SAIB Sociedad Argentina de Investigaciones en Bioquímica y Biología Molecular

CONGRESO SAIB 2023

LIX Annual Meeting of the Argentine Society for Biochemistry and Molecular Biology Research (SAIB)

MEMBERS OF THE SAIB BOARD

Eduardo Ceccarelli
President
CONICET - Universidad Nacional de Rosario

María Elena Álvarez
Vicepresident
CONICET - Universidad Nacional de Córdoba

María Isabel Colombo
Past-President
CONICET - Universidad Nacional de Cuyo

Federico Sisti
Secretary
CONICET - Universidad Nacional de La Plata

Germán Rosano
Treasurer
CONICET - Universidad Nacional de Rosario

Paula Casati
Prosecretary
CONICET - Universidad Nacional de Rosario

Alex Saka
Protreasurer
CONICET – Universidad Nacional de Córdoba

Claudia Banchio
Auditor
CONICET - Universidad Nacional de Rosario

Matías Asención Diez
Auditor
CONICET – Universidad Nacional del Litoral

DELEGATES OF SCIENTIFIC SECTIONS

Cell Biology
Pablo Aguilar
CONICET – Universidad de Buenos Aires

Lipids
Martín Oresti
CONICET - Universidad del Sur

Microbiology
Hebe Dionisi
CONICET

Plants
Elina Welchen
CONICET – Universidad Nacional del Litoral

Signal Transduction
Graciela Boccaccio
CONICET

PROGRAM AT A GLANCE

	TUESDAY , NOV 14	WEDNESDAY, NOV 15	THURSDAY, NOV 16	FRIDAY, NOV 17
8:30 a 10:30		ORAL COMMUNICATIONS **PLANTS** Room SUM **SIGNAL TRANSDUCTION/ NEUROSCIENCES** Room S04	ORAL COMMUNICATIONS **MICROBIOLOGY** Room SUM **LIPIDS** Room S04	ORAL COMMUNICATIONS **CELL BIOLOGY** Room SUM **BIOTECHNOLOGY/ ENZYMES/ STRUCTURAL BIOLOGY** Room S04
10:30 a 11			Coffee-Break	
11 a 12:30		Plenary Lecture Room SUM **Dr. Norbert ROLLAND**	Plenary Lecture Room SUM **Dra. Chiara ZURZOLO**	Plenary Lecture Room SUM **Dr. Alberto ROSA**
12:30 a 14:30			Lunch Time	
		SYMPOSIA **SIGNAL TRANSDUCTION** Room SUM **YOUNG INVESTIGATORS 1** Room S04 **PLANTS** Room S09	SYMPOSIA **MICROBIOLOGY** Room SUM **LIPIDS** Room S04 **METHODOLOGICAL ADVANCES TALKS** Room S09	SYMPOSIA **CELL BIOLOGY** Room SUM **YOUNG INVESTIGATORS 2** Room S04 **BIOTECHNOLOGY** Room S09
16:30 a 17	ACCREDITATION		Coffee-Break	
17 a 19	Opening Ceremony "Alberto Sols" Plenary Lecture **Room SUM** **Dra. Irene Díaz Moreno**	POSTERS PT-01 to PT-27 CB-01 to PT-16 ST-01 to ST-11	POSTERS CB-17 to CB-32 MI-01 to MI-18 LI-01 to LI-11 BT-01 to BT-14	POSTERS MI-19 to MI-36 CB-33 to CB-48 SB-01 to SB-10 NS-01 to NS-10 EN-01 to EN-04
19 a 20:30	Welcome Cocktail (Hall Central)	"Hector Torres" Plenary Lecture **Room SUM** **Dra. Vanesa GOTTIFREDI**	"Ranwell Caputo" Plenary Lecture **Room SUM** **Dr. Mario GUIDO**	Plenary Lecture **Room SUM** **Dr. Eduardo GROISMAN**
21 a 23			SAIB Assembly **Room SUM**	**Room SUM** **Awards and Closing Ceremony**

This meeting was supported by:

CONICET

Agencia I+D+i

Ministerio de Ciencia, Tecnología e Innovación Argentina

PABMB

INTERNATIONAL UNION OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

FEBS openbio

SEBBM

facultad de ciencias bioquímicas y farmacéuticas

UNR Universidad Nacional de Rosario

CENTRO FRANCO ARGENTINO DE ALTOS ESTUDIOS
UNIVERSIDAD NACIONAL DE ROSARIO

CONCEJO MUNICIPAL ROSARIO

Santa Fe Provincia

Tech Science Press

microlat instrumental científico

tecnolab

INBIOAR

Soluciones Analíticas Equipamiento para Laboratorios

AllScience Ciencia, Tecnología y Ambiente

OneLab

thermo scientific Representante Autorizado

Wiener lab.

BioFactors

ThermoFisher SCIENTIFIC

ESANCO

NORCES EQUIPAMIENTO CIENTIFICO

PROGRAM

TUESDAY, November 14, 2023

16:30-17:00 REGISTRATION

17:00-19:00 PLENARY LECTURE ALBERTO SOLS

Dra. Irene Díaz Moreno
Laboratorio de Proteómica Estructural y Funcional Departamento de Bioquímica Vegetal y Biología Molecular. Universidad de Sevilla. **España.**
"Crosstalk between Nucleus and Mitochondria in Cell Life and Disease"
Chairperson: Eduardo A. Ceccarelli

Room SUM

WELCOME COCKTAIL

WEDNESDAY, November 15, 2023

8:30-10:30 ORAL COMMUNICATIONS
PLANTS Room SUM
SIGNAL TRANSDUCTION NEUROSCIENCES Room S04

10:30-11:00 COFFEE BREAK

11:00-12:30 PLENARY LECTURE

Dr. Norbert ROLLAND
Laboratoire Physiologie Cellulaire & Végétale, CEA-Grenoble. Université Grenoble Alpes".
Francia.
Chloroplast biogenesis: towards the role of localized translation in Arabidopsis
Chairperson: Eduardo A. Ceccarelli

Room SUM

12:30-14:30 FREE TIME FOR LUNCH

14:30-16:30 SIGNAL TRANSDUCTION SYMPOSIUM
Room SUM
Chairpersons: Graciela Boccaccio - Mariana Melani

Dr. Diego Bustos
Instituto de Histología y Embriología de Mendoza Dr. Mario H. Burgos - IHEM CCT Mendoza, CONICET. **Argentina.**
"Allosteric regulation of phosphorylation reader 14-3-3"

Dra. Anabella Srebrow
IFIBYNE-UBA-CONICET. **Argentina.**
"Impact of dengue virus on the expression of PML mRNA isoforms"

Dr. Andrés Dekanty
Instituto de Agrobiotecnología del Litoral. IAL CCT Santa Fe, CONICET. **Argentina.**
"Dicer-p53 Axis: A Key Regulator of Systemic Adaptation to Nutrient Stress"

Dr. Ariel Kaplan
Technion - Israel Institute Of Technology, Israel
"Chromatin dynamics, one molecule at a time"

14:30-16:30 YOUNG INVESTIGATORS SYMPOSIUM I
Room S04
Chairpersons: Sebastián Klinkle / Gabriela Coux

Dr. Leonardo Acuña
Unidad de Biotecnología y Protozoarios. IPE (CONICET-UNSa)
"A Two-Pronged Attack: Dual Antigen Fusion Protein and Bacterial Adjuvant Strategies Against Trypanosomal Infection"

Dra. Victoria L. Alonso
Instituto de Biología Molecular y Celular Rosario (IBR)

"Role of α-tubulin acetylation on microtubule structure and dynamics in Trypanosoma cruzi"

Dra. Julieta Barchiesi
Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI-CONICET-UNR)
"Molecular insights into cellulose degradation by native microalgael"

14:30-16:30 PLANTS SYMPOSIUM
Room S09
Chairpersons: Clarisa E. Alvarez / María Inés Zanor

Dr. Federico Ariel
Instituto de Agrobiotecnología del Litoral, IAL, CONICET, UNL, Santa Fe, Argentina
"Plant Long Noncoding RNAs: Mechanisms and Agricultural Potential"

Dra. Ana M.Laxalt
Insituto de Investigaciones Biológicas, IIB, CONICET, UNMDP, Mar del Plata, Argentina
"Phospholipase C in plant defense"

Dr. Juan José Pierella Karlusich
FAS Division of Science, Harvard University, Cambridge, MA, USA
"Photosynthesis modulation in the global ocean"

Dr. José Miguel Alvarez,
Centro de Biotecnología Vegetal, Universidad Andrés Bello, UNAB, Millennium Institute for Integrative Biology, iBio, Chile
"Transcriptional networks integrating drought stress and nitrogen signaling in plants"

16:30-17:00 COFFEE BREAK

17:00-19:00 POSTER SESSION
Central Hall

PT-01 to PT-26
CB-01 to PT-16
ST-01 to ST-11

19:00-20:30 PLENARY LECTURE HECTOR TORRES

Dra. Vanesa GOTTIFREDI
Laboratorio de Ciclo Celular y Estabilidad Genómica La Fundación Instituto Leloir (FIL).
Argentina.
"DNA replication as a therapeutic target for cancer treatment: where we started and where are we now"
Chairperson: Ricardo Biondi

Room SUM

THURSDAY, November 16, 2023

8:30-10:30 ORAL COMMUNICATIONS

MICROBIOLOGY Room SUM
LIPIDS Room S04

10:30-11:00 COFFEE BREAK

11:00-12:30 PLENARY LECTURE

Dra. Chiara ZURZOLO
Laboratory of MEMBRANE TRAFFIC AND PATHOGENESIS. Institute Pasteur Paris. **Francia.**
"Reshaping connectivity: Tunneling nanotubes, structure function and role in neurodegenerative disease"
Chairperson: Pablo Aguilar

Room SUM

12:30-14:30 FREE TIME FOR LUNCH

14:30-16:30 LIPIDS SYMPOSIUM
Room S04
Chairpersons: Ariel Quiroga / Nicolás Favale

Dr. Roger Sandhoff
Lipid Pathobiochemistry Group, German Cancer Research Center, Heidelberg, **Alemania.**
"Endogenous and pathological functions of sphingolipids as revealed by genetic mutations"

Dra. Adriana Esteves
Universidad de la República, **Uruguay.**
"A long road with FABPs"

Dr. Mauricio Martín
Instituto de investigación Médica Mercedes y Martin Ferreyra, INIMEC - CONICET. Córdoba. **Argentina.**
"The Aging Brain and Altered Cholesterol Trafficking: Reversing the Niemann-Pick C Phenotype of Old Astrocytes with Cannabinoid Treatment"

Dra. Gabriela Gago
Instituto de Biología Molecular y Celular de Rosari, IBR, CONICET, Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR, Rosario, **Argentina.**
"Unraveling the link between the two fatty acid synthase systems in Mycobacterium tuberculosis"

14:30-16:30 MICROBIOLOGY SYMPOSIUM
Room SUM
Chairpersons: Hebe Dionisi / Fernando Soncini

Dr. Sebastian Klinke
Fundación Instituto Leloir, CONICET UBA. **Argentina.**
"Structural study of proteins involved in the biosynthesis and metabolism of* riboflavin *in Brucella abortus"

Dr. Javier M. Gonzalez
Instituto de Bionanotecnología del NOA. CONICET - Universidad Nacional de Santiago del Estero. **Argentina.**
"Extremophile microorganisms from northern Argentina as a source of enzymes of biotechnological interest"

José M. Argüello
Department of Chemistry and Biochemistry, Worcester Polytechnic Institute, Worcester, **Massachusetts, EE. UU**
"Copper homeostasis in Pseudomonas, system biology meets biochemistry"

Dra. María Eugenia Guazzaroni
Departamento de Biologia, FFCLRP-Universidade de São Paulo. **Brasil**
"Novel molecular tools for synthetic biology approaches in environmental bacteria"

Dra. Lucía B. Chemes
Laboratorio de Estructura, Función y Plasticidad de Proteínas, Instituto de Investigaciones Biotecnológicas, UNSAM, Campus Miguelete, **Argentina**
"Intrinsically disordered viral proteins: Mechanistic and structural insights on how they mediate cell cycle hijack"

14:30-16:30 METHODOLOGICAL ADVANCES TALKS
Room S09
Chairperson: Enrique Morales

Dr. Luiz Fernando Santos
Thermo Fisher Scientific. Soluciones Analíticas SA, **Argentina**
"Recent Mass Spectrometry based solutions for Proteomics"

Lic. Esteban Salvatore
OneLab Argentina. Especialista de Aplicaciones en Sartorius en el área de Bioanalítica.
"Soluciones para el descubrimiento de drogas biológicas: Anticuerpos monoclonales"

Dr. Santiago Bortolotti
Wiener Lab Rosario Argentina
"T cruzi DNA test: producto de desarrollo y fabricación nacional. Una experiencia asociativa exitosa entre el sector público y privado."

Mg. Constanza Pno
Thermo Fisher, Argentina

"High-quality nucleic acid extraction: improve extraction in your lab"

Julian Costamagna
Bio Esanco SA, Argentina
"Gilson Micropipettes: Advances, care and maintenance"

16:30-17:00 COFFEE BREAK

17:00-19:00 POSTER SESSION
Central Hall

CB-17 to CB-32
MI-01 to MI-18
LI-01 to LI-11
BT-01 to BT-14

19:00-20:30 RANWEL CAPUTTO CONFERENCE

Dr. Mario GUIDO
Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC) CONICET
Universidad Nacional de Córdoba. **Argentina.**
"A Journey through the inner retina of diurnal vertebrates. Seeking for noncanonical photoreceptors and light-inducible molecules"
Chairperson: María Elena Alvarez

Room SUM

20:45-22:00 SAIB society meeting

FRIDAY, November 17, 2023

8:30-10:300 ORAL COMMUNICATIONS

CELL BIOLOGY Room SUM
BIOTECHNOLOGY ENZYMES STRUCTURAL BIOLOGY Room S04

10:30-11:00 COFFEE BREAK

11:00-12:30 PLENARY LECTURE

Dr. Alberto ROSA
Instituto de Farmacología Experimental de Córdoba (IFEC-CONICET) Facultad de Ciencias Químicas Universidad Nacional de Córdoba (UNC). **Argentina.**
"From Neurospora to Neurology: Unravelling the Molecular Pathogenic Mechanisms of a Human Neurodegenerative Disease"
Chairperson: José Echenique

Room SUM

12:30-14:30 FREE TIME FOR LUNCH

14:30-16:30 CELULAR BIOLOGY SYMPOSIUM
Room SUM
Chairpersons: Vanesa Gottifredi / Mauricio Martin

Dr. M. Cecilia Larocca
Instituto de Fisiología Experimental (IFISE) CONICET, UNR, Rosario, **Argentina.**
"Unveiling the role of the Golgi apparatus in the maturation of the lytic NK-cell immunological synapse"

Dr. Javier Roberto Jaldin-Fincati
Unidad de Conocimiento Traslacional Hospitalaria Dr. Arturo Oñativia de Salta. **Argentina.**
"Cell Biology and Translational Research: from basic science to the treatment of non-communicable diseases"

Dra. Cecilia Alvarez
Facultad de Ciencias Químicas. Universidad Nacional de Córdoba. CIBICI-CONICET.
Argentina.
"Global cellular organization: membrane trafficking and homeostatic response"

Dr. Juan Ugalde
Instituto de Investigaciones Biotecnológicas, IIBio, Universidad Nacional de San Martin,
Argentina.
"The ins and outs of the intracellular life cycle of Brucella"

14:30-16:30 YOUNG INVESTIGATORS SYMPOSIUM II
Room S04
Chairpersons: Noelia Foresi / Javier Gonzalez

Dr. Mauricio A. Reynoso
Instituto de Biotecnología y Biología Molecular IBBM - FCE - UNLP - CONICET
"Evolutionary conserved submergence responsive transcription factors involved in the formation of lateral root organs in legumes"

Dr. Martín Roffé
CHEO Research Institute, Ottawa, ON, CANADA
"The p90 Ribosomal S6 Kinase 1 (RSK1) modulates the Translatome of Glioblastoma in an isoform-specific manner to regulate the Cell Cycle program"

Dr. Carlos Wilson
Centro de Investigación en Medicina Traslacional Dr. Severo R. Amuchástegui (CIMETSA G.V. al INIMEC-CONICET-UNC), Instituto Universitario de Ciencias Biomédicas de Córdoba
"***Homeostasis control of the histone label H3K9me2 by G9a, REST and CoREST safeguards the growth and survival of human neurons"***

14:30-16:30 BIOTECHNOLOGY SYMPOSIUM
Room S09

Chairperson: Daniela Catalano Dupuy

Dr. Marcos Morgada
I+D - Research & Development Uovotek Micro Parque Industrial, Alvear, Santa Fe, **Argentina**
"Cómo innovar desde la biotecnología en Argentina"

Dr. Hugo Menzella
Instituto de Procesos Biotecnológicos Y Químicos Rosario, IPROBYQ , CONICET, Rosario, **Argentina**
"Análisis de factibilidad de proyectos biotecnológicos"

Dr. Ignacio Smith
TREBE BIOTECH Pergamino, Buenos Aires, **Argentina**
"El poder de los Insectos: producción simple de biofármacos complejos"

16:30-17:00 COFFEE BREAK

17:00-19:00 POSTER SESSION
Central Hall

MI-19 to MI-36
CB-33 to CB-48
SB-01 to SB-10
NS-01 to NS-10
EN-01 to EN-04

19:00-20:30 PLENARY LECTURE

Dr. Eduardo GROISMAN
Yale School of Medicine/ Microbial Pathogenesis. **USA.**
"Control of Rho transcription termination by phase separation"
Chairperson: Graciela Boccaccio

Room SUM

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

ORAL COMMUNICATIONS- Wednesday, November 15th

Room SUM

PLANTS

Chairpersons: Lucila García - Corina Fusari

8:30 PL-01

Role of *At*MED17 under light and dark conditions

Giustozzi, M.; Freytes, S.; Cerdán, P.; Casati, P.

8:45 PL-02

Redesigning of photosystem I for optimizing photosynthesis/protection balance

Bultri, J.; Brugnara, C.; Miño, J. J.; Abraham, M.; Lobais, C.; Blanco, N.E.

9.00 PL-03

Insights on how light regulates alternative polyadenylation in plants

Kubaczka, M.G.; Godoy Herz, M.A.; Chen, J.; Tian, B.; Kornblihtt, A.R.

9:15 PL-04

Natural variation for flower and seed abortion in fluctuating environments

Petreli, M.V.; Herrera, C.; Segatto, L.; Blanco, N.; Fusari, C.M.

9:30 PL-05

Deciphering molecular determinants of the high levels of storage proteins and oil in the soybean seeds

Zucchetti, J.I.; Pavlovic, T.; Saenz, E.; Borrás, L.; Saigo, M.; Gerrard Wheeler, M.C.

9:45 PL-06

Optimization of high molecular weight DNA isolation and Oxford Nanopore sequencing in non-model trees

Gaischuk, S.; Leduque, B.; Servi, L.; Bermudez Salazar, L.F.; Petrillo, E.; Quadrana, L.; Bellora, N.; Arana, M.V.

Room S04

SIGNAL TRANSDUCTION - NEUROSCIENCES

Chairpersons: Diego Bustos - Graciela Boccaccio

8:30 ST-O1

Autophagy proteins are required for regulated exocytosis in *Drosophila melanogaster*

Fresco, S.M.; Suárez-Freire, S.; Wappner, P.; Melani, M.

8:45 ST-O2

The βCSP-Hsc70 complex in human spermatozoa: a key player in acrosomal exocytosis

Flores Montero, K.; Durán, N.; Fontecilla, J.; Ruete, M.C.

9.00 ST-O3

Modulation of oncogenic P53 mutants stability through drug repurposing

Cocordano, N.; Borini Etichetti, C.M.; Llorens de los ríos, M.C.; Arel Zalazar, E.E.; Soria, G.; Girardini, J.E.

9:15 ST-O4

Role of the transmembrane connector in Ire1 signaling

Cybulski, L.; Almada, J.C.; Bortolotti, A.; Colman- Lerner, A.; Dunayevich, P.,

9:30 NS-01

Characterization of bioactive extracellular vesicles secreted by human cells

Guendulain, G.G.; Gastaldi, L.; Remedi, M.; Cáceres, A.; Moyano, A.L.

09:45 NS-02

A novel player in neuronal primary cilia: the secretory pathway-associated transcription factor, CREB3L1

Rozés-Salvador, V.; Alvarez, C.

ORAL COMMUNICATIONS- Thursday, November 16th

Room SUM

MICROBIOLOGY

Chairpersons: *Julieta Fernández - Victor Blancato*

8:30 MI-01

Light modulation of phage infection in *Acinetobacter baumannii*

Arana, N.;Tomas, M.; Comité de Infecciones del Hospital Provincial del Centenario; Mussi, M.A.

8:45 MI-02

Antifungal activity and phytotoxicity of the ternary complex of copper(II) with cyanoguanidine and 1,10-phenanthroline

Guevara, L.E.; Martínez Medina, J.J.

9.00 MI-03

Physiological role and structural characterization of MabR a transcriptional regulator of mycolic acid biosynthesis pathway of *Mycobacterium tuberculosis*

Coronel Cappellari, C.; Diacovich, L.; Gramajo, H.

9:15 MI-04

Chronobiology in chemotrophic human pathogen *Acinetobacter baumannii*

Permingeat, V.; Perez Mora, B.1.; Lamberti, M.L.; Migliori, M.L.; Golombek, D.A.; Mussi, M. A.

9:30 MI-05

Identification of antimicrobial resistance genes in dairy farm soils through shotgun sequencing

Cerioli, M.F.; Moliva, M.; Sambuceti, N.; Raviolo, J.; Sánchez-Pérez, M.; Caminati, F.; Rosenblueth Laguette, M.T.; Reinoso, E.B.

9:45 MI-07

***In Vitro* Leishmanicidal activity and synergy of known drugs against *Leishmania (Leishmania) amazonensis*: a promising drug repurposing strategy**

Araoz, E.N.; Minahk, C.J.; Pérez-Brandán, C.M.; Acuña, L.; Barraza, D.E.

10:00 MI-08

Contribution of fresh river water to the dissemination of hospital-associated *Enterococcus faecium* CC17 strains in Córdoba

Blasko, E.G.; González, M.J.; Bardossy, A.C.; Sosa, E.J.; Prentiss, T.; Suleyman, G.; Jagjeet, K.; Maki, G.; Ruiz, S.E.; Grupo de Estudio de colonización por VRE de CBA; Fernández Do Porto, D.; Bocco, J.L.: Saka, H.A.; Amé, V.; Zervos, M.; Sola, C.

Room S04

LIPIDS

Chairpersons: Cecilia Casali - Ana Arabolaza

8:30 LI-01

The balance between sphingolipids and phosphoinositides as a driving factor in epithelial cell differentiation

Pescio, L.G.; Mosca, J.M.; Favale, N.O.; Sterin-Speziale, N.B.

8:45 LI-02

Protective role of the phospholipase A2-cyclooxygenase 2-prostaglandin E2-EP2/4 receptor axis in the restitution of an oxalate-damaged renal epithelium.

Sendyk, D.E.; Erjavec, L.C.; Parra, L.G.; Bonino, M.; Flor, S.; Lucangioli, S.; Fernández, M. del C.; Casali, C. I.

9:00 LI-03

Deciphering the molecular mechanisms by which PtdCho induces neuronal differentiation of NSCs, and restores neuronal phenotype (morphological and functional) under stress conditions.

Magaquian, D.; Delgado Ocaña, S.; Banchio, C.

9:15 LI-04

Lomitapide does not affect liver tumor development in mice on a high-fat diet

Comanzo, C.G.; Vera, M.C.; Oviedo Bustos, L.; Palma, N.F.; Ferretti, A.C.; Ceballos, M.P.; Álvarez, M. de L.; Quiroga, A.D.

9:30 LI-05

The Unfolding Protein Response modulates arachidonic acid metabolism under osmotic stress

Parra, L.; Erjavec, L.; Zerpa, A.; Sendyk, D.; Salafia, A.; Casali, C.; Fernández, M. del C.

9:45 LI-06

The HIF1-α and FASN overexpression by palmitic acid and fructose induced tumorigenesis in breast adenocarcinoma

Ferrero, V.; Mazo, T.; Barotto, N.N.; Don, J.A.; Moreira-Espinoza, M.J.; Yennerich, L.; Granton, F.; Mazzudulli, G.; Lagares, C.; Rodríguez, V.; Pasqualini, M. E.

10:00 LI-07

Expression and host-parasite communication studies of *Echinococcus granulosus* 2DBD nuclear receptors

Mozzo, B.; Blanco, V.; Alvite, G.

10:15 LI-08

Strategies for the production of neutral lipids enriched in medium-chain fatty acids

Pascutti, F.; Álvarez, H.M; Arabolaza, A.; Gramajo, H.

ORAL COMMUNICATIONS- Friday, November 17th

Room SUM

CELLULAR BIOLOGY

Chairpersons: Claudia Banchio - Mauricio Martin

8:30 CB-01

Effects of genetic variants in guanine quadruplexes of RNA that impact the translation of human oncogenes

Bezzi, G.; Piga, E.; Binolfi, A.; Armas, P.

8:45 CB-02

Unleashed loading of specialized DNA polymerase PrimPol to replicating DNA increases genomic instability.

Mansilla, S.F.; Calzetta, N.L.*; Venerus Arbilla, S.; Okraine, Y.V.; Siri, S.O.; Caimi, L.; Abramovici Blasco, A.; Gottifredi, V., *equal collaboration.*

9.00 CB-03

The exocyst complex controls multiple events in the pathway of regulated exocytosis

Suárez Freire, S.; Pérez-Pandolfo, S.; Fresco, S.M.; Wappner, P.; Melani, M.

9:15 CB-04

Plasma membrane nanodomains disassembly increases yeast's lifespan

Salzman, V.; Bustamante Torres, M.; Pellizza, L.; Aran, M.; Aguilar, P.

9:30 CB-05

Hyaluronan metabolism is associated with DNA repair genes in breast and colorectal cancer

Sevic, I.; Vitale, D.; Moran, C.; Brandone, A.; Rosales, P.; Icardi, A.; Romano, L.; Giannoni, P.; Cristina, C.; Alaniz, L.

9:45 CB-06

Old drugs, new uses: new perspectives for the repositioning of chemotherapy drugs and modulators of the extracellular matrix in breast cancer treatment

Vitale, D.L; Rosales, P.; Icardi, A.; Morán, C.; Sevic, I.; Alaniz, L.

10:00 CB-07

The liver X receptor interferes with estrogen receptor-dependent genomic regulation in breast cancer cells

Olszanowski, E.; Ogara, M.F.; Lafuente, A.; Nacht, A.S.; Benitez, B.; Rodríguez-Seguí, S.A.; Presman, D., Vicent, G.P., Pecci, A.

Room S04

BIOTECHNOLOGY - ENZYMES - STRUCTURAL BIOLOGY

Chairpersons: *Daniela Catalano Dupuy-Matías Asención Diez*

8:30 BT-01

Antifungal activity and toxicological profile of the ternary complex of copper with the flavonoid chrysin including 1,10-phenanthroline as secondary ligand

Bogado, L.J.; Guevara, L.E.; López Tévez, L.L.

8:45 BT-02

Ten years since the introduction of bacterial glycoengineering technology in the diagnostic algorithm of Hemolytic Uremic Syndrome

Melli, L.; Landivar, S.M.; Miliwebsky, E.; Chinen, I.; Comerci, D.; Ugalde, J.E.; Ciocchini, A.

9.00 BT-03

Developing a sustainable enzymatic degumming process for vegetable oils using a consensus sequence-designed synthetic phospholipase C

Val, D.S.; Di Nardo, L,; Marchisio, F,; Gottig, N.; Peirú, S.; Castelli, M.E.; Abriata, L.; Menzella, H.G.; Rasia, R.M.

9:15 BT-04

Use of modified algae biomass as a protector against plant osmotic stress

Marchetti-Acosta, N.; Velazquez, M.B.; Barchiesi, J.; Busi, M,V.

9:30 BT-05

Bioprocess optimization of anti-native SARS-CoV-2 Spike S1 hybridomas

Acuña Intrieri, M.E.; Pelliza, L.; Arán, M.; Cerutti, M.L.

9:45 EN-01

Biological activity of a novel fibrinolytic enzyme secreted by *Hornodermoporus martius* LBM 224

Acosta, G.A.; Fonseca, M.I.; Fariña, J.I.; Zapata, P.D.

10:00 SB-01

Studying the mechanical unfolding and refolding mechanism of Top7 at the single molecule level at different temperatures

Corrêa, C.G.; Martínez Bilbao, J.; Mjaavatten, A.; Wilson, C.A.M.

ABSTRACTS

CONFERENCES

Crosstalk between Nucleus and Mitochondria in Cell Life and Disease

I. Díaz-Moreno, L. Corrales-Guerrero, A. Guerra-Castellano, R. Giner-Arroyo, B. Baños-Jaime, J. Tamargo-Azpilicueta, A. Fernández-Veloso, M.A. De la Rosa Acosta
Institute for Chemistry Research, cicCartuja University of Seville CSIC, Seville, Spain

The crosstalk between the mitochondrion and the nucleus regulates cellular functions, including differentiation and adaptation to stress. Mitochondria supply metabolites and certain mitochondrial factors, which have been emerged as response elements to cell nucleus performance. Thus, unraveling the whole connectivity between the biomolecules involved in all this regulatory mitochondria-nucleus crosstalk and its relation to cell fate and physiological state is nowadays a major challenge. In the lecture, I will show novel mechanistic insights into electron transfer (ET) from cytochrome c1 to cytochrome c1, including gated, long-range ET in aqueous solution. Remarkably, a close contact between cytochrome c1 and cytochrome c is not essential for ET: when proteins are approaching each other, cation exclusion occurs between their active sites, enabling the building of a Gouy-Chapman charge conduit and the long-distance ET through the aqueous solution.2 Phosphorylation of cytochrome c not only affects its structure and dynamics,3,4 but also shortens the long-distance charge conduit between the partners, strengthens their interaction, and departs it from equilibrium.5
In response to DNA damage, cytochrome c escapes from its natural mitochondrial environment and, once in the cytoplasm, binds to Apaf-1 to form a complex—the so-called apoptosome—that triggers caspase activation and further leads to controlled cell dismantlement. Recent work from our group shows that cytochrome c in the cytoplasm also binds to the chaperone 14-3-3e, which is an inhibitor of Apaf-1, to block 14-3-3e-mediated Apaf-1 inhibition, thereby unveiling a novel function for cytochrome c as an indirect activator of caspase-9/3.6,7 Besides such key apoptotic roles of cytochrome c in the cytoplasm, it migrates to the nucleus soon after DNA damage, even before caspase cascade activation and apoptosome formation in the cytoplasm.8 Cytochrome c in the nucleus actually targets a variety of well-known histone chaperones involved in chromatin remodeling and DNA damage response.9-13 Our results show that nuclear/nucleolar cytochrome c inhibits the nucleosome (dis)assembly activity of histone chaperones, impairs dephosphorylation events and controls p53-mediated cell cycle arrest during the repair of injured DNA.10-12 Altogether, our recent data demonstrate that cytochrome c functions as a master, pleiotropic organellar factor, thereby playing a crucial global role in cell metabolism, both in life and death. Altogether, our recent data demonstrate that cytochrome c functions as a master, pleiotropic organellar factor, thereby playing a crucial global role in cell metabolism, both in life and death.

References
1Pérez-Mejías et al. (2022) Coord Chem Rev 450: 214233
2Lagunas et al. (2018) Nat Comm 9: 5157
3Moreno-Beltrán et al. (2017) PNAS 114: E3041
4Guerra-Castellano et al. (2018) PNAS 116: 7955
5Gomila et al. (2022) Nat Comm 13: 7100
6Elena-Real et al. (2018) Cell Death Dis 9: 365
7Elena-Real et al. (2021) Plant J 106: 74
8González-Arzola et al. (2015) PNAS 112: 9908
9González-Arzola et al. (2017) Nucleic Acids Res 45: 2150
10Rivero-Rodríguez et al. (2021) Redox Biol 43: 101967
11Casado-Combreras et al. (2022) Comp Struct Biotechnol J 20: 3695
12González-Arzola et al. (2022) Nat Struct Mol Biol 29: 1024
13Buzón et al. (2023) Adv Sci 7: e2301859

Chloroplast biogenesis: towards the role of localized translation in Arabidopsis

Norbert Rolland, 1, Laura Dimnet, 1, Sara Pullara, 1, Lucas Moyet, 1, Chloé Genevey, 1, Samantha Moulin, 1, Sabine Brugière, 2, Alexandra Launay-Avon, 3, Yohann Couté, 2, Etienne Delannoy, 3, Rémy Meret, 4, and Marcel Kuntz, 1
1 Laboratoire de Physiologie Cellulaire & Végétale, CNRS, INRAE, CEA, Université Grenoble Alpes, Grenoble, France. 2 Laboratoire Biologie à Grande Echelle, INSERM, CEA, Université Grenoble Alpes, Grenoble, France. 3 IPS2 Institut des Sciences des Plantes de Paris-Saclay, Gif sur Yvette, France. 4 Laboratoire Génome et Développement des Plantes, CNRS/UPVD, Perpignan, France

Chloroplasts are a major component of plant cells. Until recently, all nuclear-encoded proteins destined to chloroplast were believed to possess an N-terminal and cleavable chloroplast targeting peptide, and to engage the TOC/TIC machinery. However, recent studies have revealed that alternative routes also exists and identified a series of nuclear-encoded proteins imported via such pathways. Recent proteomic studies, conducted by our team (Bouchnak et al., Mol Cell Proteomics 2019), identified a list of cyto-ribosomal subunits associated to chloroplasts, thus suggesting that localized translation might occur at the chloroplast surface. We were recently able to isolate plastid-associated cyto-ribosomes and to decipher their composition when compared to purified whole cell cyto-ribosomes. Interestingly, these plastid-associated cyto-ribosomes contain a few non cyto-ribosomal proteins which might participate to the control of localized translation at the chloroplast surface. Finally, with the aim to identify the nature of the nuclear-encoded mRNAs that are translated by these chloroplast-associated cyto-ribosomes, the identification of mRNAs trapped within these plastid-associated cyto-ribosomes was performed. Surprisingly, very few of these mRNAs code for chloroplast proteins.

Acknowledgment: Work supported by the Agence Nationale de la Recherche (ANR) Grants PolyGlot (PRC ANR-18-CE12-0021) and C-TRAP (PRC ANR-22-CE12-0012).

DNA replication as a target for cancer therapy: where we started and where are we now

Gottifredi, Vanesa
Fundación Instituto Leloir

DNA replication is a source of life and disease. While we need DNA replication in millions of cells per day to maintain the homeostasis of healthy organisms, there is an intrinsic and unavoidable risk in each DNA duplication cycle. DNA is a very reactive molecule, and such a property affects the quality of the DNA templates copied in each replication cycle. If the quality of such templates is sufficiently low, the risk of incomplete DNA duplication is high, jeopardizing cell survival. Such a notion is exploited during traditional cancer therapy, which is particularly hazardous for highly proliferating cells such as those of cancer origin. While this approach is primarily successful in terms of the effectiveness of cell killing, the ability to expand from individual clones that characterize cancer tissues often prompts the survival of remnants and their adaptation to treatments. In the last decade, the increased understanding of cancer biology has given rise to many combinatorial strategies proposed by different laboratories worldwide. In fact, I will share our journey to identify a small peptide derived from the cyclin kinase inhibitor, p21, as a versatile enhancer of different therapeutic agents. However, unless the effectiveness of a given treatment reaches the almost impossible 100% efficacy, the clonal ability and adaptability of cancer remnants remain the challenge that needs to be defeated. Can we then target other properties of cancer, such as its adaptability? It is then critical to focus on the origin of such a cancer property. When highly proliferative cancer cells fail to complete DNA duplication and do not die, they accumulate unstable chromosomes in mitosis, which can recombine in a manner that facilitates the adaptation of daughter cells to the treatment and their escape from the immune system. Many researchers assume that such chromosomal instability is the unavoidable side effect of chemotherapy. The most innovative current approach is combining chemo- and immune-checkpoint therapy to kill the cancer cells attempting to adapt to the chemotherapeutic challenge. However, I will present evidence that challenges the notion of unavoidable chromosomal instability. Some therapeutic agents trigger cell death without augmenting the chromosomal instability. This observation provides tools to explore cancer cell-killing methods that may delay or avoid the adaptation to chemotherapy. Moreover, I will also discuss evidence demonstrating that even when using chemotherapies that induce both cell killing and chromosomic instability, the unwanted latter effect can be prevented without diminishing the efficacy of cell killing. Together, these pieces of evidence suggest that the chromosomal instability could be selectively reduced to prevent/delay the tumor adaptation to the treatment and its escape from the immune system.

