, amino acid transport and metabolism, chemotaxis and inorganic ion transport and metabolism were induced. Thus, the aim of this work was to analyze if the medium Pi concentration could influence the ammonium dependent-transcriptional regulation in *H. seropedicae*. For RNAseq analysis, RNA was extracted from cells grown in four conditions combining low and high Pi and NH_4^+ concentrations (+P-N, +P+N, -P-N, and -P+N). Transcriptional analysis of +P+N *vs* +P-N and -P+N *vs* -P-N revealed a total of 996 and 1051 differentially expressed genes, respectively. Variation in NH_4^+ concentration showed that the most regulated metabolic pathways belong to the Translation, ribosomal structure and biogenesis, Energy production and conversion, Inorganic ion transport and metabolism, Cell motility, Amino acid metabolism, Cell wall, and Transcription categories. Pi affected NH_4^+-dependent differential expression of some genes related to chemotaxis, flagella, transcriptional stress regulators, polyhydroxybutyrate synthesis and N_2 fixation. For instance, transcriptional stress regulators (LysR-type regulators, *oxyR*, *risA*, Hsero_2244, Hsero_2400) were up-regulated in -P+N vs -P-N, while down-regulated in +P+N vs +P-N. In summary, this study allows the identification of *H. seropedicae* candidate genes and pathways potentially involved with bacterial environmental adaptations.

MI-P23

METABOLIC RESPONSES MEDIATED BY PHOSPHATE IN *HERBASPIRILLUM SEROPEDICAE*: CHEMOTAXIS, PHB SYNTHESIS AND ROOT COLONIZATION

Grillo-Puertas M[1], Villegas JM[1]; Pankievicz VC[2], Tadra-Sfeir M[2], Mota T[2], Brusamarello-Santos L[2], Pedraza RO[3], Souza, EM[2], Rapisarda VA[1]
[1]INSIBIO, CONICET-UNT, Tucumán, Argentina; [2]UFPR, Curitiba, Brazil; [3]FAZ-UNT, Tucumán, Argentina. E-mail: marianagrillo24@gmail.com

Polyphosphate (polyP) is a molecule that plays important roles in microbial metabolism. Environmental phosphate (Pi) modulates intracellular polyP levels in *Herbaspirillum seropedicae*, a plant growth promoting bacteria (PGPB) associated with important agricultural crops. In low and high polyP accumulating conditions, RNA-seq transcriptional profile revealed several responses associated with energy production process, two component regulatory systems, and amino acid transport and metabolism, among others. The aim of the present work was to evaluate, in the differential polyP conditions, the molecular and phenotypical changes related to chemotaxis, polyhydroxybutyrate (PHB) synthesis and maize colonization. Assays were carried out in cells grown in high polyP condition (M+P, media containing 50 mM Pi,) or low polyP condition (M-P, with 5 mM Pi). In M+P cells, most chemotaxis genes (*tar*, *tsr*, and *che*), and a wide range of flagella biosynthesis, assembly and structure genes, were up-regulated. As expected, SmR1 cells presented higher motility in M+P than those grown in M-P. Also, results showed that taxis of cells grown in M+P was significantly higher than that observed in M-P, being this difference greater in the presence of root exudates and phytohormones. Several genes related to biofilm development and colonization were down-regulated in the high polyP condition, which is in agreement with the lower biofilm formation capacity and endophytic maize root colonization observed in cells grown in this condition. Genes encoding enzymes related to PHB metabolism were up-regulated in cells grown in M+P, correlating with the higher PHB levels compared to those in cells grown in M-P. Present findings provide new insights about signals triggered by intracellular polyP that could be critical for *H. seropedicae* applications as PGPB.

MI-P24
PROTEOMIC ANALYSIS OF *LACTOBACILLUS PARAPLANTARUM* CRL 1905 IN RESPONSE TO DIFFERENT PHOSPHATE CONCENTRATIONS

Araoz M[1], Grillo-Puertas M[1], Hebert EM[2], Villegas JM[1], Rapisarda, VA[1]
[1]Instituto Superior en Investigaciones Biológicas (INSIBIO) (UNT-CONICET) [2]CERELA (CONICET). Tucumán, Argentina
E-mail: marioaraoz18@gmail.com

Polyphosphate (polyP) is a linear polymer that plays important roles in microbial physiology. The metabolism of this polymer has been scarcely explored in lactic bacteria, microorganisms of great interest in the food industry and in human and animal health. Our previous results evidenced the ability of *Lactobacillus paraplantarum* CRL 1905 to maintain high levels of polyP in stationary phase in a high phosphate (Pi) medium (CDM+P). In this condition, cells increased their survival and tolerance to acidity and bile salts, compared to those grown in sufficient Pi medium (CDM-P). The aim of this work was to evaluate the influence of environmental Pi concentration in the proteomic profile of CRL 1905 strain. The comparative proteomic analysis was carried out in cells grown in CDM+P or CDM-P during 48 h. From the 51 proteins that were differentially expressed, 29 were up-regulated and 22 were down-regulated in cells grown in CDM+P *vs* CDM-P. PdhA, PdhB, PdhC, Pox3, Ack2, PflB, GlpK1, Xfp, RpiA, GalM3, RbsK, and Pgm1 proteins, involved in glycolysis, pyruvate metabolism and pentose phosphate pathway, were up-regulated in high Pi stationary phase cells, implying an active metabolism similar to that of exponential phase cells. Upp, PyrB, and PyrR, proteins involved in nucleotide transport and metabolism, were also up-regulated in the high Pi condition. On the other hand, chaperones implicated in stress response (Hsp1, Hsp3, and ClpX) were repressed in CDM+P, inferring that high polyP levels in stationary phase could act as an inorganic chaperone to maintain protein stability. Enzymes involved in cell surface modification (Lp_3421, Acm2, MltG, Alr, and FmlA) were also down-regulated in CDM+P. In accordance with the proteomic analysis, biofilm formation capacity of CRL 1905 increased in CDM-P, being ~ 3 times higher than that produced in CDM+P. Together, our results indicate that *L. paraplantarum* CRL 1905 grown in high Pi conditions presented an advantageous cellular fitness during the stationary phase by modifying different metabolic pathways.

MI-P25
PHYLOGENETIC INFERENCE IN BACTERIA: PHYLOGENOMICS VS MULTILOCUS SEQUENCE TYPING (MLST) SCHEMES

Floridia-Yapur N[1], Rusman F[1], Ragone P[1], Diosque P[1], Acuña L, Tomasini N[1]
[1] Instituto de Patología Experimental, IPE-CONICET, Salta, Argentina. E-mail: narfy89@gmail.com

The whole-genome sequence era brought a massive amount of information that helped to elucidate or clarify phylogeny relations of several organisms. It has been proposed that genome-based phylogeny is more accurate than phylogeny based in a few number of genes as MLST-based phylogeny. However, much is not always better. Here we studied the intraspecific phylogeny of 9 bacteria species, with more than 30 complete genomes sequenced: *Burkholderia pseudomallei, Campylobacter jejuni, Chlamydia trachomatis, Helicobacter pylori, Klebsiella pneumoniae, Listeria monocytogenes, Salmonella enterica, Staphylococcus aureus* and *Streptococcus pyogenes*. The phylogeny was inferred using, on the one hand, the complete genome sequences of different strains of these species and, on the other hand, their MLST schemes. Complete genomes/MLST genes were aligned. Maximum likelihood trees were constructed with FastTree2 for both complete genomes and concatenated MLST loci. In addition, genome alignments were fragmented every 5000 nt and maximum likelihood trees were built for every genome fragment (individual trees). The phylogenetic incongruence between the complete genome or MLST concatenated sequences tree and the individual trees was analyzed using MLSTest. In genome trees, most branches showed a high branch support; however, a high number of branches also showed a high percentage of topologically incongruent individual trees. For example, 90% (37/41) of the branches of the genome tree of B. pseudomallei showed a high

branch support (more than 0.8); 31 of those branches had the maximum branch support (1); however, 51% of such branches are probably artifacts because most genome fragments are topologically incompatible with them. Interestingly, genome and MLST trees showed similar levels of incongruence in the phylogeny of each bacteria specie. Both genome and MLST approaches showed that *C. trachomatis* and *S. aureus* have a tree-like evolutionary history (low levels of internal incongruence). Instead B. pseudomallei and S. pyogenes show high levels of incongruence probably caused by horizontal gene transfer. Consequently, their evolutionary history is better represented by a network. Concluding, the high branch support obtained in genome phylogenies could be an artifact probably caused by the data size. In addition, our analyses showed that MLST phylogeny is a valid method to address intraspecific phylogeny and that some species phylogenies are not properly represented by trees.

MI-P26
VIVO MURINE IMMUNE MODULATION IN RESPONSE TO ATTENUATED *TRYPANOSOMA CRUZI* INFECTION AND QUIL-A ADJUVANT ADMINISTRATION

Zabala B, Vázquez ME, Mesías AC, Acuña L, Pérez Brandán C.
Instituto de Patología Experimental (CONICET-UNSA). Salta, Argentina. E-mail: bren.zabala@gmail.com

Several immunoprophylactic attempts have been made in order to prevent the establishment and persistence in the host of *Trypanosoma cruzi*, the etiologic agent of Chagas disease (CD). Notwithstanding, up to date there are no vaccines for human application. Most assessed protocols included heterologous, prime-boost immunization schemes based on combination of subunits such us recombinant proteins and nucleic acids. These formulations are less immunogenic than live attenuated vaccines. Further, adjuvants are key components as they enhance and modulate the immune response against vaccine antigens. In our laboratory, we have extensively studied the attenuated TCC strain of *T. cruzi* and we have recorded the immune response profile of different animal models inoculated with these parasites. Our goal is to study live parasites based-formulations when adding Quil-A adjuvant (TCC+Quil-A), in order to evaluate the combination as an experimental immunogen against CD. We performed a murine immunization scheme using metacyclic trypomastigotes formulated with Quil-A. During the vaccination stage, an increase in IL-10 and IFN-γ production was detected in stimulated spleen cells of TCC-immunized animals when compared with non-immunized control group. Distinctly, we noted that IFN-γ levels increased and IL-10 levels decreased in stimulated cells of animals that received TCC combined with Quil-A (*vs*. TCC). In relation to the humoral response, levels of anti-*T.cruzi* specific IgG1 and IgG2c antibodies were higher in TCC-immunized mice sera than in non-immunized control group. IgG1 ratio was significantly higher from IgG2c when compared sera from animals treated with TCC+Quil-A in relationship to those inoculated only with TCC. After virulent challenge with Tulahuen blood trypomastigotes, parasitemias were almost undetectable in all immunized mice, whereas control groups (treated with PBS or Quil-A alone) showed high parasite load and died. Several factors are implicated in the modulation of the immune response and they still need to be tested in order to materialize the use of live parasites combined with specific adjuvants as a possible vaccine formulation. In summary, the results obtained so far indicate that Quil-A proved to be a modulator of the immune response elicited by TCC, triggering a balanced Th1/Th2 phenotype. Considering our previous results involving attenuated parasites immunizations as a prophylactic strategy for CD, we strongly support that it is worthy to continue working on multicomponent vaccines approaches.

MI-P27
CHRONIC USE OF β-LACTAMS CAN SELECT AMPC MEDIATED PRERESISTANCE TO LAST GENERATION CEFTOLOZANE IN *PSEUDOMONAS AERUGINOSA*

Colque CA[1], Tomatis PE[2], Albarracín AG[1,3], Dotta G[2], Moreno DM[4], Hedemann G[1], Moyano AJ[1], Bonomo R[5], Johansen H[6], Molin S[6], Vila AJ[2], Smania AM[1]
[1]CIQUIBIC-UNC, [2]IBR-UNR, [3]IRNASUS, [4]IQUIR-UNR, [5]Cleveland VA Med Center OH, [6]NNFCB-DTU DK. E-mail: acolque@fcq.unc.edu.ar

Cephalosporin resistance mediated by β-lactamase production remains one of the ultimate challenging problems in clinical settings. Among the most understudied, are the class C AmpC cephalosporinase, which confers high-level resistance. The recently introduced fifth-generation cephalosporin ceftolozane (CTZ), in combination with the β-lactamase inhibitor tazobactam (TAZ), has shown to be a promising therapeutic tool against *P. aeruginosa* resistant to far the most used ceftazidime, carbapenemes, and piperacillin-tazobactam. Regrettably, rapid emergence of resistants to CTZ/TAZ have been observed in patients treated with this important new therapy. We previously showed that after more than two decades of evolutionary history under long-dose of β-lactam treatment during cystic fibrosis (CF) chronic infection, *P. aeruginosa* hypermutator lineages were able to adapt by the accumulation of mutations within the *ampC* gene. Interestingly, hypermutability favored the emergence of 7 undescribed alleles consisting of differentially combined mutations (referred to as AmpC-1 to AmpC-7) shaping a highly diversified population. When expressed in an AmpC-deficient PAO1 strain and compared to wild-type β-lactamase (PDC-3), some alleles conferred 3- to 5-fold MICs increase to ceftazidime and aztreonam contributing to the high β-lactam resistance of the clinical isolates. Evenly, by enzyme-kinetic measurements, mature purified AmpC proteins displayed β-lactam hydrolysis capability 10- to 30-fold more active against ceftazidime than PDC-3. Here, we further assessed whether combinations of mutations were involved in CTZ resistance. Although the CF patient was never treated with this antibiotic, *ampC* alleles harboring 3 to 5 distinct mutations were intermediate or resistant to CTZ. Of notice, AmpC-2 (Q120K-P154L-V213A) and 4 (A89V-Q120K-V213A) were the less susceptible variants. Combination with TAZ, partially decrease MICs of AmpC-2 and 6 (A89V-Q120K-H189Y-V213A) whereas AmpC-4 and 5 (A89V-Q120K-V213A-N321S) were not affected by inhibitor addition. Mature AmpC-4, 5, 6 and PDC-3 were expressed and purified and their hydrolysis capability against CTZ was determined through enzyme-kinetic measurements. Importantly, kcat/Km values were between 8-150 times higher than that of PDC-3, showing robust CTZ hydrolysis. Molecular Docking simulations revealed key structural insights, indicating that mutations Q120K, V213A and N321S wide the substrate-binding pocket entrance, enabling accommodation of the ceftazidime, aztreonam and CTZ more bulky R1 side chain. Q120K and V213A also contribute to a conformational change in Y221 residue favoring stacking interaction between aromatic residues of the enzyme and β-lactam. This flip possibly facilitate substrate binding in AmpC-4 and

explain its higher hydrolytic capability. A deeper understanding of the mechanisms involved in AmpC protein evolution is imperative to advance in the design of novel compounds to overcome this resistance.

MI-P28
THE WSP-LIKE GENE CLUSTER OF *HALOMONAS TITANICAE* KHS3 ENCODES A METHYLTRANSFERASE SPECIFIC TO ITS COGNATE RECEPTOR

Ramos Ricciut FE, Herrera Seitz MK, Studdert CA.
Instituto de Agrobiotecnología del Litoral - Santa Fe. E-mail: claudia.studdert@santafe-conicet.gov.ar

The environmental strain *Halomonas titanicae* KHS3, isolated from Mar del Plata harbor, encodes two chemosensory systems. The canonical *che* cluster, *che1*, contains all the genes needed to control the flagellar movement in response to chemical gradients. The second cluster, *che2*, is homologous to the *wsp* cluster from *Pseudomonas* that has been implicated in biofilm formation. *Che2* consists of seven putative proteins: a chemoreceptor Htc10, a methyltransferase CheR2, an esterase CheB2, a histidine kinase CheA2, two CheW-like coupling proteins and a diguanylate cyclase. We hypothesize that Htc10 transmits information from the extracellular medium to CheA2, through CheW protein/s. This kinase activates the diguanylate cyclase by phosphorylation, affecting the levels of cyclic di-GMP and, consequently, some downstream process/es. The aim of this work was to investigate the specificity of CheR2 as a methyltransferase. To assess whether CheR2 was able to methylate the chemoreceptor Htc10, both proteins were expressed in *Escherichia coli* cells. The presence of CheR2 caused a shift in the electrophoretic mobility of Htc10, indicating that it was indeed methylated. The occurrence of Htc10 methylation by CheR2 was confirmed by mass spectrometry. MS results revealed methylation in conserved glutamate residues, within a region that corresponds to the adaptation domain of canonical chemoreceptors. In contrast, CheR2 did not change the mobility of Tsr, the serine *E. coli* chemoreceptor. Likewise, the methyltransferase CheR from *E. coli* changed the mobility of Tsr but not that of Htc10, indicating that both methyltransferases showed substrate specificity. In addition to the methyltransferase domain, CheR2 has tetratricopeptide repeats (TPR), absent in *E. coli* CheR. To assess whether the TPR domain affected CheR2 activity, we created a truncated protein, through site directed mutagenesis, with a stop codon before TPR. In SDS-PAGE, the patterns of Htc10 bands in the presence of full-length or truncated CheR2 were indistinguishable, indicating that both forms of the enzyme were capable of Htc10 modification. To find out the role of the *che2* cluster in *Halomonas*, we introduced an expression vector encoding CheR2 in wild-type *Halomonas titanicae*. Since the unbalanced expression of the methyltransferase is expected to shift the population of Htc10 towards a more methylated state and perhaps affect the downstream processes controlled by this system, we analyzed biofilm formation, motility and cell adherence in transformed *Halomonas* under induced conditions. So far, we did not detect significant changes in the analyzed behaviors. We have demonstrated that CheR2 functions as a dedicated methyltransferase that uses Htc10 as a substrate but not a canonical chemotaxis receptor. Ongoing studies are directed towards the achievement of higher overexpression systems to assess a variety of phenotypes. This might help us to find clues about the main role of this chemosensory system.

MI-P29
DIFFERENTIAL IMMUNOLOGICAL OUTCOME AFTER INFECTION WITH *TRYPANOSOMA CRUZI* NATURAL ISOLATES

Ragone PG, Diosque P, Tomasini N, Acuña L, Uncos DA, Ramos F, Pérez Brandán C.
Instituto de Patología Experimental, CONICET - Universidad Nacional de Salta – Argentina. E-mail: p_ragone@yahoo.com.ar

Chagas disease outcome depends on critical interactions between the genetic diversity of the causal agent *Trypanosoma cruzi* and host factors as the immune system. This idea was elucidated using animal models to understand how immunological processes contribute to controlling parasite levels and promoting tissue inflammation in the chronic phase. In this work we evaluated the immune response associated with the parasite load induced by two natural isolates characterized as DTUs TcVI and TcV, which are the prevalent DTUs in human infections in Argentina. For this purpose, C57BL/6 mice were inoculated with *Triatoma infestans* -derived parasite forms of two isolated obtained from an endemic area for Chagas disease in Argentina. At different time points, we measured the concentrations of total IgGs and IgG subtypes and the levels of IL-10, IFN-ɣ, and TNF-α. Besides, parasite load in blood and target organs was measured by qPCR. In our results mice infected with isolate TcVI presented a specific antibody response at 30 days post-infection (dpi) with a predominance of the subtype IgG2 and high levels of anti-inflammatory cytokine IL-10 followed by IFN-ɣ, and TNF-α. Whereas mice infected with TcV isolate presented a serological response at 90 dpi without predominance of any IgG subtype in particular and almost an undetectable cytokine response. In spite of this, circulating parasite and different parasite load in blood, heart and skeletal muscle we detected in both experimental groups. In conclusion, we detected marked differences at the immunological level product of the infection by two natural isolates of different DTUs. Isolate TcVI induces, during the acute phase of infection, a robust specific antibody response and cytokine profile that controlled the infection during the chronic phase. While isolate TcV does not seem to induce an early activation of the immune system allowing to establish a subpatent and almost undetectable infection in infected mice.

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

MI-P30
A NEW DNA BINDING SITE IN THE MISMATCH REPAIR MUTS PROTEIN INVOLVED IN THE INTERACTION WITH DNA REPLICATION STRUCTURES

Ibáñez M[1], Castell SD[1], Margara LM[1], Argaraña CE[1], Miguel V[2], Monti MR[1].
[1]CIQUIBIC-CONICET. Dpto. de Qca. Biol., FCQ-UNC, Córdoba, Argentina. [2]IIBYT-CONICET, Dpto. de Qca., FCEFN-UNC, Córdoba, Argentina. E-mail: mmonti@fcq.unc.edu.ar

MutS maintains genomic stability by recognizing mispaired nucleotides (MMs) and triggering the Mismatch Repair (MMR) pathway. Findings from our laboratory have demonstrated that the repair factor also contributes to DNA replication fidelity by regulating the access of the mutagenic DNA polymerase IV to replication sites. In this work, we showed for the first time that MutS from Pseudomonas aeruginosa can interact with DNA structures present at replication sites, i.e. primed DNA (pDNA). Moreover, MutS suffered a conformational change upon binding to a pDNA containing a GT mismatch (GT-pDNA), resulting in a more compact and stable protein structure, as determined by native gel electrophoresis, circular dichroism and trypsin digestion assays. This structural rearrangement was not observed when MutS was associated with pDNA and the MMR double-stranded DNA substrates, GT-dsDNA and dsDNA. We hypothesized that the MutS conformational change induced by its association with the mismatched pDNA could result from binding to new protein residues. In fact, using the nucleic acid binding prediction BindUP software and DNA-protein docking analysis, we identified a new DNA binding surface in MutS. Within this novel site, Arg275 appeared to directly contact the 3'-OH end of the pDNA. Mutation of this residue to Glu abolished DNA binding *in vitro* and produced a mutator phenotype *in vivo*, indicating a key role of this novel binding site in the activity of MutS. We are testing if the structural change induced by the mismatched replication substrate and the new DNA binding site are important for MutS gaining access to replication sites. In conclusion, our results reveal a novel DNA interaction site in MutS, which may play an important role in the control of Pol IV access to replication sites by MutS.

MI-P31
DNA REPAIR AND TOLERANCE PATHWAYS INVOLVED IN THE ROCESSING OF A SINGLE LESION ON THE CHROMOSOME OF *ESCHERICHIA COLI*

Margara LM[1], Pagès V[2] and Monti MR[1]
[1]CIQUIBIC-CONICET. Dpto. de Qca. Biol., FCQ-UNC, Córdoba, Argentina. [2]CRCM-CNRS, Marseille, France. E-mail: mmonti@fcq.unc.edu.ar

The genome is constantly exposed to DNA damaging agents that alter the integrity of the DNA molecule. Most of these lesions are removed by the repair mechanism called Nucleotide Excision Repair (NER). However, some lesions might escape the repair process and perturb DNA synthesis by the replicative DNA Polymerase (Pol), resulting in cell lethality. To survive DNA damage, cells have evolved Damage Tolerance (DT) pathways such as the Translesion Synthesis (TLS), which involves specialized Pols capable of inserting a nucleotide opposite the lesion. In this work, we studied both repair and tolerance pathways implied in the processing of a single alkylation lesion (N^2-furfurylguanine, FF) inserted site-specifically in the chromosome of *Escherichia coli*. The main results were: i-TLS was highly favored over other DT pathways; ii- Pol IV, but not Pol II and Pol V, catalyzed the TLS reaction; iii- Pol IV incorporated the correct nucleotide opposite the FF lesion and thus, the TLS reaction is error-free; iv-the proofreading subunit of the replicative Pol III prevented the Pol IV-catalyzed TLS, indicating a competition between exonucleolytic excision and TLS; v-the FF lesion was not removed by the NER, which is the main repair pathway implicated in the reversion of the alkylating damage; vi- the postreplicative Mismatch Repair (MMR) pathway was able to repair this damage. Finally, no differences in the repair or TLS of the FF lesion were detected when the damaged base was located in the leading or the lagging strand of replication. Our finding showed that Pol IV-catalyzed TLS and MMR become the main tolerance and repair pathways used by *E. coli* cells to deal with FF lesions on the chromosome.

MI-P32
ANTI-INFLAMMATORY EFFECTS OF *LACTOBACILLUS PLANTARUM* CRL 759 SUPERNATANT IN OCULAR INFLAMMATIONS

Layús BI[1], Gómez MA[2], Cazorla SI[1], Rodriguez AV[1]
[1] Centro de Referencia para Lactobacillus. CERELA-CONICET, [2] Hospital Ángel C. Padilla. Tucumán. E-mail: blayus@cerela.org.ar

Anti-inflammatory effect of probiotic bacteria cell free supernatant was extensively proved as therapy for different inflammatory diseases, but no investigated in ocular inflammatory disorders. Uveitis, an intraocular inflammatory disease, is a common cause of vision loss. Traditional treatments with corticosteroid present several side effects, and alternative therapies are continuously investigated. The aims of this study were to evaluate whether *Lactobacillus plantarum* CRL 759 supernatant (LplS) was able to diminish the inflammatory response triggered by LPS in ARPE-19 cells (human retinal pigment epithelium cell line); in addition, to evaluate *in vivo* its capacity to exert anti-inflammatory effect in uveitis induced by endotoxin in mice. *L. plantarum* CRL 759 was cultured in DMEM medium at 37ºC and 5% CO_2. LplS was obtained by filtration with 0.22 µm membranes. ARPE-19 cells (2.5×10^5) were treated with LplS 4 h; then, the cells were stimulated with LPS (10 µg/mL). Cytokines (by flow cytometry), NO and TBARS (by colorimetric methods) produced by ARPE-19 cells were measured in the culture supernatant. To induce uveitis, 130 µg LPS was injected subcutaneously into C57BL/6 mice. LplS was administered as drops and Prednisolone (P) was used as anti-inflammatory control. The mice were divided into six groups randomly: LPS group (LPS injection + PBS drops); LPS + LplS group (LPS injection + LplS drops); LPS + P group (LPS injection + prednisolone drops) and control groups: treated with PBS, LplS or P drops and a PBS injection. 24 h after stimulation with LPS or PBS, mice were sacrificed. The ocular inflammation was assessed by slit lamp microscopy and clinical scores were determined at the same time. The aqueous humor (AqH) was collected, and total protein (by Bradford assay), TNF-α level (by ELISA), and cell count (by Giemsa coloration) were determined. Eyes were enucleated to histopathologic evaluation. Results showed that LplS reduced the production of IL-6, IL-8, NO and TBARS in LPS-stimulated ARPE-19 cells. *In vivo* studies, the clinical score of mice treated with LplS drops

was significantly lower than the LPS group. LplS also reduced levels of TNF-α and protein concentration in AqH. Histological examination showed reduction of infiltrating inflammatory cells in the posterior segment of LPS + LplS group, however, there was no significant difference in leukocyte count in AqH in all groups. LplS anti-inflammatory effect was similar to that induced by prednisolone. In this study, we showed that LplS as ophthalmic drops attenuates the inflammatory process in an endotoxin-induced uveitis. These effects were comparable to the one achieved by prednisolone and could be proposed as a potential therapy for ocular inflammatory disorders.

MI-P33
SELECTION OF LACTIC ACID BACTERIAL STRAINS ABLE TO MODULATE THE HOST CENTRAL NERVOUS SYSTEM

Bulacios GA[1], Salazar PB[2], Posse de Chaves E[1], Hebert EM[1], Minahk C[2], Saavedra L[1]
[1]CERELA-CONICET, [2]Instituto Superior de Investigaciones Biológicas (UNT-CONICET). E-mail: gbulacios@cerela.org.ar

Current evidence indicates that modulation of the central nervous system (CNS) by the microbiome occurs primarily through neuroimmune and neuroendocrine mechanisms, often involving the vagus nerve. In fact, the gut-brain axis provides the intestinal bacteria and its metabolites a way to get access to the brain, thus regulating the expression of key effectors. Although lactic acid bacteria (LAB) represent only a small percentage of the total gut-dwelling microorganisms, they are undoubtedly important players. Pro-inflammatory cytokines are naturally upregulated in the elderly and gut microbiota undergoes changes during aging. Therefore, it has been proposed that administration of probiotics may decrease the synthesis of these pro-inflammatory cytokines, hence reducing inflammation and oxidative stress, ameliorating the effects of senescence and the progression of neurodegenerative diseases often associated with aging. These facts strongly suggest that LAB may be an invaluable tool in the treatment of aging-related pathologies such as Alzheimer's disease (AD), where microglia and non-parenchymal macrophages drive the neurodegeneration via neuroinflammation. The aim of the present work was to assess the capacity of different LAB strains metabolites to regulate the secretion of cytokines, inhibit acetylcholinesterase (AChE) and protect cells from the cytotoxic effects of the Aβ oligomers, key features of AD. For that purpose, murine RAW 264.7 macrophages were treated with conditioned media from seven selected LAB strains prior to the LPS stimulation. The mRNA expression levels of the tumor necrosis factor alpha (TNF)-α, interferon-gamma (IFN-*γ)* and interleukin IL-10 were examined by RT-PCR. The results showed that conditioned media from *Lactobacillus delbrueckii subsp. lactis* CRL 581 and *Lactobacillus reuteri* CRL 1098 significantly inhibited TNF-α mRNA expression. In addition, CRL 1098 strain increased IL-10 mRNA expression level in LPS-stimulated RAW 264.7 cells. Then, all conditioned media were evaluated in their ability to inhibit AChE from human erythrocytes (AChE-E) by Ellman's method. AChE-E constitutes a model of the isoform present in the CNS. As controls, polyphenols previously characterized as efficient inhibitors of AChE as well as strong anti-inflammatory agents were used. Conditioned media from *L. delbrueckii subsp. lactis* CRL 581 showed a 40% inhibition of enzymatic activity. Finally, APP-expressing neuroblastoma cells were preincubated with conditioned media and the expression of the APP transgene was induced by butyric acid. Viability of cells were evaluated using alamar blue assay. Results evidenced that conditioned media from *L. rhamnosus* A29 and *Enterococcus mundtii* CRL 35 protected against Aβ induced neuron cytotoxicity. These preliminary data support our ongoing investigations regarding the molecular mechanism of LAB interactions in the gut brain axis.

MI-P34
CHARACTERIZATION OF NEW ANTIMICROBIALS PRODUCED BY CLINICAL ISOLATES FROM FECAL SAMPLES FOR BIOTECHNOLOGICAL APPLICATION

Renteria J, Apaza N, Delgado MA, Pescaretti MM.
INSIBIO (CONICET-UNT) and Instituto de Química Biológica "Dr. Bernabé Bloj". E-mail: mail:justi.renteria@gmail.com

Foodborne diseases are one of the most widespread problems of the worldwide population and are produced by the ingestion of contaminated water or food. We analyzed the *Shigella* clinical isolates (CI), from patients suffered gastrointestinal infections during the summer period 2013-2017 from Catamarca, Santiago del Estero and Tucumán patients (Northwest Argentina region), in order to select those capable to produce antimicrobial compounds, using the plate's diffusion technique. We found that 11 of the total analyzed samples (371 CI) were able to produce an antimicrobial agent that inhibited the growth of the *Escherichia coli* AB1133 used as an indicator sensitive strain. In addition, in this work we characterized these compounds studying its thermotolerance (100°C), stability to pH (5 and 8) and proteinase treatments, as well its cross immunity against other bacteriocin producer strains, and its spectrum of antimicrobial action, between other characteristics. To complete the proprieties and classification of these compounds, the molecular weight was also estimated by the ability of such agents to diffuse through dialysis membranes with different cut-off size pores, mainly in the range of 10 to 12000 Da. The results indicate that the total numbers of antimicrobials are tolerant to the temperature (100°C) and sensitive to the proteinase K treatment, which are two desirable characteristics for use as food preservatives. In addition, we found that only 2 compounds are affected by pH 8 treatment. The cross-immunity test showed that at least 3 antimicrobials have a different nature and can be classified as different agents. From this study, we select producer strains of these 3 new compounds and analyzed the bacterial growth and the antimicrobial production curve through the time. On the other hand, we investigated if the production of these antimicrobial agents was induced by mitomycin C (0,5 µg/mL), an inducer agent of the SOS system required to activates the synthesis of high molecular weight bacteriocins called colicins. In this work we demonstrated that 3 of the 11 antimicrobials studied presented important characteristics that enable their use and development as new antibiotics or new food preservatives and that at least one of them is a different compound to the typical colicins produced by *Shigella*.

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

MI-P35
MreD IS INVOLVED IN CHROMOSOME SEGREGATION OF *STREPTOCOCCUS PNEUMONIAE*

Olivero NB, Yandar Barahona NY, Reinoso Vizcaíno NM, Cortes PR, Hernández Morfa M, Echenique J.
CIBICI-CONICET, Departamento Bioquímica Clínica, Facultad de Cs. Químicas, Universidad Nacional de Córdoba.
E-mail: nolivero@fcq.unc.edu.ar

The bacterial cell cycle is a highly regulated process in which both division proteins and chromosome have defined spatial and temporal localization patterns. The mechanism that connects these processes is not largely studied so far, particularly in ovococci. It has been proposed that DNA replication initiation coincides with the Z-ring formation in *Streptococcus pneumoniae* and that proper segregation of the chromosomal origin is crucial for division site selection. However, little is known about the structural support that facilitates the temporal space coordination of these processes. MreB is a bacterial actin-like protein that plays important roles in the determination of cell shape and chromosome segregation in bacteria such as *Escherichia coli* and *Caulobacter crescentus.* In these bacteria, MreB forms a membrane-bound complex with MreD and MreC which is essential to maintain cell morphology. *S. pneumoniae* lacks MreB homologs but presents MreC and MreD, although their functions are not fully known. Similarly to rod-shaped bacteria, the *mreCD* genes are essential in the virulent serotype 2 D39 strain of *S. pneumoniae*, and the conditional depletion of MreCD results in cell rounding and lysis. In contrast, the R6 strain contains suppressors that allow the growth of *mreCD* mutants. In this work, we show that in the Cp1015 strain, the *mreC* and *mreD* genes can also be mutated by insertion-duplication mutagenesis resulting in cells with smaller size compared with the wild-type strain, indicating a putative role in the elongasome. Light scattering measurements by flow cytometric analysis displayed that the *mreD* mutant shows reduced cellular complexity (granular deposits, chromosomal DNA content), in contrast, the *mreC* mutant showed similar phenotype than the wild-type strain. On the other hand, we observed by epifluorescence microscopy atypical chromosomal segregation in the *mreD* mutant but not in the *mreC* strain. We obtained the revertant strains of the *mreD* mutant and this strain showed phenotypes similar to the wild-type cells. These results suggest that MreD is involved in chromosome segregation and that both MreC and MreD are probably part of the elongasome of *S. pneumoniae*.

MI-P36
OXIDATIVE STRESS CONDITIONS INDUCE PERSISTENCE TO FLUOROQUINOLONES IN *STREPTOCOCCUS PNEUMONIAE*

Hernández-Morfa M., Olivero N, Reinoso-Vizcaíno N, Cortes P, Echenique J.
CIBICI-CONICET, Dpto. Bioquímica Clínica, Facultad de Cs. Químicas, Universidad Nacional de Córdoba. Email: nolivero@fcq.unc.edu.ar

Bacterial persisters are a clonal subpopulation of bacterial cells that shows arrested or slow growth, and a decreased susceptibility to bactericidal antibiotics. *Streptococcus pneumoniae* is a main bacterial pathogen that usually colonizes the upper respiratory tract and causes pneumonia, bacteremia, and meningitis. The persistence phenomenon had not been described in *S. pneumoniae.* The main aim of our work was to determine the formation of persisters in a population of *S. pneumoniae* in the presence of fluoroquinolones, as well as the impact of acidic and oxidative stress conditions. In this work, we found that the wild-type strain formed persisters to fluoroquinolones when cells were preincubated with H_2O_2, however this phenomenon was not detected under acidic conditions, suggesting that oxidative stress is involved in this mechanism. With the purpose to determine the contribution of oxidative stress genes in the formation of persisters to fluoroquinolones, we mutated genes coding for enzymes involved in the mechanism of oxidative stress resistance, such as such as *sodA* (codes for a superoxide dismutase that degrades superoxides), *tpxD* (codes for peroxiredoxine that degrades H_2O_2) y *nrdH* (codes for a peroxiredoxine that degrades H_2O_2). The three mutants were incubated with H_2O_2 and then with fluoroquinolones, and the *sodA* and *nrdH* mutants showed no formation of persisters, while the *tpxD* mutant showed a similar phenotype to the wild-type strain.
Here, we described for the first time the formation of persisters to antibiotics in *S. pneumoniae*, particularly persisters to fluoroquinolones. In addition, we demonstrated that the formation of persisters is associated with the mechanism of oxidative stress resistance.

MI-P37
SALMONELLA VACCINE STUDY IN AN ATTENUATED STRAIN USING AN ANIMAL INFECTION MODEL WITH *GALLERIA MELLONELLA* MOTH LARVAE

Ballesteros MF, López FE, Pescaretti MM, Delgado MA.
INSIBIO (CONICET-UNT) and Instituto de Química Biológica "Dr. Bernabe Bloj". E-mail: mflorenciaballesteros@gmail.com

The *Salmonella* RcsCDB system regulates the expression of numerous virulence genes. In previously studies, we demonstrated that the *Salmonella rcsC11* mutant is attenuated in virulence, and produces the constitutive activation of the RcsB regulator, manly during infection in an animal model. The goal of the present study was analyzed the *rcsC11* attenuation effect on *Galleria mellonella* (*G. mellonella*) larvae, which can be used as a new animal infection model. This possibility is due to the great benefits that its offer as a host model including profitability, high reproducibility, ethical approval, easy handling and maintenance. The *G. mellonella* infection model allows the study many stages of *Salmonella* infection at 37 °C, the real temperature of the human body since its innate immune response has remarkable similarities with the vertebrate. To this end, we followed the infection protocol used previously in mice to improved *G. mellonella* larvae infection assays. For this purpose, the test was organized in 3 infection groups containing 10 larvae per assay infected with: i- wild type 14028s *Salmonella* Typhimurium strain, ii-*Salmonella* clinical isolates obtained from patients suffering diarrhea iii- PBS infected as control, while the iv group were infected with *rcsC11* attenuated mutant but using 20 larvae. The number of larvae was increased in the iv lot in order to carried out a second infection challenger with 14028s virulent strain. In these assays we evaluated the protective effect of *rcsC11* mutant against the 14028s virulent strain and the clinical isolates. We here observed that

rcsC11-infected larvae as well those of the control lot, were able to survive and no disease-symptoms were manifested, in contrary to the wild type strain-infected larvae. In addition, in this control lot we observed a 90% of death after the second day of infection. On other hands, the *Salmonella* clinical isolates displayed an increased virulence than the 14028s laboratory strain. Moreover, we show that the *rcsC11*-surviving larvae were immune to a second infection challenge with the 14028s virulent strain. It is important to mention that similar results were previously obtained using a mice animal infection model. We show here that *G. mellonella* larvae can be used as a new model for the study of *Salmonella* infection, comparable to the murine infection model, but with less ethical restriction and with the possibility to used high number of sample per test. In addition, in these trials the minimum infective dose to be used in this model was determined. Moreover, we demonstrated once again the ability of the *rcsC11* mutant to attenuate virulence, generating immunity against virulent *Salmonella* strains.

MI-P38
EFFECTS OF DEACETYLASE INHIBITORS ON THE GROWTH OF *TRITRICHOMONAS FOETUS*

Luque ME[1,2,3], Rivero MB[1,2], Abdala ME[1,2,3], Di lullo D[1], Luna BE[1], Volta BJ[2,3], Assis MA[1,2,3], Scrimini S[1,2,3], Rivero FD[1,2,3], Prucca CG[4] y Carranza PG[1,2,3]
[1]IMSaTeD (UNSE-CONICET), [2]FCM (UNSE), [3]FAyA (UNSE), [4]CIQUIBIC (CONICET-UNC). E-mail: pgcarranza@gmail.com

Tritrichomonas foetus is the etiologic agent of bovine trichomoniasis, an endemic sexually transmitted disease in countries with extensive livestock with natural service as a reproductive method. Although there are few studies performed at the molecular level in this parasite, nothing is known about the existence of epigenetic regulation mechanisms. The recent sequencing of its genome has demonstrated the presence of genes homologous to enzymes that methylate, acetylate and deacetylate histones, however post-translational modifications of histones and their effects on their growth are not known. For these reasons, the aim of this work was to analyze the role of these modifying enzymes through the use of their inhibitors. Histone deacetylase enzymes can be involved in various cellular processes such as cell growth and death and can be grouped into two classes: non-NAD+- dependent (HDACs) and NAD+-dependent (Sirtuins). To evaluate their function, trichostatin A (TSA) and nicotinamide (NAM) were used, respectively. To determine the effects of HDACs inhibition by flow cytometry and immunohistochemistry, parasite cultures were incubated for 24 and 48 h at increasing concentrations of TSA (0.001 to 5uM). The IC_{50} results were: 19.01nM (24 h) and 11.65nM (48 h) and the LCM obtained was greater than 50nM. Once the IC_{50} was established, the effects at this concentration were evaluated, and the results of the cytometry analysis showed a marked change in the dispersion profile of the cells with respect to that observed in the control lots without inhibitor. This coincides with the alterations observed under the microscope: spherical cells of larger size and multinucleated. Immunohistochemistry, using the anti-acetylated tubulin α antibody, showed significant alterations at the cytoskeleton level. To assess the effects of the inhibition of sirtuins, parasite cultures were incubated for 24 and 48 h at increasing concentrations of NAM (0.1 to 25 mM). The IC_{50} values were: 5.43 mM (24 h) and 3.57 mM (48 h) and the LCM obtained was greater than 25 mM. The presence of homologous genes of these enzymes and the effects observed in this study by blocking their functions, allow us to suggest for the first time that *T. foetus* presents this type of post-translational modifications to regulate the expression of genes that would be linked to cell growth and proliferation.

MI-P39
N-ACETYL CYSTEINE IMPROVES CELLULAR GROWTH IN *SACCHAROMYCES CEREVISIAE* RESPIRATORY DEFICIENT STRAIN

Pichetto Olanda I, Grondona F G, Utge S, Burgos HI, Alonso M, Chapela SP, Stella CA.
INBIOMED (UBA – CONICET). E-mail: indypichettoolanda@gmail.com.

Reactive oxygen species (ROS) is a main factor that alters cellular physiology and functionality. Many strategies are used in order to control excessive oxidative stress. One strategy includes the use of antioxidants like N-Acetyl cysteine (NAC). Studies on *Saccharomyces cerevisiae* yeast have shown that the NAC molecule has an antioxidant effect. Using a simple system such as yeast allows assays in which current factors are more restricted or manageable than in higher organism. Due to *Saccharomyces cerevisiae* yeast metabolism, these cells can grow under both aerobic and anaerobic conditions. This is an interesting biochemical feature when planning research that mimic hypoxia and re-oxygenation cycles which are detected in pathological situations of higher organisms; such as sepsis and septic shock, pathological pregnancies and ischemia-reperfusion injury. It is known that in *S. cerevisaie* cells, NAC decreases the formation of reactive oxygen species (ROS) under aerobic growth conditions, and also prevents *cyt c* release and caspase-like activation. The aim of this study was to compare the effect of this antioxidant on ROS production and cellular growth of a wild type cell and a respiratory deficient strain. Cells of the wild strain MMY2 and its respiratory deficiency grew in YPD medium (yeast, peptone, glucose) for 48 hours. An aliquot was suspended in potassium acid phthalate buffer (pH = 4.5) in the presence of a final NAC concentration of 0.20 or 0.35 mg /mL. Incubation was continued for 72 hours and at its term cell viability was determined. As an indirect measure of the ROS level, the fluorescence signal of 2', 7'-dichlorofluorescein diacetate (DCFH-DA) was determined. In addition, the capacity of the NAC to stimulate the reducing power of the yeast was established using the change of color Methylene Blue (MB) indicator. As it is found in the present work a reducing environment exerted by NAC presence during incubation of the cells allows a deficient respiratory strain to improve its cellular growth. It seems likely that the energy production or the phenotype which characterizes a deficient strain is incapable to palliate ROS growth inhibition while NAC helps to overcome this limitation.

MI-P40
CHARACTERIZATION OF THE CELL WALL ASSOCIATED PROTEINASE ACTIVITY FROM *LACTOBACILLUS DELBRUECKII* STRAINS

Elean M, Cataldo P, Saavedra L, Hebert E
Centro de Referencia para Lactobacilos (CERELA-CONICET). E-mail: melean@cerela.org.ar

Lactic acid bacteria (LAB) have a very long history of use in the manufacturing processes of fermented dairy products. During milk fermentation process, the proteolytic system of LAB plays a key role because it enables these bacteria to grow in milk. LAB are fastidious microorganisms that require an exogenous source of amino acids or peptides, which are provided by the proteolysis of casein, the most abundant protein in milk and the main source of amino acids. The proteolytic system of LAB consists of a cell envelope-associated proteinase (CEP), amino acid and peptide transport systems and various intracellular peptidases. The CEP is the key enzyme of the system and it is responsible for casein initial degradation. In the present work, the goal is to characterize the proteinase activity of 36 *L. delbrueckii* strains belonging to the CERELA culture collection considering the major economic importance of these species as dairy starters. All strains were subjected to genotyping using the rep-PCR technique to group those isolates corresponding to clones of the same strain. One representative of each profile group was selected to further characterize their CEP enzymes. The strains were grown in a chemically defined medium (CDM) and their proteolytic activities were evaluated by two methods: the degradation of the chromogenic substrate succinyl-alanyl-alanyl-prolyl-phenylalanine-p-nitroanilide; and by the degradation profiles of alpha- and beta-casein by SDS-PAGE. Results from the hydrolysis of alpha- and beta-casein degradation evidenced six types of caseinolytic cleavage specificity. Since proteolytic activity is repressed under high peptide content, we next study the inhibitory effect of peptides concentration on the CEP activity by growing bacterial cells in CDM plus Casitone. The proteolytic activity was repressed in the presence of peptides; however the strength of repression was strain-dependent. Finally, the release of these CEPs from the cell envelope was observed after treatment with 2 M NaCl. These results contribute to enlarge the limited knowledge on thermophilic lactobacilli CEP and are important from an industrial point of view since during the manufacture of hard cheeses, high concentrations of NaCl are present, and CEPs would remain active either bound to the cell or released, maintaining the beneficial health effects of the fermented milk products.

MI-P41
ANTIFUNGAL ACTIVITY OF METALS-FLAVONOID COMPLEXES AGAINST PHYTOPHATOGENIC FUNGI WITH AGRONOMIC INTEREST

Burgos G [1], Alarcón R [1], Cristóbal H A [1,3], Céliz G [2,3].
[1]Facultad de Cs Naturales, [2]Facultad de Cs Exactas, [3]INIQUI, UNSa, Av. Bolivia 5150, Salta, Argentina. E-mail:soledadburgos1988@gmail.com

Phytopathogenic fungi are organisms that cause disease in plants; they affect agricultural production generating large economic losses in diverse crops. The need to find new environmental-friendly fungicides has led researchers to search for compounds that accomplish this concept. Naringin (NAR) and naringenin (NGE) are flavonoids obtained from wastes of the Citrus industry, with diverse biological properties. These flavonoids can form complexes with ion transition metals. Currently, the study of the antifungal properties of metal-flavonoid complexes is practically unexplored. In this work, the antifungal activity of some metal-flavonoid complexes was evaluated against three phytopathogenic fungi. This activity was quantitatively evaluated by contact assay using potato dextrose agar as culture medium. In *Sclerotium rolfsii*, solutions of Cu(II)-NGE (62 mmol/L), Cu(II)-NAR (32 mmol/L), Ni(II)-NAR (32 mmol/L) and Mn(II)-NAR (14 mmol/L) complexes were tested. Also, flavonoids NGE (124 mmol/L) and NAR (64 mmol/L) were assayed alone. For *Rhizoctonia solani* and *Sclerotinia sclerotiorum,* Cu(II)-NAR (32 and 16 mmol/L), NAR (64 and 32 mmol/L) and $CuSO_4.5H_2O$ (32 and 16 mmol/L) solutions were tested. To prepare these solutions, N, N-dimethylformamide/water 10 % (v/v) have been used as a solvent. The assays were realized with five replicates for each treatment. The results revealed that *S. rolfsii* was highly inhibited by Ni(II)-NAR complex (91 %), followed by Cu(II)-NGE (72 %), Mn(II)-NAR (36 %) and Cu(II)-NAR (3 %), whereas NGE caused only 24 % of inhibition and NAR not manifested antifungal activity. Solutions of Fe(III)-NAR (40 mmol/L), Co(II)-NAR (24 mmol/L) and Cr(III)-NAR (6 mmol/L) were also tested, but they not exhibited activity. *S. sclerotiorum* not presented inhibition by any of the compounds assayed. For *R. solani*, only the solution of $Cu(II)SO_4.5H_2O$ at 32 mmol/L caused significant inhibition (16 %). In conclusion, this study is probably the first reported about the antifungal activity of this kind of compounds, and it demonstrates that some of these coordination compounds have potential as new environmental friendly fungicides.

MI-P42
PROTEOMIC ANALYSIS TO UNDERSTAND CR(VI) HOMEOSTASIS IN *STREPTOMYCES* SP. MC1

Sineli PE[1], Aparicio JD[1,2], Pernodet Jean-Luc[3], Polti MA[1,4]
[1]PROIMI-CONICET, Tucumán, Argentina; [2]FBQF, Tucumán, Argentina; [3]I2BC, Paris, France; [4]Fac. Cs. Nat., Tucumán, Argentina.
E-mail: sinelip@gmail.com

Chromium is a heavy metal widely used in a variety of industrial processes (leather tanning, steel production, metal corrosion inhibition). Hexavalent chromium is carcinogenic and presents higher toxicity than trivalent form since Cr(VI) is more water-soluble and mobile than Cr(III). Industrial effluents containing Cr(VI) are released into water courses, mostly without proper treatment, resulting in anthropogenic contamination. Over the last years, bacteria-mediated removal or stabilization of heavy metal into no or less toxic forms has become in an effective biotechnological process. In this sense, several physiological studies on *Streptomyces* sp. MC1, an actinobacteria isolated from a polluted soil in the province of Tucumán (Argentina), demonstrated be able to grow in presence of Cr(VI) and remove the metal both in liquid medium and contaminated soils. However, the molecular mechanisms involved are unknown in this actinobacteria. MS-based proteomics have become a powerful tool to understand the mechanisms that underlie physiological processes. In the present work, we use MS-based, label-free and

quantitative proteomic analyses in order to identify enzymes involves in oxidative stress response caused by the presence of Cr(VI) in our actinobacteria strain MC1. Sampling points for proteomics analyses were established according to the growth of *Streptomyces* sp. MC1 in minimal medium (MM) amended with Cr(VI) at 50 mg L^{-1} and MM without the metal (control condition). Cells were harvested after 18 and 24 h of incubation in control condition and MM with Cr(VI) respectively. These sampling points allowed obtaining comparable and metabolically active cells (exponential phase of growth). Cr(VI) removal was 10% at the time that cells were harvested (24 h). A total of 1981 different proteins were detected in the proteome. It represents approximately 22% of the predicted protein sequences for this strain. 518 of these proteins passed our significance parameters which 186 of them were up-regulated in the condition supplemented with Cr(VI). Analysis with the software BlastKOALA showed that up-regulated proteins were distributed in metabolic pathways that result essential for a correct cellular operation. Overall, the proteins were related to carbon and energy metabolism, genetic information processing, oxidative stress response and membrane transports. Interestingly, enzymes from pentose phosphate pathway increasing significantly their abundance in presence of chromium. About, 10 different oxidoreductases enzymes were up-regulated in presence of the metal. Regarding oxidative stress response, key enzymes like superoxide dismutase, catalase, mycothiol synthase, and mycothiol amidase were identified with an increment in their abundance. The proteome analysis performed in *Streptomyces* sp. MC1 allowed us to identify the proteins involves in the homeostasis of Cr(VI). These results serve as basement to study and improve the heavy metal removal by actinobacteria.

MI-P43
BIOPOLYMER-BASED FORMULATIONS TO IMPROVE THE EFFECT OF THE ANTIMICROBIAL PEPTIDE MccJ25(G12Y)

Pioli MA[1]; Slavutsky AM[1], Koltan M[2], Acuña L[3], Vincent PA[2], Corbalán NS[1]
[1] Instituto de Investigaciones para la Industria Química (INIQUI, CONICET-UNSa), [2] Instituto Superior de Investigaciones Biológicas (INSIBIO, CONICET-UNT), 3 Instituto de Patología experimental (IPE, CONICET-UNSa). E-mail: nscorbalan@gmail.com

The antimicrobial peptide Microcin J25(G12Y) present advantageous features for its use as a food preservative, such us: temperature and pH resistant, *in vitro* and *in vivo* inactivation by digestive enzymes, no effect on coliform intestinal natural population and antimicrobial activity against important foodborne pathogens like *Escherichia coli* O157:H7, *Salmonella* and *Shigella*. It is known that the efficiency of antimicrobial peptides can decreases when they are added directly to the food since the presence of carbohydrates, proteins, fats, salts, enzymes and pH strongly influence the activity of these agents. To overcome this inconvenient the development of novel formulations like, microcapsules, hydrogels, lipid-based delivery systems, are all examples of carriers for peptide delivery that may improve the therapeutic index of antimicrobial peptides by protecting their activity and improving their bioavailability. In this report we analyze the activity of MccJ25(G12Y) included in two different carrier formulations, microcapsules and hydrogels. Two natural polymers were used to perform these formulations, brea gum was used as wall material for spray drying microencapsulation and a mix of brea gum/pectin to hydrogels preparation. *In vitro* activity for both formulations was assayed against different foodborne pathogenic and spoilage bacteria, in these assays we observed that the peptide activity was not altered by microencapsulation process or during hydrogel formulation. On the other hand, the activity of microencapsulated microcin was assayed in a food model of beef burgers artificially contaminated with *E. coli* O157:H7 observing a significant decrease respect to the initial bacterial load. In summary our results reinforce the potential of MccJ25(G12Y) as a possible food biopreservant.

MI-P44
DESIGN OF A COMPETITIVE INTERNAL AMPLIFICATION CONTROL (CIAC) FOR PCR TARGETED TO *YERSINIA ENTEROCOLITICA-YST* GENE

Mastrodonato AC, Lapadula W, Juri-Ayub M, Favier GI, Lucero Estrada C y Escudero ME.
Área de Microbiología, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis.
E-mail: annachiaramastrodonato@gmail.com

The isolation of *Yersinia enterocolitica* (*Ye*) from complex matrices such as foods presents difficulties due to the competition between this microorganism and the accompanying flora. To overcome the limitations related to culture techniques, molecular methods based on polymerase chain reaction (PCR) have been developed to detect virulence genes such as *yst* (*Ye* thermostable toxin). However, foods are complex matrices that may contain inhibitors of this reaction. To avoid false negative results, it is advisable to introduce a competitive internal amplification control (cIAC) into the PCR reaction mixture. This is a DNA molecule which is amplified by the same primers as the target sequence, but that is distinguished from it by its different molecular size. Thus, the cIAC is incorporated into the PCR reaction mixture where, together with the target DNA, will compete for the primers. In *yst* negative samples, one band corresponding to cIAC should be observed, unlike two bands (*yst* and cIAC) that will be observed in a positive sample. To *3* obtain the cIAC, a pair of oligonucleotides complementary to a DNA region belonging to the *Culex molesus* genome and flanked at their ends by the primer sequences targeted to *Ye yst* gene, were designed. This *C. molesus* sequence was analyzed by BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to verify the absence of regions complementary to the *yst* gene. The cIAC was amplified by PCR using Taq polymerase and subsequently cloned into the pGEM-T Easy® vector (pG, Invitrogen), obtaining a product of 417 bp (greater molecular weight than the *yst* amplicon of 190 bp). The cIAC will allow check the efficiency of *yst* PCR in the detection of *Ye* in foods.

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

MI-P45
STUDY OF THE PREVALENCE OF *MYCOPLASMA GENITALIUM* IN A WOMEN'S POPULATION IN CÓRDOBA

Masner M[1], , Kiguen X[2], Lujea N[1], Ganzer L,[2]Larcher J[2], Cuffini C[3], Kunda P[1]
[1] CIMETSA. IUCBC. Córdoba, [2] INVIV.FCM.UNC. Córdoba, HPUC, [3] IUCBC, Córdoba. E-mail: patricia.kunda@iucbc.edu.ar

Mycoplasmosis caused by *Mycoplasma genitalium* (MG) is considered in recent years as one of the emerging sexually transmitted diseases. The infection has been associated with vaginitis, spontaneous abortions, urethritis, etc. Little is known about the existing genotypes and the prevalence in our Province. Even less is known of the relationship between this bacterium and infertility and cancer. MG is difficult to grow because it needs special growing conditions. In addition, the growth of mycoplasma is slow, so the diagnosis based on the plate culture technique can take three or more weeks. The PCR technique, on the other hand, is fast, and highly sensitive and specific. Given the clinical and sanitary importance of MG and the difficulty of diagnosing it, we set out the PCR-based methodology for specific detection of *M. genitalium* and study the prevalence of mycoplasmosis in women in Córdoba. Genomic DNA extracted from vaginal exudates of gynecological consultation patients from the Private Hospital and the National Hospital of Clinics, were used for the study. The project has the approval from the HPUC CIEIS and it was approved by the Research and Teaching Committee from the HPUC for the retrospective analysis of samples from the National Hospital of Clinics. The presence of MG and Ureaplasma was determined by PCR using different primers directed against the DNA of the 16S ribosmal subunit of the bacteria. The results showed that MG is present in 4% of the patients analyzed while Ureaplasma has an incidence close to 50%. This is the first report of the presence of MG in these health centers, and the first in Córdoba based on molecular methods. We believe that being able to have a diagnostic method of high sensitivity, specificity and speed will result in a better treatment and patient response.

MI-P46
NISIN PURIFICATION AND EVALUATION OF ITS ANTIMICROBIAL ACTIVITY IN COMBINATION WITH EDTA AGAINST GRAM-NEGATIVE PATHOGENS

Lanza, Chalón MC, Bellomio A.
Instituto Superior de Investigaciones Biológicas INSIBIO-CONICET-UNT, Tucumán, Argentina. E-mail: lulanza_522@hotmail.com.ar

Nisin is an antimicrobial peptide, produced by *Lactococcus lactis*, as a defense system against competing microorganisms. Nisin exerts a dual mode of action to kill Gram-positive bacteria. Firstly, it inhibits cell wall synthesis by binding toward lipid II. Secondly, the peptide permeabilizes the cell membrane by forming pores that disrupt vital ion gradients. This peptide is widely used as a food preservative. Nevertheless, it is difficult for nisin to penetrate the outer membrane of Gram-negative bacteria, and thus, it cannot reach its target in the inner membrane. This leads to the inactivity against Gram-negative bacteria. Notably, nisin can inhibit the growth of these bacteria when chelating agents, like EDTA, are used to destabilize the outer membrane. Thus, the bottleneck for nisin to be active against Gram-negative bacteria appears to be its ability to pass the outer membrane. The aim of this work was to improve the purification of nisin from commercial preparations and evaluate its activity against Gram-negative foodborne pathogens. Methods: TECNIS® (commercial preparation of nisin 2.5%) was suspended in water and stirred at room temperature before dichloromethane was added. Instantaneously, a white precipitate was formed at the interface of the yellow aqueous layer and the colorless organic layer. The obtained emulsion was centrifuged, after which the desired nisin fraction formed a brown solid pellet at the interface of both layers. The pellet was dried to remove any residual solvent. The nisin extract was subsequently dissolved in water, followed by filtration. This solution was further purified by RP-HPLC in a C18 preparative column. The eluted fractions were dried *in vacuo* and stored at -20°C. Purity was checked with an analytical RP-HPLC system. Nisin activity (MIC) was evaluated against Gram-negative foodborne pathogens in liquid and solid media, using EDTA as a chelator (0.625 and 2.5 mM for *Escherichia coli* O157 and *Salmonella enterica* serovar Typhimurium 14028, respectively). *Listeria monocytogenes* FBUNT was employed as a positive activity control. The MIC was defined as the lowest nisin concentration which produces no visible microbial growth. Results: pure nisin was obtained as a brown powder and its MIC in liquid media was 1.16, 4.66 and 9.33 μM against *L. monocytogenes* FBUNT, *S.* Typhimurium 14028 and *E. coli* O157, respectively. In solid media, the MIC was 0.58 μM against *L. monocytogenes* FBUNT, and 149 μM against *S.* Typhimurium 14028 and *E. coli* O157. Conclusions: here we describe an efficient protocol for the scalable purification of nisin that allows us to obtain the enriched peptide from commercial preparations that contains only 2.5% of the desired peptide, with high antimicrobial activity against foodborne pathogens.