Journey through the inner retina of diurnal vertebrates. Seeking for non-canonical photoreceptors and light-inducible molecules

GUIDO, Mario Eduardo

1CIQUIBIC-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, 5000 Córdoba, Argentina. 2Departamento de Química Biológica Ranwel Caputto, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, 5000 Córdoba, Argentina.

Light detection is not only required for vision and image-forming activities, it is also needed for a number of non-visual tasks such as setting the biological clock, pupil reflexes or melatonin inhibition, taking place, most of them, in the vertebrate retina. In fact, the retina is a multilayer-organized tissue that is part of the central nervous system. It is composed of visual photoreceptors (PRCs) (cones and rods) responsible for diurnal/color and nocturnal vision respectively, and a group of non-canonical photoreceptors located in the inner retina specially detecting blue and UV light that were more recently identified. Among them, the first ones to be described in mammals and then, in the avian retina (results from my lab), were the intrinsically photosensitive retinal ganglion cells (ipRGCs) expressing the photopigment melanopsin (Opn4). The ipRGCs are involved in the adjustment of the biological clock by light, the pupillary light reflexes and other non-image forming tasks through their projections to different brain areas. This intrinsic photosensitivity works even in the absence of vision, as we found in the GUCY1 chickens suffering blindness since hatching. Also, we found that another inner retinal cell population, the horizontal cells (HCs) were also photosensitive and such photosensitivity in the blue region was conferred by the photopigment Opn4x (the Xenopus ortholog). My laboratory was the first to characterize the photocascade taking place in these two Opn4-expressing cells (ipRGCs and HCs) which involved a Gq protein, the activation of phospholipase C, Ca2+ mobilization, and depolarization, together with GABA release by HCs. IpRGCs also express the photoisomerase RGR that is responsible for regulating the pool of retinoids in light to provide the chromophore required for Opn4 through an alternative visual cycle. More recently, we have identified that the Muller glial cells (MGCs), the most abundant and multitasking glial cells of the retina, expressed the non-visual photopigments Opn3 (encephalopsin) and Opn5 (neuropsin) in the blue/UV region and respond to light by increasing Ca2+ and AMPc levels. This is the first report to demonstrate that a glial cell in the retina can be photosensitive, although its role is still unknown. In addition, we found that different retinal cell types contain autonomous circadian clocks regulating diverse metabolic and physiological functions. Among the metabolic processes investigated, we first demonstrated rhythms in the metabolic labeling and glycerophospholipid synthesizing enzyme activities in different retinal cell layers synchronized by light/dark cycles. In addition, we observed antiphasic rhythms in melatonin synthesis and expression and activity of AANAT (serotonin-acetyl transferase), the key regulatory melatonin enzyme, between PRCs and RGCs. Indeed, in PRCs melatonin levels and AANAT expression was high during the night whereas in RGCs, they were high during the day. All these findings together support the idea that non-visual photoreceptors and clocks converge together in the same cell populations to finely regulate

retinal function and physiology for the entire organism according to the illumination and environmental/time conditions.

From Neurospora to Neurology: Unravelling the Molecular Pathogenic Mechanism of a Human Neurodegenerative Disease

ROSA, ALBERTO LUIS

Departamento de Farmacología - IFEC-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba

The study of biological phenomena using model microorganisms has consistently made significant contributions to our understanding of the biochemical and molecular foundations of life. Among these organisms, the filamentous fungus Neurospora crassa has played a pivotal role in advancing the fields of biochemistry and molecular genetics, including the historical development of the one gene-one enzyme concept. Our laboratory has conducted research using Neurospora, exploring various aspects of biochemical processes, chromosomal topography and structure, meiotic recombination, control of gene expression, and epigenetic regulation. These studies provided a valuable framework for the development of ideas and experimental approaches to investigate the molecular mechanisms underlying certain human hereditary neurological disorders. In particular, we demonstrated that the protein DUX4, encoded by an apparent pseudogene within a 3.3 Kb repeated element (D4Z4), is cytotoxic and pro-apoptotic, playing a central role in the development of facioscapulohumeral muscular dystrophy (FSHD). Current pharmacological treatments for FSHD aim to control the toxic activity of DUX4. Additional worldwide studies have revealed that DUX4 also plays a crucial role in the early embryonic development of placental mammals, may have a potential endocrine function, and serves as a key regulator in suppressing the immune response to tumors. Scientific breakthroughs often emerge unexpectedly, highlighting the importance of fundamental, discovery-driven research. Scientists should not feel compelled to justify all research solely by its market or translational value.

SYMPOSIUMS

Recent Mass Spectrometry based solutions for Proteomics

Santos, Luiz Fernando
Thermo Fisher Scientific

This presentation will focus on recent advances in the field of proteomics based on high-resolution mass spectrometry. In addition to new equipment, it will also be shown how new accessories and software tools can leverage proteomics (and other "omics") studies.

Solutions for biological drug discovery: Monoclonal antibodies

Salvatore, Esteban
OneLab Argentina. Especialista de Aplicaciones en Sartorius en el área de Bioanalítica.

The development of biological drugs is a long, risky and demanding process, which can take up to 15 years to reach the market. Despite all the effort invested, the probability of failure of these drugs in the clinical trial stage is very high, and at that stage there is already too much effort and time invested to fail. In this way, the more biological and physiological information that can be obtained from potential candidates, the less likely they are to fail and the more likely they are to reach the market. Our bioanalytical tools help the researcher reduce time and costs in the discovery of biological drugs and increase the amount of relevant biological and physiological information.

High-quality nucleic acid extraction: improve extraction in your lab

Pino, Constanza
Thermo Fisher, Argentina

The high quality of nucleic acid extractions ensures subsequent applications in your laboratory. In this symposium, we will look at nucleic acid extraction solutions by exploring the latest magnetic bead technology.

Gilson Micropipettes: Advances, care and maintenance

Costamagna, Julian
Bio Esanco S.A.

The goal of the seminar is to provide information to users about the benefits of using Gilson pipettes for liquid handling in their laboratories. Bio Esanco S.A. has been an official and exclusive distributor for more than 35 years in the Argentine market, providing technical assistance to customers and providing technical service certified by Gilson for the maintenance and calibration of pipettes.
The presentation will begin by giving a historical review of the different models of the brand throughout history. It began in 1972 through the Classic line designed by Warren Gilson, gaining popularity over the years and acquiring the robustness and ergonomics that they present today.
In addition, we will show the different current models of pipettes according to their type of functionality and adaptation to the different samples in a laboratory. Users will know the volume ranges and increments of each of the models.
In the second part of the seminar, general advice will be given to carry out a 2-minute inspection recommended by Gilson, where we will comment on the general aspects that will allow the user to identify and verify the correct operation of the pipettes in their laboratory.
We will comment on the best practices when carrying out pipetting work and the different phases that they entail according to the type of operation that the pipette presents.
Finally, we will provide training on the proper use of pipettes, listing the main consequences that different errors in their handling could cause. These tips will allow users to extend the life of the products.

Unveiling the role of the Golgi apparatus in the NK-cytotoxic immune synapse

Larocca, Maria Cecilia
Instituto de Fisiología Experimental, Facultad de Ciencias Bioquímicas y Farmacéuticas, CONICET - Universidad Nacional de Rosario

Natural killer (NK) cells are the cytotoxic effectors of the innate immune system, which are particularly relevant for the elimination of viral infected and neoplastic cells. The NK cytotoxic effector function relies on the formation of an “immunological synapse” (IS) with the target cells. The formation and maturation of the NK-cytotoxic IS is marked by the polarized localization of NK receptors, signaling molecules and cytoskeletal elements at specific regions within the NK:target cell junction, and the translocation of the centrosome, the lytic granules and the Golgi apparatus (GA) towards this structure. Even though the role of the GA in the polarized distribution of specific proteins has been characterized in various cell types, the participation of this organelle in the maturation of the IS has hardly been investigated. Our recent results using both genetic and pharmacological approaches showed that the integrity of the GA is required for the maturation of the NK:leukemia cell IS. Those studies revealed the

existence of a GA-dependent trafficking pathway toward the IS for the lymphocyte function–associated antigen (LFA)-1, which is an integrin that participates in both NK adhesion to target cells, and initiation of signaling pathways involved in NK activation. We further aimed to characterize the mechanisms underlying GA-dependent LFA-1 polarized trafficking. In migratory cells, neurons, and hepatic-epithelial cells, the GA functions as a "microtubule organizing center" (MTOC). Different studies in those cells indicate that GA-derived microtubules support the directionality of GA-associated polarized trafficking. The fact that the GA polarizes towards the IS during NK cell activation supports a model where GA-derived microtubules contribute to directional vesicle trafficking towards NK-IS. In immune cells, microtubule nucleation has been typically attributed to the centrosome, which, in fact, is commonly denoted as the MTOC. By using ice-recovery assays, we found that NK cells also nucleated microtubules at the GA and that, similarly to what has been characterized in other cell types, A-Kinase Anchoring Protein (AKAP) 350 specifically participated in microtubule nucleation at the GA, whereas cytoplasmic linker-associated proteins 1 and 2 (CLASP1/2) stabilized GA-derived microtubules. Similarly to AKAP350KD NK cells, CLASP1/2KD NK cells exposed to leukemia cells showed a significant decrease in LFA-1 localization at the IS, which was associated with a decrease in centrosome and lytic granules polarization towards this junctional structure. Altogether, our results uncovered a novel role for the GA as a MTOC in NK cells and revealed the relevance of GA-derived microtubules in the maturation of the cytolytic IS. Currently, we are working on the identification of physiological and pharmacological modulators of the GA polarization to the IS that could enhance NK cytolytic activity against neoplastic cells.

The ins and outs of the intracellular life cycle of Brucella

Ugalde, Juan Esteban
Instituto de Investigaciones Biotecnológicas (IIB), Escuela de Bio y Nanotecnologías (EByN), Universidad Nacional de San Martín (UNSAM), CONICET

Brucella is a Gram-negative intracellular pathogen that infects mammals, including humans causing a chronic debilitating illness with great economical and human health consequences. The bacterium has evolved a plethora of mechanisms to invade host cells, avoid degradation in the endocytic pathway, actively multiply within a specialized intracellular compartment, and egress in a programmed manner to initiate a new round of infection in a naïve cell. I will present some of our latest findings on how Brucella adheres and invades host cells, modulates actin polymerization for an efficient intracellular replication and exploits multivesicular bodies to egress and initiate a new intracellular cycle. Our results show that this fascinating bacterium has specific virulence factors that act spatially and temporarily in each of these phases to finely modulate its pathogenicity.

Global cellular organization: membrane trafficking and homeostatic response

*Funes-Chaban, Macarena; Di Giusto, Pablo; Hisse, Emilia; Agüero, Marcos; De La Fuente, Sofía; Rozés-Salvador, Victoria y Alvarez, Cecilia**
Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. CIBICI-CONICET.

CREB3 transcription factors have emerged as signaling hubs for the regulation of numerous genes involved in the secretory pathway and Golgi homeostasis, integrating stimuli from multiple sources to control secretion, posttranslational modifications, and trafficking of proteins. Our laboratory is focused on studying homeostatic mechanisms that are activated when cells must adapt to an increase in intracellular protein transport. Specifically, we have focused on analyzing the participation of CREB3 factors in cellular models that represent different physiological contexts. We used a thyroid follicular cell model to analyze the adaptation of differentiated cells in response to a secretory stimulus. Additionally, we explored cellular adaptation during the neuronal differentiation process. Results indicate that CREB3L1 plays a crucial role facilitating the adjustment of the secretory pathway and the synthesis of thyroid-specific proteins in response to thyroid-stimulating hormone (TSH). Furthermore, in the context of neuronal differentiation, we observed a notable increase in the levels of both CREB3L1 and CREB3L2 throughout the differentiation process. In summary, these findings provide insights into the specific functions of CREB3 factors in cellular homeostasis, contributing to a deeper understanding of their roles in diverse biological processes

Cell Biology and Translational Research: from basic science to the treatment of non-communicable diseases

Jaldin-Fincati, Javier Roberto
Unidad de Conocimiento Traslacional Hospitalaria Dr. Arturo Oñativia (UCT-HAO)

Translation is the term frequently employed to depict the transformation of a biomedical discovery into an intervention that improves health. Translational research promotes the harmonious integration of basic research, patient-centered investigations, and population-based studies, all with the overarching goal of enhancing public health in the long run.
During my postdoctoral training and in my current position at the Unidad de Conocimiento Traslacional Hospitalaria Dr. Arturo Oñativia, my research objective has been to utilize cutting-edge cell biology techniques to generate valuable, innovative insights into common non-communicable diseases that significantly affect our local community, such as obesity, diabetes, and cancer. In pursuit of this goal, I strive to blend the latest advancements in cell biology research with the most recent developments in Translational Medicine.
This holds true when applying advanced bioimaging technologies to investigate adipose tissue disorders and cancer. Obesity stands as one of the most widespread diseases globally and is

associated with an increased risk of developing at least 13 different types of tumors, including thyroid carcinoma (TC). TC is the most prevalent endocrine neoplasm worldwide, with its incidence tripling over the last four decades and continuing to rise. Concurrently, the prevalence of overweight and obesity has also increased. Factors such as chronic low-grade inflammation, altered cytokine levels, insulin resistance, oxidative stress, and hormonal changes observed in obese patients contribute to the initiation and progression of TC.

In this regard, it is important to highlight that the Hospital Dr. Arturo Oñativia (HAO) is a regional reference center for the treatment of patients with thyroid and metabolic disorders in the province of Salta. Statistical data indicate that, over the course of a year, the HAO performs more than 1500 biopsies, about 650 punctures for oncological diagnosis and about 500 consultations for thyroid nodules and multinodular goiters. In addition, about 9000 patients are seen for consultations related to non-communicable diseases.

Hyperspectral Imaging, when coupled with spectral phasor analysis, emerges as a potent tool for investigating the capacity of autofluorescent molecules within tissues to yield molecular signatures reflecting alterations in the tumor microenvironment. These alterations can serve as indicators of progression and aggressiveness in TC among lean and overweight/obese individuals, with the purpose of developing tools that allow a personalized approach in the diagnosis, follow-up, and treatment of this condition.

The Power of Insects: Simple Production of Complex Biopharmaceuticals

Smith, Ignacio
Trebe Biotech

Trebe emerged as a response to a critical need during the COVID-19 pandemic: the production of essential supplies for the diagnosis and treatment of the disease. It is the result of a successful collaboration between a CONICET research group, specialized in the production of recombinant proteins, and the agricultural research company AgIdea, which has a significant capacity for producing insect larvae. Together, we took on the challenge and successfully produced a complex SARS-CoV-2 protein with excellent quality and at a very low cost. This protein became a critical component in the production of a diagnostic kit for a renowned national company and in the production of hyperimmune equine serum with high neutralizing power by the Malbrán Institute.

Trebe uses the BEVS platform to generate recombinant products of high complexity. Our innovative approach involves using lepidopteran larvae as biofactories, eliminating the need for expensive and complex bioreactors. This allows us to obtain high-value biological products at significantly lower costs. It is a completely safe system, as the viral vectors used only infect lepidopterans and are genetically modified not to propagate. This eliminates any environmental risk, as the only method of infection is through microinjection.

Among its advanced developments, Trebe offers antigens for the diagnosis of COVID-19 and Dengue, Sphingomyelinase D for the production of antivenom serums, canine and feline interferons for antiviral

and antitumor treatments. They are also in the development phase of an experimental vaccine and bovine hormones. The company focuses on continuously improving its platform, including an ambitious project to obtain an enhanced viral vector through genetic editing.

A Two-Pronged Attack: Dual Antigen Fusion Protein and Bacterial Adjuvant Strategies Against Trypanosomal Infection

Acuña, Leonardo
Unidad de Biotecnología y Protozoarios. IPE (CONICET-UNSa)

Chagas disease (CD), a neglected tropical disease endemic to northern Argentina that affects over eight million people worldwide, requires the development of novel therapeutics and preventive vaccines against Trypanosoma cruzi. The latest knowledge in biotechnology, immunology, and parasite-host interactions offers new possibilities for addressing this challenge. In recent years, our group has explored innovative strategies for developing an effective CD vaccine by improving both the antigens and adjuvants within the same vaccine formulation. We constructed a fusion protein, N-Tc52/TSKB20, combining the N-terminus Tc52 eliciting humoral responses and TSKB20, with two tandem TSKb20 epitopes derived from the TS protein known for its dominant cellular response. We designed, expressed, and purified a chimeric antigen, which was confirmed using mass spectrometry. Mouse immunization and infection studies showed N-Tc52/TSKB20 is a promising antigen for use in vaccine development, generating low parasitemia, parasitic burden, and organ inflammation, preventing parasite nesting with potent IFNγ-mediated and CD8+ T cell-mediated immunity. Our findings provide insight into the protective mechanisms of N-Tc52/TSkb20, indicating that TSkb20 is a valuable target for vaccine strategies against T. cruzi. We also evaluated bacterial components derived from Lactobacillus acidophilus and Escherichia coli as natural adjuvants carrying the parasitic chimeric antigen with promising results. Advances in novel multicomponent vaccine approaches could enhance the immune response against T. cruzi infection and potentially lead to the development of Chagas vaccines.

Molecular insights into cellulose degradation by native microalgael

Barchiesi, Julieta
Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI-CONICET-UNR)

Lignocellulose is the most abundant natural biopolymer on earth and a potential raw material for the production of fuels and chemicals. Main reports of cellulolytic enzymes came mostly from bacteria and fungi. In this work we have demonstrated the presence of cellulolytic activity in the supernatant of a native Scenedesmus strain. We evaluated the suitable conditions for cellulase secretion, and

demonstrate the ability of the algae to metabolize both carboximethylcellulose (CMC) and cellobiose as alternative source of carbon.
On the other hand, we verified that the hydrolytic activity of the enzyme purified from the algae culture supernatant was predominantly cellobiase. We determined its kinetic parameters and optimum temperature and pH. We observed that the enzyme presented a thermal stability up to temperatures close to 50°C.
Meanwhile, in a bioinformatics approach, we identified the presence of extracellular cellulases in the genome of five Scenedesmus species. Sequence comparison of the different identified cellulases with hydrolytic enzymes from other organisms using multisequence alignments and phylogenetic trees showed that these proteins belong to the families of glycosyl hydrolases 1, 5, 9, and 10. In addition, most of the Scenedesmus cellulases showed greater sequence similarity with those from invertebrates, fungi, bacteria, and other microalgae than with the plant homologs. Furthermore, the data obtained from the three dimensional structure showed that both, their global structure and the main amino acid residues involved in catalysis and substrate binding are well conserved.
On the other hand, with the aim of obtaining transgenic algae with modified cell wall we overexpressed carbohydrate binding modules targeted to Scenedesmus the cell wall. The transgenic lines obtained were larger than wild type, their surface-to-volume ratio was affected, and the large mass promoted sinking. Cell wall components were altered, specifically the pectin layer, which increased the union between cells forming a conglomerate. We observed a greater permeability of the cell wall and an increase in the amount of pectin. Besides, a significant effect was observed on the composition of soluble cellular metabolites, evidencing an increase in the levels of nitrogen-rich amino acids. The identified phenotypes showed the usefulness of these algae and this biotechnological strategy to obtain strains with greater biomass recovery efficiency and an enriched carbohydrate and amino acid profile for different purposes.
Further findings from this study, and recent results on polysaccharide microalgae metabolism, will also be discussed.

EVOLUTIONARY CONSERVED SUBMERGENCE RESPONSIVE TRANSCRIPTION FACTORS INVOLVED IN THE FORMATION OF LATERAL ROOT ORGANS IN LEGUMES

Reynoso, Mauricio A.
Instituto de Biotecnología y Biología Molecular IBBM - FCE - UNLP - CONICET

Oxygen distribution controls the formation of lateral roots (LR) via molecular responses to hypoxic niches that originate during development. Local hypoxia stabilizes transcription factors (TF) that repress auxin induced genes essential for LR development. In addition to LRs, legumes form specialized lateral symbiotic organs known as nodules, where rhizobia are allocated and fix nitrogen in an hypoxic environment. At early stages of the interaction, roots show a significant accumulation of transcripts belonging to the submergence response families (SURF), which are evolutionarily conserved in

angiosperms. Given this evidence and the role of the hypoxic response in the formation of lateral organs, we decided to study families of genes coding for trihelix TFs, which are located in syntenic genomic regions. This family contains the gene Hypoxia Response Attenuator in Arabidopsis, which controls the anaerobic response to hypoxia. To characterize whether these TFs are involved in root nodule symbiosis and lateral root formation, we used the model legume Medicago truncatula. Transcripts for members of this family accumulate in roots after 24 hours post inoculation with rhizobia and remain high in developing and mature nodules. Using Agrobacterium rhizogenes- mediated transformation, we generated transgenic hairy roots that express RNA interference (RNAi) constructs that induce post-transcriptional gene silencing of members of this family. We are currently characterizing the impact of silencing of individual trihelix TFs in the development and density of LRs, the progression of rhizobial infection events, formation and morphology of nodules, and overall root and shoot mass. Similarly, we are characterizing the role of a conserved TF of the Myeloblastosis (MYB) family. Transcripts coding for this TF accumulate both at 60 hours post induction of LRs, in roots after 16 hours post inoculation with rhizobia and remain high in developing and mature nodules. Early results indicate that silencing of these TFs affect the development of lateral organs in roots. Additional experimental evidence involving stable mutants will help to elucidate the role of these conserved TF families in legumes.

The p90 Ribosomal S6 Kinase 1 (RSK1) modulates the Translatome of Glioblastoma in an isoform-specific manner to regulate the Cell Cycle program

Roffé, Martín
CHEO Research Institute, Ottawa, ON

Glioblastoma (GBM), the most common and malignant brain tumor, curtails patient survival (12-15 months) despite ongoing treatments. The p90 ribosomal S6 kinase (RSK) family, downstream target of Ras/ERK signaling, can mediate cross-talk with the mammalian target of rapamycin complex 1 (mTORC1) pathway, thus connecting oncogenic pathways in GBMs. The RSK family has 4 isoforms (RSK1-4), with high homology and substrate redundancy. However, GBMs exhibit an aberrantly high expression of RSK1, but not of the other isoforms, compared to normal brain tissue and lower-grade gliomas, correlating with pro-tumoral immune infiltration. We thus investigated RSK1 isoform-specific roles in GBM cells (LN18) using CRISPR/Cas9 system to knockout RSK1 (RSK1KO), RSK2 (RSK2KO) or both (DKO). RSK3 and RSK4 are not expressed in GBM cells. RSKs are supposed to regulate mRNA translation by directly phosphorylating translation factors (most of them also regulated by mTORC1), but also by phosphorylating proteins involved in the activation of mTORC1. We noted stronger dependence of eIF4B phosphorylation at S422 on RSK1, but not on RSK2 or mTORC1. We performed high-throughput analysis of polysome-associated mRNA of RSK KO cells (translatomics), in the presence or absence of the mTOR inhibitor, Torin1. We identified mRNAs regulated by the RSK family at the isoform level, and also defined the fraction of them that depends on mTORC1 activation. We observed that RSK1, but not RSK2, is

primarily associated with DNA repair and cell cycle expression programs, which includes the translational regulation of CDC45 and TIMELESS mRNAs. Altogether our data suggest that RSK1 inhibition might be a potential therapeutic target for GBM.

Homeostasis control of the histone label H3K9me2 by G9a, REST and CoREST safeguards the growth and survival of human neurons

Wilson, Carlos
Centro de Investigación en Medicina Traslacional Dr. Severo R. Amuchástegui (CIMETSA G.V. al INIMEC-CONICET-UNC), Instituto Universitario de Ciencias Biomédicas de Córdoba.

Epigenetic regulation is critical for many aspects of neuronal life, including growth, physiology, and disease. Accordingly, we recently reported that the repressive mark H3K9me2, catalyzed by the histone methyltransferase G9a, is instrumental for axonal specification, growth, and cortical migration of rodent neurons. Nevertheless, mechanistic aspects remain elusive, including the recruitment of G9a to chromatin to adjust H3K9me2 levels in growing neurons.
The transcriptional repressor REST has been coined as the main nuclear recruiter of G9a in non-neuronal cells, along with the also repressor CoREST. The prevailing view in the field establishes that REST is silenced after neurogenesis, being undetectable in post-mitotic neurons. Nevertheless, new reports have tempered these interpretations, showing an aging-dependent expression of REST in human brains able to reduce neuronal loss by inhibiting apoptosis.
In our laboratory, by culturing human iPSC-derived neurons, quantitative fluorescence microscopy and biochemical assays, we identified a progressive expression of REST in developing hiPSC neurons, along with G9a and CoREST. Suppression of these factors leads to axonal retraction and failures on neuronal growth, suggesting developmental and survival roles. Our data also unveils that REST fine-tunes H3K9me2 levels through a crosstalk mechanism with G9a. Ongoing work aims to test the assembly of a regulatory complex involving REST, CoREST and G9a in this model of study.
In summary, our data stresses the notion that REST is not only expressed during aging but also in developing human neurons, controlling H3K9me2 balance through feed-forward mechanisms. We hypothesize a dynamic behavior of these repressors to timely satisfy the genetic demands of neurons throughout life. Moreover, exploring the regulation of H3K9me2 homeostasis could unveil new molecular insights for the growth, survival, and repair of neurons of the human brain.

Role of α-tubulin acetylation on microtubule structure and dynamics in *Trypanosoma cruzi*

Alonso, Victoria Lucia
Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario

Acetylation on K40 of α-tubulin is conserved from lower eukaryotes to mammals and is associated with microtubule stability. Trypanosoma cruzi, the causative agent of Chagas disease, has a precisely organized cytoskeleton primarily comprised of stable microtubules. Acetylated α-tubulin is the most abundant isoform in this protozoa. The primary acetyltransferase that delivers this modification was identified as Mec-17/ATAT, a Gcn5-related N-acetyltransferase, in mammals. Despite evidence supporting a role for K40 acetylation in microtubule stability, its biological function in vivo is unclear. To study α-tubulin K40 acetylation in *T. cruzi* we employed different genetic manipulation strategies (inducible over-expression of ATAT and mutated versions of K40, knock-out and endogenous tagging by CRISPR-Cas9 of ATAT). We analyzed the phenotypes of the resulting parasites using expansion, confocal and electron microscopy. We also evaluated their cell cycle progression and K40 acetylation levels using flow cytometry. We evaluated their infective capacity in cell culture and their ability to differentiate in vitro. TcATAT is located in the cytoskeleton and flagella and colocalizes with acetylated α-tubulin. We determined that ATAT is essential for the correct progression of the cell cycle, for mitochondrial duplication and for infectivity of *T. cruzi*. Acetylation on K40 of α-tubulin is also important for motility and flagellar morphogenesis. Our results support the idea that tubulin acetylation is crucial for *T. cruzi* replication and differentiation and that TcATAT is responsible for this posttranslational modification.

A long road with FABPs

Esteves, Adriana
Sección Bioquímica, Dpto. de Biología Celular y Molecular, Instituto de Biología, Facultad de Ciencias UdelaR, Uruguay. E-mail: aesteves@fcien.edu.uy

Our work is currently focused on the structure and function of FABPs, taking as models, parasitic flatworms (*Echinococcus granulosus* and *Mesocestoides corti*) and zebrafish (*Danio rerio*) as a vertebrate model.
E. granulosus, is the causative parasite of *Cystic Echinococcosis* (Hydatidosis), a serious health and economic problem that affects both man and livestock. Early diagnostic methods and effective therapies have not yet been developed. We are convinced that that key molecules for the survive of the parasite could be potential targets for drug or vaccine development. The search for key molecules led us to isolate two members of the family of fatty acid binding proteins (FABPs), EgFABP1 and EgFABP2. The crystallographic structure of the recombinant rEgFABP1 protein revealed the characteristic structure of the family. The protein has a ubiquitous localization in parasite cells.
FABPs also acquire relevance in the biology of flatworms since they do not synthesize their own fatty acids, having to import them from their hosts. This fact makes them essential proteins for the survival of the parasites.
Homologues proteins isolated from other parasitic flatworms have shown significant activity protective against experimental infection in model animals, currently being vaccine candidates; one of them is

already in phase III trials. Based on this evidence, we have started a line focused on the production of oral vaccines for dogs.
Knowing that some FABPs give up their ligands to nuclear receptors, we began the search for this type of receptors. We have identified a nuclear receptor in *E. granulosus*, specific of invertebrates, that could be candidate for anthelmintic drugs. The functional link between EgFABP1 and this receptor is under investigation.
On the other hand, the role of FABPs in higher animals is not well known, providing this fact with additional interest to the study of the structure/function of these proteins. For this purpose, we selected zebrafish as a vertebrate model. We have focused on the study of the role of the intestinal and liver type of FABPs and their role in enterocyte lipid uptake. Immunolocalization studies indicate that both proteins are located in the cytoplasm and nucleus of enterocytes. Recently we identified a structural signal that would be responsible for nuclear translocation. The role of these proteins in the nucleus is a subject under investigation as well as the study of the enterocyte transcriptome in different alimentary conditions, and the response of the FABPs before diets with different lipid content. Gaining a deeper understanding of the molecular mechanism of action of these proteins may create new therapeutic windows for the treatment of diseases characterized by disrupted lipid metabolism.

The Aging Brain and Altered Cholesterol Trafficking: Reversing the Niemann-Pick C Phenotype of Old Astrocytes with Cannabinoid Treatment

Martín, Mauricio G.
Instituto de investigación Médica Mercedes y Martin Ferreyra | INIMEC - CONICET Córdoba, Argentina.

Cholesterol is crucial for the proper functioning of eukaryotic cells, especially neurons, which rely on cholesterol to maintain their complex structure and facilitate synaptic transmission. However, brain cells are isolated from peripheral cholesterol by the blood-brain barrier and mature neurons primarily uptake the cholesterol synthesized by astrocytes for proper function. This study aimed to investigate the effect of aging on cholesterol trafficking in astrocytes and its delivery to neurons.
Using in vitro and in vivo models of aging, we found that aged astrocytes accumulated high levels of cholesterol in the lysosomal compartment, and this cholesterol buildup can be attributed to the simultaneous occurrence of two events: decreased levels of the ABCA1 transporter which impairs ApoE-cholesterol export from astrocytes, and reduced expression of NPC1, which hinders cholesterol release from lysosomes. We show that these two events are accompanied by increased microR33 in aged astrocytes, which is known to downregulate ABCA1 and NPC1. In addition, we demonstrate that the microR33 increase is triggered by oxidative stress, one of the hallmarks of aging.
By co-culture experiments we also show that aging in vitro impairs the cholesterol delivery from astrocytes to neurons. Remarkably, we found that this altered transport of cholesterol could be alleviated through treatment with endocannabinoids as well as cannabidiol or CBD. Given that reduced neuronal cholesterol affects synaptic plasticity, the ability of cannabinoids to restore cholesterol transport from aged astrocytes to neurons holds significant implications in the field of aging.

Unraveling the link between the two fatty acid synthase systems in *Mycobacterium tuberculosis*

Gago, Gabriela

Instituto de Biología Molecular y Celular de Rosario, IBR, CONICET, Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR, Rosario, Argentina.

Mycobacterium tuberculosis, the etiologic agent of tuberculosis in humans, continues to be a major health problem worldwide. One of the main features of this pathogen is the complex and dynamic lipid composition of the cell envelope, which adapts to the variable host environment and defines the fate of infection by actively interacting with and modulating immune responses. However, while much has been learned about the enzymes of the numerous lipid pathways, little knowledge is available regarding the proteins and metabolic signals that modulates lipid metabolism during M. tuberculosis infection. Our work is directed to fulfill some of these gaps by studying in detail the components and mechanisms that regulate lipid homeostasis in M. tuberculosis, how they impact in the biosynthesis and composition of its cell wall and how the host-pathogen lipid metabolisms interact.

Mycobacteria are unusual in possessing two fatty acid synthase (FAS) systems involved in fatty acid and mycolic acid (MA) biosynthesis. We have characterized FasR and MabR, transcriptional activators of the FAS I and FASII system respectively and demonstrated that these two systems are strictly co-regulated in order to maintain lipid homeostasis in mycobacteria. They are connected by the enzyme FabH, which catalyzes the condensation of malonyl-ACP with the C16-18-CoAs produced by FAS-I; giving rise to a β-ketoacyl-ACP that enters the FAS-II system. Our working hypothesis is that the coordinated regulation of the FAS I and FAS II systems is essential for the viability and normal development of mycobacterial metabolism. In order to characterize the physiological role of the FabH enzyme in mycobacteria and analyze its impact on lipid synthesis, viability and virulence, we constructed a null mutant strain in the fabH gene in M. tuberculosis H37Ra.

Our results indicated that the fabH gene is not essential for M. tuberculosis H37Ra growth and through TLC analyses we observed that the synthesis of MA is only partially decreased in the mutant strain. However, there are no reports of enzymes or alternative pathways that can generate derivatives of acyl-ACPs to initiate the synthesis of MA in the FAS-II system, suggesting that some pieces are missing in the current proposed model for MA biosynthesis. In order to identify enzymes or pathways that could overcome the absence of FabH, we performed a bottom-up proteomic experiment. We found modifications in several enzymes involved in lipid metabolism like β-oxidation and complex membrane lipid and triacylglycerol biosynthesis. In addition, a protein of unknown function, postulated as a link between MA synthesis and β-oxidation, showed increased levels in the mutant strain. We believe that this enzyme could be responsible for bypassing the FabH enzyme, which would explain the ability of this mutant to continue synthesizing MA. We are currently constructing an M. tuberculosis double mutant strain in order to analyze MA biosynthesis.

Endogenous and pathological functions of sphingolipids as revealed by genetic mutations

Sandhoff, Roger
Research Group Lipid Pathobiochemistry, German Cancer Research Center, Heidelberg, Germany

Sphingolipids are a major lipid class in vertebrates with a high structural and functional diversity. In cellular membranes sphingolipids facilitate the formation of membrane-associated protein networks, they modulate signaling processes, and they affect cell-cell interactions. Soluble sphingolipid derivatives are important direct mediators of cellular signaling processes. By that sphingolipids impact cellular growth, differentiation, apoptosis, cell-cell interactions, inflammation, and chemotaxis. In addition, sphingolipids are important components of organized tissues structures like the lipid-based skin barrier or the myelin sheaths. Consequently, sphingolipid patterns are tissue- and cell type-specific and can vary in differentiation, activation, or transformation. This implies cell-specific adaptation and regulation of sphingolipid metabolism. Therefore, genetic disorders of sphingolipid anabolism and catabolism affect certain cell types and structures more than others and lead to clinically heterogeneous pathologies. However, defects in genes affecting the same physiological process often give rise to similar pathologies.

Copper homeostasis in bacteria, system biology meets biochemistry

Argüello, José M.
Worcester Polytechnic Institute, Worcester, MA, USA

Bacterial Cu+ homeostasis enables both the precise metallation of diverse cuproproteins and the control of variable metal levels. To this end, protein networks mobilize Cu+ to cellular targets with remarkable specificity. However, there is a fragmented understanding of these processes. We are using bioinformatics, genome wide transcriptomic analysis (RNA-Seq) and mathematical models of compartmental Cu+ distribution based on uptake kinetics, to describe Cu+ homeostasis in bacteria from a whole system point of view. Experimental studies in Pseudomonas aeruginosa and Salmonella enterica will be presented to describe the influence of the various Cu+ sensors, compartmental chaperones, and transmembrane transporters on the Cu+ distribution. In turn, these observations will provide support of a comparative analysis of different molecular architectures that have evolved in micro-organisms to solve the distribution of an essential but highly reactive ion.

Extremophile microorganisms from northern Argentina as a source of enzymes of biotechnological interest

Gonzalez, Javier M.
Instituto de Bionanotecnología del NOA, CONICET, UNSE, Santiago del Estero, Argentina

In our group we explore the metabolic diversity of extremophile microorganisms native to northern Argentina in search for enzymes with biotechnological applications. In this presentation we will focus on two areas in which we have been working lately: the Structural Biology of fixation and assimilation of one-carbon compounds as a sustainable source of value-added chemical products; and the enzymatic detection of glyphosate in soil and water samples.