MI-P47
IDENTIFICATION OF A *SALMONELLA* PhoP/PhoQ SYSTEM INHIBITOR FROM A DYNAMIC COMBINATORIAL LIBRARY

Lobertti CA [1], Cabezudo I [2], Furlán RLE [2], García Véscovi E [1]
[1]Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET, [2]Área de Farmacognosia, Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR). E-mail: lobertti@ibr-conicet.gov.ar

Salmonella is an enteropathogen that causes a wide range of diseases in humans and animals. PhoP/PhoQ is a two-component system (TCS) distributed amongst several Gramnegative bacteria, consisting of the histidine kinase PhoQ, and the transcriptional regulator PhoP. In *S.* Typhimurium, the PhoP/PhoQ system regulates the adaptation to Mg2+-limiting environments and controls key virulence phenotypes such as the invasion and proliferation within host cells. As signal transduction in mammals does not involve TCS, the PhoP/PhoQ system is an attractive target to develop new antimicrobial agents. We have previously reported a methodology based on a TLC-overlay as a new strategy for the search and identification of antimicrobial agents targeting the PhoP/PhoQ system. We applied this bioguided strategy using a strain carrying a PhoP-controlled reporter gene, to the screening of a dynamic combinatorial library of hidrazones in the search for inhibitors. As a result, two libraries of hydrazones

and three libraries of thiocarbazones totalling over 370 members were screened for their inhibitory activity through a rapid inexpensive TLC strategy. Satisfactorily, a complex library of hydrazones that can repress the PhoP/PhoQ system was selected from the initial screening, to further study its members. Through iterative deconvolution of over 100 library members we identified a potential inhibitor, A25B4. This compound could be synthesized in its pure form, characterized, and it was confirmed that it does not affect the growth of *Salmonella*. By quantitative β-galactosidase assays we confirmed its inhibitory activity and it was found that the response was dose-dependent and selective as well. Once the mechanism of action of A25B4 in the system is known, a target protein domain of the TCS will be used to template a library of hydrazones, biasing the composition of the dynamic library towards A25B4. This step will further confirm its affinity and mechanism of action. This strategy allows to us to establish a novel methodology for the discovery of PhoP/PhoQ system inhibitors to fight against *Salmonella*-borne disease.

MI-P48
A GLYCOPROTEOMIC STUDY ON *XANTHOMONAS CITRI CITRI*

Landoni M[1], Carrau A[2], Orellano EG[2], Couto AS[1]
[1]UBA, FCEN, Dto.Química Orgánica, CONICET-CIHIDECAR, Buenos Aires, Argentina. [2]IBR-CONICET, FBioyF-UNR, Rosario, Argentina.
E-mail: mlandoni@qo.fcen.uba.ar

Xanthomonas citri subsp. *citri* (Xcc) is a Gram-negative bacterium that produces citrus canker, a disease that affects all citrus commercial varieties. Protein glycosylation is a post-translational modification essential to modulate protein structure and function. Glycans are involved in the pathogen-host interaction and participate in the pathogenic processes, motility, biofilm formation, cell-cell interaction and immune system evasion. A Glycoproteomic study consists in the analysis of glycoproteins, including protein identities, glycosylation sites along with the glycan structures of an organism in a specified condition. When the Xcc genome was completely sequenced, it presented three genes encoding for putative blue light receptors. As light is an important environmental signal, involving photoreceptor proteins that translate it into a biochemical signal, we have started a glycoproteomic study of Xcc grown in darkness or under white light. SDS-PAGE showed differences in the glycoprotein profile in both conditions. For this first approach, glycoproteins were fractionated by ConA chromatography and after trypsin digestion, glycopeptides were analyzed by HPLC-ESI-Orbitrap. Raw data were processed for protein identification and statistical analysis. Interestingly, from the 417 glycoproteins identified, 14 were present exclusively when the bacterium was grown under light while 110, exclusively in darkness suggesting an induction of glycosylation of proteins with high-mannose type oligosaccharides in this condition. Some of these proteins are involved in the pathogenic mechanism, motility, exopolysaccharide production and secretion systems.

MI-P49
PROTEOMIC ANALYSIS OF GAMMA AMINOBUTYRIC ACID PRODUCTION BY *LACTOBACILLUS BREVIS* CRL 2013 IN A CHEMICALLY DEFINED MEDIUM

Cataldo PG, Elean MD, Bulacios G, Saavedra L, Hebert EM
Centro de Referencia para Lactobacilos (CERELA- CONICET). E- mail: pcataldo@cerela.org.ar

Gamma-aminobutyric acid (GABA), a non-protein amino acid, plays a key role in mammals as the major inhibitory neurotransmitter of the central nervous system. Although GABA may not be able to cross the human blood-brain barrier it was approved as a food ingredient showing anti-hypertensive and anti-depressant activities as main benefits to the host after oral administration. In the last decade, several studies have demonstrated that certain lactic acid bacteria (LAB) are able to produce GABA via glutamate decarboxylase (GAD) system which consists of two important elements, the Glutamate/GABA antiporter (GadC) and a GAD enzyme (GadA or/and GadB). This system has also been associated with acid resistance in bacteria. Among the major GABA producer LAB strains, *Lactobacillus brevis* appears to be the most efficient. Hence, *L. brevis* CRL2013, a strain isolated from quinoa sourdough, was selected as the highest GABA producer strain after growth in MRS supplemented with monosodium glutamate (MSG). In this work, a chemically defined medium (CDM) was optimized to assess the effects of media composition on GABA production. The addition of fructose was essential for *L. brevis* growth in CDM (it may have a role in NADH regeneration). Interestingly, *L. brevis* CRL2013 was unable to produce GABA after incubation in a MSG- supplemented CDM (the GAD system was not completely functional). Thus, the effect of different nitrogen sources –casitone (C), casaminoacids (CA), tryptein (T) and yeast extract (YE) - on GABA production was also evaluated. A strong induction of the GAD system was evidenced in the presence of YE, while lower GABA levels were obtained in the CDM added with C and T. In order to elucidate the regulation of the GAD system, the proteome of CRL2013 strain was analyzed after cell growth in CDM plus MSG and YE (10 and 36 h). The synthesis of GadA was upregulated in the presence of YE and its expression resulted ca. 15 times higher after 36 h of incubation compared with 10 h. A third putative glutamate decarboxylase (accession number Q03NF9) was ca. 18 times upregulated in the presence of YE at 10 h. These results were validated through RT-qPCR using *recA* as the housekeeping gene. This transcriptional analysis had good correlation with the proteomic data. Nevertheless, further studies are needed to elucidate the role of Q03NF9 in GABA production. The formulation of a CDM in which the GAD system is not fully active constituted an important breakthrough to further study the regulation of this system. The proteomic approach revealed new insights to infer possible interactions with other cellular mechanisms for acid resistance. The detailed understanding of the GAD system regulation in *L. brevis* CRL 2013 coupled with the optimization of the fermentative parameters to enhance GABA productivity constitute important foundations for the development of functional foods bioenriched with GABA.

PLANTS

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

PL-P01
NBWRKY22 AND *NBWRKY25* VIGS-SILENCED PLANTS ARE IMPAIRED IN PTI AND ETI DEFENSE RESPONSES

Ramos RN[1,2], Pombo M A[1], Rosli HG[1]
[1] Instituto de Fisiología Vegetal (INFIVE, CONICET UNLP); [2] Facultad de Cs. Exactas, UNLP. E-mail: ramosrominanahir@gmail.com

Plants defend themselves against pathogens using a two layered immune system. Pattern-triggered immunity (PTI) is activated upon recognition of microbe-associated molecular patterns (MAMPs). Particularly in tomato, the detection of bacterial flagellin relies on the perception of two MAMPs: flg22 that is recognized by the receptor flagellin sensing 2 (FLS2) and flgII-28, by flagellin sensing 3 (FLS3). Virulent bacteria as *Pseudomonas syringae* pv. *tomato* (*Pst*), the causal agent of tomato bacterial speck disease, can deliver effector proteins into the plant cell to promote susceptibility. However, some plants posse resistance (R) proteins that recognize specific effectors leading to the activation of effector-triggered immunity (ETI). Resistant tomatoes such as Río Grande-PtoR (RG-PtoR) recognize two *Pst* effectors (AvrPto and AvrPtoB) through Pto/Prf complex and then activate ETI. In a previous study, we identified two tomato WRKY transcription factors (TFs), *SlWRKY22* and *SlWRKY25,* whose expression was induced specifically when ETI is activated at 6 hours post infiltration. Silencing of tomato *WRKY* ortholog genes in *Nicotiana benthamiana* (*NbWRKYs*) led to a delay in Pto/Prf-associated programmed cell death (PCD) development. Also, we observed an increase in disease symptoms in plants infiltrated with *Pseudomonas syringae* pv. *tabaci* (*P. s. tabaci*) expressing AvrPto. To confirm these results, we evaluated bacterial growth in leaf tissue and observed higher bacterial titer in plants silenced for *NbWRKYs* compared with control plants. On the other hand, we analyzed if these genes play a role in the recognition of another *Pst* effector, HopQ1-1 (effector recognized in *N. benthamiana* by Roq1 protein). Silenced plants infiltrated with *P. s. tabaci* expressing HopQ1-1 had more disease symptoms than the control ones, suggesting the participation of NbWRKYs in the recognition of more than one *Pst* effector. *Arabidopsis thaliana* WRKY22 (AtWRKY22, closest TF to SlWRKY22 and SlWRKY25), is implicated in early PTI activation. Using previously generated tomato transcriptomic data available at the Tomato Functional Genomic Database, we found that both tomato genes are induced when PTI is activated 30 minutes after treatment with flg22. To investigate the possible contribution of *NbWRKYs* to PTI, we performed a callose deposition assay and a cell death suppression assay (CDSA) in *N. benthamiana* silenced plants. Interestingly, both experiments confirmed the participation of *NbWRKYs* in PTI. Finally, we tested silencing efficiency and specificity of the different VIGS constructs used in our experiments using RT-qPCR. We observed a 90% reduction of transcript abundance using our VIGS-constructs in *N. benthamiana* plants compared with an *Ec1*-silenced negative control. Together, our results indicate the participation of both WRKY TFs as positive regulators of plant immunity.

PL-P02
STUDY OF THE ROLE OF A TOMATO WALL-ASSOCIATED KINASE (SLWAK1) IN THE DEFENSE AGAINST *PSEUDOMONAS SYRINGAE* PV. *TOMATO*

Pombo MA[1], Martin GB[2], Rosli G[1]
[1]INFIVE (UNLP-CONICET), La Plata, Argentina. [2]Boyce Thompson Institute, Ithaca, EEUU. *E-mail: mpombo@agro.unlp.edu.ar*

Plants count on a two-layered immune system to control and restrict pathogens. The first one, pattern-triggered immunity (PTI), is activated when cell surface receptors perceive microbe-associated molecular patterns (MAMPs) such as the epitope flg22 from bacterial flagellin. Certain bacterial pathogens, for example *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000), are able to deliver into the cytoplasm nearly 30 effector proteins to suppress PTI and alter metabolic processes in order to proliferate in the apoplast. The second layer of immunity, effector-triggered immunity (ETI), is activated when one of these effectors is perceived by resistance proteins and leads to a hypersensitive response that controls bacterial growth. We have previously identified using a transcriptomic-based approach, *SlWAK1* (*Solanum lycopersicum Wall-Associated Kinase*) as a gene whose expression is induced by flagellin perception and suppressed by two *Pst* DC3000 effectors, AvrPto and AvrPtoB. To test its participation in plant immunity we silenced *Nicotiana benthamiana* plants using virus-induced gene silencing (VIGS) with an *SlWAK1*-based construct. Silencing resulted in plants with a compromised PTI, higher leaf bacterial titers and more severe disease symptoms. The altered PTI phenotype was also observed when using for VIGS 2 alternative constructs based on the *N. benthamiana* orthologs. Induction with flg22 epitope, in spite of activating transcriptionally *SlWAK1*, lead to finding no difference (silenced vs control plants) in symptoms when challenged with *Pst* DC3000. Immunity induction using a mutant lacking flagellin (Δ*fliC*) and later on challenge with *Pst* DC3000 resulted in no symptom difference between *SlWAK1*-silenced and controls plants, suggesting that flagellin induction is required for full functionality of the SlWAK1-mediated pathway. Furthermore, induction with autoclaved *P. fluorescens* 55 (a strong inducer of PTI) resulted in no symptom difference between silenced and control plants, indicating that a live inducer is required for observing the phenotype of compromised PTI. Taken together our results indicate that flagellin-mediated PTI is required for *SlWAK1* transcriptional activation and that some other ligand could be perceived through SlWAK1 leading to a more robust or prolonged PTI.

PL-P03

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

ATG1 ROLE IN THE IMMUNITY OF TOMATO AGAINST *PSEUDOMONAS SYRINGAE* PV. *TOMATO*

Bekier F, Rosli H, Pombo M
Instituto de Fisiología Vegetal - INFIVE, UNLP-CONICET, La Plata, Argentina.E-mail: florencia.bekier@agro.unlp.edu.ar

The bacteria *Pseudomonas syringae* pv. t*omato* DC3000 (*Pst*) is widely recognized as a model to study plant immunity. Plants have evolved a two-layered immunity system to defend themselves from pathogens. Pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs) are detected by host's pattern recognition receptors (PRRs) during pattern-triggered immunity (PTI) activation. This leads to changes in the intracellular calcium concentration, production of ROS, activation of MAPK cascades and transcriptional alterations. However virulent pathogens as *Pst* are able to bypass PTI through the delivery of effector proteins. Resistant plants recognize some of them activating effector-triggered immunity (ETI) that mainly leads to localized programmed cell death (PCD), limiting pathogen growth. Tomato Pto protein kinase interacts with the effectors AvrPto and AvrPtoB and together with Prf lead to activation of ETI response. Using previously generated RNA-seq data we identified two tomato genes (Solyc10g084930 and Solyc09g011320) whose expression is induced during ETI activation. Sequence and phylogeny analysis allowed us establishing that they encode for proteins belonging to the autophagy-related protein 1 (ATG1) group and that each of them has two orthologs in *Nicotiana benthamiana*, the species we use to test the role of candidate genes in immunity through virus-induced gene silencing (VIGS). Plants silenced with a construct that targets all 4 *N. benthamiana* orthologs (*NbATG1*) showed a delay in the development of PCD due to co-expression of Pto and AvrPto, as compared to non-silenced *Ec1* control plants. To confirm this result, we challenged *N. benthamiana 35S::pto* silenced plants with *Pseudomonas syringae* pv. *tabaci* (*Pstab*) expressing AvrPto or an empty vector (EV). We did not observe an increase in disease symptoms with *Pstab-AvrPto* strain. However, we found a delay in the development of *Pstab*-EV disease symptoms. To test whether silencing the 4 *N. benthamiana* orthologs is required for the observed phenotype, we generated two constructs (6008 and 1011) to target them by pairs. We found a similar overall phenotype trend, but with a more marked delay in symptoms when silencing with 1011 and *NbATG1* constructs. It is worth noticing that we have not observed growth or development abnormalities in plants silenced with any of the constructs used. We chose a well-established transcriptional marker of autophagy (*ATG8a*) to investigate if silencing of our candidates affects this process. Using qPCR we did not observe differences in *ATG8a* transcript level between *Ec1*- and *NbATG1*-silenced plants, suggesting that at least in unchallenged plants, autophagy is not affected by knocking-down the genes under study. Based on our results, we believe that this group of autophagy-related proteins would be involved in two different pathways, playing a role during ETI-associated PCD and also in the development of disease symptoms.

PL-P04
COLD STORAGE INDUCES DIFFERENT METABOLIC RESPONSES IN PEACH FRUITS WITH DIFFERENT HARVEST TIME

Bustamante CA[1], Monti LL[1], Gabilondo J[2], Budde CO[2], Fernie AR[3], Lara MV[1], Drincovich MF[1]
[1] CEFOBI-UNR, Argentina, [2] INTA-EE San Pedro, Argentina, [3] MPI für Molekulare Pflanzenphysiologie, Alemania
E-mail:bustamante@cefobi-conicet.gov.ar

Peaches ripen and deteriorate quickly at room temperature. Therefore, refrigeration is used to slow these processes and to extend fruit market life; however, several fruits can develop chilling injury (CI) during storage at low temperature. CI symptoms mainly develop during fruit ripening after cold storage, so this problem is not perceived until the fruit reaches consumers. Hence, the molecular reconfiguration that takes place during cold storage impacts on the way fruits ripen during the following shelf-life, situation that limits commercialization of these fruits. In this study, a metabolite profiling study of six peach varieties with different agronomic characteristics was performed after ripening, either following cold treatment or not, in order to evaluate the effects of refrigeration on the levels of metabolites involved in organoleptic properties and protection against stress. By using GC-MS, 51 polar metabolites were detected in Flordaking (FD), Rojo 2 (R2), Springlady (SL), Red Globe (RG), Elegant Lady (EL) and Limón Marelli (LM) varieties when fruits were stored at 20ºC until reaching firmness and organoleptic characteristics suitable for consumption (SL), and after cold storage at 0ºC for 21 days followed by ripeness at 20ºC (CS+SL). The identified metabolites were divided into sugars, sugar alcohols, organic acids, amino acids, fatty acids and miscellaneous compounds. Interestingly, xylose was the only metabolite that increased in CS+SL fruits, in relation to SL samples, in all the varieties analyzed, indicating a particular reconfiguration of the cell wall after cold storage. PCA analysis revealed interesting results. The first principal component (PC1, 38.7% of the variance) separated the samples depending on harvest time, with mid & late varieties on the positive and early varieties on the negative side, independently to whether the fruits were resistant or susceptible to CI. Among the metabolites that most contribute to PC1 separation, higher levels of maltose, maltitol and fructose 6-P were found in mid & late varieties, while higher levels of Thr, Ile and Val were found in early varieties. PC2 and PC3 did not contribute to separate the samples in any biologically meaningful group. HCA showed similar results, although a clear separation of SL and CS+SL samples in EL variety could be observed, which were fruits characterized by a large increase in sugars like sucrose, glucose and fructose, and organic acids such as citrate, malate, and quinate after refrigeration. Overall, the results showed a differential restructuration of peach fruit metabolism following exposure to cold in varieties with different harvest time. In particular, the identification of the molecular basis of the particular response of EL to cold is a future challenge, since it could aid in defining strategies for the improvement of the organoleptic quality of peach fruits by increasing sugar and organic acids levels while fruits are stored at low temperatures.

PL-P05

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

PUTRESCINE PARTICIPATES IN PLANT DEFENSE IN *ARABIDOPSIS THALIANA* BY REGULATING CELL DEATH AND THE OXIDATIVE BURST

Gárriz A[1], Bach-Pages M[2], Solmi L[1], Romero F[1], Ruiz OA[1], Preston G[2], Rossi FR[1]
[1] Instituto Tecnológico Chascomús, Consejo Nacional de Investigaciones Científicas y Técnicas (INTECH/CONICET), Chascomús, Argentina, [2] Department of Plant Sciences, University of Oxford, Oxford, United Kingdom. E-mail: francorossi@intech.gov.ar

Polyamines are a group of compounds with multiple physiological roles in all organisms. In plants, the metabolism of polyamines is altered upon pathogen invasion, which generally leads to an increment in its concentration and results in the activation of different defence mechanisms. In this trend, we previously showed that infection of Arabidopsis with virulent *Pseudomonas syringae* (Pst) provokes a marked increase in the concentration of the polyamine putrescine (Put) in the apoplastic space, which is explained mainly by the upregulation of its biosynthesis and the remobilization from the intracellular compartments. In the present work, we assessed the functions played by this polyamine in plant immunity. Interestingly, a mutant plant line with reduced levels of Put showed increased cell death upon infection and showed a higher susceptibility to virulent and non-virulent strains of Pst compared to wild-type plants, suggesting that the accumulation of Put is required for plant tolerance. Interestingly, a deeper exploration of the mechanistic role played by Put demonstrated that this polyamine inhibits the calcium influx and the oxidative burst mediated by NADPH oxidases that is induced during plant defence activation. On the other hand, we also demonstrated that Arabidopsis plants infected with non-virulent strains of Pst show higher Put accumulation compared to those infected by virulent strains, which is directly correlated with the induction of plant cell death. These results suggest that a fine-tuned spatio-temporal regulation of the metabolism of Put modulates different processes associated with the defence against pathogens in plants

PL-P06
ROLE OF MEDIATOR COMPLEX IN DNA DAMAGE RESPONSES

Giustozzi M[1], Jaskolowski A[2], Cerdán P[2], Casati P[1]
[1]Centro de Estudios Fotosintéticos y Bioquímicos (CONICET-UNR), [2]Fundación Instituto Leloir (CONICET).
E-mail: gisutozzi@cefobi-conicet.gov.ar

The levels of UV-B radiation that reach the earth cause damage to proteins, lipids, RNA and DNA, inducing different responses in plants. Because of their sessile lifestyle, plants have developed defense mechanisms against this damage. One of the response pathways to DNA damage in plants involves the activation of two different protein kinases: Ataxia Telangiectasia Mutated (ATM; which mostly recognizes double strand breaks in the DNA); and Ataxia Telangiectasia mutated and RAD3-related (ATR; which mostly recognizes single strand damage and DNA crosslinks). Both kinases can activate by phosphorylation Suppressor of Gamma Response 1 (SOG1), which is a transcription factor that regulates the expression of genes involved in cell cycle transitions, DNA repair and chromatin structure. On the other hand, the Mediator complex functions as a molecular bridge between transcriptional activators and the DNA-bound RNA polymerase II. Interestingly, several reports have demonstrated that the yeast Mediator complex also acts as a regulator of DNA repair. In our lab, we recently demonstrated that *Arabidopsis thaliana med17* mutants also show higher DNA damage after UV-B exposure in the absence of photoreactivation than wild type plants, and that UV-B exposure to roots induces a lower accumulation of dead meristematic cells in *med17* seedlings than WT plants. In this work, we analyzed the role of MED17 in the DNA damage response and its possible interaction with ATM and ATR in *A. thaliana*. For this purpose, we generated crosses between *med17* and *atr* mutants, and we analyzed the effect of UV-B in *med17* x *atr* double mutants, including programmed cell death in the meristem of primary roots after exposure to UV-B radiation (2 $W.m^{-2}$). The primary root from *med17 x atr* mutants show a similar number of dead cells in the meristematic zone as *med17* mutants, but both lines have a lower number of dead cells in the primary roots than *atr* mutants after exposure. We also analyzed the primary root meristem of WT, single and double mutants after UV-B exposure. *med17* and *med17* x *atr* roots show a higher decrease in the meristematic zone length by UV-B due to a higher decrease in the number of cells in the meristematic zone than WT primary root, while the decrease in the meristematic zone length in the primary roots from *atr* mutants by UV-B is significantly higher than the decrease measured in the other mutants. In addition, we compared the fertility of *med17* x *atr*, *med17* and *atr* mutants. *med17* x *atr* mutants have a higher percentage of aborted seeds than Col-0 and *atr* mutants, but similar to those in *med17*. Together, our results suggest that *med17* is epistatic over *atr*.

PL-P07
IMPROVEMENT OF NODULATION AND BIOLOGICAL NITROGEN FIXATION IN SOYBEAN THROUGH SEED TREATMENT WITH NON-THERMAL PLASMA

Pérez-Pizá MC[1], Cejas E[2], Vallecorsa P[1], Ferreyra M[2], Zilli C, Prevosto L[2], Santa-Cruz D[1,3], Yannarelli G[3], Balestrasse K[1]
[1] Instituto de Investigaciones en Biociencias Agrícolas y Ambientales (FAUBA-CONICET, [2] Departamento de Ingeniería Electromecánica, Grupo de Descargas Eléctricas (UTN FRVT-CONICET), [3] Laboratorio de Regulación Génica y Células Madre, Instituto de Medicina Traslacional, Trasplante y Bioingeniería (Universidad Favaloro-CONICET). E-mail: kbale@agro.uba.ar

Among the most demanded nutrients by soybean crop (*Glycine max* (L.) Merr.), nitrogen ranks first. Biological nitrogen fixation (BNF), which occurs through the establishment of symbiotic associations between plants and bacteria, is one of the most important sources of nitrogen in soybean. In recent decades, great scientific efforts have been invested in studying strategies for harnessing the potential of BNF. Non-thermal plasmas (NTP) are partially ionized gases, usually generated by low-current electrical discharges that can be sprayed onto biological tissues. They are a novel and promising technology that might be employed for seed treatment before sowing as they allow enhancing seed health while promoting germination and vigor in a fast, cost-effective and eco-friendly way. In a previous research, we showed enhanced seed quality following soybean seed treatment with NPT. The NPT positive effects lasted the entire plant cycle, determining improvements in growth and yield. In this regard, the main objective of the present study was to characterize and demonstrate the effects of seed treatment with NTP on plant nodular development and on the BNF, as

mechanisms that might explain the enhanced yields. Comparing plasma treatment with the control (non-treated), our results showed important differences regarding root growth, which correlated positively with increments of about 1.7 folds in GmEXP1 gene expression in roots. On the other hand, we found improvements of 25% in the number of nodules, and improvements of 70% and 55% in nodular biomass, fresh and dry (respectively). BNF was evaluated through the determination of nitrogenase activity and leghemoglobine content in nodules, which were enhanced by 60 and 100% (respectively) through seed treatment with NTP. These parameters correlated positively with plants biometry and total and partitioned (nodular, radical and aerial) nitrogen content. We propose the NTP technology for seed treatment before sowing for improving crop yield while protecting the environment.

PL-P08
THE PAP/SAL1 RETROGRADE SIGNALING PATHWAY IS INVOLVED IN IRON HOMEOSTASIS VIA ETHYLENE ACTIVITY IN *ARABIDOPSIS*

Balparda M, Gomez Casati DF, Pagani MA
Centro de Estudios Fotosintéticos y Bioquímicos (CONICET-UNR). E-mail:balparda@cefobi-conicet.gov.ar

PAP accumulates in *Arabidopsis thaliana* plastids in response to high light or drought stress and is able to regulate stress-responsive nuclear genes. Its levels are controlled by dephosphorylation to AMP through SAL1 enzyme activity. The mode of action of PAP is the inhibition of 5′ to 3′ exoribonucleases (XRNs). Transcriptome analysis of *Arabidopsis sal1* and *xrn* mutant plants further revealed that the ferritin genes *AtFer1*, *AtFer3*, and *AtFer4* are upregulated in these genetic backgrounds, thus establishing a link between the PAP retrograde signaling pathway and the regulation of iron (Fe) homeostasis genes. In this study, we used three different mutants of the PAP/SAL1 retrograde pathway to characterize the relation of this retrograde signaling pathway with Fe metabolism. Mutant plants showed an increment in the expression level of genes implicated in Fe uptake and storage. Also, mutant lines accumulated more Fe content in shoots, roots and seeds than wild type plants when they grew in both Fe-sufficient and Fe-deficient media. Likewise, mutant plant roots did not deactivated Fe uptake when foliar Fe was applied. As reported, *AtERF1* -a transcription factor of the ethylene signaling pathway- is a target of XRN4 degradation in a posttranscriptional regulation process. In PAP/SAL1 mutant lines, there was an increment in *AtEFR1* and Fe uptake transcripts. However in the presence of ethylene inhibitors, mutant lines presented a reduction in the relative expression of genes involved in Fe acquisition and in the ferrochelate reductase activity. Furthermore, RNA decay assays showed that full XRN4 activity restored *AtFRO2* and *AtIRT1* transcripts (Strategy I genes of Fe uptake) up to wild type line levels in RNA extracts of PAP/SAL1 mutants. In summary, through these results we can infer that there is a link between PAP/SAL pathway, ethylene signaling and Fe metabolism.

PL-P09
ELUCIDATING HYL1 C-TERMINAL DOMAIN FUNCTION

Achkar NP, Manavella PA
Instituto de Agrobiotecnología del Litoral (CONICET-UNL-FBCB), Paraje El Pozo, Santa Fe, Argentina.
E-mail: nachkar@santafe-conicet.gov.ar

Gene-silencing, mediated by micro RNAs (miRNAs), is one of the most important mechanisms of post-transcriptional gene regulation. To balance gene expression/silencing, miRNA abundance is tightly regulated at transcriptional, processing and/or stability level. In plants, the main actor in miRNA processing is DICER LIKE 1 (DCL1), the endonuclease responsible for converting the primary miRNA (pri-miRNA) into the mature double stranded miRNAs. In order to achieve a precise excision of the mature miRNA, DCL1 require the molecular assistance oth the RAN-binding protein HYPONASTIC LEAVES 1 (HYL1). HYL1 is composed of two double stranded RNA binding domains (DRBM1 and DRMB2), which mediate interactions with miRNA precursors and partner proteins, a nuclear localization signal (NLS), and a C-terminal domain constituted of six tandem repetitions of a highly conserved 28 amino acids motif with unknown function. As we previously demonstrated, phosphorylation regulates HYL1 activity and controls its stability in response to light conditions. Two serine residues, located in each RNA-binding domain, were found to control HYL1 activity. However, mass-spectrometry analysis revealed that serines located in each repetition at the C-terminal region of HYL1 are also phosphorylated. Even when the function of these repeats is unknown, an alternative splicing event produces a HYL1 isoform containing only one of the repeats. Similarly, the 1001 genomes project revealed that the number of repetitions in HYL1 changes among different *Arabidopsis thaliana* ecotypes suggesting they can have a regulatory or adaptive function. Here, we report that the C-terminal domain region of HYL1, and its post-translational modifications, modulates the protein subcellular localization. Confocal microscopy allowed us to find out that this domain changes HYL1 localization pattern, especially inside the nucleus, affecting the formation of nuclear speckles. Specifically, when we remove the C-terminal repetitions one by one, HYL1 begins to disappear from cytoplasm to localize exclusively in the nucleus. Besides that, the sub-nuclear localization also changes: bigger nuclear speckles are constituted when the C-terminal domain is removed. On the other hand, when we mimic a phosphorylated or non-phosphorylated C-terminal domain, HYL1 cytoplasmic localization is not altered, but the formation nuclear speckles is promoted in the hyperphosphorylated state. Our results shows that the C-terminal region of HYL1, far from being dispensable as long thought, is important for the activity of the protein, especially by controlling its intracellular dynamics.

PL-P10

UNRAVELING THE CHLOROPLASTIC N-END RULE: SEARCHING FOR SEQUENCE DETERMINANTS FOR PROTEIN DEGRADATION

Aguilar Lucero DA, Ceccarelli EA, Rosano GL
Instituto de Biología Molecular y Celular de Rosario, IBR-CONICET-UNR, Rosario, Argentina. E-mail:rosano@ibr-conicet.gov.ar

Proteins that are to be eliminated must be proficiently recognized by proteolytic systems so that inadvertent elimination of useful proteins is avoided. One mechanism to ensure proper recognition is the presence of N-terminal degradation signals (N-degrons) that are targeted by adaptor proteins (N-recognins). The members of the caseinolytic protease S (ClpS) family of N-recognins identify targets bearing an N-terminal phenylalanine, tyrosine, tryptophan or leucine residue, and then present them to a protease system. This process is known as the 'bacterial N-end rule'. The presence of a ClpS protein in *Arabidopsis thaliana* chloroplasts (ClpS1) prompted the hypothesis that the bacterial N-end rule exists in this organelle. However, there is no experimental evidence on this and reports on the specificity of ClpS1 show conflicting data. In this work, we set up to unravel the specificity of ClpS1 by the use of peptide arrays, membrane-bound peptides synthesized directly on cellulose membranes. The arrays were ordered to the manufacturer specifying the sequence of the peptides attached to the membrane. Hundreds of different N-terminal sequences can be designed and probed simultaneously. The interactor protein (in our case, recombinant ClpS1) was incubated with the membrane, which is then thoroughly washed. Bound ClpS1 was then immunodetected by anti-ClpS1 antibodies. A colorimetric signal indicates strong interaction to the peptide in that location, whose sequence is known. Bound peptides were also ordered in their N-terminal acetylated version to analyze the effect of this post translational modification on ClpS binding. Our results show that ClpS1 can bind to peptides bearing the canonical bacterial N-degrons (Phe, Tyr, Trp and Leu). Binding to peptides starting with Phe and Tyr was readily detected. However, signals indicating binding to Leu-bearing peptides were of much lower intensity, suggesting lower affinity. Also, a second array in which the second residue was always proline or serine and the first one was systematically surveyed showed that ClpS1 did not recognize Leu-Ser or Leu-Pro; N-degrons that are readily recognized by bacterial ClpS proteins. Also, N-acetylation of the peptides in the membrane completely abolished the interaction with ClpS1. This indicates that this posttranslational modification may greatly influence protein half-life in chloroplasts. Overall, it can be concluded that ClpS1 form plant chloroplasts has a similar recognition pattern compared to bacterial ClpS but also presents differences that suggest that the chloroplastic N-end rule presents unique characteristics on its own.

PL-P11
DIFFERENTIAL PROTEOME OF ARABIDOPSIS ROOTS FROM AN *IQD30* MUTANT LINE

Triassi A[1], Drincovich MF[1], Lara MV[1]
[1]Centro de Estudios Bioquímicos y Fotosintéticos (CEFOBI-CONICET), Rosario, Argentina. E-mail: triassi@cefobi-conicet.gov.ar

Plant-specific IQ67 domain (IQD) members are part of an emerging family of calmodulin binding proteins. Although systematic characterization of this family has been carried out in various plants species, little is known about their biological roles. In this study, we made progresses in the characterization of IQD30 from *Arabidopsis thaliana*. It was previously reported that IQD30 is capable of binding to microtubules. At the same time, *in vitro* assays corroborated the interaction between IQD30 and calmodulins in a calcium dependent and independent ways. In order to evaluate the role of IQD30, a loss of function mutant (Salk 052513) and a proteomic approach were used. For this purpose, proteins were extracted from 8-day-old seedling roots and subjected to quantitative proteomics through LC/MS using CEQUIBIEM facilities (Argentina). Three biological replicates for each genotype were used. Statistical data analysis was performed using Perseus software. In total, 152 differential proteins were identified ($p \leq 0.05$; 0.5>fold change>2). Remarkably, 70% of differentially expressed proteins are induced in the mutant genotype. Functional categorization indicates differential proteins are involved in different metabolic processes; such as, protein synthesis machinery, proteasome components, hormone signaling, cytoskeleton components, calcium signaling among others. Induced expression of three auxin signaling proteins, ARF-GAP2, ZIGA4 and BIG, which usually increase their levels in response to auxins, was observed in the mutant line. This result is in agreement with lower primary root elongation of *iqd30* than Col-0 line under both normal growth conditions and IAA treatment, and with transcriptionally response of iqd30 to auxin treatment. Collectively, these results suggest IQD30 could have a role in auxin signaling. On the other hand, proteomic analysis revealed the differential expression of two calcium signaling proteins in *iqd30*. While CML42 (a calmodulin like protein) was only identified in *iqd30* line, CDPK6 (a calcium dependent kinase) was decreased in *idq30* with respect to Col-0. In addition, the 4-fold increase of MAP65.2 (a microtubule associated protein that connects microtubule organization to the polarization of auxin exporters) and the 2-fold decrease of β tubulin 6 (a structural microtubule protein) was also observed in the mutant line. According to this, previous *pull down* assays followed by MS revealed β tubulin 6 as a putative ligand of IQD30. Based on these results and according to previous evidences, auxin and calcium signaling could be integrated to modulate microtubules dynamics, by means of IQD30 protein. IQD30 might be able to interact with multiple members of signaling pathways and recruit them to cytoskeleton regulating the transduction of signals in response to various stimuli.

PL-P12
FUNCTIONAL CHARACTERIZATION OF THE miR394 PATHWAY IN *ARABIDOPSIS* AND MAIZE

Ponso MA, Vegetti A, Dotto M
Facultad de Ciencias Agrarias (UNL), Esperanza, Santa Fe y CONICET. Email: mdotto@fca.unl.edu.ar

The miR394 pathway is conserved between monocot and dicot plant species. In *Arabidopsis*, it has been shown that miR394 and its target *LCR* participate in the regulation of leaf morphology, shoot apical meristem development and in the response to abiotic stress. *LCR* encodes a protein member of the F-BOX family, which are part of SCF complexes, responsible for the addition of ubiquitin residues to a target protein, marking it for degradation by the proteasome. Work in our lab has further demonstrated that the miR394 pathway is involved in the regulation of flowering time in *Arabidopsis thaliana*. The miR394 pathway in maize has not been characterized yet. Initial studies in our laboratory indicate that there are

two genes encoding miR394 precursors in maize (*ZM-MIR394A* and *ZM-MIR394B*), which is also the case in *Arabidopsis*. However, different from *Arabidopsis*, the maize genome harbors two genes coding for F-BOX proteins that are targeted by miR394, which we named *ZmLCR1* and *ZmLCR2* accordingly. Thus, as an approach to study the role of the miR394 pathway in maize, we generated transgenic *Arabidopsis* plants overexpressing the maize precursor *ZM-MIR394B* (*2X35S::ZM-MIR394B*) as well as *Arabidopsis* mutant plants in the two endogenous *MIR394* genes (*ath-mir394a/ath-mir394b*). We compared their growth and development under normal watering conditions and determined these plant lines differ in their flowering time, number of ramifications and seed production. Furthermore, we analyzed their growth in drought conditions, both in soil and in MS plates supplemented with mannitol to simulate drought. Finally, we used a phylogenetic approach to establish there is only one protein of the MLP-like family in maize that is a putative orthologue of the Arabidopsis MLP proteins marked for degradation by the LCR-containing SCF complex.

PL-P13
IDENTIFICATION OF SMALL RNAs WITH ROLES IN THE DEVELOPMENT OF ORGANOLEPTIC PROPERTIES IN STRAWBERRY FRUIT

Sabbione A[1,2,3], Daurelio L[2,3], Vegetti A[1,2], Dotto M[1,2]
[1]Facultad de Ciencias Agrarias (UNL), Esperanza, Santa Fe; [2]CONICET; [3]LIFiBVe, FCA (UNL). Email: mdotto@fca.unl.edu.ar

Fruit's nutritional quality and sensory attraction are largely determined by the composition and amount of secondary metabolites, which contribute to the content of soluble solids, carotenoids, vitamins, antioxidants and nutrient content. An increasing interest in the production of fruits with nutritionally optimized secondary metabolite contents has emerged in the last few years, particularly through the study of regulatory networks that modulate the metabolism of these compounds. Small RNAs (smRNAs) are a broad class of RNA molecules ranging between 21 and 24-nt that participate in the regulation of gene expression at the transcriptional and post-transcriptional levels. Their role in the regulation of the content of secondary metabolites and other aspects related to fruit's organoleptic properties, such as texture and color, has been little explored so far. In this work we used a genomic approach in order to identify changes in the content of miRNAs, ta-siRNAs and phasiRNAs in strawberry fruits during ripening and identified their target genes. For this, we analyzed smRNA-seq, RNA-seq and degradome data from two different strawberry cultivars (*Fragaria x ananassa* cv. Hongjia and *Fragaria x ananassa* cv. Toyonaka) at different ripening stages and identified 42 previously uncharacterized *loci* producing phasiRNAs and 33 differentially expressed miRNAs during maturation. Among these, we identified several smRNAs regulating genes related to the development of organoleptic properties during strawberry fruit ripening, indicating a fundamental role of these regulatory molecules in this process.

PL-P14
SILENCING OF VLG, A DC1 PROTEIN, CAUSES FLAVONOID ACCUMULATION DURING SENESCENCE IN *ARABIDOPSIS*

Arias LA[1], Frik J[2], D'ippólito S, Casalongué CA, Fontana A[1], Piccoli P[1], Fiol DF[2]
[1] Instituto de Biología Agrícola de Mendoza (IBAM) [2] Instituto de Investigaciones Biológicas (IIB), CONICET -Universidad Nacional de Mar del Plata. E-mail: laarias@mdp.edu.ar

VLG (vacuoleless gametophytes) is a DC1 domain protein involved in vacuole formation during gametophytic development. DC1 domains are zinc fingers motives unique to plants that have been associated with several stress responses. In previous studies analyzing vlg promoter-GUS reporter lines subjected to different biotic and abiotic stress treatments, we showed that VLG is differentially regulated by numerous stress conditions in an organ specific manner. As *vlg* is a gametophytic mutation and homozygous mutants couldn't be recovered, we generated a miRNA silenced lines to further study VLG function during stress. Although in normal growing conditions VLG silenced plants don't seem to display abnormal phenotypes besides slight increase in leaf length, during senescence their leaves turn strong purple indicating accumulation of flavonoid compounds. Flavonoids are antioxidant compounds that prevent oxidative damage to some extent during senescence and other stress conditions. In order to investigate this, we quantified leaf pigments in a spectrophotometer, revealing that *vlg* silenced lines have less total chlorophyll content and more anthocyanin content compared to WT. Moreover, we further analyzed the metabolic profile of senescent leaf extracts through HPLC, particularly low molecular weight compounds and anthocyanins, and we show that *vlg* silenced plants display altered secondary metabolites composition compared to WT. In sum, results suggest that *vlg* silenced lines might suffer from higher oxidative stress during senescence, which causes chlorophyll degradation and flavonoid accumulation as a response. Funded by ANPCyT, Conicet, UNMdP, CIC.

PL-P15
SIGNALING EVENTS TRIGGERS BY SULFORAPHANE IN *ARABIDOPSIS THALIANA*

Arruebarrena Di Palma A, Garcia Mata C, Laxalt A
Instituto de Investigaciones Biológicas (IIB), CONICET-UNMDP. E-mail: aarrueba@mdp.edu.ar

Sulforaphane (SFN) is a compound encompassed within isothiocyanates molecules, a type of organosulfur compounds. It is present almost exclusively in cruciferous plants such as broccoli, cabbage, among others. Under no stressful conditions, SFN is present conjugated to a glucose molecule, being an inactive form called glucoraphanin. When cell damage occurs, by mechanical damage or herbivory, glucoraphanin acts as a substrate of myrosinase enzyme that cleaves it generating one molecule of SFN and one free glucose. It has been shown that SFN has a role in the immune response of plants. SFN participates in the resistance to pathogens through decrease intracellular glutathione pool affecting cellular redox

state and directly as toxic compound against bacterial and fungal pathogens. On the other hand, exogenous treatments of healthy plants with SFN, lead to priming state, a situation of "alert" in plants against possible future attacks. Mode of action of SFN it is believed rely on its electrophile nature, that allow to react whit cellular thiols, like reduced glutathione and/or protein cysteine and histidine. While there is information about its physiological effects, as mentioned above, little is known about the signaling events that lead to SFN final responses in plants. In this work we analyze the signaling responses generated by SFN treatment on *Arabidopsis thaliana*, focus on reactive oxygen species (ROS) as well as the effect of presence of free glucose in stoichiometry amounts. It was determined that SFN induces the production of ROS and this production are enhance in the presence of glucose. Using non- metabolizable analog of this sugar, we were able to determine effect responds to glucose catabolism and not to sugar signaling mechanisms. In addition, we were able to determine that calcium participates upstream for the production of ROS, since calcium blockers and chelators reduced the ROS signal. Finally, using null mutants of *A. thaliana* in NADPHox D and F (RBOHD and RBOHF) we were able to determine that isoform D is responsible for the production of ROS in response to SFN treatment. These results together indicate that SFN induces the production of ROS in *A. thaliana* through RBOHD activity dependent of calcium.

PL-P16
STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF ORGANELLAR SMALL HEAT SHOCK PROTEINS FROM TOMATO (*SOLANUM LYCOPERSICUM*)

Badia MB[1], Renzi DJ[1], Escobar MR[1], Sossi ML[1], Iusem ND[2], Boggio SB[1], Valle EM[1].
[1]Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET-UNR), [2]Instituto de Fisiología, Biología Molecular y Neurociencias (IFYBINE-CONICET-UBA). Email: badia@ibr-conicet.gov.ar

Chaperones belonging to the small heat shock protein (sHSP) family are ubiquitous and exhibit high expression levels after heat and other stress conditions. In plants, these proteins are especially abundant and diverse, and localized in the cytosol as well as in almost every organellar compartment. The presence of specific sHSP in organelles seems to be exclusive to plants, with a few exceptions. In tomato fruits, the accumulation of specific sHSP was postulated to be related to the protection against chilling postharvest injury. In this work, we focused on two organellar sHSP: sHPS23.8 and sHSP21.5, which are present in the mitochondria and endoplasmic reticulum of tomato, respectively. The mature proteins were cloned, expressed and purified to homogeneity. A structural characterization to investigate their oligomeric state *in vitro* was performed. Additionally, polyclonal antibodies against specific peptides of each of these proteins were used to detect changes in their abundance in fruit of wild type and transgenic tomato plants with altered levels of each of these proteins. In this sense, extracts from mature fruit before and after cold treatment were analyzed in order to elucidate their participation in the maturation of tomato fruit and their response to cold storage. Results indicated that the level of these proteins correlated with the chilling injury of the fruit, suggesting that the regulation of the organellar sHSP would be important for the protection mechanisms against chilling stress in tomato.

PL-P17
STUDY OF POLLEN TUBE GROWTH BY RALF-MEDIATED SIGNALING WITH RECEPTOR-LIKE KINASES IN *ARABIDOPSIS THALIANA*

Boccardo N[1], Somoza [1], Wengier D [1], Muschietti JP[1,2]
[1] Instituto de Investigaciones en Ingeniería Genética y Biología Molecular, Dr. Héctor Torres (INGEBI-CONICET), Argentina, [2] DBBE, FCEyN, UBA. E-mail: nboccardo@dna.uba.ar

In flowering plants, fertilization requires complex signal exchanges and a flawless communication between male and female reproductive tissues. This complex cell-to-cell communication that occurs in the extracellular matrix to the interior of the cell is crucial for cell's function. Receptor-like kinases (RLKs) have been implicated in various processes, including cell wall integrity, sexual reproduction, immunity and various hormone pathways. In turn, small proteins of the RAPID ALKALINIZATION FACTOR (RALF) family have been recently identified as ligands of different RLKs. RLK/RALF interaction is essential for a correct polarized growth of pollen tubes, process regulated by cytoskeletal reorganization, vesicular movement, Ca^{2+} signaling and reactive oxygen species (ROS). However, the mechanism of their perception has not been fully elucidated. Using transgenic plants that express pollen proteins followed by fluorescent proteins, we propose to study the mechanism of interactions between pollen RALFs and their respective RLKs. These results will shed light to understand the signaling pathway during pollen tube growth in *Arabidopsis thaliana*.

PL-P18

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

A *HYALOPERONOSPORA ARABIDOPSIDIS* EFFECTOR THAT ALTERS PLANT DEVELOPMENT AND INCREASES SUSCEPTIBILITY TO PATHOGENS INTERACTS WITH BIM1, AN *ARABIDOPSIS* TRANSCRIPTION FACTOR INVOLVED IN BRASSINOSTEROIDS SIGNALLING

Bogino MF, Fabro G
Centro de investigaciones en Química Biológica de Córdoba (CIQUIBIC-CONICET). Departamento de Química Biológica, Facultad de Ciencias Químicas (DQB-FCQ). Universidad Nacional de Córdoba. E-mail: florenciabogino@gmail.com

HaRxL106 is an effector from *Hyaloperonospora arabidopsidis* (Hpa), an obligated biotrophic oomycete pathogen of the plant *Arabidopsis thaliana*. It has a modular structure, with an N-terminal RxLR domain that is probably implied in its translocation to the host cytosol and a C-terminal domain through which this effector interacts with several host proteins.
Preliminar data of Yeast-two-Hybrid studies (Y2H) indicated HaRxL106 interaction with BIM1, a member of a small family of bHLH transcription factors. After brassinosteoid signal perception BIM1 heterodimerizes with BES1 activating many BR-responsive genes. We here confirmed the HaRxL106-BIM1 interaction *in planta*, by co-expressing both full-length proteins fused to either the N-terminal domain of YFP (YFPn-HaRxL106) or the C-terminal of CFP (BIM1-CPFc) and performing BiFC assays in *N. benthamiana*. Additionally, we developed Co-IP assays in the same plant using the YFP-HaRxL106 and BIM1-FLAG constructs. On the other hand, when HaRxL106 is constitutively expressed *in planta,* the transgenics phenocopy the Shade Avoidance Syndrome (SAS), and those plants are more susceptible to biotrophic and hemibiotrofic pathogens. Therefore, we are interested in elucidating if those phenotypes are dependent on BIM1. *Pseudomonas syringae pv. tomato* (Pst DC3000) and Hpa (isolate Noco2) infection assays in *bim123* knockout lines showed that these plants are more disease resistant compared to wild type (ecotype Col0), as we observed less bacterial growth, and reduced conidiophore production. Interestingly, *bim123* plants produced less callose deposition when infiltrated with Pst DC3000, and showed reduced expression of the defense-gene marker PR1 after 24 hpi compared to control plants. Our results suggest that BIM1 could be a susceptibility factor for Hpa, being modulated by the HaRxL106 effector. To assess this, we continue investigating susceptibility phenotypes in *bim123* plants transformed with HaRxL106 under the control of constitutive or inducible promoters.

PL-P19
ASSESSMENT OF A qPCR TECHNIQUE FOR THE DETECTION AND QUANTIFICATION OF *AZOSPIRILLUM BRASILENSE* IN ROOTS

Cabrera A[1], Vazquez A, Zawoznik MS[1], Benavides MP[2], Groppa, MD[2]
[1]UBA, Fac.de Farmacia y Bioquímica, Departamento de Química Biológica, Cátedra de Química Biológica Vegetal, Buenos Aires, Argentina.[2]UBA. IQUIFIB-CONICET, Fac.de Farmacia y Bioquímica, Buenos Aires, Argentina. Email:andreacab86@gmail.com

Because culture-dependent counting techniques tend to produce erroneous results mainly if the count is based on the morphology of the colony, our objective was to evaluate a molecular technique (qPCR) to perform a specific and more accurate count of *Azospirillum brasilense* Az39, a plant-growth promoting rizobacterium used in commercial inoculants. For this purpose, the sequence of the NifA transcription factor gene was searched in the Az39 genome by alignments of the same gene from other strains of the same species, and specific primers for Az39 were designed. With these primers, specificity curves were obtained using DNA extracted from Az39 and other bacteria. The expected amplicon had a Tm of 85.15 and a Ct value of 19.06 using 6.8 ng of the template of Az39. Samples from other bacteria showed non-specific amplifications with different Tm and higher Ct values, except for bacteria belonging to the *Azospirillum brasilense* species, whose Tm and Ct were close to that of Az39. Once the technique was optimized, it was used to quantify *A. brasilense* present in the roots of wheat plants inoculated with Az39 and in non-inoculated plants, and the results were compared with the data obtained using a plate counting technique. We can conclude that the primers designed were specific for *Azospirillum brasilense* species but not for Az39 strain. When comparing this method with a culture technique, we verified that the plate counting method underestimated the number of *Azospirillum* in the exorizosphere and overestimated its number in the endorizosphere.The molecular counting technique was useful to prove the endophytic nature of this rhizobacterium as well as the preferential colonization of the exorizosphere by the inoculated microorganisms.

PL-P20
EFFECT OF SALT STRESS IN ATMSH7 DEFICIENT PLANTS

Chirinos-Arias M, Spampinato C
Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI-CONICET), Universidad Nacional de Rosario.
E-mail: chirinos@cefobi-conicet.gov.ar

DNA mismatch repair (MMR) is a highly conserved biological pathway that improves the fidelity of DNA replication and recombination. MMR is initiated when MutS proteins recognize mismatches and small loops of unpaired nucleotides. Arabidopsis and other plants encode MutS protein homologs (MSH) conserved among other eukaryotic organisms, but also contain an extra MSH polypeptide (MSH7). In order to better understand the role of MSH7 in plant salt stress response, we conducted phenotypic and biochemical studies using an *msh7* T-DNA insertional mutant. Seeds were sown on agar plates containing 0.5X Murashige and Skoog medium (MS) and grown for 10 days at 22°C under a 16/8 light/dark photoperiod. Seedlings were then transferred to agar plates containing MS medium or MS supplemented with 100 mM NaCl and grown for 48 hours. Alternatively, after the treatment seedlings were transplanted to soil. Results indicate that *msh7* mutants under salt stress showed i) a lower decrease in chlorophyll and flavonoid levels, ii) a higher stomatic density and electrolyte leakage, iii) a higher 8-oxoG accumulation in DNA, iv) a higher rosette area and leaf area and a lower cell number, v) a decreased G0/G1 and an increased G2/M phase compared to those observed in WT plants under salt stress. In addition, nitroblue tetrazolium staining revealed that *msh7* mutant control plants show a higher oxidative stress than WT

control plants, consistent with its higher level of peroxidase activity. Taken together, our results suggest that MSH7 is involved in salt stress-induced DNA damage response.

PL-P21
BDPAP2 ACTS AS A FLOWERING PROMOTER IN *BRACHYPODIUM DISTACHYON*

Eusebi D. Günthardt MM, Muchut SE, Vegetti AC, Uberti Manassero N
Laboratorio de Biología Evolutiva y Molecular de Plantas - Departamento de Biología Vegetal - Facultad de Ciencias Agrarias - UNL
E-mail: delfina.eusebi8@gmail.com

Brachypodium distachyon is a model plant for temperate cereals and related grasses. In grasses, the SEPALLATA (SEP) proteins had been involved in the regulation of meristem identity and flowering time. Specifically, PAP2 (a SEP-like protein of rice) promotes the SAM phase changes, acting together with AP1-like proteins to negatively regulate *RCN4*, a *TFL1* orthologue, in a conserved pathway between *Arabidopsis thaliana* and rice. In *B. distachyon,* the AP1-like proteins BdVRN1 and BdFUL2 are able to form dimers with SEP-like proteins and to promote flowering after a prolonged cold treatment (vernalization). The aim of this study was to evaluate the putative interaction between BdPAP2 and AP1-like proteins, and its role in regulating flowering time. For this purpose, we over-expressed *BdPAP2* (PAP2-OE) in *A. thaliana* wild type and *ap1* mutant background. Transgenic PAP2-OE plants showed an early transition from vegetative to reproductive stage compared to wild type. Interestingly, no early flowering was observed in the *ap1* mutant background. The results suggest that AP1 is indispensable for the function of BdPAP2 in meristem identity regulation. We also analysed two independent *BdPAP2* mutant lines of *B. distachyon*. One of the lines carries a T-DNA insertion that introduced a 4xCaMV35S enhancer sequence in an intron of *BdPAP2*, upregulating its expression. This activation tagged line flowered earlier and developed fewer leaves than control plants. The other line was a sodium aside mutant that contains a SNP mutation in a splice-site donor that is expected to disrupt *BdPAP2* function. As expected, this mutant showed a delay in flowering time. Taking together, we can postulate BdPAP2 as a flowering promoter that seems to depend on AP1-like proteins presence in order to regulate the transition from vegetative to reproductive stage. Considering the role of AP1-like proteins in the vernalization pathway, and the assumed regulation of flowering time along with BdPAP2, we set new vernalization experiments with both *B. distachyon* mutant lines.

PL-P22
THE DC1-DOMAIN PROTEIN VLG IS INVOLVED IN *ARABIDOPSIS* FERTILITY

Frik J, Arias LA, Lombardo MC, D'Ippolito S, Terrile MC, Casalongué CA, Fiol DF.
Instituto de Investigaciones Biológicas (IIB), CONICET -Universidad Nacional de Mar del Plata. E-mail: j_frik@hotmail.com

VACUOLELESS GAMETOPHYTES (VLG) is a DC1 domain containing protein that was first characterized in our laboratory as a necessary factor to complete female and male gametophytes development in *Arabidopsis*. Its subcellular localization at the prevacuolar compartment and the ability to interact with SNARE proteins suggest a role in the vesicular fusion during vacuolar formation. *Vlg* expression was also detected in the vasculature, the filament of mature flowers and root elongation zones, implying a broader role for this protein in sporophyte tissues. Since *vlg* homozygous insertional mutants are lethal, we generated transgenic silenced lines encoding an artificial microRNA targeting *vlg* transcripts with the aim to explore the loss of VLG function. The phenotypic characterization showed a lower yield in quantity of fruits and number of seeds per fruit in *vlg* silenced plants as a result of deficient pollination. This phenotype was caused partially by undehisced anthers suggesting reduced male fertility. In addition, the first flowers on the main stem displayed excessive gynoecium elongation which resulted in stigma exsertion and thus impaired pollen-stigma interactions. Rosette leaves were also more elongated in silenced plants and the number of lateral roots was higher, suggesting that VLG may mediate processes that regulate organ morphology. We found that *vlg* promoter activity is modulated by the hormones auxin and jasmonic acid, both involved in another development and further crossed *vlg* silenced plants with auxin signaling reporter lines to explore alterations due to diminished VLG levels. Taken together, our results show that VLG participates in the processes mediating fertility and morphogenesis in Arabidopsis. Funded by ANPCyT, Conicet, UNMdP, CIC.

PL-P23
PROLINE METABOLIC DYNAMICS AND ITS IMPLICATION IN THE DROUGHT TOLERANCE OF PEANUT PLANTS

Furlan A[1,3], Bianucci E[1], Giordano W[2], Castro S[1], Becker D[3]
[1]Instituto de Investigaciones Agrobiotecnológicas (INIAB-CONICET), Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Córdoba, Argentina, [2]Instituto de Biotecnología Ambiental y Salud (INBIAS-CONICET), Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Córdoba, Argentina, [3]Department of Biochemistry, Redox Biology Center, University of Nebraska-Lincoln. Nebraska, USA. E-mail: afurlan@exa.unrc.edu.ar

Proline metabolism and turnover, rather than just proline accumulation, contributes positively in multiple ways in plant tolerance. However, under some circumstances, reactive oxygen species generated as a by-product of the catabolism of proline is responsible for oxidative damage. In order to understand the response of proline metabolism and its implication in the drought-tolerance of peanut plants, transcriptional and biochemical analyses were performed during water stress in two peanut cultivars with contrasting tolerance to drought stress (Granoleico and EC-98). To exacerbate and help identify plant responses, the amino acid proline and its analogue, thiazolidine-4-carboxylic acid (T4C), which are substrates of a proline catabolic enzyme, were added exogenously to water stressed plants. Upon exposure to polyethylene glycol (PEG), peanut plant leaves from both cultivars had significantly lower relative water content and accumulated proline; only plants from the drought sensitive cultivar,

Granoleico, exhibited oxidative damage (thiobarbituric acid reactive substances (TBARs) accumulation). Pre-treatment with exogenous proline or its analogue gave a contrasting response: proline and hydrogen peroxide accumulation and TBARs reduction. Both cultivars showed increased proline biosynthesis genes expression (*P5CS1, P5CS2a, P5CS2b, P5CR*) when exposed to water stress, but showed opposite responses in the relative expression of proline catabolism genes (*ProDH1, ProDH2*), which increased only in the sensitive cultivar. The drought tolerant EC-98 cultivar exhibited unique changes when pretreated with T4C, with mRNA levels of genes coding for proline biosynthesis (the four analyzed) and catabolism (*P5CDH*), significantly up-regulated even in the absence of water stress. Thus, higher proline metabolism in the drought tolerant cultivar, EC-98, relative to the sensitive cultivar (Granoleico), may contribute to the tolerant phenotype. Finally, T4C may be a potentially useful protecting agent in drought stress conditions for specific cultivars.

PL-P24
CHARACTERIZATION OF CALCIUM TRANSPORTERS DURING POLLEN TUBE GROWTH IN *ARABIDOPSIS THALIANA*

García Bossi J[1], Obertello M[1], Barberini ML[1], Salinas N[1], Estevez J[2,3], Muschietti J[1 4]
[1]INGEBI–CONICET, [2] FIL-IIBBA-CONICE, [3]CBV, Facultad de Ciencias Biológicas, Universidad Andrés Bello, Santiago, Chile. [4] DBBE, FCEN, UBA. E-mail:garciabossijulian@gmail.com

In plants, calcium signals are involved in multiple physiological processes such as stomatal opening, stress responses, and polarized growth of root hairs and pollen tubes. These signals are given as repetitive oscillations of cytosolic free Ca^{2+} where the intensity and amplitude correlate according to the stimulus. Pollen tube growth occurs through the concerted action of different factors such as pH, ROS, actin and calcium gradient. Any imbalance between these factors causes aborted pollen tubes and therefore, defects in fertility. In this work, we propose to perform a functional study of the P2B type calcium pumps (Autoinhibited Ca^{2+}-ATPases, ACAs), in pollen tubes of *Arabidopsis thaliana*. These pumps are mainly located in vacuole, endoplasmic reticulum and/or plasma membrane and are involved in removing calcium from the cytoplasm. Studies conducted with ACA insertional mutants have shown that these pumps have a role beyond the maintenance of Ca^{2+} homeostasis. Since some Arabidopsis ACAs are specifically expressed in mature pollen, we propose to explore pollen-pistil interactions of ACA mutant plants. We will also study calcium dynamics during pollen tube growth. These results will provide new perspectives about how pollen tube growth is regulated and it will be also applicable to other cellular polar growth models.