There are several electrochemical methods to sustainably reduce carbon dioxide into methanol, contributing to a reduction of the harmful effects of this gas on global climate. Unfortunately, methanol itself has few applications in the chemical industry. In this context, metabolic pathways for the assimilation of one carbon compounds in methylotrophs such as the Serine pathway of Methylorubrum, offer an attractive alternative because they allow the transformation of methanol into a wide variety of biomolecules with added value. In principle, the heterologous expression of key enzymes of these pathways makes it possible to transfer such metabolic potential between different organisms. However, such enzymes often require some kind of optimization because their kinetic and biophysical properties do not necessarily fit the new host biochemical environment. Therefore, the application of Protein Engineering techniques to modify the properties of an enzyme constitutes a fundamental tool for the optimization of synthetic metabolic pathways.

The Salina de Ambargasta to the south of Santiago del Estero, is a saline plain of more than 9000 Km2, with some seasonal salty lagoons that potentially harbor extremophile microorganisms adapted to metabolize xenobiotics present in aquifers contaminated with agricultural waste. Among them, glyphosate—the most widely used organophosphate herbicide worldwide, strongly questioned for its toxic effects in humans—is a xenobiotic often used excessively (partly due to the emergence of resistant weeds), giving rise to its presence in all kinds of fruits, vegetables, honey, textiles, and other derivatives. We seek to obtain an autochthonous variant of the enzyme glyphosate oxidase, capable of degrading glyphosate by oxidation with molecular oxygen, using a mechanism of action similar to that of glycine oxidases. This enzyme will be useful for development of glyphosate biosensors, such as electrochemical devices or test strips.

Intrinsically disordered viral proteins: Mechanistic and structural insights on how they mediate cell cycle hijack

Chemes, Lucía B
Laboratorio de Estructura, Función y Plasticidad de Proteínas, Instituto de Investigaciones Biotecnológicas, UNSAM, Campus Miguelete

Proteins contain intrinsically disordered regions (IDRs). Opposed to protein domains, where a defined structure mediates function, IDRs lack a defined structure and remain highly flexible under physiological conditions. IDRs represent over 40% of the human proteome and play key roles in cell signaling, transcriptional regulation and other essential cell functions. IDRs are implicated in human diseases including Alzheimer, Parkinson and Cancer. Recently, it has become clear that viral pathogens exploit protein disorder to efficiently infect and replicate within host cells. IDRs are packed with functional elements called Short linear motifs or SLiMs. SLiMs are 5-10 amino acids long and can mediate post-translational modifications, subcellular cell targeting and protein-protein interactions. Many viruses harbor proteins with IDRs containing multiple SLiMs, which allows them to pack many functions within their protein sequences. Moreover, SLiMs can be acquired quickly during evolution since they can appear and disappear with a few point mutations. Therefore, SLiMs represent good substrates for the evolution of new functions in viral proteins. In this work, we used the adenovirus E1A and papillomavirus E7 proteins from DNA tumor viruses as model systems to study the evolution of hijack functions by viral IDRs. We found that viral SLiMs evolve quickly and that changes in SLiMs are associated with viral evolutionary events such as host switches, suggesting that motif gains and losses underlie viral adaptation. Using the E1A protein as a model system, we demonstrated that two SLiMs tethered by a flexible linker evolved to optimize the binding affinity of E1A for a host transcription factor. We identify a molecular mechanism that optimizes tethering across a large family of poorly conserved E1A linkers by allowing compensatory changes in linker sequence composition and length that preserve IDR dimensions. These studies elucidate how viral proteins use IDRs to optimize binding and tethering functions, enabling an efficient hijack of the host machinery. The mechanisms we identify in viral IDRs explain the conservation of functions in variable IDRs and may underlie the evolution of many disordered protein regions.

Novel molecular tools for synthetic biology approaches in environmental bacteria

Guazzaroni, María Eugenia
Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, FFCLRP-USP, São Paulo, Brasil

Building an economy centered on biotechnology, agriculture, and biodiversity is crucial for replacing products derived from fossil sources. In order to achieve this, the search and development of new microorganisms are essential for establishing sustainable industrial processes. In this context, the concept of bioeconomy, which involves substituting non-renewable raw materials with sustainable biological resources in industrial processes, is becoming increasingly relevant. It serves as a guiding factor for industrial activity, promoting sustainability and tapping into the enormous economic potential inherent in the South American region's abundant biodiversity.
Microorganisms, referred to as "microbial cell factories" in this context, play a significant role in transitioning from a petrochemical-based economy to a sustainable one. Thus, the search and development of new biocatalysts, particularly those involving environmental bacteria, are crucial for

establishing sustainable industrial processes. In the past decade, advancements in various disciplines have enabled metabolic engineers and synthetic biologists to design and implement the production of diverse biomolecules in microorganisms. However, no microbial host currently possesses all the desirable characteristics for industrial fermentations. Even widely used microorganisms in metabolic engineering, such as Saccharomyces cerevisiae and Escherichia coli, have inherent limitations and are not universally applicable. Therefore, it is crucial to search for microorganisms with different biochemical properties and develop appropriate molecular tools for their manipulation. This presentation aims to emphasize the importance of prospecting new bacterial chassis for biotech applications, along with the search for biological parts (e.g., promoters, transcriptional terminators, genes conferring resistance to industrial stresses), and the development of molecular tools for environmental bacteria with emerging industrial properties.

Structural characterization of enzymes involved in riboflavin biosynthesis in Brucella for the rational design of drugs against human brucellosis

Klinke, Sebastián; Serer, María I.; Carrica, Mariela del C.; Bonomi, Hernán R.; Cerutti María L. ; Goldbaum, Fernando A.

Fundación Instituto Leloir, IIBBA-CONICET, Buenos Aires, Argentina

The pathogenic bacterium Brucella abortus, a Gram-negative microorganism that causes the severe zoonotic disease brucellosis, relies exclusively on its endogenous synthesis of riboflavin (vitamin B2). The absence of this metabolic pathway in mammals highlights the enzymes involved in the biosynthesis of this vitamin as attractive targets with therapeutic potential. We have demonstrated that the deletion of certain genes that encode for enzymes of this pathway results in a significantly reduced virulence phenotype, with B. abortus being rapidly eliminated by the host in cellular and animal infection models. Our laboratory has a strong background in X-ray crystallography for the determination of the three-dimensional structure of macromolecules. By means of this technique, we have thoroughly characterized three of the enzymes of the riboflavin pathway in B. abortus: Lumazine synthase, 5-Amino-6-ribosylamino-2,4(1H,3H)-pyrimidinedione 5'-phosphate reductase, and Riboflavin synthase. Based on these achievements, this project aims at the rational design of drugs against human brucellosis by the combination of high-throughput screening and structure-based drug design.

Plant Long Noncoding RNAs - From Molecular Mechanisms to Agricultural Advancements

Ariel, Federico

Instituto de Agrobiotecnología del Litoral, CONICET, Universidad Nacional del Litoral, Colectora Ruta Nacional 168 km 0, 3000, Santa Fe, Argentina.

RNA-DNA hybrid (R-loop)-associated long noncoding RNAs (lncRNAs), including the Arabidopsis lncRNA AUXIN-REGULATED PROMOTER LOOP (APOLO), are emerging as important regulators of three-dimensional chromatin conformation and gene transcriptional activity. We showed that APOLO interacts with the PRC1-component LIKE-HETEROCHROMATIN PROTEIN 1 (LHP1) and the methylcytosine-binding protein VARIANT IN METHYLATION 1 (VIM1), a conserved homolog of the mammalian DNA methylation regulator UBIQUITIN-LIKE CONTAINING PHD AND RING FINGER DOMAINS 1 (UHRF1). The APOLO-VIM1-LHP1 complex directly regulates the transcription of the auxin biosynthesis gene YUCCA2 by dynamically determining DNA methylation and H3K27me3 deposition over its promoter during the plant thermomorphogenic response. We established that the lncRNA UHRF1 Protein Associated Transcript (UPAT), a direct interactor of UHRF1 in humans, can be recognized by VIM1 and LHP1 within plant cells, despite the absence of sequence homology between UPAT and APOLO. In addition, we showed that increased levels of APOLO or UPAT hamper VIM1 and LHP1 binding to YUCCA2 promoter. Strikingly, the application of exogenous in vitro-transcribed APOLO on Arabidopsis plants leads to alterations in auxin homeostasis and modifies the plant's responsiveness to external stimuli. Collectively, our results uncover a new mechanism in which a plant lncRNA orchestrates the interplay between Polycomb-mediated repression and DNA methylation. Moreover, our study underscores the intriguing phenomenon that evolutionarily unrelated lncRNAs can serve analogous functions across diverse biological kingdoms. Beyond this, our research expands the horizons for leveraging lncRNAs as bioactive agents, presenting innovative prospects for devising sustainable agricultural strategies.

Transcriptional networks integrating drought stress and nitrogen signaling in plants

Dr. José Miguel Alvarez
Centro de Biotecnología Vegetal, Universidad Andrés Bello, UNAB, Millennium Institute for Integrative Biology, iBio, Chile

Drought and changes in nitrogen (N) availability are major factors limiting plant growth and crop productivity. The dissection of the transcriptional networks' components integrating drought stress and N responses provides valuable insights into how plants effectively balance stress response with growth programs. Given their importance, the molecular mechanisms that plants rely on to signal either N or drought have been under intense scrutiny. However, how plants sense and respond to the combination of N and drought signals at the molecular level has received scant attention. By utilizing the wealth of publicly available RNA sequencing data for Arabidopsis thaliana, we predict and compare regulatory connections between these signals. Searching online repositories, we found over 500 sequencing libraries relating to N, drought, and the stress hormoneabscisic acid (ABA) treatments. We completed and normalized their metadata profiles, allowing for universal comparisons of treatment details and

regimes. Differentially expressed genes (DEGs) were derived for each experiment and further filtered to find consistently regulated genes for a given treatment. We found high and significant overlaps for N, ABA, and drought treatment genes. More importantly, N and drought-regulatedgenes show a strong negative correlation, indicating these are opposite signals. Network-based analysis reveals common transcription factors (TFs) mediate N and drought gene responses. The NIN-LIKE PROTEIN 7 (NLP7), a master regulator of the N signaling pathway, was identified as a candidate TF to modulate N and drought interaction genes. Using a combinatorial experiment that varies N and drought levels simultaneously to quantify transcriptomes, we found that N dose impacts 55% of gene responses to drought, and water availability impacts 88% of N responses in Arabidopsis leaves. Most genes with altered expression in nlp7 mutant plants respond to N and drought, indicating NLP7 has a relevant role in integrating N and drought signals in leaves. Using a cell-based assay to capture direct TF regulation genome-wide in leaves, we demonstrated that NLP7 directly controls the expression of known N and drought TFs, such as LOB domain-containing protein 37 (LBD37), and Homeobox Protein 6 (HB6), respectively, which regulate downstream NLP7-dependent responses. Our validation studies show that NLP7-dependent pathways account for 85% of the interaction between N and drought. The nlp7 mutant plants exhibit a drought-resistance phenotype influenced by the N availability in the soil. Taken together, our results reveal that convergent regulatory circuits underlie plant responses to the conflicting N and drought signals. This advances our understanding of how plants tilt the balance toward stress responses or growth regulation and empowers targeted genetic modification strategies to enhance plant development and stress resistance, critical traits for optimizing crop yield and promoting sustainable agriculture.

PHOSPHOLIPASE C IN PLANT DEFENSE

Laxalt A.M., Robuschi L, Perk E., D'Ambrosio J.M.
Instituto de Investigaciones Biológicas (IIB-CONICET-UNMdP)

Phosphoinositide-specific phospholipase C (PI-PLC) plays an important role in signal transduction during plant development and in the response to various biotic and abiotic stresses. However, how PI-PLCs are regulated and how they control these processes remains to be fully understood. Gene families encode PLC enzymes. The hypothesis is that different PLCs participate in signaling induced by different types stress and during development. In Arabidopsis, the PI-PLC gene family is composed of nine members (AtPLC1 to AtPLC9), being AtPLC2 the most abundant isoform that gets rapidly phosphorylated upon pathogen recognition. We showed that AtPLC2 is involved in plant defense responses, stomatal closure, gametophyte development and embryogenesis. To gain insights into

PLC-regulators, we characterized the interactome of AtPLC2 by TurboID proximity-dependent biotin labeling. A total of 167 candidates were enriched. Pathway analysis showed a significantly enriched in protein modification, calcium regulation and receptor kinases. In tomato, the PI-PLC gene family is composed of seven memers (SlPLC1 to SlPLC7). Tomato plants transiently silenced in different PLC isoforms showed different susceptibility to pathogens such as Botrytis cinerea, Phytophthora infestans, Cladosporium fulvum, Verticillium dahliae and Pseudomonas syringae. However, assessing the overall plant fitness was limited in transiently silenced plants. Consequently, we employed CRISPR/Cas9 technology to generate transgene-free loss-of-function SlPLC2 mutants in tomatoes. These mutants showed reduced reactive oxygen species (ROS) levels, altered expression of defense-related genes, decreased susceptibility to Botrytis cinerea and Phytophthora infestans, while susceptibility to Pseudomonas syringae remained unchanged. Our aim is to generate transgene-free PLC loss-of-function tomato mutants, in order to improve plant resistance to pathogens and to study the role of each PLC on plant stress and development.

Photosynthesis modulation in the global ocean

Juan José Pierella Karlusich
AS Division of Science, Harvard University, Cambridge, MA, USA -

Microalgae contribute as much to global photosynthesis as terrestrial plants and are vital to aquatic food chains, as well as to the sequestration of atmospheric CO2 in the ocean depths via gravitational sinking of particles (the biological carbon pump). Certain species further enrich ecosystems by transforming atmospheric nitrogen (N2) into more usable forms, acting as natural fertilizers. Despite their profound impact on aquatic ecosystems and biogeochemistry, there are significant gaps in our understanding of their physiological responses to human-induced global changes, such as warming, increasing CO2, ocean acidification, and nutrient cycle disturbances. Leveraging massive and diverse datasets from the Tara Oceans expeditions (omics, bio-optical/imaging, physicochemistry) and satellite remote sensing, we determined patterns and drivers of microalgal abundance, diversity, adaptation, and acclimation in the global ocean. These results provide valuable information for improving our understanding of an Earth system under anthropogenic change.

Allosteric regulation of the phosphorylation reader 14-3-3

Bustos, Diego
Instituto de Histología y Embriología de Mendoza (IHEM) - Universidad Nacional de Cuyo - CONICET, Mendoza 5500, Argentina; Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Mendoza 5500, Argentina

14-3-3 proteins are a family of conserved regulatory proteins found only in eukaryotic organisms. These proteins are extraordinarily versatile and play critical roles in various cellular processes, including signal transduction, cell cycle control, and apoptosis (programmed cell death). They are involved in regulating numerous proteins and are known to interact with various targets, including enzymes, transcription factors, kinases, and receptors. As we initially discovered, 14-3-3 proteins function by binding to specific target proteins exclusively through interactions with an intrinsically disordered region containing a phosphorylatable serine/threonine residue. This binding can lead to a variety of outcomes, including changes in protein activity, localization, stability, or interaction with other molecules. By modulating the function of their binding partners, 14-3-3 proteins contribute to the regulation of various cellular processes and the maintenance of homeostasis. Originally thought to be non-regulated housekeeping proteins, we have discovered a novel allosteric site that induces a conformational change in 14-3-3 depending on its paralog. Our study reveals a dynamic conformation switch in which the affinity of 14-3-3 for its phosphorylation substrate changes dramatically in the presence or absence of a small molecule.

Impact of dengue virus on the expression of PML mRNA isoforms

Gaioli, Nicolás (1) ; Garcia Cybele (2) and Srebrow, Anabella (1)
(1) IFIBYNE-UBA-CONICET; FBMC-FCEN-UBA and (2) IQUIBICEN-UBA-CONICET; QB-FCEN-UBA

Our laboratory seeks to understand how dengue virus infection modulates host cell gene expression. We have focused on the action of this virus on promyelocytic leukemia protein (PML) pre-mRNA processing and post-translational regulation of PML protein variants. Although the extensive literature available so far only refers to alternative splicing as the source for PML isoforms, a thorough analysis indicates that they are generated by the combination of alternative splicing and alternative polyadenylation. The PML gene is not only considered an ISG (interferon stimulated gene), induced during the innate immune response, but also certain PML protein isoforms have been described as viral restriction factors. In particular, PML III and IV limit dengue virus replication in human cell lines. Results from our group show that infection with this virus differentially alters the expression of PML isoforms, significantly decreasing the levels of PML IV mRNA at early time points and limiting the increase of this transcript during the process of viral proliferation, in contrast to what we observed for the other PML mRNA variants.
Considering the antiviral role of PML IV protein mentioned above, this regulatory phenomenon could be part of the multiple strategies deployed by dengue virus to counteract or evade the innate cellular

immune response. We are exploring the molecular mechanism and the viral and host cell components responsible for the differential regulation of PML IV mRNA expression upon infection, giving special attention to the hitherto overlooked alternative polyadenylation of PML pre-RNA.

Chromatin dynamics – one molecule at a time

Kaplan, Ariel
Technion - Israel Institute of Technology, Israel

The inherent ensemble-averaging of traditional "bulk" methods poses a barrier to a mechanistic understanding of the different factors that shape the architecture of chromatin, and the mechanisms by which this architecture modulates the expression of specific genes. Using single-molecule optical tweezers experiments, we study the roles that DNA sequence, histone variants, and epigenetic markers play in modulating the structure and dynamics of nucleosomes and chromatosomes, the binding of transcription factors, and the elongation by RNA polymerase.

Dicer-p53 Axis: A Key Regulator of Systemic Adaptation to Nutrient Stress

Dekanty, Andrés
Instituto de Agrobiotecnología del Litoral, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Santa Fe, Argentina. Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral (UNL), Santa Fe, Argentina.

The adipose tissue plays a crucial role in metabolism and physiology, affecting animal lifespan and susceptibility to disease. In this study, we present evidence that adipose Dicer1 (Dcr-1), a conserved type III endoribonuclease involved in miRNA processing, plays a crucial role in the regulation of metabolism, stress resistance, and longevity. Our results indicate that the expression of Dcr-1 is tightly regulated in the Drosophila fat body, analogous to human adipose and hepatic tissues, under various stress and physiological conditions such as starvation, oxidative stress, and aging. The specific depletion of Dcr-1 in the Drosophila fat body leads to changes in lipid metabolism, enhanced resistance to oxidative and nutritional stress, and is associated with a significant increase in lifespan. Moreover, we provide mechanistic evidence showing that the JNK-activated transcription factor FOXO binds to conserved DNA-binding sites in the dcr-1 promoter, directly repressing its expression in response to nutrient deprivation. Our findings emphasize the importance of FOXO in controlling nutrient responses in the fat body by suppressing Dcr-1 expression. This mechanism coupling nutrient status with miRNA biogenesis represents a novel and previously unappreciated function of the JNK-FOXO axis in physiological responses at the organismal level.

ORAL COMMUNICATIONS

Cellular Biology

CB-01

EFFECTS OF GENETIC VARIANTS IN GUANINE QUADRUPLEXES OF RNA THAT IMPACT THE TRANSLATION OF HUMAN ONCOGENES

Bezzi, Georgina[1]; Piga, Ernesto[1]; Binolfi, Andres[1,2]; Armas, Pablo[1].
[1]Instituto de Biología Molecular y Celular de Rosario (IBR), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de Rosario (UNR), Rosario S200EZP, Santa Fe, Argentina. [2]Plataforma Argentina de Biología Estructural y Metabolómica (PLABEM), Rosario (UNR), Rosario S200EZP, Santa Fe, Argentina.
bezzi@ibr-conicet.gov.ar

Guanine quadruplexes (G4) are non-canonical secondary structures of nucleic acids formed within guanine-rich sequences, predominantly found in regulatory regions of gene expression. G4 structures have been described mainly in oncogenes as secondary RNA structures relevant for translation regulation when present in 5' untranslated regions (5'UTRs) or other mRNA regions involved in translation control. Moreover, genome-wide association studies through massive DNA sequencing revealed numerous single nucleotide variants (SNVs) associated with human diseases overlapping with 5' UTR regions. The aim of this study was to identify SNVs overlapping with G4-forming sequences (sG4) previously described as translation regulators (located within 5'UTRs) that could potentially affect G4 folding. These SNVs are referred to as G4-Vars. Initially, we conducted a bioinformatics analysis using the Ensembl database to identify SNVs overlapping with sG4 (and their flanking sequences of +/- 5 b) described as translational regulators within 14 oncogenes. For each reference sequence, we generated a collection of variant sequences representing each SNV and a sequence with mutations that disrupt G4 formation capability. Subsequently, various DNA and RNA G4 folding and stability predictors were employed to identify G4-Vars that might affect G4 folding or stability, both in sG4 and within the context of 5'UTR. Out of the 245 RNA sequences corresponding to G4-Vars from the analyzed oncogenes, 15 were selected for further in vitro analysis. Using Circular Dichroism (CD) spectroscopy, we demonstrated that certain SNVs cause quantitative and qualitative spectral changes. Additionally, CD melting assays indicated that SNVs induce stability changes in G4 structures. This finding was further corroborated by 1D1H NMR spectroscopy, confirming quantitative and qualitative changes induced by SNVs in G4-Vars of NRAS, ZIC1, and TRF2 genes. The sG4s of these three oncogenes were cloned into the psiCHECK-2 vector

along with their variants and mutated versions. Furthermore, for NRAS and TRF2, complete 5'UTRs containing the analyzed sG4 were cloned into the psiCHECK-2 vector, and their variants and mutated versions were generated. These constructs were transfected into HEK293 cells. The results revealed that SNVs alter luciferase reporter activity by affecting translation levels. The outcomes of this study suggest that G4-Vars impact G4 folding and might be the underlying cause of differential oncogene expression, leading to predisposition, establishment, progression, or metastasis of tumors. This indicates that G4-Vars could function as driver mutations in cancer and should be considered a novel molecular etiology mechanism for predisposition or establishment of human diseases.

Keywords: G-quadruplexes, Oncogenes, SNV, Translation control, HEK293

Methods: Circular Dichroism (CD) spectroscopy, CD melting assays, Nuclear Magnetic Resonance (NMR) spectroscopy, Cell Transfection, Luciferase reporter assays

CB-02

Unleashed loading of specialized DNA polymerase PrimPol to replicating DNA increases genomic instability.

Sabrina F Mansilla; Nicolás L Calzetta*; Sofía Venerus Arbilla; Yiovana Verónica Okraine; Sebastián Omar Siri; Lilen Caimi1; Ariel Abramovici Blasco and Vanesa Gottifredi. *equal collaboration.*
Fundación Instituto Leloir. Instituto de Investigaciones Bioquímicas de Buenos Aires. Consejo Nacional de Investigaciones Científicas y Técnicas
smansilla28@gmail.com

DNA replication is challenged by the frequent generation of DNA damage caused by both endogenous and exogenous sources. These lesions alter the DNA tridimensional structure in a manner that they become replication barriers that stall replicative DNA polymerases. DNA replication stalling is detrimental to cell survival. To avoid persistent blockage of replication forks, auxiliary DNA replication processes collectively known as DNA Damage tolerance (DDT) are activated. The DDT promotes DNA replication continuity by recruiting translesion DNA synthesis (TLS) polymerases that accommodate replication barriers in their active sites. Fork reversal (FR) is another DDT option which changes the fork structure from a three to a four-way junction in a manner that promotes a recombinogenic bypass of DNA lesions. Yet another DDT option involves repriming (RP) or discontinuous DNA synthesis mediated by another DNA polymerase, PrimPol (PP), with the capacity to initiate DNA synthesis downstream of the blocking lesion. When correctly activated at DNA lesions, DDT activation is key to promote cell survival

and genomic stability upon DNA damage. However, the excess participation of these processes in normal DNA replication can jeopardize DNA replication fidelity causing point mutagenesis or complex rearrangements of the genome. Intriguingly, we have recently demonstrated that another DNA polymerase, Pol iota, prevents the accumulation of chromosome instability (CIN) by favoring a DDT pathway related to FR and preventing PrimPol-mediated repriming. Also, the TLS inhibitor, p21, is a negative regulator of PrimPol participation in DNA synthesis. Partial loss of endogenous p21 levels suffices to upregulate PrimPol mediated DNA synthesis augmenting CIN. Together our results unravel that the correct interplay of different DDT factors, all converging into the preclusion of PrimPol-mediated repriming during normal DNA replication, is crucial to prevent CIN.

Keywords: DNA Replication, Nascent DNA Elongation; Pol iota, Prim Pol, Genomic instability

Methods: Cell culture, Inmunofluorescence, DNA Fiber Assay, Anaphase Aberrations

CB-03

The exocyst complex controls multiple events in the pathway of regulated exocytosis

Suárez Freire, Sofía; Pérez-Pandolfo, Sebastián; Fresco, Sabrina M.; Wappner, Pablo; Melani, Mariana
Instituto Leloir, CONICET
melanimari@gmail.com

Eukaryotic cells depend on exocytosis to direct intracellularly synthesized material towards the extracellular space or the plasma membrane, so exocytosis constitutes a basic function for cellular homeostasis and communication between cells. The exocytic process comprises several steps that include biogenesis of the secretory granule (SG), maturation of the SG, and finally, its fusion with the plasma membrane, resulting in release of SG content to the extracellular space. The larval salivary gland of Drosophila melanogaster is an excellent model for studying exocytosis. This gland synthesizes mucins that are packaged in SGs that sprout from the trans-Golgi network and then undergo a maturation process that involves homotypic fusion, condensation and acidification. Finally, mature SGs are directed to the apical domain of the plasma membrane with which they fuse, releasing their content into the gland lumen. The exocyst is a hetero-octameric complex that participates in tethering of vesicles to the plasma membrane during constitutive exocytosis. By precise temperature-dependent graded activation of the Gal4-UAS expression system, we have induced different levels of silencing of

exocyst complex subunits, and identified three temporarily distinctive steps of the regulated exocytic pathway where the exocyst is critically required: SG biogenesis, SG maturation and SG exocytosis. Our results shed light on previously unidentified functions of the exocyst along the exocytic pathway. We propose that the exocyst acts as a general tethering factor in various steps of this cellular process.

Keywords: Drosophila melanogaster, Exocytosis, Exocyst complex, Secretion, Secretory granule

Methods: Drosophila genetics, confocal microscopy, live imaging

CB-04

Plasma membrane nanodomains disassembly increases yeast's lifespan

Salzman, Valentina[1]; Bustamante Torres, Moises[1]; Pellizza, Leonardo[2]; Aran, Martín[2]; Aguilar, Pablo S[1].
[1]Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE-UBA-CONICET.) CABA. 2 IIBB (CONICET)- [2]Fundación Instituto Leloir
vsalzman@fbmc.fcen.ub.ar

The plasma membrane (PM) of eukaryotic cells is compartmentalized into domains enriched in specific lipids and proteins. Eisosomes are protein and lipid nanodomains assembled onto the PM of the budding yeast *Saccharomyces cerevisiae*. Many eisosomal proteins have been associated to stress resistance and nutrition. However, the role of proteins´ localization in eisosomes is not clear and is a hot topic under study. Using a microfluidic device, we measured the replicative lifespan (RLS) of eisosomes´ mutants. We found that the knockout strain for the main structural protein Pil1 (disassembled eisosomes) has significantly extended lifespan (unpublished results). A decrease in the concentration of glucose or certain amino acids in the culture medium extends RLS in *S. cerevisiae*. Four different amino acid's transporters are stored in eisosomes and diffuse all along the PM in the pil1 mutant. We studied if the absence of eisosomal organization leads to a nutrient imbalance state extending RLS. Measuring glucose concentration in the culture media we found that extension of longevity in the pil1 mutant is not given by a difference in glucose consumption. A metabolomics analysis using ^{1}HNMR spectroscopy coupled with multivariate statistical analysis clearly distinguished differences between pil1 and wt strains. Interestingly, the deletion of the *PIL1* gene significantly decreases the intracellular content of some amino acids transported by eisosomal permeases, suggesting for the first time an association between the extended lifespan phenotype under study and intracellular amino acids levels. As the sensitivity of the method was not sufficient to detect Trp we complete the analysis measuring ^{3}HTrp import in vivo. We found that *PIL1* deletion does not generate a decrease in Trp incorporation.

General Aminoacid Control pathway activity was determined performing reporter gene assays in eisosomal mutants. RLS experiments in starving conditions together with nutrient control pathway activity measurements will enable us to determine whether an amino acids imbalance state is underlying eisosome dissasembly-dependent RLS extension.

Keywords: lifespan, microfluidics, yeast, eisosome

Methods: NMR, microfluidics

CB-05

Hyaluronan metabolism is associated with DNA repair genes in breast and colorectal cancer.

Sevic, Ina[1]; Vitale, Daiana[1]; Moran, Candela[1]; Brandone, Alejandra[3]; Rosales, Paolo[1]; Icardi, Antonella[1]; Romano, Lucia[5]; Giannoni, Paula[4]; Cristina, Carolina[2]; Alaniz, Laura[1]

[1]Laboratorio de Microambiente Tumoral; Centro de Investigaciones Básicas y Aplicadas (CIBA), CIT NOBA, Universidad Nacional del Noroeste de la Pcia. de Bs. As. Consejo Nacional de Investigaciones Científicas y Técnicas (UNNOBA-CONICET), Junín B6000, Argentina; [2]Laboratorio de Fisiopatología de la Hipófisis; Centro de Investigaciones Básicas y Aplicadas (CIBA), CIT NOBA, Universidad Nacional del Noroeste de la Pcia. de Bs. As. Consejo Nacional de Investigaciones Científicas y Técnicas (UNNOBA-CONICET), Junín B6000, Argentina; [3]Hospital Interzonal General de Agudos Dr. Abraham F. Piñeyro, Junín B6000, Argentina; [4]Clínica Centro, Junín B6000, Argentina; [5]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)

damini7777@gmail.com

The tumor microenvironment (TME) plays an important role in the progression of cancer and represents a significant factor that should be considered in the study of this pathology. TME is composed of cellular and non-cellular components that coexist in altered homeostasis. The key non-cellular component is the extracellular matrix (ECM), a complex network of macromolecules with different biological functions. Among the components of the ECM that are altered in tumors is the glycosaminoglycan Hyaluronic Acid (HA). Several studies have reported a relationship between the level of HA and the aggressiveness of cancer. On the other hand, it has been shown that changes in the microenvironment can lead to changes in the expression of some genes. The BRCA1 and BRCA2 genes have been extensively studied in different tumors and the alterations in ECM can lead to the fixation of different mutations or epigenetic

changes. In this work, we compared mRNA levels of HA metabolism members and BRCA genes, between tumor and non-tumor adjacent tissue in breast and colorectal cancer, and its correlation with clinical biomarkers (ER, PR, HER2 and KI67). We show alteration in HA metabolism in colorectal but not breast cancer. However, we found a decrease in CD44, BRCA1 and 2 protein levels in the breast but not colorectal cancer. We also show lower HA levels in tumor compared to normal tissue that could indicate a possible influence of tumor on its surrounding "normal" tissue. Additionally, we investigated the relationship between the expression of BRCA 1 and 2 genes and HA metabolism in 3D cell culture. We observed that the decrease in HA synthase (HAS2) level increases the expression of BRCA 1 and 2 in MDA-MB-231 cells. Lastly, STRING analysis was used to evaluate a possible protein interaction network between HA metabolism members and BRCA genes. The analysis showed two clusters: HA metabolism (HAS2, HAS3, HYAL1, HYAL2, CD44) and DNA repair and regulation (BRCA1, BRCA2, TP53, EP300) with CD44 as a link between these processes. In summary, our results demonstrate the association between HA metabolism and the DNA repair genes, BRCA1 and 2 which could give us a new insight into the molecular mechanisms of the development and the progression of cancer.

Keywords: Breast cancer, Colorectal cancer, hyaluronan, BRCA1, BRCA2

Methods: 3D cell culture, RT-qPCR, fluorescent microscopy

CB-06

Old drugs, new uses: new perspectives for the repositioning of chemotherapy drugs and modulators of the extracellular matrix in breast cancer treatment.

Daiana Luján Vitale, Paolo Rosales, Antonella Icardi, Candela Morán, Ina Sevic, Laura Alaniz
Laboratorio de Microambiente Tumoral – Centro de Investigaciones Básicas y Aplicadas (CIBA) - Centro de Investigaciones y Transferencia del Noroeste de la provincia de Buenos Aires (UNNOBA-UNSAdA-CONICET).
vitaledai@gmail.com

Drug resistance is a major contributor to cancer recurrence. The tumor extracellular matrix (TECM) plays a significant role in drug resistance due to an imbalance in the synthesis and degradation of its components, including the glycosaminoglycan (GAG) hyaluronan (HA). This GAG accumulates in the TECM, impeding drug distribution and inducing pro-tumoral signals. UDP-glucuronic acid (UDP-GlcA) is

synthesized by the UDP-glucose dehydrogenase (UGDH) enzyme. Together with N-acetyl-glucosamine, UDP-GlcA is involved in HA synthesis. Furthermore, this UDP-sugar plays a crucial role in the elimination of chemotherapeutic drugs as epirubicin (EPI). EPI is primarily metabolized through glucuronidation, involving a specific transferase called UGT2B7. Besides, candidate molecules proposed for drug repositioning include 4-methylumbelliferone (4MU), an orally approved dietary supplement derived from coumarins. 4MU specifically inhibits HA synthesis by binding to UDP-GlcA and depleting the cellular pool required for HA and other GAGs synthesis. The objective was to propose a combined treatment with EPI and 4MU in two breast cancer models to reduce EPI elimination and affect HA synthesis in TECM. To achieve this, 3D cultures (spheroids) of MDA-MB-231 and MCF-7 cells were established. After 5 days, spheroids were treated with EPI, 4MU, or EPI + 4MU for 3 days, completing 8 days of 3D culture under controlled conditions. Initially, it was determined that chemotherapeutic treatment with EPI was more effective in the presence of 4MU. Specifically, EPI + 4MU treatment reduced tumor cell viability (MTS) and increased early apoptosis (flow cytometry) in both breast cancer spheroids compared to control conditions ($p<0.05$). This effect was associated with a higher intracellular accumulation of EPI (flow cytometry) and the downregulation of EPI cell membrane efflux pumps involved in EPI resistance (RT-qPCR) ($p<0.05$). Furthermore, treatment with EPI + 4MU decreased the volume of the spheroids and cell migration. Finally, as 4MU inhibits HA synthesis, a possible remodeling of TECM was analyzed. EPI + 4MU decreased the expression of the major HA synthase HAS2 ($p<0.001$) and showed a tendency to increase hyaluronidases HYAL1 and HYAL2 (RT-qPCR). Moreover, this effect was shown when HA accumulation on the cell surface of tumor cells obtained from treated spheroids was determined by flow cytometry. A decrease in extracellular HA levels was observed after EPI + 4MU treatment compared to basal conditions ($p<0.05$). In conclusion, these results indicate that treatment with 4MU promotes remodeling of the breast cancer ECM, affecting HA accumulation and modulating HA metabolism. Indeed, the combination of 4MU with EPI chemotherapy treatment reduced EPI inactivation and elimination. Additionally, the combined treatment reduced the activation of cellular mechanisms involved in drug resistance, sensitizing tumor cells to EPI treatment and enhancing the efficacy of antitumor therapy.

Keywords: Breast cancer, extracellular matrix, hyaluronan, epirubicin, 4-methylumbelliferone

Methods: 3D cell culture, flow cytometry, RT-qPCR, microscopy

CB-07

The Liver X Receptor interferes with Estrogen Receptor-dependent genomic regulation in breast cancer cells

Olszanowski, Evelyn[1,2]; Ogara, María Florencia[1]; Lafuente, Agustina[1]; Nacht, A. Silvina[3]; Benitez, Belén[1,4]; Rodríguez-Seguí, Santiago Andrés[1,5]; Presman, Diego[1], Vicent, Guillermo P.6, Pecci, Adali[1,2]

[1]CONICET-Universidad de Buenos Aires, Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE), C1428EHA, Buenos Aires, Argentina. [2]Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica, C1428EHA, Buenos Aires, Argentina. [3]Gene Regulation, Stem Cells and Cancer Program, Center for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona 08003, Spain. [4]CONICET-Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), C1428EHA, Buenos Aires, Argentina. [5]Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Fisiología, Biología Molecular y Celular, C1428EHA, Buenos Aires, Argentina. [6]Molecular Biology Institute of Barcelona, Consejo Superior de Investigaciones Científicas (IBMB-CSIC), Barcelona, 08028, Spain.

eve.olszanowski@gmail.com

Liver X Receptors (LXRs) belong to the nuclear receptors superfamily of ligand activated transcription factors, whose endogenous agonists are the oxysterols. They play a key role in the regulation of the cholesterol homeostasis, induce the de novo synthesis of triacylglycerides, and counteract pro-inflammatory effects. LXRs are also known to compromise cell proliferation in several cancer models. However, their role in breast cancer (BC) has not been studied in depth and reports are, in fact, contradictory. Here, we have examined the potential involvement of LXRs in BC cells with special emphasis on their possible crosstalk with the Estrogen Receptor alpha (ERα). We performed colony formation (CFA) and propidium iodide staining assays in MCF-7 cells treated with or without Estradiol (E2) and the LXR agonist, GW3965. Our results showed that GW3965 impaired the cell proliferation capacity induced by E2 (CFA: #colonies, Mean±SD: E2 208.7±25.7; E2+GW3965 131.3±23.7, n=3, padj<0.01, ANOVA). To understand the functional pathways involved in these effects, we performed bulk RNA-seq experiments. The differentially expressed genes between E2 and E2+GW3965 conditions revealed several genes whose expression was affected by GW3965; which are widely enriched in terms associated to DNA replication, cell cycle, G1 to S transition, and Breast Cancer (padj<0.05) including genes such as PCNA, MCM4, CCND1, POLE3, TOP2A, BRCA1, BRCA2, RAD51, RET, E2F1, E2F2 as well as the ERα (ESR1). Interestingly, the presence of GW3965 increased the expression of the Glucocorticoid Receptor (GR) (NR3C1), which is consistent with a less proliferative phenotype observed in cells treated with this ligand. We then hypothesised that the activation of LXR could affect the binding of ERα in its response elements. To address this, we used public ChIP-seq data to select sites with potential co-localization of ERα and LXR, that could regulate genes previously found with the RNA-seq analysis. We performed ChIP-qPCR analysis against ERα on several of these selected sites, and found that the combined

treatment (E2+GW3965) led to a decrease in ERα binding compared to the E2 treatment alone, in three intronic sites of genes related to cancer progression (MYB, PVT1 and SLC9A1). In addition, we studied the dynamics of the nuclear organisation of the Liver X Receptor in LXR-GFP transfected MCF-7 cells using super-resolution microscopy. We found that the combined treatment (E2+GW3965) led to a decrease in the number of nuclear condensates (foci) of LXR, compared to the GW3965 treatment alone. All these results combined could explain, at least partially, the decrease in the proliferation observed in the functional assays, and contribute to the idea of an interaction between these two receptors.

Keywords: Liver X Receptors (LXRs), Breast Cancer (BC), Estrogen Receptor (ER), Transcriptional Regulation, Nuclear Organization

Methods: Colony Formation Assay, Flow Citometry, RNA-seq, ChIP-qPCR, AiryScan Super-resolution and Confocal Microscopy

Plants

PT-01

Role of AtMED17 under light and dark conditions

Giustozzi M1, Freytes S.2, Cerdán P.2, Casati. P1
1-Centro de Estudios Fotosintéticos y Bioquímicos (CONICET-UNR). CEFOBI-UNR 2- Fundación Instituto Leloir (CONICET)
giustozzi.marisol@gmail.com

The Mediator complex is a conserved multisubunit complex that acts as a transcriptional regulator in eukaryotes at the level of assembly of the transcription preinitiation complex. Structural and biochemical studies showed that the subunits that compose it form stable subcomplexes, dividing its structure into different modules, head, middle, tail and CDK8 module (cyclin-dependent kinase). The Mediator complex plays a key role in mediating gene expression responses to changes in the environment, including light signals and UV-B radiation by coordinating the activation of specific transcription factors and the expression of genes involved in photomorphogenesis. In the head module, MED17 interacts with the middle and tail modules and participates as a structural hub of the complex. In

addition, MED17 plays an integral role in gene expression responses to various environmental and developmental signals, including in responses to ultraviolet B radiation (UV-B).
Light is one of the most important environmental signals for plants, the perception of the quantity, quality and duration of light induces different plant responses. Blue light is necessary for plant growth regulation includes stimulation of cotyledon expansion. However, excessive exposure to blue light can have negative effects, such as inhibition of root and hypocotyl growth, or epinasty of leaves. In addition, the combination of blue light and UV-B has different effects, among them, blue light protects plants from the effects of UV-B radiation. In addition, darkness is an abiotic stress that can have a significant impact on plant growth and development. In the absence of light, plants show different changes, such as reduced photosynthesis, reduced growth, altered gene expression, increased susceptibility to pests and diseases and accelerated senescence. Therefore, the objective of our work is to study the role of MED17 in plants in response to darkness and blue light combined with UV-B. We observed that med17 mutant plants showed less inhibition of hypocotyl growth than wild-type (WT) plants irradiated with a pulse of blue light, or with blue light supplemented with UV-B. The analysis of the hypocotyls showed that inhibition of primary root growth by blue light was lower in med17 mutant plants than in WT plants. med17 mutant plants did not show leaf epinasty after blue light exposure, when supplemented with UV-B light was also not observed epinasty in med17 mutant plants. On the other hand, after a 96 h dark treatment, in WT plants, chlorophyll and carotenoid content decreased. However, in med17 plants these compounds were not altered. A characteristic phenotype after darkness is leaf petiole elongation, WT plants showed larger petioles than med17 mutants. In addition, med17 plants presented less oxidative damage than WT plants. Together, MED17 acts both in response to different light qualities and also to darkness.

Keywords: MED17, dark, blue, UV-B, epinasty

Methods: phenotypic analysis - microscopy

PT-02

Redesigning of Photosystem I for optimizing photo-synthesis/protection balance

Bultri, Julián; Brugnara, Candela; Miño, Julián Jesús; Abraham, María; Lobais, Celina; Blanco, Nicolás E.
CEFOBI-UNR-CONICET, Suipacha 570, Rosario
bultrijulian@gmail.com

Photosynthesis enables autotrophic organisms to convert absorbed light energy into ATP and reducing power supporting the fixation of CO2 into biomass. These photochemical reactions are conducted by the photosynthetic apparatus located in the thylakoid membranes, the Photosynthetic Electron Transport Chain (PETC). It is composed by the light harvesting complexes, a series of protein complexes (two photosystems and the cytochrome b6f complex) and electron carriers, which couples redox reactions for the generation of proton motive force (pmf), mainly composed of the ∆pH component. The final steps of the photosynthetic electron flow involve the reduction of the stromal soluble carrier Ferredoxin (Fd) and further production of NADPH during its regeneration. An emerging picture indicates the existence of a trade-off between photosynthetic efficiency and the activation of photoprotective mechanisms. This is particularly pronounced in the frame of climate-change conditions, when plants must cope with high light intensity and drought stress. In such conditions, the availability of oxidized receptors at the end of Photosystem I is scarce, leading to what is referred to as "PSI acceptor-side limitation". This triggers the induction of different photoprotective mechanisms with a concomitant reduction in photochemical yield.

To explore approaches capable of adjusting photosynthesis/photoprotection trade-off for highly changeable conditions, we conducted a study using transplastomic tobacco plants with overexpressed pea Ferredoxin (OeFd). Our hypothesis proposes that an increased content of electron acceptor, such as Fd, can simultaneously act as a "safe valve" to avoid PSI acceptor-side limitation without affecting photochemical efficiency.

Under greenhouse conditions, OeFd plants exhibit reduced biomass, fewer leaves and smaller overall size compared with wild type tobacco plants along with an easily recognizable variegated phenotype. To better understand the physiological implications of these modifications at PSI, plants were subjected to various light conditions, ranging from 30 to 1200 μmol m-2 s-1, and photosynthetic and physiological parameters were analyzed. Low light conditions were not capable of reverting OeFd into wild type phenotype nor improve photochemical yield. Conversely, under high light conditions, overexpressors displayed uniform leaf color together with an improvement in its photochemical capacity and growth rates. The analysis of these results in conjunction with measurements focusing on ATP synthase activity suggested an alternative partition of electron flow at the PSI acceptor side, as initially hypothesized. This scenario presents new opportunities for reformulating photosynthesis/ photoprotective trade-off. Delving into the comprehension of this equilibrium certainly will allow us to engineer climate-change-resilient plants.

Keywords: Tobacco, Ferredoxin, Cyclic electron transport

Methods: Clorophyll fluorescence measurements, OJIP curves, ATP synthase conductivity

PT-03

Insights on how light regulates alternative polyadenylation in plants

Kubaczka, MG1,2; Godoy Herz, MA1,2; Chen, J3; Tian, B3; Kornblihtt, AR1,2.
1: Instituto de Fisiología, Biología Molecular y Neurociencias, CONICET, Buenos Aires, Argentina 2: Departamento de Fisiología, Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina. 3: The Wistar Institute, Philadelphia, United States of America.
guikubaczka@gmail.com

Light is an essential environmental cue for plants. We have previously shown that light generates a chloroplast retrograde signal that regulates nuclear alternative splicing (AS) of a subset of Arabidopsis thaliana transcripts, and that RNA Polymerase II (RNAPII) transcription elongation rate is involved in these alternative splicing decisions. We are now investigating the mechanism by which light and dark conditions regulate alternative polyadenylation (APA) and its differences and similarities with the control of AS. Using 3' region extraction and deep sequencing (3'READS), we mapped cleavage and polyadenylation sites (PAS) in Arabidopsis thaliana, discovering previously un-annotated PAS. We found that exposure of Arabidopsis seedlings to light promotes the usage of distal PAS, giving rise to longer 3'UTRs. With the validated data set, we are investigating the underlying mechanism. Our results suggest that, as it happens with AS, regulation of APA by light is not affected in plants with genetically disrupted phytochrome and cryptochrome pathways, is abolished by inhibition of the chloroplast photosynthetic electron transport and the effect is not observed in roots when communication with shoots is interrupted. Taken all together, these data imply that light controls different nuclear mRNA processing steps in plants through the chloroplast and throughout the whole organism.
RNAPII has a characteristic C-terminal domain (CTD) in its largest subunit RPB1. This CTD is composed of numerous repetitions (37 in Arabidopsis) of the aminoacidic heptad YSPTSPS. Post translational modifications in this CTD play a key role in the transcription cycle. Here we show that Arabidopsis mutants defective of CPL1 (cpl1-6) and CPL2 (cpl2-2), two CTD-phosphatases specific of Ser5, abolish the light/dark effect on AS but not on APA. We found that CPL1 and CPL2 are important for the changes in AS in response to light. However, the same mutants show similar to wild type seedlings with respect to the light/dark effect on APA, suggesting that these two mRNA maturation steps show a different sensitivity to the lack of CPL1 and CPL2. Western blots revealed that the RNAPII signal from wild type seedlings exposed to light is stronger than that of seedlings kept in dark conditions. Moreover, for cpl2-2, but not for cpl1-6, total RNAPII abundance is not regulated by light, showing a strong signal in both light and dark conditions. Taken together, these data suggest a role for Ser5 phosphorylation in AS regulation, but not APA regulation, in response to light. More importantly, these results demonstrate a previously undescribed regulation of RNAPII abundance in response to light and a role of a CTD phosphatase in its stability. This raises new questions regarding RNAPII pool stability and degradation that help us understand how basal transcription machinery is sensitive to environmental cues and how this controls key steps in mRNA processing.

Keywords: Alternative polyadenylation, light, *Arabidopsis thaliana,* RNAPII, alternative splicing

Methods: RT-qPCR, RT-PCR, Western blotting, 3'READS

PT-04

Natural variation for flower and seed abortion in fluctuating environments

Petreli, MV1,2; Herrera, C1,2; Segatto, L1,2; Blanco, N1,2; Fusari, CM1,2,.
1 Universidad Nacional de Rosario. Facultad de Ciencias Bioquímicas y Farmacéuticas. 2 Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI-CONICET-UNR), Argentina.
mvictoriapetreli@gmail.com

Biotechnology and agriculture have the challenge to secure food for the continuous growing population in a context jeopardized by the current climate-change scenario. Thus, crop improvement is necessary to identify varieties with improved yield performance, even under stress conditions. Yield is severely impacted by flower and seed abortion due to carbon shortfall early in the reproductive phase (Lauxmann et al., 2016). Identifying genes controlling such process would enable to develop new varieties with enhance adaptation in fluctuating environments. The era of Next Generation Sequencing and the advantages of using a diverse population in the process of mapping complex traits, have enabled Genome Wide Association Studies to be a routine toolkit for discovering new genes and alleles. In our laboratory we aim to identify genes regulating the process fruit and seed abortion, and the resuming of reproduction in Arabidopsis thaliana, through Genome Wide Association Studies.
To explore the natural variation in the process of abortion and resuming of reproduction, a set of 21 ecotypes was subjected to 4 days of extended darkness after the appearing of 2 to 3 siliques. In parallel, the same ecotypes were grown in normal conditions. We characterize: number of total aborted siliques, cm of the stem with aborted flowers, ratio of aborted/non-aborted siliques, number of days until normal reproduction is restored, seed yield (g of total seeds harvested), seed viability (germination power) and carbon status before and after the treatment. In addition, we explored the pollen physiology of stressed and control plants and evaluate photosynthetic parameters in both panels.
Overall, we found that there is a differential response among ecotypes in yield and photosynthetic traits in the two environmental setups. The increase in seed abortion seen for plants subjected to extended darkness is accompanied by a response in the photosynthetic process, i.e., a decrease in Phi2 and an increase in the NPQT. These results imply a response towards adaptation to stress. In summary, individual or a combination of several traits could be used to perform GWAS and identify candidate

genes involved in the regulation of flower and seed abortion. In the long term, we expect to deliver new tools for marker-assisted breeding and crop improvement.

Keywords: natural variation, fluctuating environments, carbon shortfall, seed abortion, yield

Methods: Genome Wide Association Studies, physiology characterization, visualization of starch in planta, photosynthetic measurements, visualization of aborted pollen by staining techniques and light microscopy

PT-05

Deciphering molecular determinants of the high levels of storage proteins and oil in the soybean seeds

Zucchetti, Juan I.1; Pavlovic, Tatiana1; Saenz, Ezequiel2; Borrás, Lucas2; Saigo, Mariana1; Gerrard Wheeler, Mariel C1.
1) Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI-CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR); 2) Instituto de Investigaciones en Ciencias Agrarias de Rosario (IICAR-CONICET), Facultad de Ciencias Agrarias (UNR)
zucchetti.juan@gmail.com

Soybean is a strategic crop due to the accumulation of high-value molecules in the seeds. Currently, the agro-industrial sector is facing a sustained decrease in protein level, a situation that shows the need to pay attention not only to seed yield but also to seed quality. In order to gain knowledge on the molecular mechanism leading to the synthesis of reserves, two closely related soybean experimental lines with different protein and oil concentration were analyzed. For this purpose, total proteins were obtained from seeds at different development stages, which were identified and quantified by mass spectrometry. Numerous proteins with differential abundance among genotypes were detected ($p < 0.05$; average fold change > 2). Functional analysis revealed changes in photosynthesis, oxidative phosphorylation, protein processing, biosynthesis of secondary metabolites, sugar metabolism, among other processes. Interestingly, the most notable differences were observed in the later filling and desiccation stages, which have the greatest differences in storage protein content. A shutdown of specific activities of organic and amino acid metabolism was observed in the high-protein line, possibly driven by changes in the expression of regulatory genes related to abscisic acid signaling. Furthermore, some heat shock proteins, responsible for protein folding, were found in greater abundance in the

high-protein line. These results clarified molecular mechanisms controlling compositional seed traits, and pointed out markers for breeding programs and/or targets for metabolic engineering.

Keywords: Soybean, oil, protein, molecular determinats

Methods: Proteomic, Bioinformatic analysis

PT-06

Optimization of high molecular weight DNA isolation and Oxford Nanopore sequencing in non-model trees

Gaischuk, Sofia1; Leduque, Basile2; Servi, Lucas3; Bermudez Salazar, Luisa Fernanda4; Petrillo, Ezequiel3; Quadrana, Leandro2; Bellora, Nicolás5; Arana, María Verónica1.
1 Instituto de Investigaciones Forestales y Agropecuarias Bariloche (INTA EEA Bariloche-CONICET), Bariloche, R8403DVZ, Rio Negro, Argentina. 2 Institute of Plant Sciences Paris-Saclay, Centre Nationale de la Recherche Scientifique, Institut National de la Recherche Agronomique, Université Evry, Université Paris-Saclay, 91405 Orsay, France. 3 Instituto de Fisiología, Biología Molecular y Neurociencias (Universidad de Buenos Aires-CONICET), CABA, C1428EGA, Buenos Aires, Argentina. 4 Instituto de Agrobiotecnología y Biología Molecular (INTA Castelar – CONICET), Hurlingham, 1686, Buenos Aires, Argentina. 5 Fundación INTECNUS. Comisión Nacional de Energía Atómica. Ruta 82, 8400, Bariloche, Rio Negro, Argentina.
gaischuk.sofia@inta.gob.ar

As a result of climate change, forest biomes are predicted to face increasing temperatures, extreme cold winters with harsh springs, and/or more frequent and severe droughts. These extreme abiotic conditions are expected to have significant consequences for biodiversity, primary productivity, and ecological functions. Revealing the genetic architecture underlying the tree responses to environment is therefore crucial for the development of conservation strategies, and for breeding stress-resistant genotypes. These studies are difficult in non-model trees, in part due to the lack of suitable biochemical protocols and the scarce availability of genomic data. The Oxford Nanopore Technology (ONT) is a sequencing platform that produces long reads, which are particularly suitable for assembling large and highly repetitive plant genomes. In addition, direct sequencing of native DNA and RNA allows simultaneous identification of base modifications alongside nucleotide sequences. We are currently working in the optimization of nucleic acid extractions and ONT sequencing of DNA and RNA of tree

species belonging to the Nothofagus genus. Nothofagus constitutes a key element of the ancient Gondwana flora, and is the dominant tree genus of the Andean Patagonian Forests. We present the advances in the adjustment of high molecular weight DNA isolation protocols and ONT sequencing in *Nothofagus pumilio* and *N. obliqua*, aiming at sharing our experience, and receive feedback in the endeavor of the elucidation of the biochemistry and ONT sequencing of non-model trees. Leaves of these species constitute 'difficult tissues' for nucleic acid extraction, due to their high polysaccharide and secondary metabolites contents. After the evaluation of at least 7 DNA extraction methods, those described by (1) Mayjonade et al. 2016, doi.org/10.2144/000114460, (2) Yang et al. 2019, doi.org/10.1007/978-1-4939-9458-8_11 in combination with a CTAB-based protocol, and (3) the HighPrep Plant DNA Plus Kit (MagBio), yielded the best results. In terms of DNA extraction, *N. pumilio* leaves resulted in a more difficult tissue compared to N. obliqua, yielding slimy solutions, and, despite of showing optimal integrity nanodrop parameters and nanodrop/qubit ratios, libraries could not be sequenced unless DNA was previously cleaned using the Monarch column kit (NEB cat T3050, blood protocol). Direct gDNA sequencing was performed using a MinION Mk1C device, yielding 7.62 Gb (N50=34.59 kb) and 7.61 Gb (N50=29.77 kb) for *N. obliqua* leaves, 1.79 Gb (N50= 9.56kb) for *N. pumilio* leaves (Flow cells R9.4.1) and 2.58 Gb (N50= 9.07 kb) for *N. pumilio* buds (Flow cells R10.4). We will discuss these sequencing results in the frame of flow cell performance, long read generation and the discovery of base modifications using ONT.

Keywords: High molecular weight DNA, base modifications, Oxford Nanopore Technology, Non-model trees species

Methods: Nuclei isolation, Direct DNA sequencing, Long reads, Oxford Nanopore Technology

Signal Transduction

ST-01

Autophagy proteins are required for regulated exocytosis in Drosophila melanogaster.

Fresco, Sabrina M. 1 3; Suárez-Freire, Sofía 1 2; Wappner, Pablo 1 2 3; Melani, Mariana 1 2 3.
1-Fundación Instituto Leloir, Buenos Aires, Argentina. 2-Consejo Nacional De Investigaciones Científicas Y Técnicas (CONICET), Buenos Aires, Argentina. 3-Departamento De Fisiología, Biología Molecular Y Celular, Facultad De Ciencias Exactas Y Naturales, Universidad De Buenos Aires, Buenos Aires, Argentina.

samifresco@gmail.com

Autophagy is the process by which eukaryotic cells undergo self-digestion of cytoplasmic components. The material to be degraded is engulfed inside a membranous structure named autophagosome that fuses with the lysosome resulting in the formation of an autolysosome, where degradation of the sequestered material takes place. Exocytosis is another vesicle-mediated process by which intracellularly synthesized material reaches the extracellular environment or the plasma membrane inside Golgi-derived exocytic vesicles. Both autophagy and exocystosis are evolutionary conserved and critical to maintain cellular homeostasis and well-functioning. Moreover, they share some common effectors and regulators, for example, the autophagic nucleation complex, composed of Vps34, Atg6, Vps15 and Atg14. In autophagy this complex marks the sites of autophagosome formation by depositing phosphatidil-inositol-3-phosphate (PI3P) on certain sites of the endoplasmic reticulum. It is known that the individual components of this complex are also required for exocytosis, although the precise requirement of its action and if they act individually or as a complex remain unknown. In this project we have characterized the requirement of the components of the nucleation complex, as well as that of other autophagy proteins (UVRAG, Atg9, Vamp7 and Tango5) in the exocytic pathway. To do this we have used the salivary gland of the Drosophila melanogaster larvae, which is an outstanding model for studying this process. These glands synthesize mucins, which are packed into secretory granules (SGs) that emerge from the trans-Golgi network. Afterwards, SGs mature by homotypic fusion, condensation and acidification of its content. At the end, SGs fuse with plasma membrane releasing its content to the gland lumen. We are currently evaluating the precise step along the exocytic pathway at which each of these proteins are required by analyzing molecular markers characteristic of each of the steps of the secretory pathway as well as SG size.

Keywords: Exocytosis, Autophagy, Drosophila melanogaster, Vps34 Complex

Methods: Confocal microscopy, Drosophila Genetics

ST-02

The βCSP-Hsc70 complex in human spermatozoa: a key player in Acrosomal Exocytosis

Flores Montero, Karina; Durán, Néstor; Frontini Lopez, Yesica; Fontecilla Escobar, Javiera; Ruete, M. Celeste
Instituto de Histología y Embriología de Mendoza (IHEM)-CONICET
kfloresmontero@yahoo.com.ar

Cysteine String Protein (CSP) is a chaperone that belongs to the DnaJ/Hsp40 family of proteins initially described in synaptic vesicles. It is known that CSP interacts with the protein Heat shock cognate 70 (Hsc70), a chaperone with ATPase activity, highly distributed in eukaryotic cells. The presence of these proteins has been demonstrated in various secretory organelles and tissues of vertebrates, such as testicular tissue, which led us to assume their presence in human sperm.
For human sperm to fertilize oocytes, it is necessary to undergo a process of exocytosis of the only large, flat granule they contain in the head, the acrosome. This process, known as the acrosomal reaction (AR), is a type of regulated exocytosis that involves the fusion of the outer acrosomal membrane with the plasma membrane. This fusion is mediated by the assembly of SNARE proteins in both membranes into a *trans* configuration, resulting in irreversible membrane coupling.
In previous studies, we demonstrated the presence and participation of the βCSP protein in the AR of human spermatozoa, but so far the role of the βCSP-Hsc70 complex in human spermatozoa is unknown. Therefore, our objective is to determine the presence and interaction of both proteins during acrosomal exocytosis.
The presence of βCSP and Hsc70 in human spermatozoa was evaluated using western blot and indirect immunofluorescence techniques. Functional assays with anti-βCSP and anti-Hsc70 antibodies demonstrated their functional activity in acrosomal secretion, as they inhibited the AR in a concentration-dependent manner. Dot blot assays confirmed the interaction between βCSP and Hsc70 in human spermatozoa.
For the first time, this study describes the formation of the βCSP-Hsc70 complex in human spermatozoa and provides evidence of its relevance in the AR.

Keywords: Acrosomal reaction, Sperm, Cysteine String Protein, Chaperones

Methods: Western blot, assay functional, dot blot, indirect immunofluorescence

ST-03

Modulation of oncogenic P53 mutants stability through drug repurposing

Cocordano, Nabila 1; Borini Etichetti, Carla M. 2; Llorens de los ríos, M. Candelaria 3; Arel Zalazar, Evelyn E. 1; Soria, Gastón 4; Girardini, Javier E. 1.

1Laboratorio de Inmuno-Oncología, Instituto de Inmunología Clínica y Experimental de Rosario, UNR-IDICER-CONICET, Rosario, Argentina. 2Instituto de Fisiología Experimental de Rosario, UNR-IFISE-CONICET, Rosario, Argentina. 3 OncoPrecision, Córdoba, Argentina. 4 OncoPrecision New York, USA.

girardini@idicer-conicet.gob.ar

The presence of missense mutations in the p53 gene is among the most frequent alterations in human cancer. These mutations lead to the expression of mutant p53 proteins, which can actively collaborate with oncogenic processes. Mutant p53 forms have attracted great interest as therapeutic targets because their elimination could reduce the development of aggressive and metastatic tumors. Targeting mutant p53 would also provide highly selective therapies, since mutations are found exclusively in tumor cells, reducing the possibility of adverse effects. Also, the high mutation frequency of p53 would make this strategy useful in different cancer types, as an agnostic therapeutic approach. In tumor cells, p53 point mutants show a remarkable increase in stability compared with the wt protein. In order to identify drugs able to induce mutant p53 degradation, we performed a high-throughput screening, using libraries of drugs approved for clinical use in humans against various pathologies. Using In Cell Western Blot, we analyzed the effect of 1760 drugs on the MDA-MB-231 cell line, derived from Triple-Negative breast adenocarcinoma, which endogenously expresses p53R280K mutant. In this way, compounds capable of significantly reducing mutant p53 levels were identified. We further characterized the effect of a selected candidate. We demonstrated that the compound reduced the levels of other p53 point mutants in different cell lines ($p < 0,001$; $n=3$). Time course analysis using western blot showed that the drug decreased the half-life of mutant p53 ($p < 0,01$; $n=3$), associated with an increase in polyubiquitination. In contrast, wt p53 levels were not affected, suggesting that the effect is selective for cells that express mutant p53. Using wound healing assays we showed that the drug reduced the migration of cancer cells ($p < 0,01$; $n=3$), a characteristic trait of metastatic cells promoted by mutant p53. In summary, we identified a compound potentially useful in antitumor strategies based on mutant p53 degradation.

Keywords: mutant p53, cancer, drug repurposing, protein degradation, chaperone machinery

Methods: Western blot, Proliferation, In vitro migration, Protein stability, qRT-PCR

ST-04

Role of the Transmembrane Connector in Ire1 Signaling

Almada, J.C.; Porrini L, Bortolotti, A.; Dunayevich, P, Colman- Lerner, A.; Cybulski, L.
1-Laboratorio de Interacciones lípido-proteína en la señalización celular-Dept Microbiología- Facultad de CIencias Bioquímicas y Farmacéuticas- UNR- CONICET, 2 : IFIBYNE-UBA-CONICET
larisa.cybulski@gmail.com

Transmembrane kinases are key for cell survival. They detect physical and chemical stimuli in the environment and activate adaptive cellular responses. We study Ire1, which is conserved from yeast to humans, and detects the presence of unfolded proteins in the lumen of the endoplasmic reticulum. Ire1 is a transmembrane sensor with three domains: a luminal domain, which senses unfolded proteins, a cytoplasmic domain with kinase and endoribonuclease activity and the transmembrane domain, which connects the other two domains.
The presence of misfolded proteins promotes dimerization of the luminal domain, which stimulates Ire1´s activity. The catalytic domain cleaves the mRNA that codes for the transcription factor, Hac1. Hac1 induces the expression of hundreds of genes involved in the biosynthesis of lipids and chaperones. In recent years, Ire1 has also been implicated in the response to lipid stress. We study how the physicochemical properties of the transmembrane segment affect signal transmission. Different mutations in transmembrane domain cause diminished or exacerbated responses to the stimulus, suggesting a role for the connector domain in Ire1 signaling.

Keywords: Saccharomyces, Ire1, protein-lipid interaction, UPR

Methods: crispr, transcriptional fusion, RT-PCR, proteomics

Neurosciences

NS-01

CHARACTERIZATION OF BIOACTIVE EXTRACELLULAR VESICLES SECRETED BY HUMAN STEM CELLS

Guendulain, Gonzalo Germán; Gastaldi, Laura; Remedi, Mónica; Wilson, Carlos; Cáceres, Alfredo; Moyano, Ana Lis.
Instituto Universitario de Ciencias Biomédicas de Córdoba (IUCBC), Centro de Investigación en Medicina Traslacional "Severo Raúl Amuchástegui" (CIMETSA). G.V. al Instituto de Investigaciones Médicas de Córdoba (INIMEC), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)
gonzalo.guendulain@iucbc.edu.ar

Extracellular vesicles (EVs) are heterogeneous nanosized vesicles (50- 250 nm) that shuttle bioactive cargoes (e.g., proteins, lipids and RNAs) between cells and participate in intercellular communication. EVs secreted by stem cells can promote tissue regeneration, regulate immunity and function as potential alternatives to stem cell therapy. Furthermore, as therapeutic agents EVs have fewer side effects and do not present risks associated with cellular transplants such as incomplete differentiation or tumorigenesis. Recent studies have shown that administration of EVs secreted by human neural stem cells (hNSC-EVs), reduce neuroinflammation, promote regeneration and restore neurological functions in animal models of central nervous system (CNS) disorders. However, it is unknown which of EVs' bioactive cargoes are responsible for these effects. Preliminary results from our laboratory reveal that EVs secreted by human neural rosettes (hNR-EVs), hNSC-enriched cells derived from human induced pluripotent stem cells (hiPSCs), promote stem cell differentiation by reducing the expression of pluripotency markers. Moreover, mass spectrometry and gene ontology analyses indicate that hNR-EVs contain neuroglial components that could partially explain their biological activity. Thus, the objective of this work is to comprehensively characterize the contribution in the transport and transfer of neuroglial components mediated by EVs to determine their biological effects in the CNS. Results from our work will help us to understand what bioactive cargoes are responsible for stem-cell derived EVs' regenerative effects and contribute to the development of new regenerative strategies for CNS diseases.

Keywords: Human Stem Cells, Extracellular Vesicles, Central Nervous System Regeneration

Methods: Cell culture, immunocytochemistry, confocal microscopy, mass spectrometry, extracellular vesicles isolation

NS-02

A Novel Player in Neuronal Primary Cilia: The Secretory Pathway-associated Transcription Factor, CREB3L1

Rozés-Salvador, Victoria; Alvarez, Cecilia
Facultad de Ciencias Químicas, CIBICI-CONICET-UNC
victoria.rozes@unc.edu.ar

CREB3L1 belongs to the CREB3 family of transcription factors implicated in the ER and Golgi stress responses as regulators of the cell secretory capacity and cell-specific cargos. In response to different signals, CREB3 proteins are transported from the ER to the Golgi where they are cleaved (activated) by S1P and S2P proteases sequentially. Although CREB3 factors have a wide range of biological functions, their role in neuronal development is poorly understood. In our study, we observed that CREB3L1 localizes to the basal bodies of primary cilia during early neuronal development. Primary cilia are sensory organelles that project from the plasma membrane of many cell types, including neurons. They play an essential role in intracellular signaling pathways and act as sensory organelle for extracellular and intracellular signals. Our preliminary results show that CREB3L1 colocalizes with γ-tubulin and Inversin (INVS), both proteins located at the basal body of primary cilia at early culture times (3DIV). This basal body localization is lost when CREB3L1 activation by S1P and S2P proteases is inhibited. Our findings suggest that CREB3L1 may have a non-canonical function in primary cilia relevant to neuronal development and function. Further research on this topic could provide new insights into the mechanisms underlying neuronal function.

Keywords: CREB3L1, transcription factor, neuronal development, primary cilia, secretory pathway

Methods: primary culture, immunofluorescence, confocal imaging, DNAs transfections, RNA interference

Microbiology

MI-01

LIGHT MODULATION OF PHAGE INFECTION IN *ACINETOBACTER BAUMANNII*

Arana, Natalia[1]; Tomas, María[2]; Comité de Infecciones del Hospital Provincial del Centenario[3]; Mussi, M. Alejandra[1].
[1]Center for Photosynthetic and Biochemical Studies (CEFOBI-CONICET), Rosario, Argentina
[2]Microbiology Department-Research Institute Biomedical A Coruña (INIBIC), Hospital A Coruña (CHUAC), University of A Coruña (UDC), 15495 A Coruña, Spain.
[3]Hospital Provincial del Centenario, Rosario, Argentina
arana.natalia@outlook.com

Acinetobacter baumannii, a Gram-negative human critical pathogen, has been recognized by the World Health Organization as a global healthcare challenge due to multi-drug resistance (MDR). It associates with pneumonia, wound, soft tissue, and bloodstream infections, especially in intensive care units. The urgent need for new antimicrobial agents has renewed interest in phage therapy. Phage therapy is now a viable option for MDR bacteria treatment, with successful cases in human patients. Phages are bacterial viruses that recognize host cells via receptors. Phage therapy only uses lytic phages. Combining antibiotics and phages has shown effective bacterial control and reduced resistance development. Phages complement antibiotics due to distinct mechanisms: narrow spectrum, local multiplication, easy isolation, and low production costs.

Our group has extensively characterized light regulation in *A. baumannii*, both at low to moderate temperatures (24°C) compatible with the microorganism's life in the environment as well as at the normal temperature of warm-blooded hosts (37°C), showing that light has a global effect on its physiology. Interestingly, we have shown that light stimulates *A. baumannii*'s ability to compete with microorganisms potentially sharing the niche, such as *Candida albicans*; as well enhances the microorganism's virulence in a skin infection model using cultured human keratinocytes. In this study, we used phage Ab105-2φΔCI, a lytic mutant derived from a lysogenic phage, to study the effect of blue light on *A. baumannii*'s susceptibility to phage infection. For this, we determined the number of lysis plaques obtained when the carbapenem-resistant strain *A. baumannii* Ab177, grown under blue light or in the dark, was infected with Ab105-2φΔCI. Our results show that light results in approximately a 15-fold increase in the susceptibility of Ab177 to infection by 2φΔCI at 37°C. It is worth noting that the individual plaques were larger when incubated under blue light than in the darkness, likely due to differential burst size under both conditions. To determine if the differential susceptibility to blue light is distributed in *A. baumannii* phage infections, we isolated phages from sewers of the Hospital Provincial del Centenario (HPC), Rosario. We found at least five morphologically different phages, which are being characterized to determine their characteristics. Additionally, we are characterizing lysogenic phages from *A. baumannii* strains of interest by induction with mitomycin-C and infection in susceptible strains, where they can manifest their lytic behavior. In particular, we were able to isolate the prophage from the clinical strain V15.

Understanding the interaction between phage infection and light not only provides insights into novel therapeutic approaches against antibiotic-resistant pathogens, but also holds the potential to revolutionize our strategies for combating infectious diseases in a more effective manner.