PL-P25
PARTICIPATION OF POLYAMINES IN THE RESISTANCE MECHANISM INDUCED BY PHOSPHITES AGAINST *PHYTOPHTHORA INFESTANS* IN POTATO

Lobato MC, Olivieri FP, Daleo GR, Andreu AB
Instituto de Investigaciones Biológicas, FCEyN, CONICET, UNMdP. Funes 3250, 4º Nivel, Mar del Plata, Argentina.
E-mail: mcandelal@gmail.com

Potato is the main horticultural crop in Argentina. Late Blight, caused by the oomycete *Phytophthora infestans*, is the most important potato disease that seriously affects crop yield. A strategy to diminish the use of toxic agrochemicals is the use of biocompatible compounds. Phosphites are metallic salts of phosphorous acid (H_3PO_3) nontoxic for the environment or human health. They are effective in increasing potato tolerance against various diseases, UV-B radiation, and also cause positive effects on physiological parameters related to crop quality and yield. These compounds would have a broad spectrum of action, however, their mode of action have not been completely elucidated yet. Previous results in our laboratory have shown that these compounds induce enzymes related to the antioxidant system and the reinforcement of the cell wall in potato. Polyamines (PAs) are aliphatic amines present in almost all organisms, including plants. Most common PAs in plants are: Putrescine (PUT), Spermidine (SPD) and Spermine (SPM). They are involved in many growth and development processes and also in the response to biotic and abiotic stresses. PAs can scavenge reactive oxygen species or directly modulate the activity antioxidant enzymes. In addition, the can also act as a source of hydrogen peroxide in response to stress through the activity of the catabolic enzymes such as diamino oxidases (DAO) and polyamino oxidases (PAO). The objective of the present work was to study the participation of PAs in the resistance mechanism induced by phosphites against *P. infestans* in potato plants. The results indicated that PAs differentially accumulate in potato leaves depending on the treatments. In the absence of infection, an increase in the content of SPD and SPM was observed in leaves 3 days after potassium phosphite (KPhi) treatment. Upon pathogen challenge, the content of PUT, SPD and SPM increased in KPhi treated leaves 48 h post-inoculation, compared to non-treated inoculated leaves. The expression of different genes involved in PA metabolism showed that many of both biosynthetic and catabolic genes decreased in non-treated inoculated leaves 48 hpi, compared to non-treated and non-inoculated leaves (control). However, this was not observed in KPhi treated and inoculated leaves, where an increase in the expression of various genes was observed. At this time, PAO enzymatic activity also increased in KPhi treated and inoculated leaves respect to the control. These results suggest that polyamine metabolism could be involved in the resistance mechanisms induced by phosphites in potato plants inoculated with *P. infestans*.

PL-P26
IS SALICYLIC ACID INVOLVED IN PHOSPHITE INDUCED POLYAMINES UPON UV-B EXPOSURE?

Lobato MC, Machinandiarena MF, Oyarburo NS, Daleo GR. Olivieri FP
Instituto de Investigaciones Biológicas, UNMDP CONICET, FCEyN. Funes 3250, 7600 Mar del Plata CC1245. E-mail: folivier@mdp.edu.ar

Phosphite compounds (metallic salts of phosphorous acid) are widely used to protect plants from pathogenic oomycetes on a wide range of horticultural crops. Since they are nontoxic for the environment or human health, they are included as biocompatible compounds into strategies to diminish the use of toxic agrochemicals. In our lab we have observed that potassium phosphite (KPhi) has positive effects in increasing potato tolerance against various diseases, UV-B radiation, and on physiological parameters related to crop quality and yield. Several signaling pathways are described to participate in phosphite induced responses. However, the molecular mechanisms behind phosphite induced resistance are poorly understood. In a previous work we have shown that KPhi primes the plant for an earlier and more intense response to *P. infestans* infection and that salicylic acid (SA) would mediate this response. On the other hand, it has been reported that pathways triggered by UV-B radiation, have a crosstalk with other signaling routes in response to biotic stress. Polyamines (PAs) are molecules involved both in biotic and abiotic stress. Previously we have showed that PAs are part of the metabolic changes produced by KPhi in potato UV-B exposed leaves. In the present work, the salicylic acid (SA) participation in the UV-B tolerance response induced by KPhi was analyzed. To this aim, transgenic tomato plants (NahG) deficient in SA accumulation, were treated with 1% KPhi and, 3 days later were exposed to 1 h/day of UV-B radiation (1.5 Wattm2) for 3 days. The pattern of the different polyamine (PA) accumulation was analyzed by TLC fractionation of dansylated PAs. As it was previously observed in potato plants, wild type tomato plants showed PA accumulation upon UV-B radiation in KPhi treated plants respect to non-treated ones. This accumulation was not observed in transgenic plants indicating that the increase in PA by KPhi could be mediated by SA. The expression of genes corresponding to PAs biosynthesis was also analyzed. The accumulation of transcripts of ADC, ODC, SPDS, SPMS and SAMDC was evaluated 3 days after KPhi and/or UV-B treatments. SPMS expression pattern correlates with its product accumulation (spermine). The other enzymes showed a variable behavior, however, in all cases, these preliminary results, showed a decrease in the expression of these biosynthetic genes in NahG plants pretreated with KPhi an exposed to UV-B, respect to their controls.

PL-P27
ALTERNATIVE SPLICING IMPACT INDUCED BY LIGHT DURING *ARABIDOPSIS THALIANA* SEEDS GERMINATION

Servi L, Tognacca RS, Petrillo E
Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE). E-mail: lucasservi@fbmc.fcen.uba.ar

Gene expression in eukaryotes is a complex process that involves different regulatory mechanisms. RNA processing is an important layer of the path genes enjoy during and after transcription. Particularly, alternative splicing (AS) brings to the game a whole new spectrum of transcripts originated from a single gene, making available a wider range of responses to the cell. This mechanism is then considered one of the main sources of protein and non-coding ARNs diversity. In plants, the relevance of AS is growing as a result of the advance in sequencing techniques. These autotrophic organisms harvest their energy from light. Being sessile organisms, plants need to adapt to a changing environment; evolution handed a vast variety of mechanisms to perceive variations in the ever-changing environment, and to respond precisely to them. In this sense, light is one of the main stimuli sensed by photoreceptor proteins. Chloroplasts—which are the organelles where photosynthesis occurs—are one of the main players in the sensing of light, modulating gene expression by retrograde signaling. Mitochondria are also, by the means of retrograde signaling, capable of modulating AS in the nucleus. In the plant life cycle, the seed is a quiescent stage where the embryo awaits favorable conditions to germinate and develop a mature plant. We are interested in understanding AS modulation during the early stages of seed development, its maturation, and future germination. Previous work indicates AS is regulated by light in the seeds. Some of the analyzed events are controlled by photoreceptors while others are not; these could be affected by retrograde signals. Our experimental approach will allow us to identify the role and the different light-sensing mechanisms interaction, like retrograde signals and photoreceptor pathways, during the whole life of the seed.

PL-P28
TRANSCRIPTOME ANALYSIS OF PEACH-*TAPHRINA DEFORMANS* INTERACTION IN A SUSCEPTIBLE GENOTYPE

Novello MA[1], Valentini GH[2], Drincovich MF[1], Lara MV[1]
[1]Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI), [2]Estación Experimental San Pedro, INTA. E-mail: novello@cefobi-conicet.gov.ar

Prunus persica (L) Batsch, is a tree of great economic importance and is susceptible to numerous diseases including the *Peach Leaf Curl*—a widely worldwide distributed disease that generates losses ranging between 2.5 and 3 million dollars per year. The causal agent of the disease is the dimorphic fungus *Taphrina deformans*. The pathogen invades new shoots in early spring when the leaves are young and have a thin cuticle. The symptoms include chlorosis, pink pigmentation, the characteristic curling of the leaf, and ultimately the senescence of the leaves and the tree's death. Normally, the disease is controlled with agrochemicals, but its high cost, its potential negative impact on animals and humans, and its insufficient control have increased the demand for farming practices that reduce risks to health and the environment, being necessary strategies based on genetically resistant materials. In this work, leaves of the susceptible genotype, DOFI-71.043.018 were inoculated with *T. deformans*. Hypha development was followed using Gueguen staining. Samples were collected at 0, 12, and 96 h post-inoculation (hpi) and used for RNA extraction. RNAseq analysis was conducted to unravel the early responses of the host. The analysis yielded 357 and 210 differentially expressed genes (DEGs) at 12 and 96 hpi compared to 0 hpi, respectively. Approximately 43% of the DEGs were induced and 57% were repressed in both comparisons. The most represented functional categories were RNA, signaling, hormones, proteins and biotic stress. Regarding RNA, transcription

factors (FT) such as WRKY, bHLH, HB, MYB, and zinc fingers FT were differentially expressed. While MYB FTs were induced both at 12 and 96 hpi, bHLH FTs were exclusively up-regulated at 12 hpi. Differences regarding phytohormones were also found; enzymes involved in the ethylene biosynthesis such as ACC oxidase (PRUPE.1G036600) and ACC synthase (PRUPE.2G176900) and involved in jasmonic acid biosynthesis like LOX1 (PRUPE.4G047800) were significantly induced at 12 hpi. Moreover, the metabolism of brassinosteroids was induced at 96 hpi. The increase in these hormones synthesis upon infection could activate defense responses. On the contrary, the abscisic acid biosynthetic pathway was repressed as an early response. The induction of auxin-responsive genes was also detected and could be responsible for some symptoms of the disease. Furthermore, many pathogenesis-related proteins (PR), like Thaumatin-like proteins (PR5), Kunitz proteinase inhibitors (PR6), lipid transfer proteins (PR14), B-1,3-glucanases (PR2), and endochitinases (PR3) were induced exclusively at 12 hpi indicating the activation of defense responses. At both times, enzymes involved in the synthesis and degradation of the cell wall were identified, suggesting a recycling and wall remodeling in order to stop the development of the fungus. These findings provide important information on the molecular responses of *P. persica* against *T. deformans*.

PL-P29
STUDY OF THE PROPERTIES OF THE FRATAXIN FROM GREEN AND BROWN ALGAE

Marchetti-Acosta NS, Barchiesi J, Gomez-Casati DF, Busi MV.
Centro de Estudios Fotosintéticos y Bioquímicos, CEFOBI – CONICET, UNR, Rosario, Argentina. E-mail: marchetti@cefobi-conicet.gov.ar

Fe-S clusters are among the oldest and most versatile cofactors used by most living organisms. They are formed by atoms of iron and inorganic sulfide. Although they can be synthesized non-enzymatically *in vitro*, their biosynthesis is catalyzed by specific enzymes. Three complete systems responsible for the biosynthesis of groups [Fe-S] were discovered in bacteria: NIF (nitrogen fixation), SUF (sulfur mobilization), and ISC (iron and sulfur cluster). On the other hand, in eukaryotes, the presence of ISC and SUF homologous systems were detected in mitochondria and chloroplasts, respectively. In this work, we address the initial characterization of this pathway in algae. First, we performed the identification *in silico* of gene and protein sequences possibly related to the biogenesis of Fe-S clusters in green and brown algae (*Chlorella vulgaris* and *Ectocarpus siliculosus*) by using the Phytozome database (https://phytozome.jgi.doe.gov). After performing sequence alignments and considering the similarity percentages found between sequences, one frataxin from *E. siliculosus* (EctsiFH) and one from *C. vulgaris* (ChlspFH) were selected to perform their functional characterization. The recombinant proteins produced in bacteria were expressed and purified to homogeneity. We evaluated their ability to attenuate the Fenton reaction by measuring the inhibition of malondialdehyde production after the addition of thiobarbituric acid. As previously suggested, frataxin could function as an iron chaperone, and in this way, its presence could attenuate oxidative damage by metals. Results showed that ChlspFH attenuated the Fenton reaction by 23%, while EctsiFH presented less attenuation. To assess whether overexpression decreases the sensitivity of *Escherichia coli* cells to oxidative and metal stress, aliquots of liquid cultures were incubated in the presence of hydrogen peroxide, nickel, cadmium, or zinc. Our results showed that the expression of frataxin allowed the cells to grow better under oxidative conditions respect to the cells that do not express the recombinant frataxins. These results suggest that algae frataxin would have a protective role against oxidative stress in algae.

PL-P30
UNCOUPLING THE ROLES OF CHLOROPLAST REDOX STATE IN LEAF DEVELOPMENT AND STRESS TOLERANCE THROUGH REGULATED EXPRESSION OF A FLAVODOXIN

Demarchi M, Saenz M*, Mayta M, Carrillo N, Lodeyro A. *These authors contributed equally to this work.*
Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET-UNR). Ocampo y Esmeralda (2000) Rosario.
E-mail: demarchi@ibr-conicet.gov.ar

In higher plants, the processes of cell proliferation and expansion are responsible for determining the final size of the leaves. Moreover, leaf size directly influences yield by affecting photosynthate production, which has important agronomic implications. Homeostasis of reactive oxygen species (ROS) has a central role in the regulation of cell expansion during leaf growth. In leaves, most ROS come from chloroplasts. The influence of ROS in cellular processes is regulated by a tight balance between their production and scavenging by antioxidant systems. Flavodoxin (Fld) is an electronic carrier isofunctional with ferredoxin (Fd). Fld is present in both algae and cyanobacteria, but absent in higher plants. Constitutive expression of a plastid-targeted can increase tolerance to multiple stresses in plants by acting as a general antioxidant specific for chloroplasts. Therefore Fld-expressing lines can be used as tools to probe the role played by chloroplast-generated ROS in different biological processes. However, constitutive expression of Fld also results in smaller leaves. The main objective of this work is to elucidate if enhanced stress tolerance and reduction of leaf size are related, by expressing Fld under the control of developmental and stress-inducible promoters. Transgenic *Arabidopsis thaliana* plants expressing Fld constructs under the control of cell proliferation or cell expansion- specific promoters were obtained using the *floral dip* technique. Additionally, plants expressing Fld under the control of two stress-inducible promoters, *PEC* and *PEC:I*, which differ in their expression strength, were obtained. Similar constructs containing the reporter gene β-glucuronidase (GUS) were used to set up the conditions of induction. NaCl and abscisic acid (ABA) were used as inducers for stress-inducible promoters, both in 15-day seedlings and in leaf discs from adult plants. Moreover, leaf size, cell size, and cell numbers were determined in non-stressed plants. Fld expression was confirmed by Western blot assays in the different stages of leaf development and under stress conditions in all lines. Preliminary results obtained provide clues to understand how chloroplast redox state determines leaf size affecting in the final instance the crop yield.

PL-P31
CADMIUM DECREASED ROOT GROWTH BY INDUCING HORMONAL IMBALANCE IN MAIZE SEEDLINGS

Matayoshi CL[1], Pena LB[1,2], Zawoznik MS[1], Arbona V[3], Gómez-Cadenas A[3], Gallego SM[1,2]
[1] UBA, Facultad de Farmacia y Bioquímica, Departamento de Química Biológica. Buenos Aires, Argentina,[2] Universidad de Buenos Aires. Consejo Nacional de Investigaciones Científicas y Técnicas. Instituto de Química y Fisicoquímica Biológicas (IQUIFIB). Buenos Aires, Argentina, [3] Universitat Jaume I. España. E-mail: matayoshi.cl@gmail.com

Plant growth is controlled by redox and hormonal balance. Cadmium toxicity is closely associated with growth reduction and previous studies showed that cadmium—although is a redox inactive metal—induced redox imbalance. The objective of the present study was to evaluate root hormonal balance response to cadmium. For this purpose, *Zea mays* L. (maize) seeds were germinated on filter paper moistened with distilled water in plastic plates. Seedlings with roots of 1–2 cm length were selected and transferred to a hydroponic culture containing diluted (1/10) Hoagland solution without (control, C) or containing 50 and 100 µM $CdCl_2$. Plants were grown in a controlled climate room at a temperature of 24 ± 2 °C in darkness. Hormonal determinations were performed after 72 h of treatment. Moreover, global SUMO1 and ubiquitin (Ub) proteins conjugation were assayed. The root apex (5 mm) segment (A) and the rest of the root tissue (R) were processed separately. Cadmium decreased root length (66 and 70 % for 50 and 100 µM $CdCl_2$, respectively) respect to the C. Jasmonic acid (JA) concentration remained similar to C, but JA-isoleucine conjugate (JA-Ile) was significantly reduced in both, A (81 and 91% for 50 and 100 µM $CdCl_2$) and R (74 and 64% for 50 and 100 µM $CdCl_2$). Gibberellins (GA) GA4 content significantly decreased in A (63 and 80% for 50 and 100 µM $CdCl_2$) as well as in R (65 and 47 % for 50 and 100 µM $CdCl_2$). The precursor GA20 was markedly enhanced in R by about 53% (without difference between concentrations), whereas GA3 and GA7 levels remained similar to C. On the other hand, indole-3-acetic acid increased significantly in a dose-dependent manner in both, A (27 and 82 % for 50 and 100 µM $CdCl_2$) and R (200 and 350 % for 50 and 100 µM $CdCl_2$). Furthermore, abscisic acid content increased in A (181 and 80 % for 50 and 100 µM of $CdCl_2$ respectively) but more remarkable in R (418 and 649 % for 50 and 100 µM $CdCl_2$). Finally, salicylic acid showed an increment of 89% in the A under 50 µM of $CdCl_2$, but a reduction of 33% with 100 µM of $CdCl_2$. Global SUMO1- and Ub-conjugated proteins were increased in A but decreased in R during the metal treatment. Summing up, cadmium induced not only redox but also hormonal imbalance, which led to root growth inhibition. Furthermore, protein conjugation with SUMO and Ub modifiers upon Cd-exposure would be involved as part of a general cell defense mechanism against metal toxicity.

PL-P32
GENOME WIDE ANALYSES OF ASPARTIC PROTEASES ON POTATO GENOME

Norero NS[1], D`Ippólito S[23], Décima Oneto CA[1], Massa GA[134], Feingold SE[1], Guevara G[23]
[1]Laboratorio de Agrobiotecnología, INTA EEA Balcarce, [2]Instituto de Investigaciones Biológicas, UNMdP, [3]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), [4]Facultad de Ciencias Agrarias – UNMdP. E-mail: gguevara@mdp.edu.ar

Aspartic proteases (AP) are proteolytic enzymes widely distributed on plants, animals, and microorganisms. They are involved in multiple functions related to protein recycling, immune response, biotic and abiotic stress, cell death, and others. They are the second largest group among proteases after serine proteases, but their specific functions are poorly understood. Some aspartic proteases have been purified from potato genome and have demonstrated to confer tolerance to *Phytophthora infestans* and diverse abiotic stresses. In addition, one of these proteases (AAT77954.1) has been associated with cell death. In this work, we characterize the aspartic protease family on the potato genome for the first time. We built a HMMER profile from 51 aspartic proteases from *Arabidopsis thaliana* L., and we performed a HMMER search on potato genome peptide database V4.1 (PGSC, 2011). We manually curated 121 proteins out of 150 sequences that included aspartic sites typical of AP, identifying 68 AP genes on potato genome (PGSC, DM V4.1). These genes were distributed on all 12 chromosomes with some preference for chromosomes VIII and VII. In several cases, tandem arrays were evidenced. Gene structural analyses showed a high proportion of intronless genes (30 out of 68). The phylogenetic study showed seven clusters named *St*AP1 to *St*AP7. The presence of aspartic proteases domains was analyzed distinguishing typical, atypical, and nucellin-like AP, previously described in *Arabidopsis thaliana* L. genome, on potato genome. A characteristic feature of some AP is the occurrence of an extra 100 amino acid long segment, known as plant-specific insert (PSI), which folds as an independent domain and generally is processed out during protein maturation. This domain was present only in proteins grouped in Cluster VII, which includes the homolog to AAT77954.1. Most aspartic proteases presented a signal peptide related to extracellular localization, and some of them presented TAXI_C and TAXI_N domains that could be related to pathogen defense. RNA-Seq expression data from double monoploid *Solanum tuberosum* group Phureja (PGSC, 2011), including libraries from different tissues, plant organs, biotic and abiotic stress conditions, reveals the expression of AP in all of them, without evident relation with cluster distribution. These analyses will help to unveil the structure and composition of the potato aspartic proteases family, providing insights for future studies.

PL-P33
METABOLIC REGULATION OF MALIC ENZYMES OF TWO PANICOIDEAE CLADE MEMBERS, *SETARIA ITALICA* AND *PANICUM VIRGATUM*

Calace P[1], Andreo CA, Gerrard Wheeler CA[1], Saigo M[1]
[1]Centro de Estudios Bioquímicos y Fotosintéticos (CEFOBI-CONICET), Rosario, Argentina. E-mail: calace@cefobi-conicet.gov.ar

C4 photosynthesis has evolved to increase the photosynthetic efficiency in conditions where photorespiration would be enhanced, such as high temperatures and drought. This was achieved through the introduction of a series of anatomical and biochemical features that allow the concentration of CO_2 around Rubisco. In most C4 species, this is done through compartmentalizing the initial and definitive CO_2 assimilation

processes into two discrete cell types, namely mesophyll (M) and bundle sheath (BS) cells. As part of this mechanism, a compound of four carbon atoms is transported from M to BS cells. *Setaria italica* (foxtail millet) and *Panicum virgatum* (switchgrass) are two members of the Panicoideae clade used for feed and biofuel production. Despite their evolutionary closeness, they have been classified into different C4 subtypes. *S. italica* belongs to the C4-NADP-ME subtype as it possesses an NADP-dependent malic enzyme (NADP-ME) as major malate decarboxylase within chloroplasts and transport mainly malate from MC to BSC. In *P. virgatum* (C4-NAD-ME subtype) the major decarboxylase is a mitochondrial NAD-ME and aspartate is the main C4 acid transported to BSC from MC. In this study, we made progress in the kinetic characterization that distinguishes each decarboxylase enzyme operating in both grasses. Furthermore, we carried out a comparative analysis in order to identify the isoforms involved in the C4 cycle and to characterize the differences between the photosynthetic and the non-photosynthetic versions. We purified recombinant NAD-MEs (α and β) and chloroplastic NADP-MEs from both species. To gain insight into the physiological roles of NAD and NADP- MEs, the activity of recombinant enzymes in the malate decarboxylation direction was evaluated in the presence of different metabolites. The general analysis of the NAD-MEs responses showed some similarities with those reported for the enzymes from Arabidopsis, the C3 model species. The NAD-ME type β (Seita.2G322000 and Pavir.2KG446000) responded positively to the presence of Krebs cycle intermediates such as citrate, fumarate, succinate, and oxaloacetate and the NAD-ME type α (Seita.9G200600 and Pavir.9KG132400) were strongly activated by both acetyl-CoA and CoA and by fructose-1,6-bP and PEP, both metabolites markers of the decrease in pyruvate glycolytic supply. These regulations agree with the functioning of NAD-ME as a pyruvate-contributing enzyme when there is a decrease in glycolysis and a high content of the Krebs cycle intermediates. On the other hand, both NADP-MEs showed inhibition by the substrate malate at pH 7, a regulatory characteristic linked to the photosynthetic function. In addition, the ATP activation and the aspartate inhibition exhibited by NADP-ME from *P. virgatum* would indicate that in this NAD-ME subtype species the NADP-ME decarboxylation could also contribute to the C4 photosynthesis.

PL-P34
METABOLOMIC RESPONSE OF ISOLATED MAIZE EMBRYONIC AXES TO OXIDATIVE CONDITIONS SUBJECTED BY CADMIUM, METHYLVIOLOGEN AND HYDROGEN PEROXIDE

Pena LB[1,2], Moratto CJ[1], Arán M[3], Méndez AAE[1,2], Matayoshi CL[1], Gallego SM[1,2]
[1]UBA, Facultad de Farmacia y Bioquímica, Departamento de Química Biológica. Buenos Aires, Argentina, [2]UBA, CONICET- IQUIFIB, Buenos Aires, Argentina. [3]Laboratorio de Resonancia Magnética Nuclear, IIBBA-CONICET Buenos Aires, Argentina. E-mail lpena@ffyb.uba.ar

The germination process starts with the uptake of water (imbibition) by the dry seed, followed by metabolism reactivation, and ends when radicle protrudes. Successful seed germination will depend on the abilities of the embryo to gain its metabolic activity and for its capacity to cope with adverse environmental conditions. Although high levels of reactive oxygen species (ROS) produce oxidative stress, a basal level of ROS plays fundamental roles in the early stage of embryonic axes imbibitions. The objective of this study was to contribute to understanding the metabolomic adjustment that needs to be established for a successful equilibrium between growth and resistance. Intact embryo axes isolated from *Zea mays* cv. Chalqueño seeds were imbibed with 50 mM Tris-HCl pH 7.6, 50 mM KCl, 2% (w/v) sucrose, 10 mM $MgCl_2$ without (C) or containing 10 µM $CdCl_2$, 0.5 µM methylviologen (MV) or 1 mM H_2O_2. The study was focused on 24 and 48 h of imbibition. The absolute elongation rate of 0.43 mm d^{-1} for control axes was reduced 60, 62, and 49 % in Cd-, MV- and H_2O_2-treated ones, respectively. Also, treatments disrupted cell redox balance by inducing the accumulation of O_2^- and H_2O_2 and modifying antioxidant enzymes defense system (catalase, superoxide dismutase, ascorbate and guaiacol peroxidases, glutathione and dehydroascorbate reductases). Oxidative cell damage was determined by the increase in protein carbonylation. Increment in proteolytic activities was determined during the axes imbibitions and also during the oxidative conditions generated by treatments. Water-methanol extracts were used to evaluate the polar metabolites profile using nuclear magnetic resonance (H^1-NMR) spectroscopy. Hierarchical cluster analysis showed that after 24 h of imbibition, the metabolome of Cd-treated axes showed lower response respect to MV and H_2O_2 since its cluster was closer to the control. After 48 h of imbibitions, stress conditions clustered in a group that was distinct to the control. Our results are indicating that during growth arrest, metabolic reorganization of the maize embryonic axes depends on an intense protein degradation to produce free amino acids. This metabolic module would be necessary to improve the cell defense systems, by allowing the synthesis of new proteins and by maintaining metabolic homeostasis.

PL-P35
CHLOROPHYLL AND STORAGE SUBSTANCE CONTENT IN SOYBEAN SEEDS FROM DIFFERENT GENOTYPES

Poeta MF[1], Pavlovic T[1], Sáenz E[2], Calace P[1], Borrás L[2], Saigo M[1], Gerrard Wheeler M[1]
[1]Centro de Estudios Fotosintéticos y Bioquímicos, [2] Instituto de Investigaciones en Ciencias Agrarias de Rosario.
E-mail: florenciabpoeta@gmail.com

Soybean seed is a product of central importance in the agro-economy of Argentina, so it is necessary to attend its quality by generating strategies in order to obtain improved genotypes. The soybean seed derivatives mainly marketed are protein flour and oil, which are used for human and animal feeding and biofuel industry. In this work, a characterization of the organic composition of soybean cultivars selected from 1100 F7 lines belonging to 9 segregating populations is presented. This subsample comes from crossings between the high-performance commercial genotype DM3100 and high-protein concentration parental with large (PI538376) or small (PI818757) seed size. The content of reserves and chlorophyll in the grains were evaluated in samples from plants at different phenological stages, which corresponded to four different values of grain moisture: 74, 66, 46, and 9 %. Gravimetric analyses revealed that the dry grains (9% moisture) contained a range of 15–22 % of lipids depending on the genotype. In addition, the proteins accounted for between 36 and 44% of the final weight of mature soybean seeds, according to the Kjeldahl method. A negative correlation and a temporary offset in the accumulation of the different components were observed, while oil deposited at the end of the filling, the proteins accumulated earlier. On the other hand, the levels of chlorophyll decreased as maturation progressed in accordance with the decrease in the light that reached the growing embryos. These results allowed the selection of pairs of soybean isogenic lines contrasting

in terms of protein and oil content. This work constitutes a starting point in order to detect metabolic differences that explain the different levels of reserve compounds and will help us identify molecular components involved in oil and/or protein biosynthesis, including enzymes, transporters, transcription factors, and other proteins.

PL-P36
NITROGEN METABOLISM IS ALTERED IN *ARABIDOPSIS* PLANTS WITH MODIFIED POLYAMINE LEVELS SUBJECTED TO NITROGEN DEFICIENCY

Recalde L[1], Pena L[1,2], Rossi F[3], Arbona V[4], Groppa MD[1,2], Benavides MP[1,2]
[1]UBA, Facultad de Farmacia y Bioquímica, Departamento de Química Biológica, Argentina. [2]CONICET-IQUIFIB. Facultad de Farmacia y Bioquímica, Buenos Aires, Argentina. [3]INTECH-CONICET. [4]Universitat Jaume I, Castelló de la Plana, España.
E-mail: laurarecalde7@gmail.com

Nitrogen (N) is one of the most important nutrients in plant growth and is a constituent of many stable compounds such as amino acids, proteins, and polyamines (PAs). In *Arabidopsis thaliana*, where the PA putrescine (Put) is produced exclusively through the arginine decarboxylase (ADC) pathway, two genes encode ADC (*ADC1* and *ADC2*). The objective of this work was to study the interrelation between N and PAs metabolisms in *Arabidopsis* plants under N deprivation. Leaves of wild type plants (WT), a line defective in Put biosynthesis (adc1) and a line overexpressing ADC (7.2) were used. Plants were grown in a Hoagland basal nutrient media containing normal (C) or reduced (Low N) total nitrogen content, in hydroponics for 21 days. In C media, free Put content in adc1 and 7.2 lines was 35% lower and 2.5-fold higher, respectively, than in WT line, whereas in low N medium, Put content decreased close to 30% in WT and adc1 plants but did not change in 7.2. Spermine content was greatly increased in adc1 respect to WT plants in both media. The mutant adc1 has a slightly higher fresh biomass and protein content in N7 medium but seemed to be the more affected one when grown under N deprivation in terms of growth, protein or chlorophyll content and showed different behavior in some aspects of N metabolism respect to WT, in basal and deficient media. Nitrate reductase activity was lower in the adc1 line and slightly changed in 7.2 line compared to WT in C medium, while in low N medium, its activity decayed in WT and adc1, but it maintained its level in 7.2. Ammonium and nitrate levels were reduced in low N respect to C at a comparable rate in all lines, but the decay was less pronounced in 7.2. The amino acids glutamate, glutamine, arginine, proline, and GABA were higher in C respect to low N media in the three studied lines. However, adc1 showed elevated levels of the five compounds respect to WT in C, but all compounds decreased in the mutant line compared to WT in low N media. In 7.2 line, only GABA and glutamate showed higher levels, respect to the WT in C medium. Altogether, these results suggest that N depletion affected PAs or nitrogenated metabolites formation differently in WT, mutant or transgenic plants, highlighting the importance of N intermediates flux through the major network of nitrogen-metabolizing pathways in plants under N deficiency.