Keywords: Phages, Acinetobacter baumannii, Light

Methods: Plaque assay, Spot test, Titulation, Serial dilution

MI-02

ANTIFUNGAL ACTIVITY AND PHYTOTOXICITY OF THE TERNARY COMPLEX OF COPPER(II) WITH CYANOGUANIDINE AND 1,10-PHENANTHROLINE

Guevara, Lorena Elizabeth; Martínez Medina, Juan José
Universidad Nacional del Chaco Austral, Comandante Fernández Nº 755, Presidencia Roque Sáenz Peña (3700), Chaco, Argentina
fiorillaleguevara02@gmail.com

Copper is an essential trace element for many biological functions, but in certain amounts, it can have toxic effects against microorganisms. The ligand 1,10-phenanthroline (phen) has antimicrobial activity on several species of bacteria and fungi, whereas cyanoguanidine (cnge) is a nitrogenated ligand without antimicrobial action. It has been reported that coordination with copper(II) cation improves the biological properties of ligands. We previously reported the molecular structure, bioavailability and bioactivity of the ternary $[Cu(phen)(cnge)(H_2O)(NO_3)_2]$ complex (Cu/phen/cnge). The antifungal activity of this complex was determined against one strain derived from the American Type Culture Collections (ATCC) namely *Candida parapsilosis* ATCC 22019 and two strains from clinical isolates (*C. albicans* and *C. tropicalis*). It is known that the strains responsible for infectious diseases in hospitals could show different resistance profiles than the ATCC strains. In this context, we decided to extend the antifungal evaluation of the Cu/phen/cnge complex including another ATCC strain and clinical isolates of *Candida* genus, which were kindly provided by hospitals of Chaco. The antimicrobial studies were coupled with the phytoxicity evaluation, in order to broaden safety studies previously carried out for this complex. The Cu/phen/cnge complex was prepared following the procedure described in our paper and the coordination was checked by vibrational infrared spectroscopy (FTIR). The antifungal activity of the complex was studied by the agar dilution method against *C. albicans* ATCC 10231, *C. parapsilosis*, *C. glabrata* and *C. krusei*. The minimum inhibitory concentration (MIC) is defined as the lowest dilution of the complex that inhibits the visible growth of the microorganism. MIC determinations against *C. parapsilosis* ATCC 22019, *C. albicans* and *C. tropicalis* were repeated and these results were included in this report for comparative purposes. On the other hand, the phytotoxicity was evaluated by the *Allium cepa* test, in order to determine the 50% inhibitory concentration (IC50). IC50 is defined as the concentration that inhibits 50% of root length from the negative control (with DMSO). The results showed that the Cu/phen/cnge complex has antifungal activity clinically relevant with MIC values between 3.9 and 15.6 μg/mL for most of the tested strains (except for the strains of *C. parapsilosis*). The MIC values for both ATCC and clinical isolate of *C. albicans* are the same, whereas the MIC value for *C. parapsilosis* from clinical isolate was higher than that for the ATCC strain. This result suggests a higher resistance profile for the clinical isolate strain. The MIC values for antifungal activity are slightly higher than the

IC50 of the complex in *A. cepa* (IC50= 3.4 μg/mL). Nevertheless, we previously demonstrated that the complex did not exert acute toxicity on *Artemia salina* until 750 μg/mL. In summary, the complexation is a useful strategy for designing novel antifungal agents.

Keywords: Phenanthroline and cyanoguanidine ligands, copper cation, coordination compounds, antimicrobials, safety

Methods: Agar dilution method, Allium cepa test

MI-03

PHYSIOLOGICAL ROLE AND STRUCTURAL CHARACTERIZATION OF MabR, A TRANSCRIPTIONAL REGULATOR OF MYCOLIC ACID BIOSYNTHESIS PATHWAY OF *MYCOBACTERIUM TUBERCULOSIS*

Coronel Cappellari, Chiara[1], Diacovich[1], Lautaro; Gramajo, Hugo[1]
[1]Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas (FBIOyF), Universidad Nacional de Rosario (UNR)
coronelchiara@gmail.com

Mycobacterium tuberculosis, the etiologic agent of tuberculosis, continues to be a major health problem worldwide. Mycolic acids, one of the most important lipids of the outer membrane of mycobacteria, have been associated with bacterial virulence and antibiotic resistance. Biosynthesis of mycolic acids involves two structural distinct fatty acid synthase systems, FAS-I and FAS-II. MabR is an essential transcriptional regulator that activates the expression of the fasII operon, modulating in this way the biosynthesis of mycolic acids. Its activity was found to increase in the presence of long-chain acyl-CoA, products of FAS-I and substrates of FAS-II. At the same time, these effectors prevent the binding of the transcriptional activator FasR to the *fasI* promoter, inhibiting fatty acid biosynthesis, suggesting the importance of maintaining lipid homeostasis and a regulated coordination between the two pathways.
One of the aims of this project is to perform structural studies on MabR in order to analyze its physiological mechanism at the molecular level. For this, we overexpressed and purified from *Escherichia coli* and *Mycobacterium smegmatis* two versions of this protein, since we found by bioinformatic studies that de N-terminal portion of the protein (19 aa) is probably unfold. Full-length and MabRΔ19 form a tetramer of 180 and 172 kDa respectively in solution. This result was corroborated by size exclusion chromatography.
The protein presented different stability and aggregation problems, and required high concentrations of glycerol, which is not compatible with structural trials. In this context, different buffers and additives were tested to improve protein concentration in solution and to replace the presence of glycerol in the samples to perform crystallization and cryo-microscopy assays. At the moment, in order to obtain images that provide preliminary information on the condition of the samples and to optimize the

conditions for future electron cryomicroscopy, we observed the sample with negative staining by electron microscopy.
With the aim of use an alternative strategy for the stabilization of the MabR protein, which will allow future structural, detection and inhibition studies, we started a protocol for the generation of nanobodies, in collaboration with a group from INQUIMAE at UBA. Among many applications, nanobodies can be used as crystallization or Cryo-Em partners, that is, as specific chaperones that bind and stabilize the three-dimensional conformation of the protein. Currently, a library of antibodies has been generated, and the selection and biopanning will be carried out in November, followed by their characterization and coexpression tests.

Keywords: Tuberculosis, Mycolic acids, transcriptional regulator, lipid homeostasis

Methods: protein purification, fast protein liquid chromatography, negative staining for electron microscopy, biopanning of nanobodies, crystallization trials

MI-04

CHRONOBIOLOGY IN CHEMOTROPHIC HUMAN PATHOGEN *ACINETOBACTER BAUMANNII*

Permingeat, Valentín[1]; Perez Mora, Barbara[1*]; Lamberti, Melisa Luciana[2]; Migliori, María Laura[2]; Golombek, Diego Andrés[2,3]; Mussi, María Alejandra[1]*
[1]Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI), CONICET, Rosario, Argentina. [2]Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Bernal, Argentina. [3]Universidad de San Andrés, Victoria, Argentina
permingeat@cefobi-conicet.gov.ar

Acinetobacter baumannii belongs to the ESKAPEE group due to its ability to "escape" antibiotic treatment and this pathogen is associated with high morbi-mortality infections. In this context, the World Health Organization included *A. baumannii* within the critical group of bacteria that constitute serious threats to human health.
We have extensively shown that *A. baumannii* perceives and responds to light, modulating global aspects of its pathophysiology. Most interestingly, light modulates its persistence in the environment, antibiotic susceptibility and virulence. We postulate that these bacteria could respond to light to synchronize their physiology with the host, to optimize infection outcome.
In this work, we demonstrate that the expression of the *blsA* gene presents oscillations along the day, when cells are entrained in light-dark cycles of 12 h each (12L: 12D) at 23ºC. These oscillations persisted when the cultures were further incubated under constant darkness. These observations are compatible with two criteria that define a circadian rhythm: free-running and entrainment.
These results were first obtained at discrete points of the day and were determined by RT-qPCR, then luminescent and fluorescent reporters were used under the control of the promoter of the gene under study to achieve a better temporal resolution of the phenomenon under study.

On the other hand, we have recently revealed that the BfmRS two-component system is directly involved in light signal transduction at 23 and 37°C. Sequence comparisons revealed that BfmR is 42.93% identical to RpaA, a response regulator involved in the circadian clock in Cyanobacteria. We hypothesize that BfmRS could be a component of a circadian clock that integrates environmental signals, including light. BfmS could antagonize BfmR function in light or dark, and let it function in the other condition, as occurs in the cyanobacterial KaiABC system.
These results could contribute to establish a new paradigm, with potential impact on biomedicine, since the current evidence regarding the circadian rhythms in nonphotosynthetic prokaryotes is scarce.

Keywords: circadian, fluorescence, luminescence, expression, light

Methods: fluorescence, luminescence qrt pcr, bioinformatic

MI-05

IDENTIFICATION OF ANTIMICROBIAL RESISTANCE GENES IN DAIRY FARM SOILS THROUGH SHOTGUN SEQUENCING

Cerioli Maria Florencia[1], Moliva Melina[1], Sambuceti Nicolas, Raviolo Jose[2], Sánchez-Pérez Mishael[2], Caminati Florencia[1], Rosenblueth Laguette Monica Teresa[2], Reinoso Elina Beatriz[1]
[1]Facultad de Ciencias Exactas, Fco, Quimicas y Naturales de la Universidad Nacional de Rio Cuarto, Córdoba-Argentina. [2]Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México. florenciacerioli@gmail.com

The natural habitat of opportunistic agents that can cause bovine mastitis is the environment where cows reside, with soil being one of the most microbiologically complex settings. The growing concern about antimicrobial resistance affects the general population. Detecting this resistance in soil samples is crucial, as recent studies have identified antibiotic-resistant genes in dairy facility waste, and these genes persist as reservoirs in soil bacteria, potentially contributing to resistant infections. The system involving soil, microorganisms, animals, and plants serves as a critical link between the natural environment and human health, both directly and indirectly. Therefore, it is essential to investigate processes related to antimicrobial resistance genes (ARGs) and relevant pathogens as they move through the soil environment to prevent potential adverse consequences for human health. The aim of this work was to employ metagenomic techniques, including sequencing and bioinformatic analysis, to identify antimicrobial resistance genes in DNA samples extracted from dairy farm soils in the province of Córdoba. Two farms, referred to as M1 and M2, with different prevalences of mastitis were selected: M1 with a high prevalence of contagious pathogens, while M2 had a high prevalence of environmental pathogens. Independent soil samples were taken from each farm, and DNA was extracted from each sample. Subsequently, sequencing was performed using the Illumina Novaseq 6000 platform, and the resulting reads were analyzed using the RGI software to identify the presence of antibiotic resistance genes. The results revealed that the DNA sample from farm M2 had twice as many genes related to antimicrobial resistance compared to M1. Genes encoding resistance to glycopeptides (*vanY*, *vanW*,

vanT, *vanR*, *vanH*), beta-lactamases (TEM, *cfxA*, *cfxA*6), and tetracycline resistance genes (*tet* genes) were identified. Additionally, numerous genes involved in the formation of multidrug efflux pumps, such as *mex*, *acr*, mdt, among others, were identified. Bioinformatic analysis based on a single DNA sample enabled the identification of a wide range of antimicrobial resistance genes. This underscores the significant advantage of sequencing in genome studies and highlights the importance of identifying these genes to understand the relationship and prevalence of pathogens, their potential impact on bovine mastitis, and the monitoring of the persistence of these genes to safeguard the health of humans, animals, and plants.

Keywords: Antibiotic-resistant genes, soil, dairy farm, metagenomic techniques

Methods: Extraction of DNA , sequencing, bioinformatic analysis

MI-07

IN VITRO LEISHMANICIDAL ACTIVITY AND SYNERGY OF KNOWN DRUGS AGAINST *LEISHMANIA* (LEISHMANIA) *AMAZONENSIS*: A PROMISING DRUG REPURPOSING STRATEGY

Araoz Emilse Natalia[1], Minahk Carlos Javier[2], Pérez-Brandán Cecilia Maria[1], Acuña Leonardo[1], Barraza Daniela Estefanía[1]

[1]Universidad Nacional de Salta, IPE-CONICET, [2]Universidad Nacional de Tucumán, INSIBIO-CONICET

barrazade86@gmail.com

American Tegumentary Leishmaniasis (ATL) is a neglected tropical disease, endemic in Argentina, caused by the flagellate protozoan Leishmania. Although not fatal, ATL leads to significant morbidity in affected patients. Current reference drugs for various forms of leishmaniasis include glucantime and amphotericin B. However, many treated patients experience adverse effects, intolerance, or unresponsiveness. Additionally, pharmaceutical investments and interest in drug development are scarce for neglected tropical diseases such as ATL.

In this study, we investigated the leishmanicidal effects of drugs already authorized in Argentina for the treatment of another disease, with the aim of exploring their potential repurposing for activity against Leishmania (Leishmania) amazonensis in vitro. The goal of evaluating existing approved medications is to identify candidates that could be redirected from their current therapeutic uses to address neglected diseases like leishmaniasis, thereby expanding treatment options through drug repurposing approaches. First, a screening of the drugs at their marketed doses was performed on L. (L.) amazonensis

(MHOM/BR/73/M2269) and RAW 264.7 macrophages. We determined the 50% inhibitory concentration (IC50), 50% cytotoxic concentration (CC50), for each drug tested, and fractional inhibitory concentrations (FICs) for amphotericin B in combination with selected drugs. An overall mean ΣFIC ($\chi\Sigma$FIC) was calculated to classify drug interactions. Parasites and macrophages were cultured and treated with individual drugs or amphotericin B combinations for 48 h. The WST-1 method was used to measure cellular respiration according to manufacturer instructions.
As a result, we established IC50 and CC50 values and observed synergistic $\chi\Sigma$FIC effects for all drug combinations against promastigotes parasites. Further experiments were then carried out to begin elucidating the mechanisms of action of the selected drugs. Tests included evaluating the action of the drugs in the absence of auxotrophic substances in the culture medium such as choline, in order to determine if the drugs could be acting at the level of Leishmania parasite metabolism. In conclusion, this work provides the first evidence that drugs approved for another disease demonstrate leishmanicidal activity and synergize with amphotericin B against L. (L.) amazonensis in vitro. Follow-up studies offered insight into potential parasitic metabolic modes of action. Significantly, these findings suggest that with optimization, the investigated medications may be repurposed as novel ATL therapeutics. Overall, this study demonstrates how drug repurposing can expand treatment options for neglected tropical diseases with limited resources.

Keywords: neglected tropical disease, Leishmaniasis, drug repurposing, parasite

Methods: Cellular culture, WST, Spectrophotometry

MI-08

CONTRIBUTION OF FRESH RIVER WATER TO THE DISSEMINATION OF HOSPITAL-ASSOCIATED *ENTEROCOCCUS FAECIUM* CC17 STRAINS IN CÓRDOBA

Blasko, Enrique G[1]; González, María J[1]; Bardossy, Ana C[2]; Sosa, Ezequiel J[5]; Prentiss, Tyler[3]; Suleyman, Geehan[2]; Jagjeet, Kaur[2]; Maki, Gina[2]; Ruiz, Susana E[4]; Grupo de Estudio de colonización por VRE de CBA[1]; Fernández Do Porto, Darío[5]; Bocco, J Luis[1]; Saka, H Alex[1]; Amé, Valeria[1]; Zervos, Marcus[23]; and Sola, Claudia[1]

[1]Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI) CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina; [2]Division of Infectious Disease, Henry Ford Health System, Detroit, MI, USA; [3]Global Health Initiative, Henry Ford Health System, Detroit, MI, USA; [4]LACE Laboratorios, Córdoba, Argentina; [5]Instituto de Cálculo-CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, CABA, Argentina.
enrique.blasko@unc.edu.ar

E. faecium is a bacterium capable of causing serious infections mostly acquired in hospitals. This bacterium is commonly found in the intestine of healthy humans and animals, allowing it to enter the environment through feces. The rise of vancomycin-resistant Enterococcus (VRE) has led to treatment challenges in hospital settings worldwide.

Our study aimed to investigate whether fresh river water contributes to the transmission of hospital-associated VRE clonal lineages.

We conducted a comprehensive analysis of the antibiotic resistome, virulome, and phylogenomic lineages of vancomycin-resistant *E. faecium* (VREfm) isolates using whole-genome sequencing (WGS) and bioinformatics tools. The isolates were obtained from various sources, including hospital-acquired infections (n: 3), hospital-associated colonization samples [from rectal swabs (n: 9) and chronic ulcers (n: 1)], and isolates collected from freshwater sources (Suquia River, n: 5) in an area downstream of the Córdoba city and the wastewater treatment plant (WWTP).

The antibiotic susceptibility was determined by diffusion/Vitek2, CLSI2019 and the presence of vanA/B genes by PCR. All the VREfm isolates were resistant to vancomycin (vanA+), teicoplanin, ampicillin and ciprofloxacin, with 77.8% presenting resistance to minocycline. VREfm belonged to clonal complex (CC) 17, ST17 (n= 6), ST736 (n= 6), and ST792 (n = 6). Ampicillin resistance was associated with predicted amino acid changes in the PBP5 protein. Most isolates harbored the following resistance genes: macrolides/lincosamides [msr(C), erm(B)], minocycline [tet(M), and tet(L)], aminoglycoside [aac(6)-aph(2), ant(6)-Ia, aph(3)-III, aac(6)-Iid] and trimethoprim (dfrG). Fluoroquinolone resistance was associated with amino acid substitutions in GyrA (Ser84Tyr: 66.7%; n =12) or Ser84Ile: 33.3%; n=6 and ParC (Ser82Arg: 100%; n=18). A total of 23 different virulence genes were identified including those encoding for adhesion (acm, scm, esp, sgrA, fms6 and fms22), capsule (cpsA/uppS, cpsB/cdsA) and biofilm formation (bopD).

Phylogenetic analysis revealed two clearly defined groups: one consisted of isolates with the ST17 profile (which grouped the 3 infection isolates and 3 environmental isolates), while the other showed ST736 and ST792 profiles (which grouped the 10 colonization isolates and 2 environmental isolates). Consequently, in both groups, genomes of environmental origin were identified. Moreover, no significant differences were observed in terms of genes related to resistance or virulence between them. These results support the hypothesis that the environment may act as a reservoir and/or means of dissemination for these hospital associated high-risk clones. Therefore, it is essential to prioritize the monitoring of resistance in environmental sources from urban areas and implement wastewater treatment strategies aimed at preventing the release of VRE into the environment.

Keywords: Enterococcus, faecium, clones, river, resistance

Methods: whole genome sequencing, bioinformatics tools, PCR, antibiotic disc diffusion

Lipids

LI-01

The balance between sphingolipids and phosphoinositides as a driving factor in epithelial cell differentiation

Pescio, Lucila Gisele; Mosca, Jazmín María; Favale, Nicolás Octavio; Sterin-Speziale, Norma Beatriz
Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. IQUIFIB – CONICET
pesciolucila@gmail.com

Epithelial cell differentiation starts with apicobasal polarization and asymmetric expression of the proteins and lipids of the membrane. Glycosphingolipids and phosphoinositides are key polarity lipids. The modulation of the distribution of the phosphoinositides is dependent on the location of specific kinases and phosphatases, and the distribution of these lipids within the plasma membrane changes during the process of epithelial cell polarization. In fully polarized cells, PI(4,5)P2 is enriched in the apical membrane, whereas PI(3,4,5)P3 is basolateral. The apical localization of PTEN allows the local synthesis of PI(4,5)P2 and the consequent apical recruitment of the PAR/aPKC complex, an important apical polarity complex.

Glycosphingolipids are enriched at the apical membrane of epithelial cells. Previous results from our laboratory showed that glycosphingolipid synthesis is essential for MDCK cell differentiation, expressed by a mature apical membrane and primary cilium formation, in cells subjected to hypertonicity. Also, we showed that PTEN knockdown or inhibition impairs MDCK cell polarization inducing aberrant lumens at the lateral domain. In this study we used a pharmacological inhibitor of PTEN (SF1670) in addition to an inhibitor of Glucosylceramide synthase (D-PDMP). Cells treated with SF1670 + D-PDMP showed a fully polarized phenotype with apical accumulation of the apical marker gp135 and basolateral distribution of α catenin. These results suggest that the treatment with D-PDMP reverted the deleterious effect caused by the inhibition of PTEN. We hypothesized that the treatment with D-PDMP induces an accumulation of sphingomyelin, responsible for rescuing the phenotype. The lipid extraction and separation of sphingolipids by Thin Layer Chromatography showed an accumulation of sphingomyelin in SF1670 + D-PDMP treated cells in comparison with SF1670 treated cells. These results suggest that the enrichment of sphingomyelin cause by the inhibition of glucosylceramide synthase improve the apical – basal polarity. Previous studies showed a transbilayer colocalization between sphingomyelin rich domains in the outer leaflet and PI(4,5)P2 – rich domains in the inner leaflet. Based on these evidence, we propose that the accumulation of sphingomyelin induced by SF1670 + D-PDMP treatment promotes the clustering of this sphingolipid with PI(4,5)P2, allowing the recruitment of the proteins that are part of the polarity complexes. In conclusion, cell differentiation requires regulated mechanisms to correctly distribute the sphingolipids and phosphoinositides to proper localize the polarity proteins.

Keywords: SPHINGOLIPIDS, PHOSPHOINOSITIDES, SPHINGOMYELIN, EPITHELIAL CELL DIFFERENTIATION

Methods: Cell culture, Sphingolipid extraction, Thin Layer Chromatography, Immunofluorescence, Confocal microscopy

LI-02

PROTECTIVE ROLE OF THE PHOSPHOLIPASE A2-CYCLOOXYGENASE 2-PROSTAGLANDIN E2-EP2/4 RECEPTOR AXIS IN THE RESTITUTION OF AN OXALATE-DAMAGED RENAL EPITHELIUM.

Sendyk, Dylan E.[1,4,6]; Erjavec, Luciana C.[1,4,6]; Parra, Leandro G.[1,4,6]; Bonino, Martina [1]; Flor, Sabrina [3,5,6]; Lucangioli, Silvia[2,5,6]; Fernández, María del Carmen [1,4,6]; Casali, Cecilia I. [1,4,6].
[1]-UBA, FFyB, Dpto Ciencias Biológicas, Biología Celular y Molecular. [2]-UBA, Facultad de Farmacia y Bioquímica, Departamento de Tecnología Farmacéutica. [3]-UBA, Facultad de Farmacia y Bioquímica, Departamento de Ciencias Químicas. [4]-IQUIFIB- Instituto Dr Alejandro Paladini -UBA-CONICET. [5]-InTecFyB (Instituto de Tecnología Farmacéutica y Biofarmacia)-UBA. [6]-CONICET.
sendykdylan@gmail.com

The renal inner medulla is responsible for the hydro-saline equilibrium maintenance through water and electrolyte excretion in urine. The collecting ducts, involved in the urine concentration, are immersed in an extracellular matrix with the highest body osmolarity and are exposed to wastes coming from blood filtration. There are several nephrotoxic agents such as antibiotics, diuretics, antineoplastic and cytostatic agents, and renal stones. Calcium oxalate stones are the most common type of kidney stones. The crystal aggregates are harmful to epithelial renal cells and tubular structures, and that damage could lead to the development of chronic kidney disease. Our previous results showed that renal differentiated cells treated with oxalate (Ox) for 24 h acquire a spindle-shaped morphology characteristic of an epithelial-mesenchymal transition (EMT) and a decrease in cell number. Then, cells started to recover their morphology reaching a restituted epithelium after 72 h of Ox. We also observed that Ox treatment modulates the expression of mRNA and protein Cyclooxygenase 2 (COX2), and COX2 inhibition with 10 μM NS398 prevents epithelial restitution. However, when the inhibition was bypassed by adding 10-6 M PGE2, EMT was not allowed after 24 and 48 h, and cells exhibited a morphology characteristic of the renal epithelium (cobblestone). The cPLA2 expression was also modulated by Ox. The aim of the present work is to evaluate the role of the cPLA2-COX2-PGE2-EP axis in the damage and restitution of a renal differentiated epithelium during the first 24h of Ox treatment. To do that, the renal

epithelial cells MDCK were grown in a hyperosmolar environment (512 mOsm/Kg H2O) for 72 h to get a differentiated epithelium, and then subjected to 1.5 mM Ox for 0, 4, 8, 16, and 24 h. Cell number and morphology were evaluated. Cell number started to decrease at 4 h, becoming significant at 8h after Ox addition and reaching the lowest value at 24 h. After 8 h, cells began to acquire a spindle-shaped morphology observing a complete EMT at 24 h. *cPLA2* and *COX2* mRNA and protein levels were evaluated. *cPLA2* mRNA and protein levels decreased at 4 h of Ox reaching control values at 24 h. However, an increase in *COX2* mRNA levels was observed after 4 h of treatment, peaking at 8 h and remaining elevated at 16 and 24 h. Also, COX2 protein level increased after 8 h of Ox reaching control values at 24 h. Also, PGE2 levels were quantified by mass spectrometry, showing an increase after 4 h, reaching their highest value after 8 h of treatment and remaining elevated after 24 h. Finally, the expression of EP2 and EP4 receptors was assessed by RT-PCR, showing a tendency to increase in the case of EP2 from 8 h onwards and a peak at 8 h for EP4. These results reinforce the importance of the cPLA2-COX2-PGE2-EP axis in the restitution of damaged differentiated renal epithelium caused by oxalate, highlighting the significance of studying it in the early hours following Ox addition.

Keywords: epithelial restitution, cPLA2, calcium oxalate, COX2, EP receptors
Methods: cell culture, western blot, PCR, fluorescent microscopy, mass spectrometry.

LI-03

DECIPHERING THE MOLECULAR MECHANISMS BY WHICH PTDCHO INDUCES NEURONAL DIFFERENTIATION OF NSCs, AND RESTORES NEURONAL PHENOTYPE (MORPHOLOGICAL AND FUNCTIONAL) UNDER STRESS CONDITIONS

Magaquian, Dario; Delgado Ocaña, Susana; Banchio, Claudia
Instituto de Biología Molecular y Celular de Rosario
darioem1994@gmail.com

Neuroinflammation is a common feature of many neurodegenerative diseases, and causes an imbalance in neural stem cells (NSCs) growth and differentiation, preventing important processes such as neurogenesis. Thus, it is essential to generate a favorables condition for NSCs and conduct them to differentiate towards functional neurons. Interestingly, we demonstrated that phosphatidylcholine (PtdCho)-enriched media enhances neuronal differentiation and restores the population of healthy normal neurons under inflammatory stress. We demonstrate that choline and CDP-Choline regulate the fate of NSCs and induce neurogenesis by its conversion into PtdCho. Experiments demonstrated that when the Kennedy pathway was blocked, Choline is unable to induce neurogenesis suggesting it has to be converted in PtdCho to affect the fate of NSCs. Subsequently, we evaluated the effect of this molecule under inflammatory stress conditions. As expected, like PtdCho treatments, Choline clearly

alters the balance between healthy/normal and dystrophic neurons, driving them toward the normal population. PtdCho induces neuronal differentiation of NSCs by a PKA-dependent mechanism, but restores neuronal morphology by an independent mechanism. To investigate the underlying mechanism, a variety of inhibitors targeting diverse molecular pathways were employed. Furthermore, changes in lipid composition of NSCs incubated under inflammatory conditions in the presence and in the absence of PtdCho was investigated. Here we provide evidence that reinforces the role of this lipid as a signaling molecule, opening new avenues to propose new medical therapies

Keywords: Lipids, neural stem cell, phosphatidylcholine, inflammation
Methods: Immunocytochemistry, western blot, morfological analysis, confocal microscopy

LI-04

LOMITAPIDE DOES NOT AFFECT LIVER TUMOR DEVELOPMENT IN MICE ON A HIGH-FAT DIET

Comanzo, Carla Gabriela [1,2,3]; Vera, Marina Cecilia [1,3]; Oviedo Bustos, Lucía [1]; Palma, Nicolás Francisco [1,2]; Ferretti, Anabela Cecilia [1,2]; Ceballos, María Paula [1]; Álvarez, María de Luján [1,2,3]; Quiroga, Ariel Darío [1,2,3].
[1]Instituto de Fisiología Experimental (IFISE-CONICET), [2]Área Morfología (FCByF – UNR, [3]CAECIHS (UAI)
cgcomanzo@gmail.com

Dysregulation in lipid metabolism is a general molecular phenomenon during the progression of hepatocarcinogenesis. It has been shown that lipid accumulation occurs during liver cancer development; however this mechanism is not fully understood. Microsomal triacylglycerol transfer protein (MTP) locates in the lumen of the endoplasmic reticulum and participates in the secretion of lipids from the liver as VLDL. MTP inhibitor lomitapide binds directly to MTP thereby inhibiting the synthesis of triglyceride-rich VLDL in the liver. In a previous work, we demonstrated that mice subjected to a chemical model of hepatocarcinogenesis, and treated with lomitapide, presented with increased liver/body weight ratio (2-fold) and with more tumors (2-fold) than control mice. The objective of the present work was to study the effect of the inhibition of VLDL secretion on liver tumor development in mice with dyslipidemia. Two-week old C57BL/6 male mice were subjected to a model of chemical hepatocarcinogenesis and kept on a chow diet. At week 16, mice were changed to a 60% fat diet (high fat diet – HFD). At week 29, mice were randomly divided into two groups. Control group received a vehicle (methylcellulose, gastric probe), and the other group received 5 mg/kg bw/day lomitapide (gastric probe) for 3 weeks. At week 32, mice were euthanized, livers were excised and weighed and tumors counted from the surface of the liver. There were no differences on body weight of lomitapide-treated mice compared to control mice. HFD induced an increase in plasma triacylglycerol (TG) and total cholesterol levels; as expected, plasma levels of TG and cholesterol were decreased

(-25%, and -20%, respectively) in lomitapide-treated mice compared to control mice. Lomitapide-treated mice showed no differences on liver/body weights ratio or number of tumors compared to control mice. Conclusion: Lomitapide administration did not present disadvantages regarding tumor growth in a model with metabolic alterations, unlike what we had observed in a model of chemical hepatocarcinogenesis with chow diet. These studies demonstrate that metabolic context could significantly change the effect of a treatment in a given disease.

Keywords: liver, cancer, high-fat diet, VLDL, MTP
Methods: biochemical determinations, animal handling, surgical procedures

LI-05

THE UNFOLDING PROTEIN RESPONSE MODULATES ARACHIDONIC ACID METABOLISM UNDER OSMOTIC STRESS.

Parra, Leandro[1,2]; Erjavec Luciana[1,2]; Zerpa Andrea1; Sendyk Dylan[1,2]; Salafia Ailén[1]; Casali Cecilia[1,2]; Fernández María del Carmen[1,2].
[1] Cátedra de Biología Celular y Molecular, Facultad de Farmacia y Bioquímica, UBA. [2] Instituto de Química y Fisicoquímica Biológicas, IQUFIB, UBA-CONICET.
le.parra@live.com

Renal medullary cells are constantly exposed to abrupt changes in environmental osmolality. To adapt and survive under these conditions, renal cells implement different osmoprotective mechanisms that include the upregulation of cytosolic phospholipase A2 (cPLA2) and cyclooxygenase 2 (COX2) expression and activity, and an increase in triacylglyceride (TG) synthesis.
We previously showed that hyperosmolality induces ER stress and activates the unfolding protein response (UPR) through IRE1α-XBP1s pathway, and that XBP1s induces TG synthesis and accumulation in renal epithelial cells. We also demonstrated that arachidonic acid (AA) released by cPLA2 activity stimulates TG synthesis in a COX2-independent manner. In this work we evaluated whether XBP1s regulates cPLA2-AA-COX2 axis under osmotic stress. MDCK cells were subjected to hyperosmolality (298-512 mOsm/kg H2O) for 24 h and treated in the presence or not of IRE1α RNase activity inhibitor 4μ8C, which blocks XBP1s expression. RT-qPCR results showed that 4μ8C prevented hyperosmolality-induced *cPLA2* and *COX2* mRNA upregulation. WB experiments showed that cPLA2 mRNA downregulation was accompanied by a decrease in protein abundance. In contrast, COX2 protein was significantly increased when cells were treated with 4μ8C. Confocal microscopy analysis demonstrated that IRE1α inhibition caused COX2 accumulation in granules that colocalized with ER marker calnexin, suggesting an impairment in protein degradation. Moreover, metabolic labeling

experiments showed that 4μ8C treatment decreased ^{3}H-AA incorporation into phospholipids under hyperosmolar conditions, indicating a reduction in cPLA2 activity. However, even though IRE1α inhibition induced an increase in COX2 protein, we did not observe changes in prostaglandin synthesis. Our results demonstrate that IRE1α-XBP1s UPR pathway has an osmoprotective role in renal epithelial cells by regulating AA metabolism and TG synthesis.

Keywords: Phospholipase A2, Hyperosmolarity, Unfolded Protein Response, Renal Cell
Methods: RT-qPCR, Western Blot, Confocal Microscopy, Metabolic Labeling

LI-06

THE HIF1-α AND FASN OVEREXPRESSION BY PALMITIC ACID AND FRUCTOSE INDUCED TUMORIGENESIS IN BREAST ADENOCARCINOMA

Ferrero, Victoria[1]; Mazo, Tamara[1]; Barotto, Nelso Neri[2]; Don, Julieta Aldana[1]; Moreira-Espinoza, María José[2]; Yennerich, Laura[2]; Granton, Florencia[2]; Mazzudulli, Gina[1]; Lagares, Clarisa [1]; Rodríguez, Valeria[1]; Pasqualini, María Eugenia[1].
[1] Instituto de Investigaciones en Ciencias de la Salud (INICSA-CONICET) - Facultad de Ciencias Médicas, Universidad Nacional de Córdoba. Ciudad Universitaria, 5000 Córdoba, Argentina; [2] Cátedra de Biología Celular, Histología y Embriología, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba. Ciudad Universitaria, 5000 Córdoba, Argentina
victoria.ferrero@unc.edu.ar

Cancer cells are characterized by their ability to maintain uncontrolled cell proliferation. The fatty acid synthase (FASN) is a key enzyme in the synthesis of membrane components necessary for cell division since it mediates de novo lipid synthesis catalyzing the production of fatty acids such as palmitic acid (PA) (1,2). Activation of the *FASN* gene is regulated by the alpha subunit of hypoxia-inducible factor 1 alpha (HIF1-α), which contributes to increased glycolysis in breast cancer (3). We proposed to investigate the effects of dietary palmitic acid (PA) and fructose (Fr) on FASN and HIF1-α expression during breast cancer development in BALB/c mice. Mice were divided into four dietary groups (n=20 each): CONTROL (6% corn oil + 30% Fr), PCS (20% palm oil + 15% Fr), PBA (20% corn oil + 45% Fr) and PCS+PBA (20% palm oil + 45% Fr). After 90 days on dietary treatment, mice were inoculated with murine breast adenocarcinoma cells (LM3: 1x106 cells). At 120 days, we evaluate biochemical blood profile and cancer growth parameters as tumor size, metastasis, tumor necrosis and infiltration (histopathology), Ki67 tumor expression (immunohistochemistry), tumor lipid profile (gas chromatography), and tumor FASN and HIF1-α expression (by western blot, qPCR and immunohistochemistry). In vitro experiments were performed using LM3 cells treated with AP (40μM), Fr (2.5mM) and the combination of both

treatments(AP+Fr). We analyzed cell viability, proliferation (using Resazurin and Hoechst), FASN expression (by western blot) and lipid profile (by gas chromatography). Experiments were repeated at least three times and analyzed by ANOVA ($p<0.05$). Mice that were fed with PCS+PBA diet showed a significant increment in tumor growth, infiltration and necrosis ($p<0.05$). The PCS group presented the highest AP content in tumor tissue, while the PBA group showed highest ω-6 PUFAs ($p<0.05$). The PCS+PBA diet induced an increment in FASN expression (by immunohistochemistry and western blot, $p<0.0001$) and high HIF1-α expression (by immunohistochemistry, $p<0.0001$). Fr-rich diet (PBA) increased *FASN* mRNA expression in tumor tissue ($p<0.05$, by qPCR) and Ki67 by IHQ ($p<0.0041$). Cell treatments with AP (40μM) and Fr (2.5mM) reduced apoptosis and increased viability compared to the control group ($p<0.05$). The combination of AP and Fr (40μM/2.5mM) of cell treatments increased FASN expression (by western blot, $p<0.006$). In conclusion, diets rich in palmitic acid and fructose promote higher FASN and HIF1-α expression, which in turn, stimulates cell proliferation in murine breast cancer.

Keywords: Palmitic acid, fructose, breast cancer, fatty acid synthase (FASN), HIF1-α.
Methods: Western Blot, qPCR, Immunolabeling, Gas chromatography, Resazurin Fluorimetric Assay.