PL-P37
INTERACTION BETWEEN *PSEUDOMONAS STUTZERI* MJL19 AND SOYBEAN PLANTS IN SALINE STRESS

Lami MJ[1], Caram di Santo MC[1], Zenoff AM[1], Rubio Molina AC[1], Bulacio Gil NM[1], Pomares MF[1], Adler C[1], Vincent PA[1], Espinosa Urgel M[2], de Cristobal RE[1]
[1]INSIBIO (CONICET-UNT), Tucumán, Argentina. [2]Estación Experimental del Zaidín, Granada-España. E-mail: vincent.paula@gmail.com

Root surrounding is a highly complex ecosystem influenced by roots secretions and by microbial activity in the soil. During colonization and adaptation to life in the rhizosphere, bacteria modify gene expression affecting metabolism, mobility, chemotaxis, EPS synthesis, and secretion. The interaction of the bacterium *Pseudomonas stutzeri* MJL19 with soybean root was studied by RT-qPCR transcriptional analysis of genes potentially related to colonization and abiotic stress response. We analyzed the expression of genes involved in root colonization (*algA*, *bcsA*, *oprF*, *clpP*, *bcsA*, *putA*, and *putP*) and genes coding for chaperones responsible for the proper folding of stress-damaged proteins, superoxide dismutase and production of protective osmolites (i.e., *dnaJ*, *proC*, and *sodC*). We found higher expression of *clpP*, *oprF*, *algA*, *bcsA*, *putA*, and *putP* when MJL19 was attached to soybean root in saline conditions compared with MJL19 growing in the absence of the plant. In the case of *clpP* and *oprF*, the difference in root expression was about 5-fold greater in the saline condition with respect to the non-saline one. In contrast, NaCl in the absence of soybean roots did not generate significant changes in the expressions. The expression of genes related to abiotic stress, *dnaJ* and *proC*, showed greater expression in populations of MJL19 attached to the soybean root in saline stress, meanwhile *sodC* decreased its expression under this condition. In *P. stutzeri* MJL19 genes related to colonization and formation of biofilms such as *oprF, clpP, algA*, and *bcsA* are expressed in the presence of soybeans roots exposed to saline stress, indicating that the bacterium is prone to grow as a biofilm when it is in the presence of the soybean root. Expression of the gene involved in the proline biosynthesis (*proC*) and expression of the chaperone DnaJ (*dnaJ*) are low when MJL19 is in the presence of soybean in non-saline conditions. However, when MJL19 is in contact with soybean roots in saline conditions, the expression of both genes is substantially enhanced.

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

PL-P38
DECREASED SUSCEPTIBILITY TO *PSEUDOMONAS SYRINGAE* INFECTION IN *MSH6 ARABIDOPSIS THALIANA* MUTANT PLANTS

Ramos RS, Spampinato CP
CEFOBI-CONICET, Rosario, Argentina. E-mail: ramos@cefobi-conicet.gov.ar

DNA mismatch repair (MMR) proteins have been implicated in sensing and correcting DNA-replication-associated errors and in regulating cell cycle progression. In addition, MMR proteins are also involved in the recognition of nucleotide lesions induced by different stresses. Besides abiotic stresses, all organisms are also threatened by various pathogens. To avoid infection, organisms rely on their immune systems. Even though both DNA damage and immune responses have been studied in depth separately, whether and how they are connected are largely unknown. The aim of this work was to study the contribution of the MMR system in the susceptibility/resistance of *Arabidopsis thaliana* plants during the immune response. Initially, MutS homolog 6 (MSH6) was studied because it forms the major mismatch recognition complex (MSH6-MSH2). First, we spray infected 14-day-old WT and *msh6* mutant plants with the bacterial pathogen *Pseudomonas syringae* pv. *tomato* strain DC3000 (*Pst* DC3000). Colony-forming units (CFU) were quantified in leaves from inoculated plants, and growth parameters like rosette area, cell number, and cell area were analyzed. We found that disruption of *MSH6* in *A. thaliana* plants results in less susceptibility to *Pst* DC3000 infection, which could be evidenced by a lower CFU amount, a lower plant growth decrease, a lower number of cells and a higher cell area in mutant than in WT plants at 3 days after inoculation. Additionally, the homologous recombination rate was measured in both genotypes. The recombination assay construct contains two overlapping 1.6% divergent halves of the β-glucuronidase (*GUS*) reporter gene and upon recombination leads to the formation of an active *GUS* gene. We observed that *Pst* DC3000 infection led to an increase in the homologous recombination rate in WT plants. This increase was not evident in *msh6* mutant plants, which already show a high recombination rate under control conditions. These results suggest that the tolerance to *Pst DC3000* in *msh6* mutant plants could be associated with increased programmed cell death. Future experiments will allow testing this hypothesis.

PL-P39
THE APOPLASTIC SECRETOME OF *PRUNUS PERSICA* IN RESPONSE TO *TAPHRINA DEFORMANS* INOCULATION

Butassi E[1], Valentini G[2], Drincovich MF[1], Lara MV[1]
[1]Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI), [2]Estación Experimental San Pedro, INTA. E-mail: butassi@cefobi-conicet.gov.ar

The dimorphic fungus *Taphrina deformans* is responsible for the "Leaf Curl Disease" that affects *Prunus persica* L. trees around the world. The pathogen on the lower surface penetrates through the stomata or into the cuticle. The hyphae grow in the intercellular spaces and do not penetrate the cell. To gain insight into the molecular mechanisms involved in plant resistance to the disease, we studied apoplastic proteins differentially expressed in a resistant genotype (DR) in comparison with a susceptible one (Flavorcrest FL) during fungal infection. For this, leaves were inoculated with the fungus and collected at 0, 12, and 96 hpi (hours post-inoculation) and used for apoplastic protein isolation using the infiltration-centrifugation methodology. Proteins were analyzed by LC-ESI-MS, and differential proteins were identified using the Perseus software. In DR we identified 49 and 133 differentially expressed apoplastic proteins at 12 and 96 hpi with respect to 0 hpi, respectively. With respect to FL, 42 and 104 proteins with different levels were found at 12 and 96 hpi, respectively. The most represented functional categories of differential proteins detected in both genotypes were miscellaneous (30%), biotic and abiotic stress (27%), cell wall degradation (16.5%), protein degradation (8.5%), and signaling (5%). Regarding miscellaneous category, glucosidases, galactosidases and mannosidases, phosphatases, oxidases, GDSL-motif lipases, peroxidases, among others, were detected in both genotypes at 12 and 96 hpi. Many pathogenesis-related (PR) proteins such as beta-1,3-endoglucanases (PR2), chitinases (PR3 and PR4), thaumatin-like proteins (TLP, PR5), defensins (PR12), lipid transfer proteins (LTP, PR14) and gemin-like proteins (PR16 and PR17) were induced, mostly at 96 hpi in both genotypes. Additionally, other PRs were only induced in DR at 96 hpi, like a chitinase (M5XTV3), two TLPs (M5WTQ8 and M5WV03), a defensin (M5Y0D4), and two LTPs (M5W0V2 and M5WW86). In the cell wall degradation category, cellulases, pectinesterases, pectate lyases, polygalacturonases, and expansins were expressed in DR and FL at 12 and 96 hpi. Some of these proteins were induced only in DR at 96 hpi, such as a pectinesterase (M5VV51) and an expansin (M5XKK6). Of particular interest is the induction in the resistant genotype of proteins that are repressed or not induced in the susceptible genotype. Among these are a rapid alkalinization factor (M5WB80), a dirigent protein (M5WZH0), a gibberellin-regulated family protein (M5XFZ3), a protein with GASA domain (M5XNP6), a PR5K putative transmembrane receptor protein serine/threonine kinase (M5WA70) and a glyoxal oxidase (M5XBM0), which are currently under study. This study allowed to identify candidate resistance proteins involved in the plant pathogen response and to dissect the early molecular processes in the apoplast during the *P. persica–T. deformans* interaction in resistant and susceptible genotypes.

PL-P40
ZMS5H, A NOVEL ENZYME INVOLVED IN SALICYLIC ACID HYDROXYLATION

Righini S, Serra P, Falcone Ferreyra ML, Casati P
Centro de Estudios Fotosintéticos y Bioquímicos, Universidad Nacional de Rosario. E-mail: righini@cefobi-conicet.gov.ar

Salicylic acid (SA) has been described as an important signaling molecule in plants, regulating growth, development, senescence, and responses to biotic and abiotic stresses. 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. Hydroxylated SA is the major degradation product of SA. Recently, the *Arabidopsis thaliana* enzyme catalyzing SA to 2,3-dihydroxybenzoic acid (2,3-DHBA; *At*S3H) has been identified, and SA was found to accumulate in *s3h* mutants. In this study, we report the discovery and functional characterization of a novel maize salicylic acid 5-hydroxylase (*Zm*S5H), a 2-oxoglutarate dependent dioxygenase that catalyzes the formation of 2,5-DHBA by hydroxylating SA at the C5 position of its phenyl ring. Once identified, we carried out *in vitro* activity assays in order to kinetically characterize this enzyme. His-tagged *Zm*S5H was heterologously expressed in *Escherichia coli* and then purified. The reaction product 2,5-DHBA was identified by HPLC by comparison with authentic standards. Interestingly, according to sequence similarity analysis, *Zm*S5H and *At*S3H are closely evolutionarily related, though we could not identify 2,3-DHBA as a product of the studied reaction. Kinetic parameters of the recombinant *Zm*S5H were also determined by HPLC. In addition, its activity *in planta* was demonstrated, as transgenic *Arabidopsis* plants expressing *Zm*S5H were more susceptible to *Pseudomonas syringae* pv. *tomato DC3000* pathogen infection than WT plants, suggesting that these plants would have decreased SA levels due to higher hydroxylation of the hormone. In order to further confirm this, we investigated the expression level of three different genes modulated by SA in *s3h*, wild-type and transgenic Arabidopsis plants expressing *Zm*S5H that were treated with this hormone compared to plants treated with a mock solution. *PR1*, *EDS-1*, and *SAG13* showed a decreased expression level in plants overexpressing *Zm*S5H compared to *s3h* mutants and wild-type plants, suggesting that in fact, *Zm*S5H hydroxylates SA *in planta*. We are now analyzing the possible crosstalk between SA hydroxylation and flavonoids synthesis, a model proposed in our laboratory, based on previous results. So far, transgenic plants expressing two different maize flavone synthases recently characterized, (*ZmFNSI* and *ZmFNSII*), which accumulate flavones, in a mutant *s3h* background, show increased susceptibility towards infection with *P. syringae* compared to wild-type and mutant plants, suggesting that flavones regulate SA levels *in vivo*.

PL-P41
EFFECT OF FOLIAR APPLICATION OF PHOSPHITES IN "HAYWARD" KIWIFRUIT IN STORAGE AND SHELF LIFE: ANALYSIS OF PECTIN COMPOSITION

Rivas-Velásquez M[1], Paz CD[2], Dal Lago CC[2], Martínez-Noël GMA[3], Yommi AK[4], Andreu AB[5]
[1]FCEyN-UNMdP [2]IIDEAGROS, FCA-UNMdP [3]INBIOTEC-CONICET [4]EE Balcarce-INTA [5]IIB, FCEyN-UNMDP.
E-mail: cosmepaz@mdp.edu.ar

The kiwifruit (*Actinidia chinensis* var *chinensis* cv. Hayward), a climacteric fruit, can be harvested at physiological maturity and maintain its quality for up to six months at cold storage. The length of the storage period depends, among others, on cell wall composition and structure, which impacts on texture and softening. Indeed, solubilization and degradation of pectins occur during fruit softening, leading to the disintegration of the cell wall. Pectin is a complex heterogeneous polymer that can have different interactions within the cell wall as free pectin, bound to starch, attached by calcium bridges and bound to cellulose via hydrogen bonds. The phosphites activate the synthesis of compounds that reinforce cell walls, like pectin and lignin. The aim of this work was to study the effect of foliar application of phosphite on pectin composition in cold storage and shelf life. Plants were foliar sprayed (six weekly applications) 100 days after blooming, with 0.3% potassium phosphite (KPhi; 30% P_2O_5, 20% K_2O) or water (Control). Fruits were harvested at physiological maturity and stored for 5 and 6 months (5M and 6M) at 0°C and 90–95 % RH. Kiwifruit was analyzed at the end of each storage period (ES) and its shelf life (SL, 7 days at 20°C). Samples of outer pericarp tissue were frozen and ground using liquid N_2. A chemical solvent method was used to successively extract cell walls and determine the composition of pectin. The cell wall material (CWM) was obtained by the inactivation of the enzymes with a mixture of phenol/acetic acid/water (PAW) and water-soluble pectin fraction (W-SP) was recovered. To remove the kiwifruit starch and extract their bound pectin (S-SP), a solution of dimethyl sulfoxide was used. The Na_2CO_3 was added to obtain the pectin attached by tightly bonds and calcium bridges (C-SP). Each extract was dialyzed 5 days, lyophilized, and weighed. The results showed that KPhi treatment increased the yields of total pectins respect to the Control and also resulted lower at 5M respect to 6M of storage. At shelf life, pectin yield decreased in all treatments compared to the end of storage. The yield of W-SP fractions was lower in SL than in ES and resulted highest in KPhi treatment at 6M. The yield S-SP fraction was lowest in KPhi and SL. This could be due to the enzymatic degradation of starch. The proportion of C-SP yield also was lower in KPhi but was higher in SL. The yield of CWM decreased from the 5M to 6M and increase at SF with the application of KPhi. These results suggest that KPhi treatment promotes the pectin biosynthesis and their release in shelf life. After 5–6 months of cold storage, "Hayward" kiwifruit enters in its last ripening/over-ripening stage related to senescence, led by the cell wall disintegration. In conclusion, KPhi treatment is suggested to be used in order to maintain the firmness "Hayward" kiwifruit, at least until 5 months in cold storage prior shelf life and its consumption.

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

PL-P42
POTENTIAL FUNCTIONS OF *ARABIDOPSIS THALIANA* HEAT SHOCK PROTEINS IN THE PLANT APOPLAST

Rossi F[1], Pérez Rojas S[2], Sánchez López E[2], Deng B[3], Zhang F[3], Gárriz A[1], Clemente M[2], Corigliano MG[2]
[1]Unidad de Biotecnología INTECH, [2]Laboratorio de Biotecnología Vegetal. INTECH, [3]Marsh Life Science Building, University of Vermont Burlington. E-mail: marianacorigliano@intech.gov.ar

Hsp90s are found in all plant cellular compartments examined to date, including cytosol, nucleus, endoplasmic reticulum, mitochondria, and chloroplasts. Besides, Hsp90s have also been found in the apoplast, even though their roles in this location remain elusive. Previously, we added some light to this issue by assessing the regulation of defense-associated mechanisms in *Arabidopsis thaliana* plants infiltrated with the recombinant Hsp90 protein rAtHsp81.2. In this trend, we showed that ROS production and callose deposition were incremented upon rAtHsp81.2 infiltration. Besides, a 24-h pre-treatment of leaves with rAtHsp81.2 resulted in a reduction in the proliferation of *Pseudomonas syringae* pv. *tomato* DC3000, whereas the *in vitro* growth of the bacteria is not affected in the presence of rAtHsp81.2, suggesting that this protein may participate in the induction of defense. In the present study, we attempted to gain a deeper insight into the role played by apoplastic-localized Hsp90s by evaluating their effects on defense gene expression. We observed that rAtHsp81.2 infiltration downregulated the expression of *PR1* and endogenous *AtHsp81.2*, while *Pdf1.2* expression showed no statistical differences compared to mock-inoculated controls. However, pre-treatment of leaves with rAtHsp81.2 followed by infiltration with *P. syringae* provokes an opposite response to that in leaves infiltrated with AtHsp81.2 alone, as *PR1* and *AtHsp81.2* gene expression resulted up-regulated. Our results suggest that Hsp90s localized in the plant apoplast play an active role in the activation of the plant defense mechanisms against pathogens.

PL-P43
SHADE AVOIDANCE SIGNALLING IS AFFECTED BY DROUGHT STRESS IN *ARABIDOPSIS THALIANA*

Semmoloni M, Costigliolo Rojas C, Casal JJ
IFEVA, UBA, CONICET, Facultad de Agronomía, Buenos Aires, Argentina. E-mail: msemmoloni@agro.uba.ar

Neighboring vegetation modifies the light environment by absorbing red and blue light whilst more efficiently transmitting and reflecting far-red light. These cues are perceived mainly by the photo-sensory sensors phytochrome B (phyB) and cryptochrome 1 (cry1). Reduced phyB and cry1 activities trigger a set of morphological changes, known as shade avoidance responses, which include the elongation of hypocotyls, stems, and petioles. The enhanced cellular growth that mediates these responses is mainly mediated by elevated signaling of the growth hormone auxin, induced by the transcription factors PHYTOCHROME INTERACTING FACTORs (PIFs), repressed by phyB and cry1 in the absence of shade. The integration of neighbor and other environmental cues on the control of plant growth is an emerging topic. Despite our profound knowledge of the mechanisms of perception and transduction of shade signals, whether shade avoidance is affected by water availability remains unclear. To investigate this issue, we exposed seedlings of tomato, peas, and *Arabidopsis thaliana* to the combination of light treatments simulating neighbor cues and different levels of water availability. In all three species, water restriction impaired the shade-avoidance response. Mutations at the *PIF3*, *PIF4*, *PIF5*, and/or *PIF7*, which reduce the magnitude of shade-avoidance responses, also impaired the growth response to water availability. Several pieces of evidence indicate that water restriction did not simply limit growth at the biophysical level, by affecting cellular turgor pressure required for expansion. Rather, it affected signaling events involved in the shade-avoidance response. First, a meta-analysis of published transcriptome data of *Arabidopsis* seedlings exposed to shade or drought treatments showed that the gene ontology term auxin transport was enriched among the genes induced by shade and repressed by drought. Since auxin-transport is required for shade avoidance, drought could affect this step of the response. Second, drought reduced the promotion of expression of shade-avoidance marker genes when both treatments were combined. Third, confocal microscopy of hypocotyl cells revealed that the promotion of PIF4 nuclear abundance by neighbor cues was reduced by drought. In these cells, which are responsible for the growth response, drought reduced auxin signal as revealed by an auxin sensor in combination with confocal microscopy. We propose that drought inhibits shade avoidance by modulating signaling downstream the photo-sensory receptors. These findings provide insight into the molecular mechanism of signal integration that contributes to adjust growth responses to the complex environmental conditions that plants experience in the field.

PL-P44
IMPACT OF UV-B RADIATION ON PRIMARY ROOT ELONGATION: EFFECT ON MERISTEM CELL PROLIFERATION AND CELL ELONGATION

Sheridan M L, Casati P
Centro de Estudios Fotosintéticos y Bioquímicos (CONICET-UNR). E-mail: sheridan@cefobi-conicet.gov.ar

Two common phenotypes of plants after UV-B exposure are the inhibition of leaf growth and primary root elongation. In proliferating leaves, the reduction in leaf area is in part a result of the inhibition of cell proliferation mediated by miR396, a microRNA that downregulates the expression of Growth Regulating Factors (GRFs) transcription factors (FTs). These FTs regulate numerous developmental processes acting redundantly. In leaf primordia, miR396 is expressed at low levels and increases during organ development, whereas GRFs are expressed in an opposite way. MicroRNAs (miRNAs) are short RNA molecules that control gene expression at the post-transcriptional level by targeting the cleavage of complementary mRNAs or by inhibiting their translation. In plants, miRNAs are produced from RNA polymerase II transcripts in a multi-step process. At least four proteins are involved in the processing of primary miRNA precursors (pri-miRNAs) in *Arabidopsis thaliana*: DCL1, HYL1, SE, and HEN1. DCL1 participates in the early stages of pri-miRNA maturation by consecutive trimming at the 5′ and 3′ ends leading to the so-

called pre-miRNAs. HYL1 protein forms a nuclear complex with DCL1 and is important for precise and efficient cleavage of at least several pri-miRNAs, SE is crucial for the accumulation of multiple miRNAs and trans-acting small interfering RNAs (ta-siRNA) and is found in nuclear bodies together with DCL1 and HYL1. Finally, the nuclear protein HEN1 specifically methylates miRNA:miRNA* and siRNA:siRNA* duplexes. In this work, we analyzed the participation of miR396 in particular, and the microRNA processing pathway in general, in the response of *A. thaliana* roots to UV-B exposure. For this, plants were irradiated with a single UV-B treatment during 1 h at an intensity of 2 W/m^2 5 days after stratification, and then they allowed continuing their growth in the absence of UV-B. The studies were carried out using WT lines, and using lines deficient in microRNA processing pathway (*hyl1-2*, *se-1*, and *hen1-8*), and also transgenic lines with and altered miR396 pathway (*MIM396* line, which expresses a target mimic that competes with GRFs for the binding of miR396 and rGRF3 a transgenic plant resistant to the regulation by miR396). The root growth rate was analyzed by monitoring primary root elongation, and by means of confocal microscopy, analyzing the number of meristematic dead cells after exposure, and also the length and number of cells in the meristematic and elongation zones. The results show that all lines show differences in at least one of the parameters analyzed, indicating that proteins involved in the biosynthetic pathways of miRNAs, and in particular of miR396, modulate the response of *Arabidopsis* roots to UV-B radiation, acting in the development of the meristematic zone.

PL-P45
SUPPLY OF NUTRIENTS FROM THE FOLIAR TISSUE TO THE SEED FILLING IN SOYBEAN GENOTYPES WITH DIFFERENT RESERVE COMPOSITION

Pavlovic T[1], Sáenz E[2], Poeta FB[1], Borrás L[2], Saigo M[1], Gerrard Wheeler MC[1]
[1] Centro de Estudios Fotosintéticos y Bioquímicos, [2]Instituto de Investigaciones en Ciencias Agrarias de Rosario
E-mail: pavlovic@cefobi-conicet.gov.ar

Protein and lipid content of soybean seed is a trait of great economic value. The ratio of these two components is variable and likely to be modified, so its study is essential for future biotechnological improvements. The accumulation of storage compounds depends on the supply of nutrients from autotrophic tissues and the internal seed metabolism. Maternal tissues provide mainly sucrose and amino acids produced during the senescence in the leaves. These precursors needed to build the storage compounds reach the seed through the phloem. Once in the seed coat, they enter the embryo through the apoplast, as there is no vascular connection between the coat and the embryo. The aim of this work is to study this maternal provision and its influence on the seed final composition. For this purpose, we analyzed the polar metabolites that reach the soybean embryo in experimental isogenic lines with contrasting contents of proteins and lipids. The experiments were carried out with plants at the onset and in the middle of seed maturation. The embryos were removed and replaced with melted agar. The polar metabolites including sugars, amino acids, and organic acids were extracted from agar and run in gas chromatography coupled to mass spectrometry (GC-MS), using ribitol as the internal standard. In the samples of the high lipid and low protein line, we could identify several amino acids (Ala, Ser, Thr, Asp, Gln, Asn, GABA, among others), organic acids (malate, fumarate, and citrate) and carbohydrates (fructose, glucose, sucrose, among others). In contrast, in the samples from the line with low lipid and high protein content, we were able to detect mostly carbohydrates. These results may be observed because proteins are deposited earlier than lipids in the seeds (see the poster of Poeta et al.), thus indicating that the carbon and nitrogen supplies are coordinated with the metabolic demands or regulate the metabolic program of the growing embryo.

PL-P46
CHARACTERIZATION OF THE FRATAXIN HOMOLOG FTX1 FROM THE GREEN UNICELLULAR ALGA *CHLAMYDOMONAS REINHARDTII*

Terenzi A, Busi MV, Gomez-Casati DF
Centro de Estudios Fotosintéticos y Bioquímicos, (CEFOBI–CONICET), UNR. E-mail: terenzi@cefobi-conicet.gov.ar

Frataxin plays a key role in cellular iron homeostasis of different organisms. It has been implicated in iron storage, detoxification, and delivery for iron sulfur-cluster assembly and heme biosynthesis. However, its role in iron metabolism remains unclear, especially in photosynthetic organisms. In recent years, the green unicellular alga *Chlamydomonas reinhardtii* has been used as an excellent experimental system for understanding iron metabolism. In this work, we explore the functions of the frataxin homolog (FTX1) from *C. reinhardtii*. To perform *in vitro* studies, FTX1 was cloned, expressed, and purified. The molecular mass and the oligomerization state were determined using native gel electrophoresis. Results showed that FTX1 forms tetramers in the presence of Fe, Zn, and Cu. Octamers were only produced in the presence of Cu. Furthermore, the addition of FTX1 to a mixture of Fe^{2+} and H_2O_2 resulted in significant attenuation of Fenton reaction. In addition to this, bacteria expressing FTX1 grew better in LB medium supplemented with a high concentration of metals (Cu, Cd, Fe, Zn, Ni, As) and also after incubation with 20 mM H_2O_2. Altogether, our results indicate that FTX1 is an essential protein in *C. reinhardtii*, involved in Fe homeostasis, protection against metal oxidative damage, and the maintenance of redox cellular state.

PL-P47
COLLETOTRICHUM ACUTATUM PRODUCES A COMPOUND THAT EXERTS OPPOSITE EFFECTS ON THE DEFENSE RESPONSE OF STRAWBERRY PLANTS DEPENDING ON THE PATHOGEN LIFESTYLE

Tomas-Grau RH, Díaz-Ricci JC
Instituto Superior de Investigaciones Biológicas (INSIBIO, CONICET-UNT). Facultad de Bioquímica Química y Farmacia, Universidad Nacional de Tucumán, Tucumán, Argentina. E-mail: rhtomasgrau@fbqf.unt.edu.ar

The fungal pathogen *Colletotrichum acutatum,* the causal agent of anthracnose disease causes important economic losses in strawberry farming worldwide. Crop fungal diseases are usually controlled with fungicides that despite the different origins and chemical nature, are contaminants of the environment and dangerous for human and animal health. The use of crude or barely purified microbe-derived extracts containing defense elicitors are interesting alternatives, as they can induce plant defenses with the advantage that they do not require highly demanding and expensive purification procedures. In this context, we have evaluated the effects on the defense response produced *in planta* of the supernatant obtained from a liquid culture of a virulent isolate (M11) of the hemibiotrophic fungus *C. acutatum* (M11-CF). Results obtained showed that M11-CF is capable to suppress the accumulation of the reactive oxygen species (ROS), and the defense response induced by the protein AsES against the hemibiotrophic fungus *C. acutatum*. Since M11-CF exhibits the capacity to suppress the oxidative burst, and taking into account that there is a direct correlation between the occurrence of the oxidative burst and the pathogenicity of necrotrophic fungi, we hypothesized that M11-CF may further confer protection against a necrotrophic pathogen such as *B. cinerea*. Results indicate that strawberry plants treated with M11-CF induces ethylene accumulation, and up-regulates ethylene-related genes (e.g., *FaETR1*, *FaERS1*, *FaERF1*) causing the induction of a defense response against *Botrytis cinerea*. The latter was evidenced by the decrease of the lesion diameter in M11-CF-treated plants as compared to control plants. In contrast, salicylic acid-related genes (e.g., *FaPR1*, *FaOGBG*, *FaCHI2-2*) did not show changes after the treatment. This outcome confirms the above-mentioned hypothesis about the capacity of M11, and its extracts, to exert opposite effects depending on the pathogen tested. That include, to activate a defense response against a necrotrophic pathogen by activating the ethylene pathway, and to suppress a defense response against a hemibiotrophic pathogen by the negative regulation exerted by ethylene on the salicylic acid pathway, and the suppression of the oxidative burst. The results presented here highlight the necessity to make an integral study of the microbiome present in soils and plant biosphere before applying any bioproduct that would activate the defense response to control crop diseases.

PL-P48
THE SECRETOME OF THE GREEN ALGA *SCENEDESMUS SP.*

Velázquez MB, Barchiesi J, Gomez-Casati DG, Busi, MV
Centro de Estudios Fotosintéticos y Bioquímicas, CEFOBI – CONICET, UNR, Rosario, Argentina. E-mail: busi.conicet@gmail.com

The generation of food wastes includes from agricultural scale, passing by industrial factories and up to trade and domestic uses. The principal component of wasted forest is lignocellulose, the most abundant natural biopolymer, and cheap renewable energy and carbon resources, which mainly is composed of cellulosic and lignin materials. The use of fermentable sugars produced from these materials by enzymatic treatments would add value to these wastes. Nevertheless, no practical process has still been reported for the enzymatic hydrolysis of cellulose. The main reason for this is the high cost of the required enzyme because free cellulase presents low specific activity, is susceptible to inactivation, and difficult to recycle. Cellulase is mostly studied from microbes, more commonly from fungal sources than bacterial. However, there are fewer reports of cellulase from algae. Obtaining these hydrolytic enzymes from algae would help to reduce CO_2 emissions and represents economic advantages due to algae are the organisms with the higher photosynthetic yield, and can be grown in closed containers (photobioreactors) or tanks in fields not suitable for agriculture by using waste products such as derivatives of wastewater treatment and flue gas as nutrients. At the same time, the determination of the optimal conditions for its higher photosynthetic efficiency would help to increase the productivity in microalgae, which could be used to increase the food and/or biofuels production. The relationship between photoprotective thermal dissipation and productivity is the key for a biorefinery to manage to compete with petroleum products and materials. Recently, we have detected the presence of extracellular cellulase activity in photoheterotrophic microalgae of freshwater. This work is based on the *Scenedesmus quadricauda* strain. It is a genus of green algae, freshwater, not mobile, which are usually grouped forming colonies of four cells. For the evaluation of the suitable conditions for cellulose secretion, we grew the algae at different temperatures (from 4 to 30 °C) and acidity (from pH 3 to pH 7) in agar plates containing TAP, TAP-MINIMUM and B3N medium in presence and absence of carboxymethyl cellulose (CMC) and with or without light. After five days the plate was revealed with congo red. The results demonstrate that cellulolytic activity can be inducible by substrates since activity is only observed in those media supplemented with CMC. The identification and molecular characterization of these cellulases are being carried out because they constitute biotechnological targets for the treatment of effluents, CO_2 capture, and production of biofuels.