LI-07

EXPRESSION AND HOST-PARASITE COMMUNICATION STUDIES OF *Echinococcus granulosus* 2DBD NUCLEAR RECEPTORS

Mozzo, Bruno; Blanco, Valentina; Alvite, Gabriela
Sección Bioquímica, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay.
gabial@fcien.edu.uy

The larval form of *Echinococcus granulosus* s. l. (cestode platyhelminth) is the causative agent of Cystic Echinococcosis, a cosmopolitan zoonosis that constitutes an important public health problem and causes economic losses. Treatment of this disease is often expensive, sometimes requiring major surgery and/or prolonged drug therapy. Despite the considerable socioeconomic impact, this parasitism continues to be a neglected zoonotic disease lacking effective therapeutic strategies. It is necessary to identify and study new specific targets of the parasite, as could be the Eg2DBDs proteins. The Eg2DBDs are nuclear receptors (NRs) belonging to the new 2DBD subfamily whose members contain two DNA binding domains and are not present in the parasite hosts. Since the NRs of parasitic helminths are considered as potential targets for new anthelmintic drugs, we propose to elucidate the biological functions of the Eg2DBDs in protoscoleces (PE) of *E. granulosus* s.l. (larval stage). Recently, we have identified four 2DBD-NRs in the parasite database: Eg2DBDα, Eg2DBDα.1, Eg2DBDβ and Eg2DBDγ; Eg2DBDα.1 is probably an Eg2DBDα isoform. We advanced in the characterization of Eg2DBDα.1 through in silico analyses of its 3D structure and putative ligands, as well as the description of its dimerization

mode by an in vivo approach. In this work, we analyze the *Eg2DBDs* expression levels in PE through RT-qPCR assays, and we study the ability of bovine fetal serum (BFS) to stimulate Eg2DBDα.1 homodimerization using modified yeast two-hybrid assays. In addition, the localization of Eg2DBDα and Eg2DBDβ proteins in PE was described by whole mount immunohistofluorescence experiments. Our results show that *Eg2DBDα* and *Eg2DBDβ* are expressed in PE with expression levels 121.5 and 35.4 times higher than that of *Eg2DBDα.1*, respectively; but it was not possible to amplify *Eg2DBDɣ* suggesting a low expression in this stage. Furthermore, we determined that BFS (intermediate host serum) stimulates the dimerization of Eg2DBDα.1, suggesting that at least one lipofilic molecule (probably an unsaturated long-chain fatty acid) binds to this NR, establishing a putative host-parasite communication. Finally, Eg2DBDα and Eg2DBDβ protein expression are detected in PE with specific antibodies, Eg2DBDα mainly localized in the parenchyma and sub-tegumentary region while Eg2DBDβ presents a high signal in PE stalk and tegument. In the future, we plan to study the ability of the Eg2DBDs to interact with the fatty acid binding protein EgFABP1, since EgFABPs could be involved in the distribution of fatty acids obtained from the hosts due to the platyhelminths cannot synthesize FA de novo. Supported by ANII (ANII-FCE 2017_1_136527), CSIC (CSIC I+D_2020_C112-347) and PEDECIBA, from Uruguay.

Keywords: nuclear receptors, Echinococcus granulosus, Cystic echinococcosis
Methods: RT-qPCR, modified yeast two-hybrid, whole mount immunohistofluorescence

LI-08

STRATEGIES FOR THE PRODUCTION OF NEUTRAL LIPIDS ENRICHED IN MEDIUM-CHAIN FATTY ACIDS

Pascutti, Federico [1]; Álvarez, Héctor M. [2]; Arabolaza, Ana [1]; Gramajo, Hugo [1]
[1] Instituto de biología molecular y celular de Rosario (IBR-CONICET). Facultad de Ciencias Bioquímicas y Farmacéuticas. UNR. [2]Instituto de Biociencias de la Patagonia (INBIOP-UNPSJB-CONICET), Comodoro Rivadavia, Chubut, Argentina
fedepascutti450@gmail.com

Since the escalating cost of crude oil and the increasing concerns about its finite supply, there is an emerging strategic need to develop additional renewable products from plants, or microorganisms. In this context, there are considerable environmental and economic drivers to develop new and improved bio-based chemicals. Particularly, oleochemicals such as free fatty acids (FA), triglycerides (TAG) and wax esters (WE) are considered as viable alternatives to most of the currently used petrochemicals.
On the other hand, oleochemicals generally derived from plants, are comprised mostly of long-chain fatty acids (LCFA), whilst medium-chain fatty acids (MCFA) are restricted only to a limited number of crops that are not suitable for their mass agronomic production. Neutral lipids, such as TAG and WE,

comprised of MCFA are gaining relevance in the food, jet-fuel and lubricants industries, given their differential physicochemical characteristics. Furthermore, the lubricant industry also relies on the use of petrochemicals derived components for the production of synthetic WE. These molecules present several advantages over vegetable oils in their use as lubricants, mostly due to their high oxidation stabilities and resistance to hydrolysis. Nevertheless, due to the high costs of obtaining WE from existing sources, their use is limited to specialized and high value products.
Microbial-cell-factories are an attractive model for the production of specific products, as it provides the opportunity to convert sustainable biomass into high-value chemicals. The assembly of metabolic activities derived from different organisms allows the reconstitution of designed biosynthetic pathways for the production of novel molecules with desired features. With the aim of producing novel lipids, such as TAG and WE containing MCFA, we propose the synthesis of these molecules using *Escherichia coli* as a platform. To achieve this, we first sequenced the genome of a *Rhodococcus sp.* strain isolated from contaminated sources, with the ability to synthesize neutral lipids esterified with medium-chain acyl FA. Wax ester synthase/diacilglycerol: acyltrasnferase (WS/DGAT) are the key enzymes for production of WE and TAG in bacteria. We found 13 different WS/DGAT homologs from the sequenced genome; 10 of them were selected based on phylogenetic analysis and conserved active-site motif. These 10 enzymes were heterologously expressed in a genetically modified *E. coli* (ΔdgkA ΔfadE ΔaraBAD, named MPS15) in order to analyze their ability to incorporate MCFA into the neutral lipids. Thin layer chromatography, GC-MS and LC MS confirmed the synthesis of TAG containing a high proportion of MCFA when WS/DGAT 602-3 was expressed in this recombinant *E. coli* strain. As a means to increase the strain MCFA tolerance, we generated an *E. coli* mutant strain derived from MPS15 named MPS16. This strain has a deletion in the AAS encoding gene whose function is to recycle 2-acyl-PE by re-acylating it.

Keywords: Triglycerides, Wax Esters, Metabolic engineering, medium chain fatty acids, biofuels
Methods: GC-MS, LC-MS, Gene deletion, Genome sequencing, Recombinant protein expression

Enzymes

EN-01

Biological activity of a novel fibrinolytic enzyme secreted by Hornodermoporus martius LBM 224

Acosta, Gabriela Alejandra 1; Fonseca, Maria Isabel 1; Fariña, Julia Inés 2; Zapata, Pedro Dario 1
1 Universidad Nacional de Misiones. Facultad de Ciencias Exactas Químicas y Naturales. Instituto de Biotecnología Misiones "Dra. María Ebe Reca" (INBIOMIS). Laboratorio de Biotecnología Molecular.

Misiones, Argentina. 2 Laboratorio de Micodiversidad & Micoprospección. PROIMI-CONICET. Tucumán, Argentina.
gaby_acosta@hotmail.com

Cardiovascular diseases represent global disorders that impact the circulatory system, constituting primary causes of morbidity and mortality. Fungal fibrinolytic enzymes offer promising advantages over conventional therapies, facilitating targeted clot lysis and mitigating hemorrhagic risks.The aim of this study was to characterize the biological activity of a novel fibrinolytic enzyme secreted by Hornodermoporus martius LBM 224. The plasminogen activator activity of the purified enzyme was studied using agarose-fibrin plates (0.5 % w/v) with and without plasminogen. The difference in the area of lytic zones between those plates indicated plasminogen activation activity.

The thrombin-like activity and the ability to degrade fibrinogen in vitro was evaluated. To do this, 1 mL of 10 mg/mL bovine plasma fibrinogen, 500 µL of enzyme in 50 mM tris-HCl buffer pH 7.4 were added and incubated at 37 ± 1 °C examining the formation of fibrin clot. After 1 h, 500 µL of 500 U/mL thrombin were added and the formation of the fibrin clot was observed. The in vitro anticoagulant effect was studied using human whole blood. To this end, 500 µL of purified enzyme, 500 µL of 100 U/mL sodium heparin, 500 µL of 50 mM pH 7.4 tris-HCl buffer, and 500 µL of saline solution were placed in four different sterile tubes. To each tube, 1 mL of blood was added, gently mixed by inversion, and incubated at 37 ± 1 °C. The tubes were tilted once every 30 s, and the time required for blood coagulation was recorded. Heparin, tris-HCl buffer, and saline solution were used as controls.

In the evaluation of the plasminogen activator activity, halos of fibrin degradation were observed both in the plates with and without plasminogen. However, the plates with plasminogen showed larger areas (66.36 ± 4.36 mm^2 and 50.10 ± 1.21 mm^2, respectively; $p < 0.05$), suggesting that the enzyme can degrade the fibrin clot both directly and indirectly by activating plasminogen into plasmin.

After incubation, for the enzyme with fibrinogen solution no fibrin clot formation was observed, indicating that it does not exhibit thrombin-like activity. However, the subsequent addition of thrombin prevented the formation of fibrin clots. These results suggest that the enzyme could act, not only as a fibrinolytic agent for direct degradation of the fibrin clot, but also for the prevention of clot formation.

The in vitro anticoagulant capacity showed blood clot formation in the same time as negative controls. However, after 1 h of incubation, the blood clots previously formed in the tubes containing the enzyme were degraded indicating that the enzyme has no anticoagulant activity but has the ability to degrade blood clots in vitro.

The results obtained demonstrate that the enzyme secreted by H. martius LBM 224 is a promising non-conventional alternative for clot degradation with potential application as a novel thrombolytic agent.

Keywords: Fibrinolytic enzyme, Hornodermoporus martius, Biological activity.

Methods: Plasminogen activation on agarose-fibrin plates, Evaluation of thrombin-like activity and fibrinogen degradation, In vitro anticoagulant effect study using human blood.

Structural Biology

SB-01

Studying the mechanical unfolding and refolding mechanism of Top7 at the single molecule level at different temperatures

Camila Graziele Corrêa[2], Javiera Martínez Bilbao[1,3], Are Mjaavatten[4], Christian A.M. Wilson[1]
[1]Laboratory of Biochemistry and Mechanobiology of Individual Molecules, Department of Biochemistry and Molecular Biology, Faculty of Chemical Sciences and Pharmacy, Universidad de Chile, Santiago, Chile. [2]Ph.D. Program in Biophysics and Computational Biology, Faculty of Sciences, Universidad de Valparaíso [3]Ph.D. Program in Biochemistry, Faculty of Chemical Sciences and Pharmacy, Universidad de Chile. [4]University of South-Eastern Norway, Porsgrunn, Norway
cami.grazi@usp.br

Top7 is a globular protein of 92 aminoacids. The tridimensional structure was designed with precision at the atomic level with computational design. As this protein does not carry the weight of evolutionary history, due to its artificial construction, it can be used as a model for proteins of similar size. Although biochemistry knowledge is significant in protein folding, few studies investigate the folding mechanics of proteins at the single molecule level at different temperatures. Those studies can provide the complete thermodynamics characterization of protein. Therefore, this study aims to investigate with optical tweezers (OT) the folding and refolding mechanics of Top7 with temperature variation, to elucidate the energetic profile of this protein. We can obtain the kinetic unfolding and refolding constants (ku and kr), the free energy difference of unfolding and for the transition state (ΔG and ΔG‡), the distance to the transition state (x‡) and the specific heat (ΔCp). We performed the experiment doing a force ramp at different speeds (10 nm/s, 100 nm/s, and 1000 nm/s), and temperatures (3º, 10º, 16º and 25ºC). Preliminary results show that the Top7 at 3º, 10º, and 16º performs unfolding at low and high forces, this could be because this protein explores different conformations or structures at low temperature. In room temperature (25º) experiments, the protein exploits only the high force conformation. In future experiments, we want to verify whether its secondary structure changes at different temperatures and obtain the stability curves for each temperature by circular dichroism to compare with OT.

Keywords: Top7, optical tweezers , folding, refolding

Methods: optical tweezers

Biotechnology

BT-01

Antifungal activity and toxicological profile of the ternary complex of copper with the flavonoid chrysin including 1,10-phenanthroline as secondary ligand

Bogado, Luciana Judith; Guevara, Lorena Elizabeth; López Tévez, Libertad Leonor
Universidad Nacional del Chaco Austral, Comandante Fernández Nº 755, Presidencia Roque Sáenz Peña (3700), Chaco, Argentina.
lucianabogado19@gmail.com

Chrysin is a flavonoid widely distributed in nature, which has important pharmacological and therapeutic properties. The ligand 1,10-phenanthroline is an organic molecule with a broad antimicrobial spectrum but without therapeutic applications due to its high toxicity (medium lethal dose on Artemia salina= 93.7 μg/mL). Flavonoids possess the ability to form complexes with essential metal ions as copper, and in some cases the complex exhibit improved pharmacological properties. We previously studied the antimicrobial activity and safety profile of the ligand chrysin and its binary complex (CuChrys). The aim of this work was to evaluate the antifungal activity of the copper complex with chrysin and 1,10-phenanthroline (CuChrysPhen) against strains derived from the American Type Culture Collections (ATCC) and strains from clinical isolates. This biological property was coupled with the safety evaluation at two experimental levels (Level 1: assays with bacterial strains; Level 3: assays with complete organisms). The antimicrobial activity of the complex has been studied by the agar dilution method. The minimum inhibitory concentration (MIC) was determined firstly against ATCC strains (Candida albicans ATCC 10231 and Candida parapsilosis ATCC 22019) in order to detect antifungal activity, and then against clinical isolates of four fungal strains (C. albicans, C. parapsilosis, C. tropicalis and C. glabrata). Mutagenicity was evaluated on two strains of Salmonella typhimurium by

the plate incorporation method (Ames test), acute toxicity was determined by the Artemia salina assay, and phytotoxicity was evaluated by the Allium cepa test. The CuChrys and CuChrysPhen complexes were prepared following the reported procedures and the coordination was checked by vibrational infrared spectroscopy (FTIR). The ternary CuChrysPhen complex showed antifungal activity clinically relevant (MIC values between 7.8 and 15.6 μg/mL) against both ATCC and clinical isolate strains. On the contrary, the binary CuChrys complex showed no antifungal activity. The CuChrysPhen complex induces neither mutagenicity (frameshift mutations on *S. typhimurium* TA98 or base-pair substitution mutations on *S. typhimurium* TA100) until 600 μg/plate nor acute toxicity on A. salina nauplii until 150 μg/mL. This concentration is higher than that required for antifungal action. Nevertheless, the MIC values for antifungal activity are close to the 50% inhibitory concentration in A. cepa (concentration that inhibits the 50% of root length from the negative control with DMSO). In summary, complexation of chrysin with copper modified the antimicrobial and toxicological profiles of the flavonoid. Moreover, the inclusion of the secondary ligand 1,10-phenanthroline improved the antifungal activity of the binary complex. In this context, the complexation is a useful strategy for designing novel and safe antimicrobial agents.

Keywords: chrysin flavonoid, copper cation, coordination compounds, antimicrobials, safety

Methods: Agar dilution method, Ames test, Artemia salina assay, Allium cepa test

BT-02

Ten years since the introduction of bacterial glycoengineering technology in the diagnostic algorithm of Hemolytic Uremic Syndrome

Melli, Luciano1; Landivar, Stella Maris2; Miliwebsky Elizabeth3; Chinen, Isabel3; Comerci, Diego2; Ugalde, JuanE2 and Ciocchini Andrés2.
1CHEMTEST ARGENTINA S.A.; 2Instituto de Investigaciones Biotecnológicas "Dr. Rodolfo A. Ugalde", UNSAM-CONICET; 3Servicio de Fisiopatogenia, INEI-ANLIS "Dr. Carlos Malbrán"
lmelli@chemtest.net

Human infection with Shiga toxin-producing *Escherichia coli* (STEC) is a major cause of postdiarrheal hemolytic uremic syndrome (HUS), a life-threatening condition characterized by hemolytic anemia, thrombocytopenia and acute renal failure. E. coli O157:H7 is the dominant STEC serotype associated with HUS worldwide although non-O157 STEC serogroups can cause a similar disease. In Argentina, O157, O145, O121, and O103 are the most prevalent serogroups. Detection of anti-O157 LPS antibodies in

combination with bacteriological procedures considerably improves diagnosis of STEC infections. Ten years ago, we have exploited the bacterial glycoengineering technology for the development of recombinant glycoproteins consisting of the O157, O145, O121 or O103-polysaccharide attached to a carrier protein. Then, an indirect ELISA was developed by using these serogroup-specific bacterial engineered glycoproteins (Glyco-iELISAs). We demonstrate that using these antigens it is possible to clearly discriminate between STEC O157, O145 and O121 infected patients and healthy children, even at early stages of the disease, as well as to confirm the diagnosis in HUS patients in which the classical diagnostic procedures failed. The Glyco-iELISAs were transferred to the National Reference Laboratory (NRL) where more than 1800 samples were analyzed over a period of 10 years. The implementation of Glyco-iELISAs in the NRL allowed to increase the association of HUS / DS to STEC from 25 to 75%. In recent years, advances in the field of nanobiotechnology and their application for the development of lateral flow immunoassays (LFIA) took on great relevance as tests for the rapid and in place diagnosis of different infectious diseases. Using the LFIA platform, we developed CHEMSTRIP® E. coli O157/O145 test that allows the detection of specific anti-O157 and anti-O145 IgM antibodies using the recombinant glycoproteins AcrA-O157 and AcrA-O145, respectively. By analyzing sera from patients with information from the days post-onset of symptoms, it was possible to detect 100% of positive cases after 3 days of onset of symptoms and up to 80% of positive cases before 3 days. The intervention of these antigens in the diagnostic algorithm produced a significant improvement in the association of HUS / DS to STEC and we consider that the implementation of the newly developed test could become an effective tool to avoid delays in supportive treatment or the application of a specific treatment when this becomes available.

Keywords: hemolytic uremic syndrome, Shiga toxin-producing Escherichia coli, in vitro diagnosis

Methods: bacterial glycoengineering technology, ELISA, lateral flow,

BT-03

Developing a Sustainable Enzymatic Degumming Process for Vegetable Oils Using a Consensus Sequence-Designed Synthetic Phospholipase C

*Val, Diego Sebastián (1); Di Nardo, Luisina (2); Marchisio, Fiorela (1); Gottig, Natalia (1); Peirú, Salvador (3); Castelli, María Eugenia (1); Abriata, Luciano (4); Menzella, Hugo Gabriel (1) and Rasia, Rodolfo Maximiliano (2, 5, *)*

(1) Instituto de Procesos Biotecnológicos y Químicos (IPROBYQ), FbioyF-UNR-CONICET. Mitre 1998, 2000-Rosario, Argentina. (2) Instituto de Biología Celular y Molecular de Rosario (IBR), FbioyF-UNR-CONICET. Ocampo y Esmeralda, 2000-Rosario, Argentina. (3) Keclon S.A. Cerrito 847, 2000-Rosario, Argentina. (4)

Ecole Polythechnique Federale de Lausanne, Rte Cantonale, 1015 Lausanne, Switzerland. (5) Plataforma Argentina de Biología Estructural y Metabolómica, Ocampo y Esmeralda, 2000-Rosario, Argentina. valdiegosebastian@gmail.com

The ever-growing demand for food and biofuels has exerted increasing pressure on the vegetable oil industry to innovate and adopt more efficient and sustainable refining processes. One promising approach involves the use of type C phospholipases (PLCs) for degumming, a green technology that yields extra oil. However, natural PLCs often fall short of meeting the demanding conditions found in oil refining plants, requiring additional operational steps to soften the process.

To address these challenges, protein engineering studies have frequently sought to enhance the stability of proteins, offering solutions to both environmental and industrial concerns. Enzymatic degumming, in particular, stands out as a pivotal enabling technology for achieving cleaner and more efficient methods in the industry. However, the widespread adoption of this sustainable refining technique has been hindered by the associated upfront capital expenditures and operational cost increases.

Here we achieved a substantial thermal stabilization of a bacterial Zn(II) dependent phospholipase C by consensus sequence design. We retrieved and analyzed sequenced homologs from different sources, selecting a subset of examples for expression and characterization. A non-natural consensus sequence, hare named ChPLC, showed the highest stability and activity among those tested. Comparison of activity and stability parameters of this stabilized mutant and other natural variants bearing similar mutations allow us to pinpoint the sites most likely to be responsible for the enhancement. Point mutations in these sites alter the unfolding process of the consensus sequence. We show that the stabilized version of the protein retains full activity even in the harsh oil degumming conditions, making it suitable for industrial applications.

Besides, we developed an enzymatic degumming process based on ChPLC. Using the synthetic enzyme crude soybean oil degumming was completed at 80 °C in only 30 minutes, the temperature and residence time used for water-degumming in most of the oil refining plants, saving the investment required to implement enzyme-based oil degumming. Remarkably, an extra yield of oil of 2 % was obtained with ChPLC using 60 % of the dose recommended for PLCs marketed today, reducing also the operational cost of degumming. A techno-economic analysis based on the obtained results indicates that, for medium size plants, ChPLC can reduce the overall cost of soybean oil enzymatic degumming by 58 %. The sustainable process presented here was conceived to be accessible for all vegetable oil producers, regardless of their processing capacity and the geographical location of their plant, bringing a potential annual benefit to the global economy in the billion-dollar range.

Keywords: vegetable oil, phospholipase C, enzymatic degumming, consensus sequence design, industrial enzymes

Methods: Hydrophobic HPLC purification, enzymatic oil degumming, thermal shift assay, circular dichroism, splicing by overlap extension PCR

BT-04

Use of modified algae biomass as a protector against plant osmotic stress

Marchetti-Acosta, Noelia; Velazquez, María Belén; Barchiesi, Julieta; Busi, María Victoria
CEFOBI, Centro de Estudios Fotosintéticos y Bioquimicos, CONICET/UNR
busi.conicet@gmail.com

Frataxin is a nuclear-encoded protein that has functions in the mitochondria. It is one of the components of the machinery responsible for the assembly of Fe-S complexes to different proteins. In addition, it would participate in the regulation of bioavailable iron levels and the attenuation of oxidative damage. Our group has characterized several Frataxins from different organisms, mainly from Arabidopsis thaliana, Zea mays, and Chlamydomonas reinhardtii. Recently, in gain-of-function tests with transgenic algae that overexpress CrFH we detected greater survival under conditions of oxidative and metal stress. When analysing the algal biomass by metabolomics techniques, we found an increase between 2 to 3-fold in amino acids content (mainly branched-chain and proline). Because it has been reported that the exogenous supply of branched chain amino acids increases tolerance to osmotic stress, we evaluated the application of algal biomass as stress protector.

Keywords: algae, plants, stress, frataxin

Methods: algae transformation, metabolomics, cell culture

BT-05

Bioprocess optimization of anti-native SARS-CoV-2 Spike S1 hybridomas

Acuña Intrieri, María Eugenia1; Pelliza, Leonardo2; Arán, Martín2; Cerutti, María Laura1
1Centro de Rediseño e Ingeniería de Proteínas (CRIP-EByN_UNSAM)), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). 2Fundación Instituto Leloir-IIBBA (CONICET),

macunaintrieri@iib.unsam.edu.ar

Monoclonal antibodies (mAbs) are one of the cornerstones of science involved in a broad range of applications such as research, diagnostic, and medical therapy. To meet the global demand, large quantities of mAbs are continuously produced, both from academic institutions and life sciences companies. Traditionally, the main method to obtain milligrams of mAbs at low cost and short times had relied on ascites extraction from mice carrying a peritoneal tumor. However, the use of this in vivo system presents several disadvantages, including animal ethical concerns, contamination with non-specific immunoglobulins, proteases, and/or adventitious agents, and tedious purification protocols. In fact, actual European regulations demand the use of animal-free replacement methods for antibody production and development. Thus, to obtain milligrams of high-purity antibodies under ethical procedures, mAbs production must evolve to in vitro methods using high-density hybridoma suspension cultures, a technological leap that not only fulfils international regulatory requirements but allows more robust and scalable bioprocesses and higher mAb product quality. We here present the adaptation and production yield of a hybridoma cell line grown in suspension shake flasks in chemically defined media (CDM) along with the set-up of NMR spent media experiments for -omic analysis of the bioprocess. Quantitative NMR was first validated by comparison of classical cell culture media analysis with reported composition. As model hybridoma, we used our 80E12 cell line which produces a high affinity mAb against native RBD and S1 Spike antigens. Cell culture parameters -viability and viable cell density- were routinely analysed as well as mAb productivity, this data was compared to static hybridoma cultures grown with fetal bovine serum (FBS) supplemented RPMI media. CDM hybridoma cultures produced 3-fold mAb product than static cultures. Furthermore, in contrast to mAb production in static cultures which were highly contaminated with FBS proteins, CDM cultures allowed simple product identification and quantification in coomassie blue stained SDS-PAGE. Altogether these results showed that in a short time, milligrams of mAb can be simply obtained from hybridomas cultured in vitro in high-density suspension cultures to accomplish both regulatory requirements and manufacturing demands.

Keywords: mAb, hybridoma, chemically defined medium, bioprocess

Methods: suspension culture, NMR, spent media analysis

POSTERS

Cell Biology

CB-01

Lactate as a mediator of autophagy in *Drosophila melanogaster*

Borrat, Agustina; Pérez-Pandolfo, Sebastián; Suárez, Sofía; Melani, Mariana; Wappner, Pablo
Instituto Leloir, Buenos Aires, Argentina
agustina.borrat@gmail.com

The cell recycles and degrades its cytoplasmic components through the process of autophagy. While this process is always active at basal levels, autophagy is strongly activated in stress conditions, including exposure to oxidizing agents, starvation and hypoxia. The adaptive response to hypoxia requires autophagy, yet the detailed molecular mechanisms by which it is activated remain elusive. During hypoxia, eukaryotic cells obtain all the energy they require from glycolysis, modifying their metabolism to inhibit mitochondrial oxidative phosphorylation; lactic acid is released as a byproduct. Using the fruit fly Drosophila melanogaster as a model, we have discovered that overexpression of Lactate Dehydrogenase (LDH), the enzyme that produces lactic acid, triggers the activation of autophagy in various larval organs.To identify the tissue source of the autophagic induction, we performed a screen of genetic drivers. We utilized the Gal4/UAS binary system to overexpress LDH in various tissues, after which we analyzed the activation of autophagy using the mCh-Atg8 reporter. We found that overexpression of LDH in the tracheal system (respiratory system), but not in other organs, triggers autophagy in the fat body (analogous to the mammalian liver). We are currently trying to define which is the biochemical signal that emanates from the tracheae to trigger autophagy at the fat body. By this project, we hope to contribute to the understanding of the mechanisms that activate autophagy under hypoxic conditions.

Keywords: Autophagy, lactic acid, Drosophila, metabolism

Methods: Gal4/UAS, confocal microscopy, mChAtg8

CB-02

QUERCETIN EXHIBITS ANTI-TUMOR ACTIVITY IN AN IN VIVO MODEL OF KAPOSI'S SARCOMA

Principe, Gabriel[1,2]; Tiburzi, Silvina[1,2]; Lezcano, Virginia[1,2]; García, Betina N. [2,3]; Gumilar, Fernanda[1,2]; González-Pardo, Verónica[1,2]

[1]Instituto de Ciencias Biológicas y Biomédicas del Sur (INBIOSUR); UNS-Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Bahía Blanca, Argentina. [2]Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur (UNS), Bahía Blanca, Argentina. [3]Bioquímica Austral, Laboratorio de Análisis Clínicos y Gestión, Bahía Blanca, Argentina.

vgpardo@criba.edu.ar

Quercetin (QUE) is a flavonoid with well-known anticancer properties, although its effect on viral-induced cancers is less studied. Kaposi's sarcoma is a viral cancer caused by the human herpesvirus-8, which during its lytic phase expresses a constitutively activated G protein-coupled receptor (vGPCR) able to induce oncogenic modifications that lead to tumor development. Our previous work showed that QUE inhibits cell proliferation and promotes apoptosis in endothelial cells stably expressing vGPCR (vGPCR cells). To follow up with this research, the aim of this study was to evaluate the anti-tumor activity of QUE in an in vivo model of Kaposi's sarcoma. For this purpose, tumor allografts from vGPCR cells were subcutaneously induced in N:NIH nu/nu mice. After 15 days of tumor development, mice were treated with QUE (50 or 100 mg/kg/d) or PBS (as control) administered by IP injection three times a week for 30 days. During the treatment, mice weight and behavior was monitored, and tumor volume was measured. The results showed that tumor progression was retarded in mice treated with QUE (100 mg/kg/d) compared to control ($p < 0.001$); whereas tumor weight was reduced by both QUE treatments (50 and 100 mg/kg/d) at the end of the test ($p < 0.05$ and $p < 0.01$, respectively). Immunohistochemical staining of tumors with the proliferation marker Ki67 showed that QUE treated tumors (50 and 100 mg/kg/d) exhibited a decline in proliferation compared to control ($p < 0.05$). In addition, serum biochemical parameters were measured to evaluate QUE toxicity. Neither kidney nor liver damage was detected since creatinine, alanine aminotransferase, and aspartate aminotransferase levels remained unchanged. Additionally, uremia was reduced by QUE treatment (50 and 100 mg/kg/d; $p < 0.05$, $p < 0.01$, respectively) and ALP levels decreased in mice treated with QUE (100 mg/kg/d) compared to control ($p < 0.01$). Moreover, a reduction in glycemia was found in mice treated with QUE (100 mg/kg/d) compared to control ($p < 0.05$), whereas not significant differences were found in total cholesterol and triglycerides. In conclusion, this study suggests that QUE exhibits antineoplastic activity and is devoid of toxic effects in an in vivo model of Kaposi's sarcoma, being a suitable candidate for the treatment of this pathology.

Keywords: PHYTOESTROGEN, KAPOSI'S SARCOMA, ANTI-TUMOR ACTIVITY

Methods: TUMOR ALLOGRAFTS, INMUNOHISTOCHEMISTRY, SERUM BIOCHEMISTRY

CB-03

GUANINE QUADRUPLEXES AS REGULATORY ELEMENTS OF THE SARS-COV-2 VIRUS

Diedrich, Lucas[1] ; Bezzi, Georgina[1] ; Binolfi, Andres[1,2] ; Armas, Pablo[1] .
[1] Instituto de Biología Molecular y Celular de Rosario (IBR), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de Rosario (UNR), Rosario S200EZP, Santa Fe, Argentina. [2] Plataforma Argentina de Biología Estructural y Metabolómica (PLABEM), Rosario (UNR), Rosario S200EZP, Santa Fe, Argentina.
lucasdiedrich00@gmail.com

The coronavirus disease 2019 (COVID-19) pandemic was triggered by the RNA virus that causes severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The replication cycle of this virus depends on different RNA molecules that act not only as viral genome but also as intermediates and templates for several processes such as viral protein translation, RNA dependent RNA synthesis for replication and transcription. Guanine quadruplexes (G4s) are non-canonical secondary structures formed by nucleic acids (DNA or RNA), that function as transcriptional and translational regulatory elements originally and mainly described in human oncogenes. Additionally, G4s have been implicated in the control of a variety of biological processes, including viral replication. Using various G4 computational prediction tools, we found putative G4-forming sequences (PQS) within the SARS-CoV-2 RNA genome (positive sense genomic RNA or +gRNA) and the negative sense genomic RNA (-gRNA) that acts as an intermediate RNA during viral replication. Some of the identified PQS are conserved in various betacoronaviruses related to SARS-CoV-2 while others are exclusive to SARS-CoV-2. Using multiple spectroscopic and biophysical techniques, we confirmed the formation of three G4s in the +gRNA and five G4s in the -gRNA of SARS-CoV-2, several of which had not been previously experimentally characterized. We performed CD melting assays in the presence and absence of pyridostatin (PDS), a specific ligand that binds and stabilizes G4s, and demonstrated that the ligand stabilizes most assayed G4s in both +gRNA and -gRNA. On the other hand, spectroscopic and biochemical approaches were used to demonstrate that CNBP, the major human cellular protein bound to the SARS-CoV-2 RNA genome, binds to and promotes the unfolding of most of the G4s formed both in the +gRNA as well as in the -gRNA of SARS-CoV-2. Employing molecular and cellular biology techniques, we assayed the function of the G4s formed by three PQSs of +gRNA on translation in a cellular context. In addition, we

studied the effect of one G4 formed by a PQS of the +gRNA on the programmed ribosomal frameshifting, an essential mechanism for viral gene expression. Finally, a computational search for sequence variations on the studied SARS-CoV-2 PQSs allowed us to identify two high-frequency variations that could affect G4s formation in viral variants of interest or concern, encouraging further study on the effects of these variations. Our results suggest that the G4s found in the SARS-CoV-2 RNA genome and their negative-sense replicative intermediates, as well as the cellular proteins and/or ligands that interact with them, are relevant factors for the regulation of viral gene expression and/or for the control of the viral replication cycle, and may constitute interesting targets for the development of antiviral drugs.

Keywords: G-quadruplexes, RNA, SARS-CoV-2, Translational control, Programmed ribosomal frameshifting

Methods: Circular Dichroism (CD) spectroscopy, CD melting assays, Nuclear Magnetic Resonance (NMR) spectroscopy, Cell Transfection, Luciferase reporter assays

CB-04

Consequences of disease-related genetic variations in guanine quadruplexes on post-transcriptional gene expression

Bayon, Candela[1]; Gismondi, Mauro[2]; Margarit, Ezequiel[2]; Armas, Pablo[1].
[1]Instituto de Biología Molecular y Celular de Rosario (IBR), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de Rosario (UNR), Rosario S200EZP, Santa Fe, Argentina. [2]Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Rosario, Santa Fe, Argentina.
bayon@ibr-conicet.gov.ar

Guanine quadruplexes (G4s) are non-canonical nucleic acid structures playing pivotal roles in modulating gene expression mechanisms ranging from transcription to translation, as well as other DNA- and RNA-associated processes. G4s are formed within G-rich single-stranded sequences characterized by a pattern of four tracts of 3 or more Gs separated by loops of 1 to 12 nucleotides (G3+N1-12G3+N1-12G3+N1-12G3+). Each G within a G-tract interacts with Gs in the other tracts to form a G-tetrad through Hoogsteen bonds, which can subsequently stack adopting the G4 structure. The stability of a G4 is closely related to the number of stacked G-tetrads and the length and composition of the loops as well as the adjacent sequences. Putative G4-forming sequences (PG4s) are overrepresented within the human genome's in gene regulatory regions, particularly those associated

with proto-oncogene transcription and translation control. G4s folding dynamics can be affected by short genetic variations within these PG4s. Moreover, disease related variants in noncoding regions can alter gene expression, prompting the hypothesis that these variants affect regulatory motifs, possibly G4s. To identify genetic variants in PG4s impacting G4 formation and stability in translation control, we conducted a genomic search on 5' UTRs and CDSs using the ENSEMBL database. The downloaded metadata were processed using R and Perl programming languages, which allowed us to identify flanking sequences to short genetic variants containing RNA PG4s. We then classified the variants into two groups. Group "A" corresponds to variants that present PG4 only in the sequence with the reference allele and not in the sequence with the alternative allele, thus includes variants that may cause the disruption of G4s formed in the reference sequences. On the other hand, group "B" corresponds to variants that present PG4 only in the alternative allele-sequence and not in the reference allele-sequence, thus includes variants favoring the formation of G4s not formed in reference sequences. PG4s associated with variants from groups “A” and “B” were assigned G4 formation scores using the G4RNAScreener predictor, which were used together with bibliographic data for selecting five candidates from group "A" to characterize in vitro the effect of the identified variants on the formation and stability of G4s. By means of Circular Dichroism spectroscopy, we observed that the PG4s found in the reference sequences can form G4 structures and present quantitative differences in the spectroscopic signals and structural stabilities compared to the alternative sequences. Our work suggests that variants affecting G4 folding may impact differential gene expression at the translational level, potentially contributing to human disease predisposition or onset. The effects of these variants on G4-mediated translation will be assessed through reporter gene expression assays in cultured cells.

Keywords: RNA, G-quadruplex, Short Genetic Variations, Translation, Disease

Methods: Bioinformatics sequence analysis, Circular Dichroism (CD) Spectroscopy, CD melting assays.

CB-05

Coupling between transcription and alternative splicing in C. elegans

Monti, Gonzalo; Martín, M.Eugenia; Buggiano, Valeria; Kornblihtt, Alberto; Godoy Herz, Micaela
Instituto de Fisiología, Biología Molecular y Neurociencias, CONICET y Departamento de Fisiología, Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina.
gonzalo.monti00@gmail.com

In eukaryotic cells, the RNA molecule resulting from transcription contains both coding (exon) and noncoding (intron) sequences. Through the process of splicing the intron sequences are removed from the newly synthesized RNA: the mature RNA transcript is composed only of exon sequences. Alternative splicing is a process that explains how multiple mRNA variants can be produced from a single gene. Our goal is to study coupling between transcription and alternative splicing in whole organisms, using *C. elegans* as our model organism. We have chosen to study the effect of starvation on alternative splicing in L1 animals. Alternative splicing patterns of representative splicing events (hrpf-1 and uaf-1) show changes in response to starvation. Furthermore, L1 animals were treated with camptothecin (a drug that inhibits transcription elongation): treatment with camptothecin increases hrpf-1 exon inclusion, whereas treatment with trichostatin A (drug that causes histone acetylation and therefore increased transcription elongation) produces the opposite effect on hrpf-1 splicing pattern. In addition, we have decided to silence specific elongation factors using a technique known as RNAi by feeding, used in *C. elegans*, with the objective of studying changes in splicing associated with them. This technique is based on the transformation of bacteria with a vector containing dsRNAs. We then feed the bacteria to the worms, which will incorporate the dsRNAs and pass them on to the progeny. We have chosen to knock down the transcription elongation factors RTFO, PAFO-1 and TFIIS.
These approaches allow us to study the role that transcription elongation plays in the regulation of alternative splicing in *C. elegans* and the importance of coupling for a whole organism to respond to environmental cues.

Keywords: Alternative splicing, C. elegans, Transcriptional elongation, RNAi

Methods: RT-PCR, RNAi, qPCR

CB-06

Guanine Quadruplexes Involved in Post-Transcriptional Regulation of Gene Expression Relevant to Embryonic Development

Bezzi, Georgina[1]; Gismondi, Mauro[2]; Margarit, Ezequiel[2]; Armas, Pablo[1].
[1]Instituto de Biología Molecular y Celular de Rosario (IBR), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de Rosario (UNR), Rosario, Santa Fe, Argentina.
[2]Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Rosario, Santa Fe, Argentina.
bezzi@ibr-conicet.gov.ar

Genomic DNA as well as RNA molecules may transiently fold as G-quadruplexes (G4s), non-canonical structures of nucleic acids associated with gene expression control and genome integrity. Although G4s formation and function was demonstrated in vitro and in cultured cells, the in vivo biological relevance of these structures is still elusive. Here, we used zebrafish embryonic development as an in vivo model to assess the role of G4s on the translation of developmentally regulated genes. The objective of this study was to identify conserved putative G4 forming sequences (PG4s) among H. sapiens, M. musculus, and D. rerio in genes related to embryonic development and located in regions probably implicated in the translational regulation of these genes. Firstly, we conducted a bioinformatic analysis using the Ensembl database to identify ~1100 genes related to the Gene Ontology (GO) term 'embryo development' for each species. Subsequently, PG4s were identified within the 5' untranslated region (5'UTR), coding sequence (CDS), and 3' untranslated region (3'UTR) using an algorithm to search PG4s. We found four developmentally regulated genes containing conserved PG4s within their 5´UTRs (zeb1a), CDSs (megf8 and col2a1) and 3´UTRs (smad3a) and assayed by circular dichroism spectroscopy that they are able to fold as G4s in vitro. Then, we cloned human and zebrafish PG4s of zeb1a and megf8a upstream and within, respectively, Renilla luciferase reporter CDS, and transfected the constructs in HEK293 cell line. Results reveal that all tested G4s alter Renilla luciferase activity by affecting translation levels. Finally, for the case of megf8, the in vivo role of the G4 was analyzed by microinjection experiments in zebrafish embryos of in vitro generated mRNAs containing the PG4s fused to GFP reporter coding sequence and specific disruption by co-microinjection of antisense oligonucleotides (ASOs) complementary to the PG4s thus disrupting G4 formation. This strategy led to translational decrease of the analyzed fluorescence expression, indicating that in this biologically relevant environment, the G4 of megf8 may act as a translation activator. Overall, this study indicates that G4s function regulating translation in vivo and may act as conserved fine-tuning elements of gene expression during embryonic development.

Keywords: G-quadruplexes, Embryonic Development, Zebrafish, Translation control

Methods: Circular Dichroism (CD) spectroscopy, CD melting assays, Cell culture, Cell Transfection, Embryo Microinjection.

CB-07

Sequential control of RNAPII levels by TC- and GG-NER in response to UV irradiation

Muñoz, Juan Cristobal; Beckerman, Ines; Etchepareborda, Joaquín Andrés; Alvarez, Gonzalo Gabriel; Rebottaro, María Luz; Bouvier, León Alberto; Muñoz, Manuel Javier

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

cristobaljuan.m@gmail.com

Our skin is regularly exposed to UV radiation that reaches the Earth's surface and damages cellular components. Since DNA is the only biopolymer that is neither disposable nor recyclable, it must be repaired when damaged. Among the various repair systems human cells have, the nucleotide excision repair (NER) system is the most relevant for repairing UV-induced lesions. Damage detection by the NER system occurs through two distinct lesion-recognition mechanisms that then use the same machinery to repair the damage: Transcription-Coupled Repair (TC-NER) and Global-Genome Repair (GG-NER). Given that RNA polymerase II (RNAPII) cannot transcribe a UV-induced DNA lesion, TC-NER rapidly detects and eliminates damage in the template strand of transcriptionally active genes. On its part, GG-NER detects damaged DNA thanks to the recognition factors XPC and XPE and eliminates UV-induced damage throughout the genome. Both detection systems converge in a single repair system that involves factors needed for double helix opening (XPB/XPD), excision of the fragment containing the lesion (XPA/XPG/XPF), and gap-filling of the single stranded DNA (ssDNA) by DNA polymerases. Regarding the connection between DNA damage and gene expression, it has been known for many years that RNAPII is specifically degraded by the ubiquitin-proteasome system in response to UV. Recently, the residue responsible for the ubiquitination and degradation of the major subunit of RNAPII was identified, and its replacement by a non-ubiquitinable amino acid prevented RNAPII degradation which, in turn, suppressed the transcriptional shutdown upon UV. The mechanisms leading to RNAPII degradation have not yet been elucidated. One hypothesis, known as the "last resort", suggests that RNAPII degradation is dependent on TC-NER. According to this theory, the stalling of RNAPII on DNA - due to its inability to transcribe the lesions - would lead to its degradation, allowing access to the repair machinery. Thus, it is speculated that RNAPII degradation is an in cis process dependent on TC-NER. While these ideas might explain some of RNAPII degradation observed after UV, preliminary results from our group showed that the majority of RNAPII degradation is controlled by the GG-NER system. Skin cells unable to recognize lesions through the GG-NER system (XPC/XPE double KO cells) showed a marked inhibition of RNAPII degradation at late time points after UV. On the contrary, skin cells unable to complete damage repair (XPA, XPG, XPF or XPD deficient cells), but proficient on its recognition, exhibited an enhanced degradation of RNAPII. These results suggest that signaling for RNAPII degradation lies between DNA damage recognition and the completeness of the repair. Having in mind that TC-NER is active immediately after UV, all together these results suggest a sequential control of RNAPII levels, first by TC-NER acting in cis and then by GG-NER acting in trans.

Keywords: UV, NER, RNAPII, GENE EXPRESSION

Methods: UV IRRADIATION, CRISPR-CAS9, WESTERN BLOT

CB-08

PARPi efficacy: the relevance of tissue origin, drug durability and off-targets

Castro, María Victoria[1]; Moyano, Ginette[1]; Menay, Florencia[2]; Cayrol, Florencia[2]; Sterle, Helena[2]; Iglesias, Brenda[3]; Sharma, Geeta[4]; Colmann, Kevin[4]; Stronach, Euan[5]; Madauss, Kevin[6]; Cremaschi, Graciela[2]; Mansilla, Sabrina Florencia[1]; Gottifredi, Vanesa[1]

[1]Fundación Instituto Leloir, Instituto de Investigaciones Bioquímicas de Buenos Aires, CONICET; [2]Instituto de Investigaciones Biomédicas UCA, CONICET; [3]GlaxoSmithKline, Oncology R&D, London, United Kingdom; [4]GlaxoSmithKline, Synthetic Lethal Research Unit, Waltham, MA, United States; [5]GlaxoSmithKline, Global Health R&D, Stevenage, United Kingdom; [6]GlaxoSmithKline, Global Health R&D, Upper Providence, PA, United States.

maria.victoria.castro95@gmail.com

Synthetic lethality is a therapeutic strategy based on the combination of genetic and pharmacologic factors that promote a selective cell death used to treat cancer. A well-known example of this is the appearance of PARP inhibitors (PARPi) to treat homologous recombination (HR) deficient cells. Nowadays, four PARPi – Olaparib, Rucaparib, Talazoparib and Niraparib – are approved by FDA to treat HR deficient tumors. However, Niraparib also showed great efficiency treating HR proficient ovarian tumors, indicating that although the four PARPi work by trapping PARP to DNA, its chemical differences affect their effectiveness. While there are limited reports including side-by-side comparison of PARPi, there is one report indicating that different cell lines from breast origin are more sensitive to niraparib than olaparib, an observation limited to cancers originated from these tissues. To extend this analysis, we performed survival experiments in presence of these PARPi using different cancer cell lines from several tissue origins and explored GDSC database to obtain data about the response of cell lines to niraparib and olaparib. With the exception of three cell lines, the fold difference (FD) in IC50 (olaparib/niraparib) was higher in breast/ovarian cancer cells - independently of their BRCA status - than in cancers from other tissue origin. Database analysis showed similar results indicating that Niraparib was more effective than olaparib on samples from breast/ovarian origin, an advantage not evident in samples from other tissue origin. One possible explanation is that these inhibitors have different durability, but we do not favour this possibility, as we did not see an improvement in the olaparib treatment when the survival protocol changed from single (previously mentioned results) to daily supply of the PARPi. Interestingly, a difference between olaparib and niraparib is their off-targets. Niraparib inhibits PARPs but also but also other enzymes including dCK, ALDH2, and Dyrk1A. Our current experiments are focus on determining the contribution of these off-targets to the cell killing power of niraparib.

Keywords: Ovarian cancer, PARP inhibitors, cell survival

Methods: Cell culture, survival assays, bioinformatic analysis

CB-09

Use of ascites fluid from patients to evaluate PARPi mechanisms of action

Moyano, Ginette[1]; Castro, María Victoria [1]; Menay, Florencia[2]; Ernst, Glenda[3]; Real, Jorgelina[3]; Korbenfeld, Ernesto[3]; Iglesias, Brenda[4]; Madauss, Kevin[4]; Sterle, Helena[2]; Cayrol, Florencia[2]; Cremaschi, Graciela[2]; Mansilla, Sabrina Florencia[1]; Gottifredi, Vanesa[1]

[1]Fundación Instituto Leloir, Instituto de Investigaciones Bioquímicas de Buenos Aires, CONICET; [2]Instituto de Investigaciones Biomédicas, UCA, CONICET; [3]Hospital Británico, Buenos Aires, Argentina; [4]GlaxoSmithKline, Oncology R&D, London, United Kingdom; [5]GlaxoSmithKline, Global Health R&D, Upper Providence, PA, United States.

ginymoyano@gmail.com

High-grade serous ovarian carcinoma (HGSOC) is the most common subtype of epithelial ovarian cancers (EOCs), representing 70% of them. Approximately 50% of HGSOC are associated with abnormalities in the homologous recombination (HR) pathway, as occurs with mutations in the BReast Cancer Genes 1 and 2 (BRCA1 and BRCA2), resulting in defective DNA repair known as "homologous recombination deficiency". In addition, advanced stage ovarian cancer is frequently associated with the accumulation of ascitic fluids in the abdomen, known as ascites, which is made up of cellular and acellular components.

Once removed, the ascitic fluid has no value from the clinical perspective but provides a source of tumor cells directly from patients, which can be used to validate results obtained from commercial cell line assays. They are also useful for evaluating their sensitivity to different drugs, in our case, Poly ADP-ribose polymerase inhibitors (PARPi), a therapeutic strategy used for the selective killing of HR-deficient tumor cells.

We have set up a work protocol with the aim of generating primary cultures from ascitic fluid-derived tumor patient cells. Compared to other types of cancer, primary cultures of ovarian cancer cells are relatively easy to obtain due to their high viability, strong surface adhesion, and rapid cell division. With serial trypsinization, we were able to select the tumor cells and eliminate the rest of the cell populations. We carried out in vitro assays on the already established primary cultures, which allowed us to characterize key markers for this tumor type, such as HR, BRCA, p53, and CK7 status. Currently, we are evaluating the effect of different PARPi, looking for possible correlations between the sensitivity to these drugs and the previously mentioned markers.

Keywords: Ovarian cancer, primary culture, ascites

Methods: Primary culture, Western Blot, Immunofluorescence

CB-10

Delving deeper into RNAPII degradation upon UV-induced DNA damage

Beckerman, Inés[1]; Muñoz, Juan Cristóbal[1]; Rebottaro, María Luz[1]; Álvarez, Gonzalo Gabriel[1]; Etchepareborda, Gonzalo[1]; Bouvier, León Alberto[1]; Muñoz, Manuel Javier[1,2]
[1]IFIBYNE - UBA - CONICET; [2]Facultad de Ciencias Exactas y Naturales - UBA
inesbeckerman@gmail.com

Among the numerous responses that occur in the event of DNA damage by ultraviolet (UV) light, RPB1, the major and catalytic subunit of human RNA Polymerase II (RNAPII), is specifically degraded by the ubiquitin-proteasome system. However, the mechanisms that control RPB1 degradation are not yet fully understood. The “last resort” model proposes that RPB1 is degraded at the damage site, or in cis, in order to facilitate access to the repair machinery.

However, RPB1 degradation might not necessarily occur only as a last resort. In this sense, unpublished results from our group indicate that RNAPII degradation is controlled by a DNA damage-recognition system which does not rely on the stalling of RNAPII in front of a lesion.

Here, we show that, accordingly with an in trans mechanism, UV-induced RNAPII degradation is not restricted to active RNAPII molecules. Indeed, the carboxy-terminal domain (CTD) of RPB1, which serves as a platform for post-translational modifications that regulate gene expression, is not required for RNAPII degradation. In this sense, two mutant versions of RPB1, one lacking the entire CTD and another one incapable of being phosphorylated on residues which are relevant for transcription, are both degraded upon UV-induced DNA damage.

On another hand, we have studied the relationship between chromatin structure and RNAPII degradation. Unlike exposure of cells with UV light, transfection of in-vitro damaged DNA did not trigger RNAPII degradation, suggesting that chromatin structure might be involved in the signaling pathway controlling RPB1 levels. With this in mind, treatment with trichostatin A, a histone deacetylase inhibitor, partially inhibited RNAPII degradation.

Altogether, these results indicate that RNAPII degradation is not restricted to RPB1 molecules that are engaged in transcription, and that the chromatin structure modulates the signaling pathway involved in the control of RPB1 levels in response to DNA damage.

Keywords: DNA damage, UV, RNAPII, chromatin structure

Methods: Western blot, immunofluorescence, RT-qPCR

CB-11

Role of heterogeneous ribosomes on translational activity from quiescent exist cells.

Fernández, Martín; Ortolá, María Clara; Rossi, Silvia; Galello, Fiorella and Portela Paula.
Universidad de Buenos Aires. Facultad de Ciencias Exactas y Naturales. Departamento de Química Biológica. Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales-Consejo Nacional de Investigaciones Científicas y Técnicas (IQUIBICEN-CONICET). Buenos Aires, Argentina.
pportela@qb.fcen.uba.ar

Cellular quiescence is the predominant state of all cells. S. cerevisiae is a useful model to study the molecular basis of quiescence and aging. Previously, we described that the ribosome composition of monosomes and polysomes changes during translational activation of quiescent cells. Now, we evaluated the role of individual ribosomal proteins (RPs), assessing cell growth and global translational activity of mutant cells (Δ) lacking one RP gene (RPG). First, we evaluated the consequences of a single RPGΔ paralog or non-paralog on growth fitness during quiescence exit. All RPGΔ strains reach the same level of stationary phase growth and exhibit similar cellular viability. However, strains rpp2BΔ or rps7BΔ show delayed cellular growth, and a strain rpl38Δ partially reduces lag phase length during quiescence exit. The results suggest that RP-specific effects control the efficiency of outgrowth from quiescence. Next, the role of RPs on global translational activation of nutrient-stimulated quiescent cells was evaluated by puromycin incorporation and Renilla luciferase translation reporter assays. We verified that the RPGΔs strain does not affect rRNA biogenesis. Compared to wild-type cells, rpl38Δ strains show a decrease in translational activity. Single deletions of the RPS7A/B, RPL16A/6B, and RPL37A/B paralog genes affect translational activity during quiescence exit. From the Rps7A/B pair, the paralog Rps7B is the most heavily expressed both in stationary phase and after nutrient stimulus, which differs from expression in exponential phase cells. Rps7B is associated with polysomes, whereas Rps7A remains associated with 80S fractions. The rps7AΔ strain reduces global translation activity in comparison to rps7bΔ. Therefore, both Rps7 paralogs carry out different roles in translation. The Rpl16A/B paralogs exhibit different behaviors. Rpl16 paralogs show similar distribution in polysomes upon fresh medium addition to stationary phase cells. Pull-down assays of Rpl16A-GST suggest that a mixed population of Rpl16A and Rpl16B ribosomes exists on individual mRNAs. As the level of the Rpl16B paralog is generally

higher than that of Rpl16A, polysomes containing only the Rpl16B paralog might also be expected. The rpl16BΔ strain shows lower translational activity than rpl16AΔ, suggesting a different role for these paralogs in translation. Lastly, Rpl37A associates exclusively with the monosome fraction in quiescent cells, and its deletion decreases global translational activity, while RPL7B gene deletion has no effect. For RP paralogs, it cannot be distinguished if the changes in global translation activity upon RPGΔ are a consequence of gene dose or gene type requirements. Altogether, the differential expression of RPs and their association with ribosomes might give rise to the formation of heterogeneous ribosomes, allowing a diversity in translation functionality during quiescence exit.

Keywords: Quiescence, translation, riboproteome, ribosome heterogeneity, Saccharomyces cerevisiae

Methods: RT-qPCR, polysome analysis, gene tagging, pull-down assay

CB-12

ALPHA 2-MACROGLOBULIN INDUCE LRP1-DEPENDENT AUTOPHAGY IN TWO HUMAN ERITRHOID LINES

Jacob, Julieta[1]; Castro Pérez, Santiago[1]; Reta, Pablo[1]; Vieyra, Cynthia[1,2]; Fader, Claudio Marcelo[1,2]
[1]Laboratorio de la Fisiología y la Fisiopatología del Glóbulo Rojo, Instituto de Histología y Embriología (IHEM), Universidad Nacional de Cuyo, CONICET, Mendoza, Argentina; 2Bioquímica General y Estomatológica, Facultad de Odontología, Universidad Nacional de Cuyo, Mendoza, Argentina
santiagorcastrop@gmail.com

The erythroid precursors go through physiological changes during erythropoiesis that are important for the maturation of the red blood cells. These changes involve the remodelling of the membrane, the decrease in cell volume, the production of hemoglobin and organelle clearance, such as mitochondria and ribosomes, after enucleation. Autophagy is a cellular process related to the engulfment of cytosolic macromolecules and whole organelles into double membrane vesicles called autophagosomes, which then fuse with lysosomes for the cargo degradation. During erythropoiesis, mitophagy and ribophagy play a crucial role in enabling the proper maturation of red blood cells. A transmembrane receptor known as LRP1 (low density lipoprotein receptor-related protein 1) engages in a variety of cell processes, including proliferation, differentiation, metabolism, apoptosis, autophagy, and the degradation of the hemin-hemopexin complex. To address the role of LRP1 in autophagy stimulation, we generated two different LRP1 knockdown hematopoietic cell lines (K562 and UT7), using the lentivirus approach. Both cell lines were derived from patients with chronic myeloid leukemia and are widely used as models for erythroid maturation and differentiation. We demonstrate that alpha-2 macroglobulin (LRP1 ligand)

triggers the autophagic pathway in a LRP1 dependent manner using confocal immunofluorescence, electron microscopy, and western blot. The study of autophagy in relation to the role of the LRP1 receptor during differentiation and erythropoietic maturation is crucial for the creation of potential treatments for several hematopathologies, including anemia and leukemia.

Keywords: AUTOPHAGY, LRP1, ERYTHROPOIESIS, LEUKEMIA

Methods: confocal immunofluorescence, electron microscopy, western blot

CB-13

Assessing the impact of single nucleotide variants in promoters of genes involved in neuronal differentiation.

Pinkasz Marina[1]; Iungman Martin[1], Salaberry J Pedro[1]; Arcuschin D Camila[1]; Fededa Juan Pablo[2]; Schor E Ignacio[1,3].

[1]Instituto de Fisiología Biología Molecular y Neurociencias (IFIBYNE-UBA-CONICET); [2]Instituto de Investigaciones Biotecnologicas (IIBIO-CONICET); [3]Departamento de Fisiología Biología Molecuar y Celular (DFBMC-FCEN-UBA)

marina.pinkasz@gmail.com

Metazoan development relies on the coordinated action of multiple transcriptional programs that determine cell fate. A crucial aspect of these programs is their ability to maintain functionality consistently, even in the presence of perturbations and noise. Non-coding variants, particularly those affecting transcriptional regulatory elements, play a significant role in contributing to phenotypic variability by modulating the expression levels of numerous genes.

With the aim of assessing the extent and phenotypic impact of regulatory genetic variants in a key developmental process, we used expression data from various human stem cell neuronal differentiation (Yao et al., Cell Stem Cell, 2017; Strano et al., Cell Reports, 2020; Floruta et al., Stem Cell Reports, 2017) to identify drivers of this process. We then analyzed the distribution of common single nucleotide variations (SNVs) (NCBI dbSNP) in the promoter regions of genes involved in neuronal differentiation and use the Combined Annotation Dependent Depletion tool (CADD) to identify a subset of potentially functional SNVs. To better understand the distribution of non-coding SNVs in neural differentiation, we evaluated how this is affected by different promoter- and gene-associated features. We found a positive association between the presence of TATA-box, Inr motif and CCAT motif with the

presence of high-impact variants, whereas CpG islands showed a negative association. In addition, when considering a neuronal differentiation gene regulatory network, we found that the promoters of network regulators have more deleterious variants than those of network targets. However, the minor allele frequency (MAF) of these variants was significantly smaller, suggesting that different selective forces are acting in the promoter regions of these two groups. We have selected four key differentiation genes, SRY-box 2 (SOX2), paired box gene 3 (PAX3), SIX Homeobox 3 (SIX3) and Frizzled Class Receptor 5 (FZD5) that harbor multiple potentially deleterious SNVs in their promoters, in order to experimentally test the effects of the different variants on the resulting promoter activity. We used a dual fluorescent reporter system that allows us to measure reporter expression at the single-cell level, enabling the detection of variations in both transcriptional activity and noise. We observed small but significant changes in the mean activity of PAX3 and FZD5 promoters when comparing the WT promoter with the one containing one or two SNVs. These results indicate that natural genetic variation cause differences in the expression level of key regulators of cell fate. We are currently studying the relevance of this transcriptional modulation, assessing the impact of these variants on the outcome of the neuronal differentiation process using the pluripotent human NT2 cell line as a cell culture model.

Keywords: Gene Regulatory Networks, Neuronal differentiation, Transcription, Genetic Variation, Transcriptional Robustness

Methods: Cloning, Cell culture, Flow Citometry, Genomics

CB-14

The inhibition of several mitosis regulators causes selective cell killing of BRCA2-deficient cells

Siri Sebastian Omar; Mares Ahlers Candelaria; Calzetta Nicolas , Abramovici Blasco Ariel; Okraine Yiovana Veronica; Caimi Lilen, Venerus Arbilla Sofia; Gottifredi Vanesa
Fundación Instituto Leloir, Instituto de Investigaciones Bioquímicas de Buenos Aires- CONICET
sebastian.siri11@gmail.com

Cells deficient in the tumor suppressor BRCA2 are highly sensitive to poly-ADP-ribose polymerase inhibitors (PARPi) due to the trapping of PARP on DNA. PARPi augments the focal organization of phosphorylated histone H2AX, which is considered a marker of replication stress (RS) and it is accompanied by increased micronuclei frequency. RS is a source or chromosomal rearrangements

that trigger adaptation of cells to PARPi. We are exploring alternative strategies to cause BRCA2-deficient cell killing. Strikingly, BRCA2-deficient cells are very sensitive to the inhibition of proteins with a key role in mitosis such as ROCK or PLK1 (Martino and Siri et al., 2023; Carabajosa et al., 2019). Such a cell killing is free of RS but is preceded by aberrant M phase progression. In addition, our more recent results show that the M phase-triggered selective cell killing of BRCA2-deficient cells is also observed after inhibition of Citron Rho-interacting kinase, Aurora A kinase, deoxycytidine kinase and other proteins with a key role in mitosis, as well as the mitotic poison paclitaxel. Supporting the relevance of M phase for the killing of BRCA2-deficient cells, the skipping of mitotic entry by early mitotic inhibitor 1 (EMI1) depletion promotes survival of BRCA2-deficient cells treated with ROCKi. Such a strong dependency of BRCA2-deficient cells on proteins that control the M phase reveals a potential therapeutic strategy to kill BRCA2-deficient tumors in a manner that could prevent RS induction, which could be advantageous for either preventing or postponing resistance to treatments.

Keywords: BRCA2, Mitosis, ROCK, EMI1, Synthetic Lethality

Methods: Cell Survival, Immunofluorescense

CB-15

Dialogue between molecular chaperones SRP and NAC during the biosynthesis of the urea transporter UreA in Aspergillus nidulans.

Gómez, Ania; Sanguinetti, Manuel; Ramón, Ana.
Sección Bioquímica, Departamento de Biología Celular y Molecular, Instituto de Biología, Facultad de Ciencias, Universidad de la República
agomez@fcien.edu.uy

UreA is an *A. nidulans* integral membrane protein (IMP) which mediates urea transport for its utilization as a nitrogen source. Our team has shown that two non-optimal codons encoding amino acids in the positions 24-25, situated in the limit between the N-terminal domain and the first transmembrane segment (TMS), are essential for its correct biosynthesis. Mutant strains bearing synonymous replacements of these non-optimal codons for their optimal counterparts (ureA2425), show a defective growth on urea as sole nitrogen source and an improved resistance to the toxic analogue 2-thiourea (2-TU), at optimal growth temperature (37ºC). The wild type phenotypes are partially restored when the ureA2425 strains are grown at 25ºC. Our hypothesis is that the non-optimal 24-25 codons would

determine a translational pause at very early stages, fundamental for the effective recognition and binding by specialized chaperones.

The signal recognition particle (SRP) plays a relevant role during the synthesis of IMPs, by recognizing signal sequences (SS) in the nascent peptides during early stages of translation, and guiding the translating ribosome-nascent polypeptide chain complexes (RNC) to the endoplasmic reticulum (ER) membrane, where it resumes its synthesis. NAC (nascent-polypeptide associated complex) is a general chaperone that acts as a negative regulator of the SRP pathway, unless the nascent polypeptides carry a SS. In such cases SRP displaces NAC, thanks to its high affinity to SSs. Some models propose that NAC aids this interaction, by recruiting and/or provoking conformational changes in SRP. This means that there is a dialogue between the two chaperones to determine effectively and efficiently the fate of translating proteins.

We have proved that deleting the alpha subunit of NAC provokes a lightly exacerbated defective growth on urea as sole nitrogen source in all *A. nidulans* strains, wt and ureA2425; as well as a slight augmented resistance to 2-TU. At the same time, the overexpression of the SrpA subunit of SRP has shown to partially revert the ureA2425 phenotype.

In this work we seek to deepen in the understanding of the relevance of the non-optimal codons 24-25 in the interaction of ribosomes translating UreA, with the molecular chaperones SRP and NAC, during its biosynthesis.

Keywords: SRP, membrane proteins, *A. nidulans*, NAC, translation regulation

Methods: Western blot, plate growth assays, mutagenesis

CB-16

ASSESSING THE CONSEQUENCES OF NON-CODING SOMATIC MUTATIONS IN THE MALIGNANT CHARACTERISTICS OF TRIPLE-NEGATIVE BREAST CANCER CELLS

Salaberry, Pedro J.[1]; Pinkasz, Marina[1]; Arcuschin, Camila D.[1]; Iungman, Martín[1]; Schor, Ignacio E.[1,2]

[1]Instituto de Fisiología, Biología Molecular y Neurociencias (UBA-CONICET), Buenos Aires, Argentina;

[2]Departamento de Fisiología, Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina

salaberrypedro.97@gmail.com

Whole-genome sequencing of tumor samples has revealed the high frequency of somatic mutations in non-coding regions. However, limited effort has been made to understand the oncogenic potential of these mutations. In order to understand the impact of non-coding somatic mutations and their contribution for disease characterization, we examined their distribution among promoters of genes which are relevant for malignancy of triple-negative breast cancer (TNBC), the subtype of worst prognosis among breast cancers, with high rates of metastasis and lack of targeted treatments. We first used gene expression data from isogenic TNBC cell lines with different metastatic ability to identify master regulators and pathways associated with a malignant phenotype. We combined a widely-used differential expression analysis method (DESeq2) and a breast cancer gene regulatory network (aracne.networks) with an algorithm (VIPER) which allowed us to infer changes in the activity of specific regulators through the interrogation of the expression levels of their target genes. This led us to a list of 354 differentially active regulators (FDR < 0.001). From this candidate set, we obtained an expanded network using the STRING database, and we delimited their active promoter regions in breast tissue using CAGE data from the FANTOM5 project. Then, we analyzed the frequency of potentially pathogenic regulatory mutations in their promoters, in order to prioritize candidates through regulatory mutation recurrence. We retrieved reported TNBC mutations from COSMIC and PCAWG databases, and we used three different functionality prediction scores (FATHMM-MKL, CADD and DeepSEA) to robustly assess their pathogenic potential. By means of a network propagation analysis, we found recurrently mutated subnetworks and functionally characterized them, identifying the chromatin remodeler complex SWI/SNF as a protein subnetwork recurrently affected by high-score non-coding somatic mutations in their promoters. We are currently assessing experimentally the consequences of the reported regulatory mutations on promoters' activity using single-cell reporter assays. Preliminary experiments showed a significant difference between the activities of wild-type and mutated promoters of the gene SMARCA4/BRG1 (p-value < 0.05, Wilcoxon test), encoding for a catalytic subunit of SWI/SNF. Finally, we are evaluating mutations of other promoters from SWI/SNF genes, as well as the influence of the transcriptional deregulation of these candidates in the malignant characteristics of TNBC cells.

Keywords: non-coding somatic mutations; gene regulatory networks; triple-negative breast cancer

Methods: differential activity analysis; network propagation analysis; flow cytometry

CB-17

COMPARATIVE GENOMICS REVEALS AN ASCOMYCETE H4 HISTONE VARIANT OF UNKONOWN FUNCTION

Flipphi, Michel[1]; Harispe, Laura[2]; Hamari, Zsuzsanna[3]; Kocsubé, Sándor[3]; Scazzocchio, Claudio[4]; Ramón, Ana[5]

[1]Department of Biochemical Engineering, University of Debrecen, Hungary; [2]Instituto de Profesores Artigas, Consejo de Formación en Educación (CFE, ANEP), Uruguay; [3]University of Szeged, Faculty of Science and Informatics, Department of Microbiology, Szeged, Hungary; [4]Institut de Biologie Integrative de la Cellule, Université Paris-Saclay and Imperial College London, Department of Life Sciences; [5]Sección Bioquímica, Dpto. de Biología Celular y Molecular, Facultad de Ciencias, UdeLaR, Montevideo, Uruguay

anaramon@fcien.edu.uy

The eukaryotic genome is organized in a chain of nucleosomes that consist of 146 bp of DNA wrapped around an octamer of H2A, H2B, H3, and H4 histones. Histone variants leading to altered nucleosome structure, dynamics and DNA accessibility have been described for all histones but are rare in the case of histone H4. Through a thorough scrutiny across ascomycete genomes, we identified a gene with a peculiar, well conserved intron-exon organisation encoding a novel H4-like histone (H4E, the E for "enigmatic") that is present throughout the sub-phylum Pezizomycotina, in two basal species of the sub-phylum Taphrinomycotina and in the phylum Glomeromycotina. At the protein level, H4E shows a conserved central core domain, but both N- and C-terminal domains show variations in length and sequence, with variable number and distribution of Lys and Arg residues with respect to canonical H4.

We found that in *Aspergillus nidulans* (Pezizomycotina, Eurotiomycetes) the H4E-coding gene is expressed under nitrogen starvation conditions. H4E localizes to the nucleus and it is able to interact with H3, but the deletion or overexpression of the encoding gene does not produce any detectable phenotype. Deletion of one of the canonical H4 genes resulted in a severe deleterious phenotype, which could not be corrected by H4E. Thus, we report a fungal H4 variant that is present throughout a whole subphylum of the ascomycetes, but whose function could not be revealed. The differences found in the terminal extensions of the H4E and the canonical H4 histones may result in novel post-translational histone modifications, which can alter the regulation of nucleosomal structure and function.

Keywords: histone variant- H4- Aspergillus nidulans

Methods: Comparative genomics, Fusion-PCR, fluorescence microscopy, RT-PCR, BiFC

CB-18

The bromodomain factor 5 (BDF5) is essential and participates in the compaction of the chromatin of Trypanosoma cruzi

Attala, Lucila; Araya Rodríguez, Elvio; Cribb, Pamela; Serra, Esteban
Instituto de Biología Molecular y Celular de Rosario, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario. (IBR – UNR - CONICET)
luliattala@gmail.com

Trypanosoma cruzi is a flagellated protozoan, the causative agent of Chagas disease. Like other trypanosomatids, it is characterized by an unusual genomic arrangement and gene expression primarily regulated through epigenetic mechanisms. In this regard, post-translational modifications of histones play an essential role, with acetylation of lysine residues being one of the key modifications. Bromodomains (BDs) are the only domains capable of recognizing these modifications and are found in proteins with various architectures known as Bromodomain-Containing Factors (BDFs).

Eight proteins with BDs have been identified in T. cruzi, referred to as TcBDF1-8. Among them, TcBDF5 is the only BDF that possesses two BDs, in addition to a copy of an MRG domain, defined as a protein-protein interaction module. Using a gene knockout assay with the CRISPR/Cas9 technique for the first 7 BDFs, it has been demonstrated that at least five of these proteins are essential. After three independent transformation and selection events, only hemizygous parasites could be obtained. Notably, hemizygous parasites for TcBDF5 exhibited an increased duplication time compared to the wild-type strain.

To gain a better understanding the function of BDF5, different versions of the protein were inducibly overexpressed using the pTcINDEX plasmid. From these experiments, it was observed that the expression levels of this factor are closely related to the parasite's growth phenotype. The expression of the wild-type protein drastically inhibited parasite growth, similar to the expression of a mutant version affecting an essential residue for the MRG domain's function. In contrast, mutants in the asparagine considered essential for each bromodomain's function had a slight impact on growth. These results suggest that the MRG domain might be more critical for TcBDF5 function than its BDs.

Furthermore, nuclear resistance assays to detergent lysis indicated that the overexpression of different versions affected nuclear resistance, showing a strong correlation between the initial growth rate effect and the number of recovered resistant nuclei. Transmission electron microscopy experiments suggested that the effect on nuclear resistance is the result of a high degree of chromatin compaction resulting from the overexpression of this factor.

On the other hand, metacyclogenesis and UV radiation exposure assays demonstrated that intracellular expression levels of this protein affect the parasite's transformation capacity and survival.

In summary, the results confirm that this factor is indispensable for the development and survival of the parasites and indicate that this could be a consequence of its potential regulatory role in chromatin compaction.

Keywords: Bromodomains, Epigenetics, Chromatin

Methods: Microscopy, Growth curves, Chromatin precipitation, Western Blot, Transfections

CB-19

LRP1 EXPRESSION IN THE MONOCYTE-MACROPHAGE DIFFERENTIATION PROCESS

Casale, Rocio; Campos Londero, Agustin; Saragusti, Alejandra; Chiabrando, Gustavo; Ceschin, Danilo
CIMETSA-IUCBC g.v. INIMEC-CONICET-UNC. Córdoba, Argentina
rociocasale95@gmail.com

Peripheral blood monocytes (PBMs) acquire pro-inflammatory profiles and participate in the early stages of atherosclerosis development, referred to as subclinical atherosclerosis (ASC) in asymptomatic individuals. LRP1 (low-density lipoprotein receptor-related protein 1) is an intracellular trafficking receptor that regulates endocytosis and intracellular signaling pathways and plays a significant role in atherosclerosis. In inflammatory processes, this receptor regulates the function of several membrane proteins such as the urokinase receptor (PLAUR), membrane metalloproteinases, β-integrins, and Toll-like receptors (TLRs), primarily TLR2 and TLR4. LRP1 is abundantly expressed in macrophages involved in the formation of atherosclerotic plaques. However, in previous studies, we have demonstrated that both gene and protein levels of LRP1 are decreased in pro-inflammatory PBMs of individuals with ASC. Nevertheless, the molecular mechanism that regulates LRP1 expression in PBMs and macrophages related to atherosclerosis development remains unknown. The aim of the present work is to study LRP1 expression using an in vitro cellular model of monocyte-macrophage differentiation. In this study, the THP-1 human monocytic cell line was cultured with Phorbol-12-Myristate-13-Acetate (PMA) at different concentrations and times to obtain three differentiation stages: monocytes, basal macrophages or M0, and pro-inflammatory macrophages or M1. At each stage, the protein and transcriptional expression of LRP1 and pro-inflammatory factors such as TNFα, IL1β, CCL2, and IL6 were measured using flow cytometry, Western blot, and quantitative RT-PCR (qPCR). The results show that LRP1 expression levels in the monocyte and M0 stages are lower than those observed in the pro-inflammatory M1 stage. Furthermore, this differential pattern of LRP1 expression positively correlates with the expression of pro-inflammatory mediators. These findings suggest that LRP1 could be involved in monocyte-macrophage differentiation and pro-inflammatory macrophage activation.