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

SIGNAL TRANSDUCTION

ST-P01
THE EFFECT OF AMINO ACIDS ON THE UNFOLDED PROTEIN RESPONSE IN *SACCHAROMYCES CEREVISIAE*

Gulía JF[1], Muñoz SA[1], Birenbaum J[1], Bermúdez-Moretti M[1], Correa-García S[1]
[1]Laboratorio de Regulación Génica en Levaduras, Dpto de Química Biológica, FCEN, UBA. IQUIBICEN, CONICET.
E-mail: fgulias@qb.fcen.uba.ar

In eukaryotic cells, folding and modifications of membrane and secretory proteins take place in the endoplasmic reticulum (ER). When the protein folding capacity of the ER is exceeded or experimentally impaired, unfolded proteins accumulate in the ER and activate the unfolded protein response (UPR). Amino acids are the building blocks of proteins and their levels are sensed by at least two evolutionarily conserved and intersecting mechanisms: one involving Gcn2 kinase and the other involving the TORC1 kinase. Gcn4, the main target of Gcn2, is a key transcriptional activator of multiple amino acid biosynthetic genes, and Leu3 which acts mainly as a regulator of branched-chain amino acid metabolism is itself under the control of Gcn4. In *Saccharomyces cerevisiae* amino acids are also sensed by the sensor system SPS. The aim of this work was to study how an excess of amino acids affects the UPR pathway and also to establish the signaling pathways involved. UPR was measured by using a reporter gene assay in cells grown either in the absence or in the presence of all amino acids. It is expected that the addition of amino acids increases protein synthesis. We used wild type cells and cells deficient in the kinase Tor1, in the factors Gcn4 and Leu3, and in the essential SPS protein Ssy1. We observed that both DTT and tunicamycin, two ER stressors, induce UPR to different extents and that the presence of amino acids triggers UPR except in cells lacking Ssy1 and Leu3. We also found that Leu3 is necessary for UPR. When ammonium is used as a nitrogen source instead of proline, UPR activation is always observed. UPR response decreases in the presence of rapamycin, a TORC1 inhibitor, in cells grown in both nitrogen sources independently on the presence of amino acids and is nearly abolished in *tor1* cells. Tunicamycin has no effect on UPR in rapamycin-treated cells. We analyzed autophagy, a process that produces an increase in the intracellular amino acid pool. We observed that *tor1* and *leu3* cells present higher autophagy than wild type and that the addition of amino acids reduces the autophagic activity in all genotypes tested. In order to assess a link between UPR activation and chronological life span (CLS), we measured CLS using the colony-forming unit spot assay. Longevity was affected by growth conditions and genotypes assayed; however, we could not establish a direct correlation between UPR and CLS in all genotypes. Finally, we measured *GCN4* expression as a marker of protein synthesis and we found that it decreases in cells lacking Tor1 and wild type cells treated with rapamycin whereas increases in *leu3* cells. Altogether, these results allow us to conclude that the addition of amino acids or the use of a rich nitrogen source activates UPR and that TORC1 and Leu3 are required in this process. We demonstrate that TORC1 and Leu3 are also involved in autophagy, amino acid biosynthesis, and longevity, but in some of these processes, they act in an opposing manner.

ST-P02
NOVEL FUNCTION OF THE TRANSCRIPTION FACTOR UGA3 AS AN ACTIVATOR OF BRANCHED-CHAIN AMINO ACID PERMEASE *BAP2* GENE EXPRESSION

Muñoz SA[1], Gulias JF[1], Valencia Guillén J[1], Correa-García S[1], Bermúdez-Morett M[1]
[1]Departamento de Química Biológica, FCEN, UBA - IQUIBICEN, CONICET. CABA, Argentina. Email: munozsebastiananibal@gmail.com

Transcription regulation of most genes in yeast occurs at the level of activation, i.e. the basal level of expression is very low and increased transcription requires gene-specific transcription factors allowing the recruitment of basal transcriptional machinery. *Saccharomyces cerevisiae BAP2*gene encodes the permease responsible for the major part of leucine, valine and isoleucine uptake, amino acids that this yeast can use as nitrogen sources. Moreover, *BAP2* expression is known to be induced by the presence of amino acids such as leucine. However, *BAP2*regulation by nitrogen source quality has rendered controversial results and remains unclear. In this context, we analyzed the transcriptional regulation of *BAP2* in response to extracellular leucine in the presence of both a poor and a rich nitrogen source. Our results show that *BAP2* expression is inducible by leucine in the poor nitrogen source proline and that *BAP2* expression is constitutive in the rich nitrogen source ammonium, with high values unaltered by the addition of leucine. We also demonstrate here that an active SPS pathway is necessary for *BAP2* expression in both nitrogen conditions and in the presence or absence of the inducer leucine. Transcription factors Leu3, Gcn4, and Dal81are also involved in *BAP2* regulation in both a direct and an indirect way depending on the quality of the nitrogen source. We further demonstrate here that a physical interaction occurs between the transcription factor Uga3 and the regulatory region of the *BAP2* gene, which leads to a strong positive regulation. We thus conclude that the transcription factors involved in *BAP2* regulation affect its expression to different extents depending on the quality of the nitrogen source. We also demonstrate for the first time that Uga3, until now known as a transcriptional activator responsible for the induction of gamma-aminobutyric acid (GABA) genes, is one of the main positive regulators of *BAP2* transcription. So, we found that*BAP2*, a gene expressed under environmental conditions quite different from those of *UGA* expression, is also regulated by Dal81, Uga3, and Leu3 factors together with Gcn4 and Stp1/2.

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

ST-P03
RELATIONSHIP BETWEEN THE GAAC AND TORC1 PATHWAYS WITH THE EXPRESSION OF AMINO ACID PERMEASES ALONG WITH AGING IN BUDDING YEASTS

Birenbaum JM[1], Gulías JF[1], Muñoz SA[1], Bermúdez-Moretti M[1], Correa-García S[1]
[1]Departamento de Química Biológica, FCEN, UBA; IQUIBICEN, CONICET. CABA, Argentina. E-mail: joaquinbiren@gmail.com

Protein biosynthesis, lifespan and autophagy induction are processes where the transcription factor Gcn4 has been described as an important regulator in the budding yeast *Saccharomyces cerevisiae*. This factor is mainly induced when amino acid starvation occurs. Several Gcn4 target genes belong to the GAAC (General Amino Acid Control) or the TORC1 (Target Of Rapamycin) pathways. Important proteins that are downstream targets of these regulatory pathways are the amino acid permeases that internalize amino acids through the plasmatic membrane. Uga4 and Bap2 are two amino acid permeases induced by their substrates, γ-aminobutyric acid (GABA) and the branched amino acid leucine, respectively. The aim of this work was to analyze the transcriptional regulation of these permeases and *GCN4* in cells deficient in the GAAC (*gcn4Δ* and *leu3Δ* cells) and TORC1 (*tor1Δ*, *gln3Δ*, and *sch9Δ* cells) pathways, grown in the absence or in the presence of amino acids, during growth and along with aging. For this purpose, we used the lacZ reporter gene assays. We observed that in wild type cells *GCN4* expression decreases after cells reach the stationary growth phase; and when TORC1 is inhibited by rapamycin, *GCN4* expression slightly decreases and then remains constant along time. In *leu3Δ* cells, high levels of *GCN4* expression are detected at the exponential phase and they drastically diminish during growth, but when these cells are treated with rapamycin, *GCN4* remains high. The analysis of *GCN4* expression in cells deficient in *GLN3* and *SCH9*, two downstream targets of TORC1, showed that this expression varies in the opposite direction. These results suggest that *GCN4* expression along growth depends on the TORC1 pathway. We also found that the expression of Uga4 and Bap2 permeases is differentially regulated in wild type cells. Whereas *UGA4* expression increases along cells aged, even though it has been described that protein translation is generally reduced in aged yeast cells, *BAP2* remains low, and Gcn4 seems to be needed for *UGA4* full expression. The deficiency in genes of the TORC1 pathway does not affect *UGA4* expression, and only in *tor1Δ* cells, we observed an increase in *BAP2* expression. Further research must be done to understand how Gcn4, Uga4, Bap2 and other permeases such as *GAP1*, a general amino acid permease, are regulated during aging both at transcriptional and translational levels by the nutrient-sensing signaling pathways TORC1 and GAAC.

ST-P04
A REGULATORY AXIS CONNECTING PKCA AND ZEB1 MODULATES EPITHELIAL–MESENCHYMAL TRANSITION AND INVASIVENESS OF BREAST CANCER CELLS

Llorens MA [1], Rossi FA[2, 3], García IA[1], Cooke M[4], Rossi M[2, 3], Bocco JL[1], Kazanietz MG[4], Soria G[1]
[1]CIBICI-CONICET. [2] IBioBA-CONICET. [3] IIMT-CONICET. [4] Perelman School of Medicine, University of Pennsylvania, USA
E-mail: candellorens@gmail.com

The Epithelial–Mesenchymal Transition (EMT) is an essential program of embryogenesis and tumor progression, ZEB1 is a master regulator of the EMT. While extensive evidence confirmed the importance of ZEB1 as an EMT transcription factor that promotes tumor invasiveness and metastasis, little is known about its regulation. The aim of this work was to explore the signaling pathways that regulate ZEB1 levels and functionality, and how this regulation impacts on the dynamics of the EMT in cancer cells. We screened for potential regulatory links between ZEB1 and multiple cellular kinases. Our preliminary in silico studies revealed a plethora of potential phosphorylation sites for several kinases. Due to this level of complexity, we decided to follow up this analysis using ZEB1 deletion mutants (ZD1-HD and NZEB1), these constructs represent 60% and 10% of the full-length protein, respectively, and both retain the capacity to repress the E-cadherin promoter in cells, as determined with a luciferase reporter assay in cells. Intriguingly, we found that NZEB1 is enriched in PKC-specific sites and a substrate of p-PKC antibodies in cell extracts, thus suggesting an unforeseen regulatory role of PKC kinases on ZEB1 biology. Our initial experiments showed that NZEB1 and full-length ZEB1 (ZEB1-FL) levels were actively reduced when NMuMMG-NZEB1 or MDA-MB-231cells were treated with the pharmacological inhibitors of PKCs GF109203X and Gö69761. To study the penetrance of this phenotype with ZEB1-FL, we investigated the levels of three well-known PKCs paralogs (α, δ, and ε), ZEB1 and EMT makers in a group of 9 breast cancer cell lines. Strikingly, we found that PKCα and ZEB1 had a significant positive correlation, being both proteins overexpressed in cell lines with more aggressive phenotypes. Subsequent validation experiments using siRNAs against PKCα in MDA-MB231 cells revealed that its knockdown leads to a concomitant decrease in ZEB1 levels, while ZEB1 knockdown had no impact on PKCα levels. Remarkably, PKCα-mediated downregulation of ZEB1 recapitulates the inhibition of mesenchymal phenotypes, including inhibition in cell migration and invasiveness. These findings were extended to an *in vivo* model, by demonstrating that the stable knockdown of PKCα using lentiviral shRNAs markedly impaired the metastatic potential of MDA-MB-231 breast cancer cells. Conclusion: We demonstrated for the first time that the PKCα signal transduction pathway regulates the biological function of ZEB1, defining a novel regulatory axis of the EMT program in breast cancer cell lines, which might stimulate the evaluation of PKC inhibitors for metastatic breast cancer therapy

ST-P05
VDR AGONISTS TRIGGER WNT/B-CATENIN DOWNREGULATION IN A CELLULAR MODEL OF KAPOSI'S SARCOMA

Tapia C[1], De Genaro P[1], González Pardo V[1]
[1]INBIOSUR-CONICET, Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, AR. E-mail: biocin@live.com.ar

We have previously shown that 1α,25(OH)$_2$D$_3$ exerts antiproliferative effects in endothelial cells stably expressing Kaposi's Sarcoma-associated Herpesvirus G Protein-coupled Receptor (vGPCR) through NF-κB pathway inhibition and apoptosis induction. β-catenin, a multifunctional protein,

is required for cell–cell adhesion and gene expression regulation in response to Wnt. Aberrant activation of this pathway provokes β-catenin accumulation in the nucleus and induces cell proliferation. Since it is well documented that vGPCR activates the canonical Wnt/β-catenin signaling pathway, we investigated if β-catenin and its target genes are regulated by $1\alpha,25(OH)_2D_3$. Firstly, Western blot studies showed an increase in β-catenin protein levels, which were not affected by the presence of Actinomycin D, a transcription blocker, after $1\alpha,25(OH)_2D_3$ (10 nM, 24 h) treatment. Secondly, β-catenin localization, investigated by immunofluorescence and subcellular fractioning techniques, was increased in the nucleus and plasma membrane. This event was accompanied by an increase in VE-cadherin in the cell membrane. Furthermore, a β-catenin/VDR interaction was observed by co-immunoprecipitation, which correlated with a decrease in the expression levels of β-catenin target genes *c-myc*, *cyclin D1*, and *MMP-9* mRNA after $1\alpha,25(OH)_2D_3$ (10 nM, 48 h). Finally, Dkk-1, the extracellular inhibitor of the Wnt/β-catenin pathway, elicited an initial upregulation of mRNA expression accompanied by lower β-catenin protein levels (0.5–1 h). Altogether, β-catenin/VDR interaction may account for non-transcriptional accumulation of β-catenin protein levels and downregulation of its target genes in response to $1\alpha,25(OH)_2D_3$.

ST-P06
DIFFERENT SUGARS EXERT NON-OVERLAPPING EFFECTS ON THE GROWTH REGULATION OF *ARABIDOPSIS THALIANA*

Pereyra C[1], Rodriguez M[2-3], Martinez-Noël G[1]
[1]INBIOTEC, Vieytes 3103, 7600 Mar del Plata, Argentina. [2]IFRGV-CIAP-INTA, [3]UDEA- CONICET, Camino 60 cuadras km 5.5 X5020ICA, Córdoba, Argentina. E-mail: gnoel@inbiotec-conicet.gob.ar

Sugars are not only energy sources, but also important molecular signals that regulate growth, development, and response to environmental stresses. In plants, sucrose (Suc) is the main sugar for systemic transport, and a specific signaling pathway for this disaccharide has been described. Although, the underpin sensor and mechanism are still unknown. Besides, it was proposed than Suc could be an antioxidant metabolite, especially when it is present at high concentrations, acting as ROS scavenger. On the other hand, glucose (Glc) is sensed through both dependent and independent mechanisms of hexokinase. To gain insights into these subjects, we studied the effects of different sugars on the activation of root meristems (RAM) and redox homeostasis in *Arabidopsis thaliana*. The results showed that exogenous Suc and Glc differently induced the root growth, and fructose in a minor extent, depending on their concentrations. In contrast, trehalose (Tre) was not able to affect the root length. Besides, the endogenous increment of Tre/Tre-P levels using a trehalase inhibitor, validamycin-A, did not induce the quiescent meristem. To test the putative protective role of sugars, we analyzed the effect of the different sugars on the inhibition of RAM produced by oxidative stress. The data indicated that only the addition of Suc could override the negative effect of methyl-viologen on root growth. Moreover, we measured the activity of Target Of Rapamycin (TOR), a master integrator of external and internal signals that regulates growth and development in eukaryotes. Results revealed differences in the kinase activity in seedlings grown with distinct sugars. In summary, we propose that sugars regulate growth differently, being their nature and concentration crucial for the effect, through a diverse action on ROS homeostasis. *Supported by CONICET, ANPCyT (PICT2014-551, PICT2016-0173), UNMdP (EXA841/17, EXA947/19) and FIBA.*

ST-P07
REGULATION OF SEX TYPE IDENTITY IN *SACCHAROMYCES CEREVISIAE*

Beldorati-Stark G[1], Grande A[1], Colman-Lerner A[1]
[1]IFIBYNE (UBA-CONICET). E-mail: gerbeldo@gmail.com

Sex type determination in the yeast *Saccharomyces cerevisiae* depends on the combinatorial expression of different transcription factors, encoded by two alleles of the *MAT locus*. Among the genes that are expressed differentially, there are those that code for the pheromones needed for mating and their specific receptors. Previous high throughput studies suggested that other transcription factors, induced during the mating response, could be important for the maintenance of sex type identity since their deletion resulted in the induction of opposite-sex specific genes upon stimulation with pheromone. To test this hypothesis, we deleted these transcription factors in a *MAT*a strain that also contained a fluorescent reporter for two *MATα* specific genes and determined reporter abundance by quantitative fluorescence microscopy. Upon *α*-factor pheromone stimulation, some of these strains showed strong reporter induction compared to wild type, in which there was none. In addition, we compared the dose-response and the temporal dynamics of expression of the *MATα* specific genes with those of *PRM1* (a heavily studied pheromone response gene, regulated directly by Ste12). We found that the dose-response curves were identical, which suggested that the induction mechanism is shared. However, the induction of the *MATα* specific reporter genes was delayed with respect to *PRM1*, which is indicative of the existence of an additional step in the activation pathway. We hypothesized that the physical interaction between Ste12 and these transcription factors prevents Ste12 from ectopically activating *MATα* specific genes by interfering with its binding to their promoter regions. To test this hypothesis, we overexpressed Ste12 in wild type strains and observed strong reporter induction. Likewise, when expressing mutant transcription factors, which cannot bind to Ste12, the *MATα* specific reporters were induced as well. These findings, taken together, suggest that specificity of pheromone-induced gene expression via Ste12 is finely tuned by the cell through the expression of helper transcription factors that form Ste12 heterodimers with altered specificity. In this case, the mechanism enforces correct sex type identity during the mating response.

ST-P08
shRNA-MEDIATED KNOCKDOWN OF 14-3-3γ REVEALS ITS ESSENTIAL ROLE IN REGULATING ADIPOGENIC DIFFERENTIATION OF UCMSCs

Rivera L[1], Santoni DN[3], Bustos DM[1,2], Uhart M[1]
IHEM[1] (CONICET-UNCuyo). FCEN[2], UNCuyo, Argentina. Clínica Santa Clara[3], Mendoza, Argentina. E-mail: lautarivera08@gmail.com

Human umbilical cord-derived mesenchymal stem cells (UCMSCs) are self-renewing multipotent progenitors that can differentiate into cells of the mesoderm-lineage, which makes them attractive targets for regenerative medicine. However, the lack of understanding of the molecular mechanisms that regulate cell differentiation is currently an obstacle to such applications. In this sense, 14-3-3 proteins have received tremendous attention since these phospho-serine/threonine binding proteins have a pivotal role in the regulation of metabolism and signal transduction networks. A recently published work in our lab has shown that both at the mRNA and protein level, 14-3-3 β and γ were the isoforms that most changed after adipogenic differentiation of 3T3-L1, a well-established murine pre-adipocyte cell line. Since we have developed a method for UCMSCs isolation and also obtained the efficient adenoviral transduction of these cells, the aim of this study was to analyze the effect of decreased expression of 14-3-3γ by using shRNA on the adipogenic potential of UCMSCs. The recombinant adenoviruses (Adv) were E1/E3-deleted type 5 Advs expressing shRNA for 14-3-3γ, under the control of the small nuclear RNA (snRNA) U6 promoter. The Pac I-digested vector was used to transfect 293A cells to produce Adv-shRNA 14-3-3γ stock. Then, the Advs were amplified by infecting the 293A cells with the crude viral lysate. UCMSCs were isolated and expanded from Wharton Jelly of the human umbilical cord, using a culture explant method. For transduction experiments, cells at 80–90% confluence were incubated with Adv-shRNA 14-3-3γ for 2 h at 37ºC, and then the transduction media was replaced with standard growth media (high glucose DMEM; 10% FBS). To investigate the effects of 14-3-3γ in adipogenesis, we induced UCMSCs (transduced or not- with Adv-shRNA 14-3-3γ) with adipogenic differentiation medium (ADM; an optimized drug cocktail that includes high glucose DMEM, 10% FBS, Dexamethasone, Insulin, Rosiglitazone, and IBMX) for 10 days. We also used untreated and Adv-GFP transduced cells as controls. Lipid droplets accumulation was examined using Oil Red O staining. UCMSCs transduced with Adv-shRNA 14-3-3γ exhibited an increased lipid droplets accumulation, compared to control cells. These data suggest an essential role for 14-3-3γ in the process of adipogenesis in UCMSCs. Our finding proposes the existence of a previously unknown regulatory mechanism of UCMSCs adipogenic differentiation, and that 14-3-3γ could be an interesting candidate to be evaluated as a therapeutic target molecule to treat chronic diseases, such as obesity and type 2 diabetes.

ST-P09
PKA AND HOG1 ROLE IN GENE EXPRESSION AND CELL SURVIVAL IN RESPONSE TO OSMOSTRESS IN *SACCHAROMYCES CEREVISIAE*

Ojeda L[1], Dunayevich P[2], Rossi S[1], Colman-Lerner A[2], Portela P[1]
[1]Departamento de Química Biológica FCEN UBA-IQUIBICEN, [2]IFIBYNE-CONICET, UBA. E-mail: lucaseoj@gmail.com

The stress-adaptation response involves several signaling pathways that combine stimuli to coordinate responses ensuring cellular homeostasis. We focus on crosstalk between HOG1-MAPK and cAMP-PKA pathways in osmostress cellular responses. We compared the growth rate, glucose consumption rate and osmotic stress survival of the WT, *hog1Δ, tpk2*Δ and *tpk1*Δ, *hog1*Δ*tpk2*Δ and *hog1*Δ*tpk1*Δ strains. The *hog1*Δ strain shows growth defects, reduced efficiency of glucose metabolism, and cell viability under osmotic stress. The *hog1*Δ*tpk2*Δ double mutant shows higher duplication time than *hog1*Δ and *hog1*Δ*tpk1*Δ strains and similar to WT cells. However, under osmotic stress, the *hog1*Δ*tpk2*Δ double mutant strain shows a maximum growth level similar to *hog1*Δ and *hog1*Δ*tpk1*Δ that was about 50% less than the WT strain. The *hog1*Δ*tpk2*Δ double mutant improved the glucose consumption rate and cell viability compared to the *hog1*Δ strain. These results indicate that *TPK2* deletion improves the stress tolerance of *hog1*Δ mutant strain. An increase in external osmolarity causes loss of turgor pressure and cell volume due to water efflux, which triggers a homeostatic response activated by the HOG pathway. Consequently, there are glycerol accumulation and cell volume recovery during osmostress-adaptation. HOG pathway activation requires transient phosphorylation of the activation loop and the subsequent nuclear accumulation of Hog1. In the nucleus, Hog1 controls the expression of several stress-response genes. *TPK2* deletion did not affect the levels of Hog1 activation loop phosphorylation, neither Hog1 nuclear accumulation, nor HOG transcriptional reporter induction, nor cell volume recovery in response to osmotic stress. These results indicate that osmostress activation of HOG-MAPKwas not affected by the Tpk2 catalytic subunit. The *in vivo* kinetic recruitment of Hog1 to the *STL1* (an osmostress-activated gene) promoter was slightly affected in strains lacking Tpk2. Then, we analyzed the Hog1 contribution on gene expression of two osmostress genes, *HSP42* and *RPS29B*, that were previously described as targets of PKA binding. Analysis of the transcriptional time curse was performed in WT, *hog1Δ*, *tpk2Δ*, *tpk2Δhog1Δ* strains. Our results suggest that the adaptive response to transient stress could be regulated by the opposite roles of Hog1 and PKA. Tpk2 recruitment on *HSP42* coding region and *RPS29B* promoter was also assessed in response to osmotic stress. Tpk2 binding was affected in cells lacking Hog1, suggesting crosstalk between the chromatin recruitment of Tpk2 and Hog1. Altogether, our results suggest that both Tpk2 and Hog1 are key regulators in osmostress adaptation that could act in parallel in order to perform opposite roles in this stress response.

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

ST-P10
TPK1 PROMOTER REGULATION BY INOSITOL PHOSPHATES DURING HEAT STRESS IN *SACCHAROMYCES CEREVISIAE*

Ortolá Martínez MC, Reca S, Galello F, Rossi S
[1]Dpto. Qca. Biológica, FCEyN, UBA; IQUIBICEN-CONICET. E-mail: marita.ortola@hotmail.com

In *Saccharomyces cerevisiae,* Protein Kinase A (PKA) pathway controls several functions and biological processes in response to different stimuli. PKA from *S. cerevisiae* is composed of two catalytic subunits encoded by *TPK1*, *TPK2* and *TPK3* genes, and two regulatory subunits, encoded by *BCY1* gene. The specificity of the PKA pathway depends on several factors as substrates specificity and the PKA localization by interaction with anchoring proteins (AKAPs). However, transcriptional regulation and expression levels of PKA subunits are also events involved in maintaining specificity. In response to environmental variations, cells must respond through changes that occur at transcriptional and post-transcriptional levels in order to adapt to the new conditions. We have previously demonstrated that a negative mechanism of autoregulation directs *TPKs* and *BCY1* gene expression and that only *TPK1* promoter activity is positively regulated during heat stress. Results from our group showed that the *TPK1* promoter presents three positioned nucleosomes that upon heat stress they are no longer detectable, according to *TPK1* promoter activation. On the other hand, it has been described that inositol phosphates can modulate the activities of several chromatin remodeling complexes. In order to identify additional factors involved in PKA subunits transcriptional regulation, we analyzed *TPK1* promoter activity, mRNA levels and nucleosomes positioning in null mutant strains for the components of the inositol phosphates pathway (*Δplc1*, *Δipk1*, *Δipk2*, *Δkcs1*) in heat stress. Our results showed that *TPK1* is not upregulated and there was not chromatin remodeling during heat shock in null mutant strains for the components of the inositol phosphates pathway. It has also been demonstrated that Plc1p regulates the recruitment of the SAGA complex to stress-responsive genes promoters in order to facilitate transcriptional initiation. Then, we decided to evaluate the relationship between the inositol phosphates pathway and chromatin remodelers on the expression regulation of *TPK1*. Using β-galactosidase reporter assays and RT-qPCR in null mutant strains for subunits of the SAGA complex, we evaluated *TPK1* promoter activity and mRNA level. We demonstrated that *TPK1* promoter activity and mRNA levels decreased in *Δgcn5*, *Δada2*, and *Δspt20* strains during heat shock. We evaluated the recruitment of the SAGA complex to the *TPK1* promoter and the dependence on Plc1p using ChIP assays. Finally, other authors have demonstrated that the inositol phosphates pathway modulates the expression of several stress-responsive genes through the activation of the HDAC Rpd3L. Thus, we evaluated the participation of HDAC Rpd3L in *TPK1* and *BCY1* transcriptional regulation during heat stress. Taken together, these results strongly suggest that the inositol phosphate pathway is involved in the regulation of *TPK1* gene expression during heat stress.

ST-P11
UNDERSTANDING PROTEIN KINASE PDK1 REGULATION USING SMALL MOLECULES TARGETING DIFFERENT SITES

Sacerdoti M[1], Froese K[2], Schulze J[2], Pastor-Flores D[2], Bauer A[2], Herbrand A[2], Riley A[3], Czier G, Potter B[3], Suess E[2], Gross LZF [1], Leroux AE[1], Biondi RM[1,2]
[1] Instituto de Investigación en Biomedicina de Buenos Aires – CONICET – Instituto Partner de la Sociedad Max Planck; [2]Department of Internal Medicine I, Universitätsklinikum Frankfurt, Germany; [3]University of Oxford, United Kingdom. E-mail: msacerdoti@ibioba-mpsp-conicet.gov.ar

Overactivation of the phosphoinositide 3-kinase (PI3K) pathway is one of the most frequent events in cancer, a disease that is becoming increasingly frequent because of the increase in life expectancy. A key PI3K downstream event is the phosphorylation of PKB/Akt, S6K, SGK, and RSK by master kinase phosphoinositide-dependent protein kinase-1 (PDK1), which also phosphorylates other protein kinases constitutively. Throughout the years, we have investigated different mechanisms used by PDK1 to specifically and timely phosphorylate its substrates. Phosphorylation of most substrates, like S6K, SGK, PKC, PRK/PKN, relies on a docking interaction where a C-terminal hydrophobic motif (HM) interacts with a regulatory site, PIF-pocket, located on the small lobe of the kinase domain of PDK1. Since some substrates interact better with PDK1 when their HM is phosphorylated, this acts as a regulated docking interaction. In addition, the interaction with the PIF-pocket allosterically "activates" PDK1 itself, stabilizing a closed-active structure of the catalytic domain. We have described in the past that the binding of the HM or small compounds to the PIF-pocket *allosterically* affects the ATP-binding site. Allostery implies that the reverse modulation, i.e. from the ATP-binding site to the regulatory PIF-pocket, should also be possible. Indeed, we have shown that small molecules and metabolites binding at the ATP-binding site can inhibit or enhance the docking interaction at the PIF-pocket. This is important for the mechanism of action of drugs. Interestingly, the interaction with the PIF-pocket of PDK1 is not a requirement for the phosphorylation of PKB/Akt after PI3K activation. Although the HM of PKB/Akt does interact with the PIF-pocket of PDK1, we believe that other mechanisms must regulate that interaction. PDK1 has been described to dimerize. We will here provide preliminary data and discuss if dimerization could also be part of the mechanism by which PDK1 phosphorylates its substrates. We describe the effect of different inositol poliphosphorylated molecules and present results of a screening performed in order to find small compounds to regulate dimer formation.

ST-P12
USE OF FLUORESCENT REPORTERS FOR THE ANALYSIS OF AKT AND UPR ACTIVATION DYNAMICS IN HUMAN SINGLE CELLS

Sánchez G[1,2], Suaya M[1,2]*, Cotarelo M[1,2], Amoroso A[3], Colman-Lerner A[1,2], Blaustein M[1,2]*
**These authors contributed equally to this work.*
[1]Departamento de Fisiología, Biología Molecular y Celular, FCEN, UBA, Buenos Aires, Argentina, [2]IFIBYNE, CONICET-UBA, Buenos Aires, Argentina, [3] Facultad Ciencia, Universidad San Sebastián, Santiago, Chile. E-mail: marusuaya@gmail.com

In order to process the information from the extra- and intracellular milieu, cells have evolved a multiplicity of signaling systems. At least 15% of the vertebrate's genome protein-coding capability is linked to receptors, components of signaling systems, and transcription regulatory proteins. Our general goal is to study the regulation of cell signaling systems and the key mechanisms of tumor cell decision-making using a mixed approach that combines quantitative fluorescence techniques in individual cells with classical techniques of molecular biology. Particularly, we study the PI3K/Akt pathway—or cell survival pathway—and the Unfolded Protein Response (UPR) pathways. Akt, a serine/threonine protein kinase member of the AGC family, plays a central role in growth, proliferation, glucose uptake, metabolism, angiogenesis, protein translation, and cell survival. Not surprisingly, a variety of human cancers exhibit deregulated Akt activity, and several mouse models with activated Akt develop cancer. The UPR is a cellular stress signaling cascade essentially triggered by the accumulation of misfolded proteins in the Endoplasmic Reticulum (ER). Three mechanistically distinct pathways (IRE1, PERK, and ATF6) make up this collective response aimed at restoring homeostasis. Tumor cells, however, evade this outcome and exploit the UPR pathways for proliferation and metastasis. Crosstalk between Akt and UPR pathways has been described. Particularly, we have previously shown that Akt is a PERK kinase, influencing UPR activation. Here, we designed and characterized a set of fluorescent reporters for each of these pathways and tested them in cells treated with traditional activators or inhibitors. In the case of UPR, we co-transfected these reporters into different tumor cell lines and were able to follow the activation of all three UPR pathways at the same time in every single cell for the first time. Specifically, we analyzed the pattern of activation of these pathways by automated segmentation of single cells and quantitative measurement of subcellular fluorescence using Cell Profiler. We validated our results by comparing reporters' behavior with the activation of endogenous counterparts, confirming that our reporters allow us to study these pathways in an accurate and efficient way. Finally, we used our fluorescent reporters for Akt and UPR pathways in order to address whether environmental pollutants linked to cancer onset regulate these pathways.

ST-P13
ANALYSIS OF AKT MOLECULAR, SUBCELLULAR AND TUMORAL CODE AS AN EXPLANATORY AND PREDICTIVE TOOL FOR THE EFFECTIVENESS OF THERAPIES AGAINST BREAST CANCER

Vila A[1,2], Amante A[1,2], Colman-Lerner A[1,2], Blaustein M[1,2]
[1]Departamento de Fisiología, Biología Molecular y Celular, FCEN- UBA, Buenos Aires, Argentina, [2] IFIBYNE, CONICET-UBA, Buenos Aires, Argentina. E-mail: antonella.s.vila@gmail.com

Cancer is a highly heterogeneous disease and even within each tumor, there is significant cell-to-cell variability. This heterogeneity has been traditionally considered as 'noise'. However, understanding the sources of cell-to-cell variability might help to improve existing therapies and design new ones. Akt kinase is an attractive therapeutic target for cancer treatment and it is known to be regulated through numerous post-translational modifications as well as to be recruited to different subcellular compartments to fulfill its functions. Little is known about how a cell determines—for a given stimulus or under pathological conditions—which substrates and which functions Akt should regulate. Our hypothesis is that the Akt molecular code, i.e., the profile of post-translational modifications of Akt, can determine Akt subcellular localization and vice versa, thus establishing the subset of Akt substrates and the set of functions that Akt displays after each stimulus and in each cellular context. The general objective of our research is to determine the subcellular compartments to which Akt and its substrates are recruited, the phosphorylation patterns of Akt and Akt substrates in different mammary cell lines, both normal and tumor, and to analyze if a correlation between these variables and the resistance/sensitivity of these cell lines to different antitumor drugs can be established. Our long-term goal is to determine which modification and localization patterns of Akt and its substrates are useful for predicting the evolution of mammary tumors. Here, using a strategy that combines automated imaging and quantitative measurement of Akt localization, we discovered novel Akt modifications and subcellular localizations. Our preliminary results show that phosphorylation and localization patterns of Akt and its substrates differ between different normal and tumor mammary cell lines. Finally, we performed a bioinformatic analysis using the DisGeNET platform to associate subcellular locations of Akt and its substrates with different pathologies, particularly cancer. Our data shed light on the Akt molecular code, improving our understanding of complex cell and tumor behaviors.

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

WORSHOP DRUG DISCOVERY

WS-P01
DESIGN OF *1,2,3*-TRIAZOLES AS INHIBITORS OF CRUZIPAIN (CZP): TARGET VALIDATION, *IN SILICO* MODELING AND *vHTS* SCREENING

Cerutti JP[1], Ribone SR[2], Opsomer T[2], Dehaen W[2], Quevedo MA[1]
[1]UNITEFA-CONICET, Dpto. Farmacia, Fac. Ciencias Químicas, Universidad Nacional de Córdoba, Argentina, [2]Molecular Design and Synthesis Laboratory, Department of Chemistry, KU Leuven, Celestijnenlaan 200F, 3001, Leuven, Belgium. E-mail: alfredoq@fcq.unc.edu.ar

Chagas disease constitutes a relevant public health issue throughout the world. Unfortunately, limited pharmacological options are available for its treatment, with only two drugs currently approved (i.e., benznidazole (BZN) and nifurtimox (NFX)). Both BZN and NFX, exhibit important side effects, thus the development of new antichagasic agents constitutes an urgent need. Different druggable targets have been identified for the development of therapeutically useful antichagasic compounds. Among them, cruzipain (CZP) has been actively studied, with its modulation being possible by both reversible and irreversible inhibitors. The latter ones have gained significant interest due to their potential of eliciting high inhibitory potencies. Structurally, CZP belongs to cysteine proteases family, bearing a catalytically active cysteine (CYS25) residue required for its proteolytic activity. Also, at least three subdomains (S1-S3) have been identified within its catalytic site. The rational design of CZP inhibitors is focused towards the obtaining of molecules able to bind to this catalytic site with high affinity and selectivity. Derivatives of *1,2,3*-triazoles constitute very promising compounds for the design of CZP irreversible inhibitors due to its bioisosteric relationship with the peptidic bond. Noteworthy, the chemical space accessible for the preparation of *1,2,3*-triazole derivatives is huge, representing both an opportunity for the design of bioactive molecules, but also signifying a challenge regarding the requirements for the screening of candidates. Thus, the development and validation of an *in-silico* screening platform constitute an important requirement. In this work, we present an *in silico* workflow designed for the preparation and validation of a CZP receptor model based on available crystallographic structures, followed by automated procedures for ligand modeling, molecular docking, molecular dynamics and free-energy of interaction analyses, with the aim of providing a platform able to describe the recognition and binding mode of *1,2,3*-triazole derivatives to CZP. A set of 20 previously reported *1,2,3*-triazole derived irreversible inhibitors (IC_{50}: 5-23000 nM) was used as a training set, with the corresponding structure-activity relationships being established. The workflow led to the identification of key pharmacophoric contacts driving inhibitors binding, elucidating also the structural basis for the observed ligand enantioselectivity. The methodology was afterward extended to the *vHTS* of a library containing above 100K *1,2,3*-triazoles derivatives, which was virtually constructed based on commercially available reagents and feasible synthetic procedures. Overall, the presented methodology constitutes a valuable *vHTS* platform for the automated screening of large *1,2,3*-triazoles databases, constituting a valuable structure-guided aid to our ongoing synthetic efforts towards the obtention of new antichagasic agents.

WS-P02
SCREENING OF ANTIGEN CROSS-PRESENTATION POTENTIATING DRUGS FOR VACCINE DEVELOPMENT

Crespo MI, Guantay ML, Pascual MM, Harman MF, Dho N, Pistoresi MC, Maletto B, Soria G, Morón G
CIBICI-CONICET. Departamento de Bioquímica Clínica - Facultad de Ciencias Químicas – UNC. Córdoba, Argentina
E-mail: micrespo@fcq.unc.edu.ar

The cellular immune response mediated by the induction of cytotoxic $CD8^+$ T lymphocytes (CTLs) is crucial for therapeutic interventions in tumor immunotherapy and for the induction of protective immunity against intracellular pathogens. Dendritic cells (DCs) are the only antigen (Ag) presenting cells of the immune system with the ability to activate *naïve* $CD8^+$ T cells to generate CTLs, and are particularly fitted to internalize and present exogenous Ag (from infected, tumor, or dead cells) bound to MHC I molecules to *naïve* $CD8^+$ T cells. This process, known as cross-presentation, is essential for antimicrobial and antitumor immunity. Non-living vaccine Ag, especially in subunit vaccines, is often poorly immunogenic and adjuvants are required to boost immunity. Adjuvants enhance the immunogenicity of vaccines and experimental Ag by a variety of mechanisms. One of the current major challenges is to develop adjuvants that help in the generation of protective CTLs responses to soluble proteins. So far, many adjuvants activate DCs and other Ag presenting cells to provide proper co-stimulatory signals to T cells. We have found that some adjuvant compounds can also activate Ag cross-presentation, increasing T cells activation levels. The complexity of Ag cross-presentation pathways challenges the identification of therapeutic targets in DCs to stimulate Ag cross-presentation. In this work, we performed an *in vitro* screening using 1760 drugs approved by international agencies such as the FDA to identify chemical entities and molecular pathways capable of enhancing Ag cross-presentation in DCs. To achieve this goal, we developed a high-performance screening method by adapting the colorimetric B3Z presentation assay using the JAWSII DC cell line and OVA as a soluble Ag model. B3Z is a $CD8^+$ T cell hybridoma specific for the $OVA_{257–264}$ epitope in the context of H-$2K^b$ (MHC I) which is activated by the detection of $OVA_{257-264}$ peptide associated with MHC I on the DC surface. The primary screening revealed an increase in Ag cross-presentation with approximately 1% of the assayed drugs. Among the active drugs, most of the hits had antiallergic, antimalarial, antiemetic, or antipsychotic effects. Although these hits are currently under validation, it is interesting to highlight that several active compounds have biological activities that are compatible with a potential modulatory capacity of Ag cross-presentation (i.e., we found a chloroquine derivative and it has been previously reported that chloroquine favors soluble Ag cross-presentation, presumably by the delay of endosomal maturation). In conclusion, we established a sensitive, fast, and robust screening platform for the search of compounds capable of stimulating Ag cross-presentation in DCs. The hits that pass the dose-response, structural, and functional validation will be evaluated as adjuvants for both preventive and therapeutic vaccination strategies that require a CTL response.

WS-P03
BRCA-DEFICIENT CELLS CAN BE KILLED WITHOUT TRIGGERING DNA REPLICATION STRESS

Paviolo N[1], Pansa M[2], Siri S[1], Martino J[1], García M[2], Nicotra V[2], Garro C[2], Guantay L[2], Mülbaier M [3], Drewes G[3], Madauss K[3], Gloger I[3], Gottifredi V[1], Soria G[2]
[1]FIL/IIBBA, [2]CIBICI-UNC, [3]GSK. E-mail: npaviolo@leloir.org.ar

The identification of compounds with selective tumor cytotoxicity can improve anticancer therapies. A promising strategy that takes advantage of the frequent impairment of DNA repair pathways in tumor cells is synthetic lethality (SL). For example, loss of the Homologous Recombination Repair (HRR) capacity is frequent in breast and ovarian cancer, and the accumulation of HRR substrates should selectively kill such cancer cells. Such a hypothesis has been validated in BRCA1- and BRCA2-deficient cell lines treated with different poly (ADP-ribose) polymerase inhibitors (PARPi). Several pharmaceutical companies evaluated PARPi in more than 400 clinical trials, and to date, the FDA has approved 4 PARPi. However, evidence of acquired resistance to PARPi has prompted the search of other synthetic lethal interactions in HRR-deficient cells. To this end, a cell-based phenotypic screening of several thousands of compounds was carried out using a high-throughput method recently described by us. The compounds were from different sources, including a library kinase inhibitors (PKIS2), natural products and extracts from plant species from Argentina, and a library of 13K natural products and natural products-like compounds from GSK. After the screening, 4 hits were validated in different BRCA-deficient cell lines. The two first hits, Polo-like kinase-PLK1 (hit 1) and Rho-associated protein kinases ROCK1/2 (hit 2), were validated using commercially available inhibitors for these two kinases. The other two hits were identified from the other compound sources and will be referred to as hit 3 and hit 4. Intriguingly, the mechanisms of cell killing associated with the PARPi differs mainly from the mechanisms of cell killing triggered by the 4 hits identified in our screening. PARPi-induced cell death in BRCA1- and BRCA2-deficient cells is accompanied by replication stress (revealed by replication stress markers such as γH2AX and 53BP1 nuclear foci) and acute chromosome instability (CIN) revealed by micronuclei accumulation and chromosomic aberrations analysis. In contrast, cell death triggered by the hits 1–4 was not associated with acute replication stress or CIN. Another difference was observed when evaluating the genetic backgrounds affected by the hits. In agreement with the literature, PARPi induce SL in both BRCA1- and BRCA-deficient cells. In contrast, hits 1–4 were, in most cases, not effective in both BRCA-deficient backgrounds, but showed SL either on BRCA1- or BRCA2-deficient cells. We conclude that novel types of synthetic lethal interactions may be achieved without increasing CIN, which *in vivo* might prevent the acquisition of resistance mechanisms. On the other hand, our data also surprisingly reveal that many novel SL interactions may not broadly apply to all type of HR deficiencies.

WS-P04
DRUG REPURPOSING SCREENING STRATEGY TO IDENTIFY MODULATORS OF MUTANT P53 LEVELS IN CANCER CELLS

Llorens MC[1], Borini EC [2,3], Garcia IA[1], Girardini J [2,3], Soria G[1].
[1] CIBICI-CONICET. Dpto de Bioquímica Clínica, FCQ-UNC. [2]IFISE-CONICET, Rosario. [3] IDICER-CONICET, Rosario.
E-mail: candellorens@gmail.com

Mutation of the *TP53* gene is the most frequent genetic alteration found in human cancers. Tumor-associated p53 missense mutants act as drivers of cancer progression, leading to gain-of-function (GOF) phenotypes that promote metastatic potential and drug resistance. Given that mutant p53 protein stabilization is a prerequisite for GOF, the aim of this work was to develop a high-throughput screening assay to identify drugs and molecular pathways that induce destabilization of mutant p53. We set up an In-Cell Western (ICW) assay using MDA-MB-231 cells and performed a primary screening with a collection of 1760 FDA-approved drugs. Our preliminary results let us identify 34 drugs that induced mutant p53 destabilization, most of them belonging to four major functional groups (1) nucleotide synthesis mediators, (2) agonists and antagonists of steroid hormones, (3) antidepressants and antipsychotics, and (4) beta-adrenergic agonists. In this work, we will show the design of the screening strategy, the screening results, and the early validation of the most robust hits. Our project is focused on the preclinical study of cancer drugs that destabilize mutant p53, using a drug repositioning strategy. Future collaborations can be established for the validation of the most promising hits.

WS-P05
DRUG REPOSITIONING FOR NEW TREATMENTS AGAINST CHAGAS DISEASE. OXIDATIVE DAMAGE REPAIR ENZYMES FROM *TRYPANOSOMA CRUZI* AS MOLECULAR TARGETS

Sasoni N[1,2], Davies C[3], Campos EM[3], Garay AS[2], Herrea F[2], Rodrigues D[2], Bürgi MDLM[2], Zago P[3], Iglesias AA[1,2], Guerrero SA[1,2] and Arias DG[1,2]
[1]LEM, IAL (CONICET - UNL), Santa Fe – Argentina, [2]FBCB, UNL, Santa Fe, Argentina, [3]IPE, UNS - CONICET, Salta – Argentina
E-mail: darias@fbcb.unl.edu.ar

Methionine is an amino acid susceptible to be oxidized to methionine sulfoxide (MetSO). The reduction of MetSO to methionine is catalyzed by methionine sulfoxide reductases (MSR), enzymes present in almost all organisms. Recently, we characterized MSR proteins from *Trypanosoma cruzi,* which would be relevant for the survival of these pathogens in the various stages of their life cycle. Chagas is a neglected disease caused by the parasite *T. cruzi*, which affects underdeveloped countries. The current drugs are nifurtimox and benznidazole, but both have severe adverse effects and less effectivity in chronic infections; therefore, the need to discover new drugs is essential. Drug repositioning is an interesting option within the international drug development community. Through molecular modeling and virtual screening using the commercially available approved drugs extracted from the ZINC database (2924), we identified drugs with a potential binding capacity to *T. cruzi* MSR. From a preliminary molecular docking analysis, a set of ten compounds with the best binding energies were selected and tested by *in vitro* and *in vivo* assays. Epimastigote and metacyclic trypomastigote cells were used to test the trypanocidal effect of ten selected compounds. Among these drugs,

flunarizine, trifluoperazine, estradiol-benzoate, domperidone, and itraconazole showed better or similar trypanocidal effects (IC_{50} range 1 to 50 μM) in comparison with nifurtimox or benznidazole (IC_{50} of 6 and 20 μM, respectively). *In vitro* enzymatic assays confirmed the inhibitory effect of these drugs over methionine sulfoxide reductase activity of *T. cruzi* MSRs, which exhibited similar inhibitory potency. This work suggests that five known drugs could be used to design new therapy strategies against Chagas disease. Granted by ANPCyT (PICT2014-2103, PICT2016-1778, and PICT2017-2268) and Fundación Bunge y Born (Subsidio para investigación de la enfermedad de Chagas – 2016)

WS-P06
MICRO-VOLUME ASSAYS FOR DEVELOPMENT AND APPLICATION OF BIOCHEMICAL BIOMARKER CLUSTERS IN PRECLINICAL TRIALS

Santiago GM, Cabaña ER, Marelli BE, Hein GJ, Ortega HH
Centro de Medicina Comparada, ICiVet-Litoral (UNL-CONICET), Esperanza, Santa Fe, Argentina. Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, Esperanza, Santa Fe, Argentina. E-mail: hhortega@fcv.unl.edu.ar

The evaluation of new pharmaceutical products requires data about preclinical tests to ensure that the benefits will exceed any potential risk. Recently, clinical biomarkers are part of the diagnosis and monitoring of different diseases in experimental models and concept proof. In this sense, a biomarker is defined as a substance made by a specific tissue that can be detected in the circulation or tissues. To be useful from the clinical approach, it must be released in an amount proportional to the evolution of the specific pathological process and provide information on the presence, severity, and prognosis of the injury. Biochemical biomarkers have been used around for a long time to elucidate the physiologic, toxicologic, pharmacologic, or significance results to fill in gaps of uncertainty about disease targets or variability in drug response. However, the few volumes of biological samples from small laboratory animals (rats and mice) limit the possibility of carrying out commercial methods. Therefore, we aimed to develop and validate an analytical platform for micro-volume assays (less than 50 μL of total serum) to design biomarker packages useful in preclinical trials. All analytical techniques were developed under an ISO 9001 certified Quality Management System in accordance with the principles of Good Laboratory Practices (BPL-OECD recognized facility). Mice, rats, and rabbits were sampled and serum concentrations of glucose (Glc), triglycerides (Tg), cholesterol (Col), high (HDL) and low density lipoprotein (LDL), total protein (TP), albumin (Alb), uric acid (UA), and urea (U) were quantified using the ultra-fast UV/Vis spectrometers SPECTROstar Nano and CLARIOstar (BMG LABTECH GmbH, Germany) and commercial kits (Wiener Labs, Argentina) designed for higher volumes of human samples. The figures of merit were limit of detection, limit of quantification, quantifiable range, intra- and inter-precision test, accuracy, inter-laboratories comparison, matrix effect, dilutional linearity and stability at −20°C and −80°C for 60 days. All these variables were analyzed and validated in accordance with EMA Guideline on bioanalytical method validation and FDA Guidance for Industry: Bioanalytical Method Validation. The results obtained with these validated micro-volume analytical techniques could contribute to the development of preclinical trials in small species, reducing the amount of necessary testing substance and adding complexity to the studies carried out. This development also establishes a scientific framework that contributes to elucidate the pathophysiological changes in different animal models for the study of subclinical changes occurred in certain organs, such as liver, pancreas, kidney, and heart not detectable by other techniques such as pathology. Furthermore, the reductions of sample and reagent volumes agree with the 3Rs principle and sustainability.

WS-P07
ORGANIC SYNTHESIS PLATFORM. DESIGN AND SYNTHESIS OF NEW SMALL MOLECULES AND KILOLAB-SCALE MANUFACTURING FOR PRECLINICAL TRIALS

Gandolfi L, Bellomo A, Elhalem E, Ciarlantini M, Touloumdjian C, Scravaglieri A, Comin J
Instituto Nacional de Tecnología Industrial, Departamento de Ingredientes Activos y Biorrefinerías. San Martín, B1650WAB, Buenos Aires, Argentina. E-mail: jcomin@inti.gob.ar

Our chemical development team works on the synthesis of different types of molecules in collaboration with academic institutions and industrial partners. We focus on the development of new modulators of relevant therapeutic targets involved in cancer processes and other related diseases. In particular, we work on the synthesis of DAG-lactones as selective and potent agents capable of discriminating between protein kinase C (PKC) isoforms and related proteins containing a C1 domain, in order to achieve a specific cellular response with potential therapeutic value. In addition, we target Rac1, a small GTP-binding protein that belongs to the Rho GTPases family that has emerged as an innovative molecular target for the generation of new oncological drugs. We center our efforts towards hepatocellular carcinoma and fibrosis through collaborative approaches. We have recently set up a High-Throughput Experimentation (HTE) facility at INTI, which is the first of its kind in South America. This pioneer, potent technology allows for running rapid screening of multiple reaction conditions working in parallel and at micro-scale, thus preserving valuable materials. As pharmaceutical companies come under more pressure to accelerate the timelines of drug discovery and development, process chemistry departments must contribute to meeting this demand by delivering robust and efficient manufacturing routes to active pharmaceutical ingredients (APIs) faster than ever. This pressure extends to the academic space through increased demand for novel chemical transformations. Our manual and automation technology can also be used to respond to these challenges by accelerating process development and we have several tools to enable experimentation in this sense. Another aim of our work is to develop efficiently scalable manufacturing processes. Working in state-of-the-art laboratories equipped with the latest process and analytical instrumentation, we efficiently conduct route scouting, rapid robust and efficient process development, optimization of reaction conditions for scale-up materials for preclinical trials or large-scale manufacturing non-GMP. Our complete suite of capabilities includes custom synthesis, scale-up capabilities non-GMP purposes, route evaluation and development of new synthetic routes for target molecules, optimization of reaction conditions to reduce costs, development of reliable methods for in-process testing and analysis of intermediates and APIs, rapid screening of reaction conditions using parallel equipment.

WS-P08
PRESENT ADVANCES IN STRUCTURE DETERMINATION BY X-RAY CRYSTALLOGRAPHY AND CRYO-EM AT THE ARGENTINIAN PLATFORM PLABEM

Klinke S[1,2], Cerutt ML[1,2], Otero LH[1,2], Goldbaum FA[1,2,3]
[1] IIBBA-CONICET, Buenos Aire, [2] PLABEM, Buenos Aires, [3] Centro de Rediseño e Ingeniería de Proteínas CRIP, UNSA, Buenos Aires, Argentina
E-mail: sklinke@leloir.org.ar

The Argentinian Platform for Structural Biology and Metabolomics (PLABEM, www.plabem.gob.ar) is a facility focused on innovation, which provides the newest technology generation with applications in scientific research, biotechnology, translational medicine, and food control. Structure determination by protein crystallography and cryogenic electron microscopy (Cryo-EM) is one of our main research activities at the Crystallography Node located at the Leloir Institute in Buenos Aires. The Node is equipped with a Honeybee 963 crystallization robot and a controlled temperature room for crystal growth. Additionally, we operate a Bruker D8 QUEST copper microfocus diffractometer with a CMOS PHOTON 100 detector and a low-temperature attachment device that allows routine macromolecular X-ray diffraction data collection at 100 K. We also facilitate the access to the macromolecular crystallography beamlines PROXIMA 1 and 2A from the SOLEIL Synchrotron (France) through an Argentinian Block Allocation Group and organize the access *via* periodic proposals to high-performance TEMs at the LNNano (Brazil) and CNB-Instruct (Spain) for Cryo-EM. In addition, in our recently founded Center for Protein Re-design and Engineering (CRIP) located at the National University of San Martín, we offer scientific assessment and laboratory capacities for upstream and downstream bioprocess development and optimization of bacterial and animal cell culture expressed products, along with a full package of analytical techniques for industrial partners and governmental technological projects. PLABEM offers the possibility of achieving the resolution of three-dimensional structures of macromolecules by X-ray diffraction and Cryo-EM for the entire Argentine academic system. The activities carried out are not only what is offered by a classic facility (this is, to enable the access to expensive equipment and *know-how* to end users), but also the Platform is consultative, collaborative, and provides an important intellectual capital promoting the training of human resources by means of stays and courses.

WS-P09
IDENTIFICATION OF NOVEL INHIBITORS OF *MYCOBACTERIUM TUBERCULOSIS* ACCASE 5 VIA HIGH-THROUGHPUT SCREENING

Fiorito M , Collaccini F, Bazet Lyonnet B, Banchio C, Gago G, Gramajo H.
Instituto de Biología Molecular y Celular de Rosario (IBR - CONICET). FBIOyF- UNR. E-mail: gago@ibr-conicet.gov.ar

Tuberculosis (TB) is one of the top ten causes of death and the leading cause arising from a single infectious agent. The emergence of multidrug-resistant TB (MDR-TB) presents an increasingly difficult therapeutic challenge and is now the main cause of death due to antimicrobial resistance. In this context, the effective control of this major health problem requires the identification of novel drug targets and new chemical entities suitable for the development of new anti-mycobacterial drugs. *Mycobacterium tuberculosis* (*Mtb*) produces a large number of structurally diverse lipids that have been implicated in the pathogenicity, persistence, and antibiotic resistance of this organism. Most building blocks involved in the biosynthesis of all these lipids are generated by acyl-CoA carboxylases (ACCase). Bioinformatic, biochemical, and structural analysis of the *Mtb* ACCase 5 complex indicated that the main catalytic activity of this enzyme corresponds to that of a propionyl-CoA/acetyl-CoA carboxylase. This complex is formed by the biotinylated α subunit AccA3, the carboxyltransferase β subunit AccD5 and the small ε subunit AccE5. Previous studies indicated that this enzyme complex is essential for the viability of mycobacteria, inferring that the ACCase 5 complex has an essential activity for this bacterium. Moreover, the analysis of a conditional mutant demonstrated that AccD5 and AccE5 are part of an essential ACCase involved in lipid biosynthesis, and proposed ACCase 5 as an attractive target for tuberculosis drug discovery. In this work, high-throughput screening assays were implemented to test millions of compounds belonging to Glaxo SmithKline and Novartis and found several molecules that inhibited more than 85% of the ACCase 5 activity. We further analyzed these candidates by conventional enzymatic methods and found five compounds that inhibited ACCase 5 at low μM concentrations. For these compounds IC_{50}, MIC on *Mtb* H37Ra and cytotoxicity assays were obtained. To start elucidating the mechanism of action of these compounds *in vivo*, we studied their effect on lipid biosynthesis by analyzing ^{14}C-acetate incorporation on fatty acids and mycolic acids and found that all of them inhibited lipid biosynthesis. These results validated the high-throughput screening assay as a powerful tool for identifying novel enzyme inhibitors that could be developed as anti-tuberculosis drugs.

WS-P10
GLYCOGEN SYNTHASE KINASE 3 PLAYS A CENTRAL ROLE IN THE TRANSCRIPTIONAL RESPONSE TO UV-INDUCED DNA DAMAGE

Nieto Moreno N[1], Villafañez F[2], Soria G[2], Muñoz MJ[1], Kornblihtt AR[1]
[1] IFIBYNE-UBA-CONICET, Argentina. [2] CIBICI-UNC-CONICET, Argentina. E-mail: nnietomoreno@fbmc.fcen.uba.ar

DNA integrity is a major requisite for life and, therefore, cells develop a concerted response upon DNA damage. At the gene expression level, this response consists of a genome-wide downregulation of transcription initiation and elongation rates, as well as the modulation of alternative splicing (AS) patterns. In particular, we have described that UV-induced DNA lesions trigger an *in trans* signaling cascade that promotes hyperphosphorylation of the carboxyl-terminal domain (CTD) of RNA polymerase II (RNAPII), a decrease in transcription elongation rates and changes in AS patterns in the context of the kinetic coupling model between transcription and splicing. In order to identify kinases mediating this response, we developed an alternative splicing fluorescent reporter system and performed a screening with the Public Kinase Inhibitors Library (PKIS2) from GlaxoSmithKline. This allowed us to identify glycogen synthase kinase 3 (GSK-3) as a central player: GSK-3 inhibition prevents

UV-induced RNAPII hyperphosphorylation, as well as the changes in AS patterns and the decrease in transcription elongation rates. Using RNAPII ChIP analysis we detected that while intragenic RNAPII occupancy is increased upon UV irradiation, consistent with a decrease in RNAPII elongation rates, GSK-3 inhibition prevents this increase. In addition, GSK-3 inhibition prevents UV-induced apoptosis. Finally, since RNAPII CTD adjusts to the GSK-3 consensus site and that it was reported that GSK-3 translocates to the nucleus in stress conditions, we are currently testing the hypothesis that GSK-3 may be phosphorylating RNAPII in a direct manner. Altogether, our results set GSK-3 as a central kinase in the transcriptional response to DNA damage.

AUTHOR INDEX

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

Y

Z

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

Scientific Institutions and Gubernamental Sponsors

*Agencia Nacional de Promoción Científica y Tecnológica

**National Science Foundation

*Grants RC-RPN-2019-00097 to Moreno, Silvia M and RC-TW-2019-00067 to. Biondi, Ricardo M
**Disclaimer: A portion of this workshop is supported by the National Science Foundation under grant #1346567 to Anderson, Pelaez, and others for the Advancing Competencies in Experimentation - Biology (ACE-Bio) Network. Any opinions, findings, and conclusions or recommendations expressed in this workshop are those of the authors and do not necessarily reflect the views of the National Science Foundation.

BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

Academic Endorsements

Private Sponsors