Keywords: LRP1, MONOCYTES, MACROPHAGES, ATHEROSCLEROSIS, INFLAMMATION

Methods: Cell cultures, flow cytometry, Western Blot, and qPCR

CB-20

Activated Alpha 2-macroglobulin and aggregated LDL promote differential subcellular distribution mediated by LRP1

Campos Londero, Agustín[1]; Casale, Rocio[1]; Castro Pérez, Santiago[2]; Jacob, Julieta[2]; Ceschin, Danilo[1]; Fader, Claudio[2]; Chiabrando, Gustavo[1]

[1]CIMETSA-IUCBC g.v. INIMEC-CONICET-UNC Córdoba, Argentina. [2]IHEM-CONICET Mendoza, Argentina

gustavo.chiabrando@iucbc.edu.ar

Low-density lipoprotein receptor-related protein-1 (LRP1) is an intracellular trafficking receptor that regulates endocytosis and intracellular signaling pathways in several types of cells. This receptor binds more than 50 different ligands, included α2-macroglobulin-proteinase complex (activated α2M or α2M*) and aggregated low-density lipoprotein (agLDL). Depending on the ligand LRP1 can follow different intracellular trafficking routes and mediates diverse cellular functions, such as cellular differentiation, migration and proliferation as well as autophagy and apoptosis. Previous data of our laboratory and other authors demonstrated that all ligands that interact with LRP1 undergo endocytosis and both, ligand and receptor, are accumulated in early endosomes mediated by a common clathrin-internalized process. In addition, we showed that α2M* induced the LRP1 endocytic recycling to plasma membrane, whereas agLDL led this receptor to locate in late endosomes and lysosomes. These results suggest that the intracellular trafficking route of LRP1 to different membrane compartments of the cell is regulated from its endocytosis and accumulation in early endosomes. Thus, the aim of this study is to determine whether α2M*and agLDL promote changes of subcellular vesicles and compartments in cells treated with α2M*and agLDL. In this way, HeLa cells were transiently transfected with a plasmid codifying GFP-Rab5 to identify early endosomes by fluorescent microscopy. After ligand stimulation (α2M*60 nM and agLDL 100 µg/ml) at 37 oC for different times (up to 30 min), colocalization analysis of LRP1 and Rab5 were carried out by confocal microscopy and the intracellular signaling activation (phosphorylated ERK1/2 and Akt) was examined by Western blot analysis. The subcellular endosomal analysis (subcellular vesicles and compartments) were performed by transmission electron microscopy (TEM). The results demonstrated that both α2M* and agLDL promotes a rapid accumulation of LRP1 in GFP-Rab5+ early endosomes (maximal proportion of colocalization to 10 min of ligand stimulation) and induced a similar profile of intracellular cell signaling activation: MAPK-ERK and

PI3K/Akt. On the other hand, by TEM we observed that HeLa cells treated with α2M* for 30 min configured a scenario of vesicular distribution rich in early endosomes and clathrin-coated vesicles compatible with recycling activity, whereas cells treated with agLDL for 30 min promoted an accumulation of late endosomes and myelinic bodies which may be associated with dysfunctional lysosomal activity. These data suggest that LRP1 ligands produce differential membrane trafficking at early endosome level from the ligand-LRP1 endocytosis and signaling activation, which in part may explain non-redundant functions of α2M and agLDL.

Keywords: Endocytosis, Endosomes, Membranes, Signaling, Traffic

Methods: Cell Culture, Confocal Microscopy, Transmission Electron Microscopy (TEM), Flow Cytometry assays; Protein Analysis Assays

CB-21

14-3-3 proteins regulate the condensation of Smaug1 membraneless organelles downstream of Smoothened/AMPK activation

María Gabriela Thomas[1], Francisco Corvetto Aristarain[1], Ana Julia Fernández Álvarez[1], Diego Bustos[2], Graciela Boccaccio1

[1]Instituto Leloir y IIBBA- CONICET, Buenos Aires; [2]Instituto de Histologia y Embriologia de Mendoza IHEM, Fac Exactas y Naturales UNCUYO

fcorvetto@leloir.org.ar

Membraneless organelles (MLO) are novel players in cell compartmentalization. In both the nucleus and the cytoplasm the condensation of macromolecules in discrete bodies termed biocondensates or MLOs helps chemical reactions, buffers intracellular concentrations and contributes to signaling pathways, among other functions. We have previously described that the post-transcriptional regulator Smaug –which is conserved from fly to mammals- condenses into cytosolic MLOs that are different from PBs and SGs (JBC 2009, JCB 2011). MLO formation typically involves low-complexity regions (LCRs) and a few LCRs are present in Smaug proteins. Drosophila Smaug MLOs dissolve upon activation of Smoothened (SMO), a key molecule in the Hedgehog pathway (EMBO Rep 2020). MLOs of the mammalian Smaug1/Samd4a dissolve upon exposure to metformin, an antidiabetic drug that activates AMPK. Smaug1 MLOs contain mRNAs for metabolic enzymes and their dissolution upon metformine exposure correlates with mRNA release (JCS2022). Here we show that Smaug1 MLOs respond to the pharmacological stimulation of a non-canonical SMO pathway, which involves AMPK activation and doesn´t require transcriptional changes. The molecular mechanisms controlling Smaug1 condensation

downstream of AMPK are unknown and we speculate it involves changes in Smaug1 phosphorylation and 14-3-3 proteins, which are chaperones of phosphoproteins. We found that overexpression of difopein, a non-natural peptide that inhibits 14-3-3 proteins, completely blocked the dissolution of Smaug1 MLOs upon SMO activation. The non-canonical SMO pathway affects metabolism and relevantly, a point mutation H86P in mouse Smaug1, referred to as "Supermodel", leads to a strong metabolic phenotype characterized by resistance to fat diet-induced obesity and defective adipogenesis. The H86P mutation is located in a LCR conserved in vertebrates and we investigated whether this mutation affects the condensation or dynamics of Smaug1 membraneless organelles (MLOs). We found that Supermodel forms a greater number of MLOs and a higher proportion of Smaug is recruited to the condensates relative to the soluble fraction. Preliminary results indicate that the response to SMO activation is impaired by the supermodel mutation. Collectively, these results suggest that condensation of Smaug1 MLOs regulates the energetic metabolism downstream of the SMO/AMPK axis involving 14-3-3 chaperones.

Keywords: Membraneless organelles, Smaug 1, Low-complexity regions, AMPK, 14-3-3 proteins

Methods: Confocal Microscopy, Cell Culture, Cell Transfection

CB-22

Exploring the Role of Vault RNAs in Cellular Stress Responses

Macarena Giménez, Peters Tomás, Graciela L. Boccaccio, Ana J. Fernandez
Instituto Leloir y IIBBA- CONICET, Buenos Aires, Argentina
tpeters@leloir.org.ar

Non-coding RNAs constitute a diverse family of molecules present in eukaryotes. Among them, Vault RNAs, a highly conserved subclass, warrant special attention. Despite being discovered over three decades ago, the molecular functions of Vault RNAs remain an enigma. Initially identified as components of the Vault particle, which is a ribonucleoprotein linked to transport across the nuclear envelope, the vast majority of Vault RNAs (~95%) are not part of the Vault particle and are involved in various cellular processes, including the activation of RIG-I, a key player in the interferon response. The human genome includes four Vault RNAs on the same chromosome at two different loci, all under the control of type 2 polymerase III-promoters. Vault RNAs usually have a short half-life, approximately 1 hour, making them candidates for rapid cellular responses. Recent research has revealed their involvement in autophagy and a prominent role in the antiviral response. During viral infections, such as

those caused by influenza, herpes, or Epstein-Barr viruses, a significant increase in Vault RNA 1-3 levels is observed. We investigated changes in Vault expression levels and half-life in response to acute cellular stress modeled in cultured HEK and A549 cells treated with sodium arsenite, and we found a moderate increase in their level. This work sheds light on the mysterious Vault RNAs, suggesting that they may be involved in the connection between the cellular stress response and innate immunity.

Keywords: Vault RNAs, Cellular Stress, Immunity, RNA

Methods: RT-PCR, Cell culture

CB-23

Quantifying the Lifespan of *Saccharomyces cerevisiae* Aging Model using Microfluidics

Bustamante-Torres, Moises[1]; Salzman, Valentina[1]; Godas, Maria José[2]; Bravo, Joaquín[2]; Estrada, Laura[2]; Aguilar, Pablo S.[1].

[1]Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE-UBA-CONICET). Bs. As., Argentina. [2]. Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Física, Buenos Aires, Argentina.

moisesbustamante819@gmail.com

Replicative Lifespan (RLS) is defined as the number of cell divisions that occur before a cell reaches senescence. Using *S. cerevisiae* as a model organism, RLS is traditionally measured by separating daughter cells from mother cells using a manual micromanipulator equipped with a fiber optic needle. This process takes from 3 to 5 weeks, making it a particularly challenging and error-prone endeavor. To address this limitation, we use a microfluidic single-cell analysis chip in conjunction with time-lapse microscopy to observe hundreds of individual cells throughout their lifespans. We were able to monitor cell lifespan in real-time of multiple positions over the array were recorded at 8.5 min intervals with a 40× objective lens for 96 h. Trapped yeast cells were cultured with continuous flow of SC media and maintained at 28 ºC. Using FIJI software (ImageJ), we manually reconstructed the entire lifespan of each trapped cell. This approach allowed us to successfully generate survival curves for both the well-known Wild-Type (WT) and long-lived tor1Δ strains, highlighting the device's capability for automated lifespan measurements. Notably, we determined the single-cell generation times for each strain throughout the entire experiment. Furthermore, these devices enabled us to assess phenotypic changes that occurred during aging and correlate them with different lifespan trajectories. Additionally, we employed curve fitting techniques to the experimental data using several parametric survival

distributions (Weibull, Gamma, Gompertz). This enabled us to reliably predict our RLS curves even in shorter experiments. To improve the resolution of the RLS quantification we calibrated the age distribution of initially loaded cells. For this we determined the number of bud scars in recently trapped cells through calcofluor staining by fluorescent microscopy. Consistently, this device allows us to measure the RLS of yeast strains and, using fluorescence microscopy, to study multiple changes that occur as the cell ages throughout its lifespan. Besides, using our microfluidic device, we found that the disassembly of plasma membrane nanodomains known as eisosomes extends yeast's replicative lifespan. These findings are currently unpublished and hold significant implications for our understanding of cell aging and lifespan regulation.

Keywords: Eisosome, replicative lifespan, microfluidics, *Saccharomyces cerevisiae*

Methods: Fluorescent microscopy, Microfluidics.

CB-24

Screening of heterologous cell fusogens in *Saccharomyces cerevisiae*

Gudiño, Virginia; Aguilar, Pablo Sebastián
Laboratorio de Biología Celular de Membranas, Instituto de Fisiología Biología Molecular y Neurociencias (IFIBYNE, UBA-CONICET)
gudvirginia@gmail.com

Specific proteins, called fusogens, catalyze the fusion of the biological membranes. In contrast to enveloped virus fusion and intracellular fusion, the fusogens that catalyze cell fusion are hardly known. In this sense, different research works show that the HAP2/GCS1 protein is involved in the fusion of gametes in diverse organisms (Speijer et al. 2015, doi: 10.1073/pnas.1501725112; Liu et al. 2008, doi: 10.1101/gad.1656508; Cole et al. 2014, doi: 10.1016/j.cub.2014.07.064; Von Besser et al. 2006, doi: 10.1242/dev.02683). In collaboration with others, our group showed that HAP2 is a bona fide fusogen. This protein is homologous to somatic cell fusogens (FFs) and class II viral fusogens (Valansi et al. 2017, doi: 10.1083/jcb.201610093). This superfamily of proteins was named FUSEXINS: fusion proteins essential for sexual reproduction and exoplasmic merger of plasma membranes. Fusexins are present in most eukaryotic lineages and therefore it is assumed they were present in the last eukaryotic common ancestor. However, they have not been detected in fungal and vertebrate genomes yet. Therefore, it seems very likely that during evolution other proteins replaced fusexins for gamete fusion in these lineages.

We aim to identify and characterize candidate genes to participate in the gamete fusion step. For this, we use the mating process of *Saccharomyces cerevisiae* as a gamete fusion model. To develop a functional complementation screening, we evaluated, in yeast, the performance of different cell fusion proteins from plants and viruses. On the other hand, we tested the effect of over-expression of different yeast proteins involved in gamete fusion. For this screening we used a S. cerevisiae gene overexpression library, which involves large amounts of cell-cell fusion assays, for this reason we trained a convolutional neural network to automatic segmentation of mating pairs and cell fusion quantification.

Keywords: fusogens, membrane proteins, screening, yeast

Methods: cell fusion assays, PCR, cloned, transformation

CB-25

Metformin Mitigates Craniofacial Abnormalities and Reduces Oxidative Stress in a Zebrafish Model of Treacher Collins Syndrome

Torres, Mercedes; Graziati, Sebastián; Coux, Gabriela
Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET-UNR)
mercedestorres98@hotmail.com

Treacher Collins Syndrome (TCS) is a hereditary mandibulofacial dysostosis resulting primarily from mutations in the TCOF1 (treacle ribosome biogenesis factor 1) gene. TCS is characterized by distinctive craniofacial abnormalities ranging from almost unnoticeable to severe even among individuals with identical mutations. However, the molecular mechanisms underlying the variable expressivity of TCS remain unclear. Previous research has indicated increased levels of reactive oxygen species (ROS) together with reduced CNBP protein expression in a zebrafish model of TCS. CNBP (CCHC-type zinc finger nucleic acid binding protein) is a ROS-sensitive protein involved in the regulation of neural crest cells (NCC) proliferation and apoptosis. Phosphorylation of CNBP by AMP-activated protein kinase (AMPK) plays a role in its stability. The anti-diabetic biguanide metformin is an AMPK activator with antioxidant properties. We propose that metformin may ameliorate TCS-related manifestations by modulating cellular metabolism.
In this study, we employed a zebrafish TCS model generated via specific Morpholino (designed to inhibit the translation of tcof1 transcript) injection into 1-cell staged zebrafish embryos. Negative controls were injected with the standard universal Morpholino, which does not bind to any zebrafish transcript. Incubations with varying concentrations of metformin diluted in embryo medium were carried out from 6 to 24 hours post-fertilization (hpf). Craniofacial cartilage morphology of the embryos was analyzed by

Alcian Blue staining of 120 hpf larvae. Remarkably, treatment with 1 mM metformin resulted in statistically significant improvements in craniofacial cartilage morphology of TCS-like larvae. Analysis of ROS levels (using DCFH-DA) revealed a normalization of these in TCS-like embryos treated with 1 mM metformin. The expression of redox-response genes (sod2, cat, nfe2l2α), assessed by qPCR, corroborated these findings. Additionally, metformin-treated embryos displayed a decrease in neuro-epithelial cell death (detected by Acridine Orange staining) and a restoration of NCC populations (evaluated by flow cytometry analyses with GFP-labelled NCC from Tg(-4.7sox10:GFP) embryos). Furthermore, metformin treatment led to the restoration of CNBP protein levels.
This study sheds light on the mechanism of action underlying metformin's protective effects, emphasizing its role in mitigating oxidative stress and preserving craniofacial development in TCS. Furthermore, thanks to the conservation of proteins and processes involved in vertebrate development, and although more in-depth studies are required, our findings suggest that metformin could be a therapeutic alternative to moderate the TCS phenotype. Finally, our results highlight the role of external factors (as oxidative stress) in TCS manifestations.

Keywords: CNBP, NEURAL CREST CELLS, AMPK, MANDIBULOFACIAL DYSOSTOSIS, TCOF1

Methods: Western Blot, RT-qPCR, Flow Cytometry, Microscopy

CB-26

Acetylation of PKA modulates both its catalytic activity and anchoring to scaffolding AKAP4 protein.

Gentile, Iñaki[1]; Novero, Analía[1]; Curcio, Catalina[1]; Buffone, Mariano[2]; Binolfi, Andres[1]; Krapf, Dario[1]; Stiva, Cintia[1]

[1] Laboratory of Cell Signal Transduction Networks. IBR (CONICET-UNR) and Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR), Argentina [2] Laboratory of Cellular and Molecular Biology of Reproduction. IBYME (CONICET), Argentina

gentile@ibr-conicet.gov.ar

After ejaculation, mammalian sperm require a period of residence in the female reproductive tract to gain fertilizing competence, in a process named “capacitation”. Capacitation can also be achieved in vitro by incubating sperm in a defined culture medium that induces an increase in cAMP levels which directly activates Protein Kinase A (PKA). Current knowledge positions PKA as a key player of sperm capacitation. In vivo, the resulting PKA activity depends on its catalytic activity, proper positioning, and activity of ser/thr phosphatases. Understanding its fine-tuned regulation becomes even more relevant

after we recently demonstrated how cAMP levels decrease after 10 minutes of incubation in a capacitating medium, while enzymatic levels of PKA remain constant. Two studies have identified 576 and 456 acetylated proteins in capacitated and non-capacitated human spermatozoa respectively, revealing a pool of proteins that are acetylated either only in capacitating or in non-capacitating conditions. Among these proteins, the catalytic subunit of PKA (cPKA) was identified to be acetylated in human capacitated sperm. We have confirmed its acetylation in murine sperm by western-blot experiments. Moreover, a recombinant his-tagged version of the mouse cPKA ("cPKArec") was purified in order to analyze the effect of lysine acetylation in this kinase. The recombinant cPKA was acetylated using whole sperm extracts supplemented with Acetyl CoA and inhibitors of deacetylases. Our results showed that acetylated cPKArec increased its catalytic activity, as measured in vitro using the peptidic substrate Kemptide and a gel mobility shift assay. In addition, the acetylating extract not only induced acetylation of cPKA but also stimulated phosphorylation of cPKArec in Thr197, which has been associated with PKA activation. We then aimed at understanding structural modifications promoted by Lys acetylation of cPKA in the recombinant protein. We conducted NMR experiments on a 600 MHz Nuclear Magnetic Resonance Spectrometer and observed that acetylation induced spectral shifts in amino acids of the catalytic site, which might account for its augmented activity.
Acetylation of cPKArec also affected anchoring since affinity purification of His-tagged cPKA showed an increased interaction of AKAP4 with acetylated cPKArec. This interaction protected AKAP4 from degradation during capacitation, when mouse sperm cells were incubated with deacetylase inhibitors, as shown by western-blot experiments. Our results shed light onto the molecular modulation of PKA promoted by Lys acetylation that are independent of cAMP, and might have key roles, not only in sperm, but in general cell physiology.

Keywords: PKA, sperm capacitation, acetylation, PKA activity

Methods: Western Blot, NMR, gel mobility shift assay

CB-27

Tracking APP in the endo-lysosomal pathway in a model of AD

Antonino, Magdalena[1], Piconêz Pereira, Tiago Miguel[2], Lorenzo, Alfredo1, Guimas Almeida, Claudia[2], Bignante, Elena Anahí[1]
[1]Instituto de Investigación Médica Mercedes y Martín Ferreyra, INIMEC-CONICET; [2]iNOVA4Health, Chronic Diseases Research Center (CEDOC), NOVA Medical School (NMS).
mantonino@immf.uncor.edu

Alzheimer´s Disease is a neurodegenerative disorder characterized by brain deposition of amyloid-β (Aβ). We recently found that Aβ assemblies, oligomers and fibrils, increase APP and BACE1 convergence and interaction in recycling endosomes of human neurons derived from iPSCs through Go/Gβγ signalling, which leads to an increase in APP processing by BACE1 and intracellular accumulation of Aβ1-42. Now, we are interested in deepen on Aβ effects on the APP trafficking in the endocytic pathway. We started transfecting N2A cells with whether APP:YFP or BiFC (a Bimolecular Fluorescent System to asses APP and BACE1 interaction) and amyloidogenic compartments markers fused to the RFP. Next, we treated the cultures with gallein, a Gβγ subunit inhibitor, and Aβ assemblies. We found that Aβ induced an increment of APP levels and its interaction with BACE1 in recycling endosomes and Golgi Apparatus and decreased APP levels and its interaction with BACE1 in lysosomes, effects that were abrogated by gallein. These results suggest that Aβ induces a redirection of APP to endosomes, avoiding its default route to degradation in lysosomes. Also, in order to test if this enrichment of APP in endosomes could be due to an increase in the APP endocytosis rate, we performed a pulse-chase assay that consisted of starving cells with serum free medium, incubating with anti-APP (clone 22C11) in complete medium (pulse) and chasing for 0, 30 and 60 min. We found that Aβ notably enhanced APP endocytosis rate by a mechanism dependent on Gβγ signalling. Furthermore, Aβ significatively increased the number of endocytosed 22C11-positive vesicles by a Gβγ signalling pathway. Finally, we evaluated if this accumulation of APP and its fragments in endosomes is related to the endosomal dysfunction largely described in AD patients so we measured the size and number of endosomes and we found that Aβ increased the number and size of both recycling and early endosomes by a Gβγ signalling. In conclusion, Aβ assemblies induce an increase in the APP endocytosis rate and a its redirection to endosomes avoiding lysosomes by a Gβγ signalling, which leads to APP accumulation in endosomes where it interacts with BACE1 favouring its amyloidogenic processing. Also, this accumulation of APP and BACE1 in endosomes leads to the enlargement and increase the number of endosomes, another early pathological sign of AD.

Keywords: Alzheimer´s Disease, Aβ assemblies, endo-lysosomal pathway, Gβγ signalling

Methods: Transfection, Colocalization, Pulse-Chase assay, Bimolecular Fluorescent System

CB-28

Antitumor activity of copper(II) complexes with 1,10 phenanthroline and dipeptides in cultured breast cancer cells

Katherine S. Muñoz Garzón[1], Natalia Álvarez[2], Gianella Facchin[2], Delia B. Soria[1], Ana Laura Di Virgilio[1]
[1]CEQUINOR (CONICET-UNLP) Bv120 N1465 e 60 y 64, La Plata, Argentina. [2]Área de Química Inorgánica, DEC, Facultad de Química Gral. Flores 2124, Montevideo.

kseneth22@gmail.com

Non-toxic antitumor therapies are the focus of numerous scientific investigations, during which various metal complexes linked to essential molecules such as amino acids are being developed. In this study, the antitumor activity of two copper(II) complexes with 1,10-phenanthroline (phen) as the primary ligand was examined in the human breast cancer cell line MCF-7. The complex [CuCl2(phen)] (1) was studied in comparison to its ternary counterpart with a dipeptide (Ala-Phe) as an auxiliary ligand, denoted as [Cu(L-Ala-Phe)(phen)]·4 H2O (2). Functional analysis revealed that both complexes induce antiproliferative effects and clone inhibition; however, neither of them exhibited an increase in the production of reactive oxygen species (ROS). On the other hand, the phenomenon of cell death induced by 1 occurs through the process of necrosis, while 2 triggers early apoptosis. Likewise, only at low concentrations of 2 (1 μM) were double and single-stranded DNA breaks detected, suggesting that this might be one of the molecular targets associated with its antitumor activity. This compound is a promising candidate for further in vivo studies to demonstrate its potential as an alternative therapy for breast cancer.

Keywords: Breast cancer, copper, antitumor agent, dipeptides, coordination complexes.

Methods: Spectroscopy fluorescence, Flow Cytometry, Comet Assay, Colorimetric Cell Viability Assay.

CB-29

KLF6 Tumor Suppressor is involved in cell death signaling triggered by Vibrio cholerae Cytolysin

Ochoa Denise, Salazar Villarreal Rocío, Scarzello Facundo, Saka Héctor Alex and Bocco Jose Luis
Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba (UNC), Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET-UNC). Córdoba. Argentina
denise.ochoa@unc.edu.ar

KLF6 belongs to the Krüppel-like/Sp1 family of transcription factors, which play diverse roles in cell proliferation, signal transduction, apoptosis and oncogenesis. Notably, mutations or downregulation of the klf6 gene have been identified in several human cancers, indicating its function as a tumor

suppressor. In support of this, our research has revealed that reducing KLF6 expression predisposes cells to spontaneous transformation and enhances the tumorigenic potential of NIH3T3 cells.
Furthermore, when KLF6 is overexpressed, it significantly impedes the growth of H-RasG12V-driven tumors. This effect is achieved through mechanisms involving Jun N-terminal Kinase activity, a G1-phase cell cycle arrest and induction of senescence. However, this cytostatic response was associated with resistance to apoptosis induced by DNA-damaging chemotherapy drugs. This suggests that KLF6 induction might be a protective mechanism activated in response to oncogenic stimuli, but also contributes to the survival of transformed cells. Therefore, molecules capable of reducing KLF6 protein levels may sensitize tumor cells death upon drug treatment, offering potential therapeutic benefits.
In this context, the field of cancer therapy is currently exploring engineered bacteria and the toxins they produce as promising avenues. Our laboratory has obtained results demonstrating that Vibrio Cholerae Cytolysin (VCC) exhibits potent cytotoxic activity at picomolar concentrations across multiple tumor cell lines. VCC triggers cell death through necrosis, apoptosis and autophagy in a dose-dependent manner.
We observed that exposing a colon carcinoma cell line to different doses of wild-type or VCC-defective mutant Vibrio cholerae culture supernatants, or purified VCC, led to an early and transient activation of JNK (Jun N-terminal Kinase) within 15 minutes, peaking at 45 minutes, and declining by 60 minutes of VCC exposure. A similar kinetic response was observed for the activation of ERK and p38 MAPK (Mitogen-Activated Protein Kinases) upon treatment with VCC. Notably, c-Jun, a preferential JNK substrate, was also phosphorylated shortly after VCC treatment, a process blocked by specific JNK inhibition (SP600125). Intriguingly, KLF6 protein levels were efficiently and progressively reduced starting at 15 minutes following VCC treatment.
Regarding the significance of MAPK signaling pathways for cell fate, we observed that inhibiting JNK, but not ERK or p38, resulted in decreased cell survival after toxin exposure. This involvement of JNK activity in cell survival upon VCC intoxication was further confirmed in similar experiments using Mouse Embryo Fibroblasts (MEF), both wild-type and JNK-deficient derivatives. These findings, combined with our previous observations of DNA damage accumulation and signs of genomic instability upon KLF6 silencing, suggest that early targeting of KLF6 is essential for VCC-mediated cell death.

Keywords: Bacterial Toxin, Tumor Therapy, Oncogenesis, Tumor Suppression

Methods: Cell Culture, Western blot, Cell viability assay

CB-30

Resveratrol exerts different effects on renal epithelial cells depending on media osmolality.

Erjavec, Luciana C.[1,2]; Parra, Leandro G.[1,2]; Casali, Cecilia I.[1,2]; Sendyk, Dylan[1,2]; Tulino, María Soledad[3]; Recabarren, Manuel[1]; López Nigro, Marcela[3,4]; Carballo, Marta[3,4]; Favale, Nicolás O.[1,2]; Fernández, María del Carmen[1,2].

[1]Cátedra de Biología Celular y Molecular, Facultad de Farmacia y Bioquímica (FFyB), Universidad de Buenos Aires (UBA). [2]Instituto de Química y Fisicoquímica Biológicas Prof. Alejandro C. Paladini (IQUIFIB), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). [3]Departamento de Bioquímica Clínica, FFyB, UBA. [4]Instituto de Fisiopatología y Bioquímica Clínica (INFIBIOC), FFyB, UBA.

lucianaerjavec@gmail.com

Resveratrol (RSV) is a polyphenol naturally present in several plants. Nowadays it is sold as an over-the-counter dietary supplement due to its antioxidant, anti-inflammatory and antitumoral effects. Paradoxically, it was proven that RSV may also present pro-oxidizing and pro-proliferative effects. Likewise, RSV effects on renal tissue are still controversial, as some injury models described beneficial effects, while others observed nephrotoxicity, without apparent cause. Renal medullary interstitium presents an elevated osmolality due to sodium and urea accumulation. Depending on the hydric state of the body, this osmolality can abruptly vary, reaching values up to 800-1200 mOsm/kg H2O. Initially, cells activate protective mechanisms to survive in such an adverse environment, but then this high osmolality is also a physiological signal for cell differentiation. We demonstrated that the renal epithelial cell line MDCK undergoes an adaptive process during the first 24h of hyperosmolarity, and after 48h cells are already adapted and begin to differentiate, acquiring a polarized morphology. Considering the controversial effects of RSV in the kidney, in this work we evaluate RSV effect both on cells cultured in isosmolality or subjected to physiological hyperosmolarity, particularly on osmoadaptation and differentiation mechanisms. MDCK cells were pretreated with different concentrations of RSV (1-25 μM) and then cultured in isosmolar (ISO, ~298 mOsm/kg H2O) or hyperosmolar media (HYPER, ~512 mOsm/kg H2O) for 24 and 48h. Cells were counted to obtain cell number. Viability using neutral red assay, cell cycle, DNA damage through comet assay, immunofluorescence (IF), western blot and qPCR analysis were performed. We found that RSV significantly reduced cell number and viability in concentration-dependent manner after 24 and 48h in HYPER, but not in ISO cells. Cell cycle analysis revealed that RSV increased S-phase and sub-G0 population in HYPER, but not in ISO cells. Comet assay showed a significant increase in DNA damage with 25 μM RSV in HYPER cells, which can be associated with the S-phase arrest; in this condition PARP cleavage was also higher, explaining the increase in sub-G0 apoptotic population. Additionally, RSV-treated HYPER cells did not attain typical epithelium morphology; instead, high RSV concentrations induced a mesenchymal phenotype. IF showed that RSV increased cytoplasmic accumulation of E-cadherin and β-catenin nuclear translocation in concentration-dependent manner. RSV treatment significantly increased mRNA expression of

mesenchymal genes (N-cadherin, α-SMA) and decreased E-cadherin mRNA expression in HYPER cells, supporting the previous results. Our results suggest that RSV concentrations higher than 5 μM exert significant toxic effects on renal cells exposed to physiological hyperosmolarity, but not on cells cultured in isosmolar media. These results can contribute to explain RSV controversial effects in different renal models.

Keywords: Resveratrol, kidney, osmoprotection, hyperosmolality, epithelial-mesenchymal transition

Methods: Cell culture, Western Blot, qPCR, comet assay, fluorescence microscopy

CB-31

CHOLESTEROL ENDO-LYSOSOMAL DISTRIBUTION RESEMBLING NEURONAL AGING FAVORS ALZHEIMER'S DISEASE LINKED PROCESSES

Martinez Navarro, Rosario; Almirón, Romina Soledad; Antonino, Magdalena; Martín, Mauricio; Bignante, Elena Anahi.
Instituto de Investigación Médica Mercedes y Martín Ferreyra. INIMEC-CONICET-UNC
rosario.martinez.navarro@mi.unc.edu.ar

Aging is the main risk factor for the Alzheimer's disease (AD) occurrence. It was reported that aging of hippocampal neurons is associated with a decrease in membrane cholesterol. Taking into account that cholesterol levels in the membrane affect multiple molecular processes, in this work we study how the redistribution of cholesterol emulating aging, affects the interaction of protein linked to AD. We particularly analyzed the effect of lowering cholesterol levels on events related to the amyloid precursor protein (APP) focusing in both: its role as Aβ precursor interacting with BACE1, as well as in its role as a membrane receptor analyzing its degree of homodimerization. One of the mechanisms that underlie the reduction of cholesterol in neuronal membranes is a decline in the levels of the NPC1 protein which is involved in the release of cholesterol from lysosomes following its endocytosis. We used the compound U18666a to induce re-distribution of cholesterol, emulating aging, and analyzed the interaction between APP and BACE1 and the dimerization of APP by bimolecular fluorescence complementation. We found that U18666a treatment increased the degree of APP dimerization, which could be related to activation of pathological signal transduction downstream. Likewise, we observed that U18666a treatment incremented the interaction between APP and BACE1, an effect that was avoided by incubation with the endocannabinoid anandamide which restores cholesterol efflux from lysosomes. In conclusion, the reduction on cholesterol levels on neurons favors events linked to AD since induces: an increment in APP/BACE1 interaction which could relate to an increment on APP amyloidogenic processing and Aβ

production; and also promotes APP dimerization which are linked to augmented APP signaling and amyloidogenesis.

Keywords: Alzheimer's disease, cholesterol, U18666a, amyloidogenesis

Methods: Bimolecular fluorescence complementation (BiFC), cell transfection, cell cultures, fluorescence microscopy

CB-32

Biological Activity of Copper(II) Complexes with Ligands Derived from 1,10-Phenanthroline and Dipeptides in Breast Cancer Cells

Katherine S. Muñoz Garzón[1], Natalia Álvarez[2], Gianella Facchin[2], Delia B. Soria[1], Ana Laura Di Virgilio[1].
[1]CEQUINOR (CONICET-UNLP) Bv120 N1465 e 60 y 64, La Plata, Argentina. [2]Área de Química Inorgánica, DEC, Facultad de Química Gral. Flores 2124, Montevideo.
kseneth22@gmail.com

In recent years, complexes formed by transition metal ions with amino acids as ligands have gained momentum as potential candidates for cancer treatment due to their interaction with various biological systems. In this study, three pairs of copper(II) complexes were analyzed: CuCl2(phen)(1), CuCl2(neo)(2), and CuCl2(tmp)(3), with primary ligands such as 1,10-phenanthroline (phen), neocuproine (neo), and tetramethylphenanthroline (tmp). Their effects were compared with their homologous complexes: Cu(L-Ala-Phe)(phen) (4), Cu(L-Ala-Phe)(neo)(5), and Cu(L-Ala-Phe)(tmp)(6), formed with a dipeptide (Ala-Phe) as an auxiliary ligand in MCF-7 breast cancer cells. It was observed that the IC50 at 24 hours for 1, 2, and 3 were 5.8 μM, 3.2 μM, and 1.8 μM, respectively. It was evident that the IC50 decreases with an increase in methyl groups on the primary ligands, enhancing the antiproliferative effects. Furthermore, the assays demonstrated clone inhibition starting at a concentration of 2.5 μM, genotoxic damage as assessed by the Comet assay in complexes 1 and 2 at concentrations of 1.5 μM and 1.0 μM, and their analogs 4 y 5 at a concentration of 1.0 μM y 0.5 μM respectively. On the other hand, it was found that the presence of dipeptides in the complexes leads to an increase in cell death through apoptosis. There was no evidence of an increase in reactive oxygen species (ROS) levels, indicating that oxidative stress is not the mechanism of action for these complexes. Thus, their molecular target would possibly be their direct action on DNA.

Keywords: dipeptides, copper, methyl groups, transition metals, breast cancer.

Methods: Spectroscopy fluorescence, Flow Cytometry, Comet Assay, Colorimetric Cell Viability Assay.

CB-33

Adaptive laboratory evolution induces a transitory tolerance to sulfite in wine yeast

Agustina, Caffaratti; María Laura, Raymond Eder; Alberto Luis, Rosa
Laboratorio de Genética y Biología Celular y Molecular, Departamento de Farmacología Otto Orsingher, IFEC-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba
acaffaratti@mi.unc.edu.ar

S. cerevisiae exhibit significant genetic plasticity to adapt to the environments created by human industry. Exposure of S. cerevisiae to SO 2 (i.e., SO 3 H - ; colloquially referred to as “sulfite”), in winemaking ecosystems, has led to the domestication and/or selection of strains tolerant to this compound. Cellular efflux of sulfite, mediated by the membrane protein Ssu1p, is considered a major phenomenon associated with SO 2 tolerance. In this sense, certain S. cerevisiae SO 2 -tolerant strains carry chromosomal aberrations which can eventually increase SSU1 gene expression (i.e., VIII-t-XVI, XV-t-XVI, and inv-XVI). Furthermore, it has recently been proved that shared metabolic pathways for both sulfur and selenium (i.e., SeO 3 =), require from the activity of Ssu1p. In this work, we analyzed the potential cross-tolerance to SeO 3 = of SO 2 -tolerant indigenous S. cerevisiae strains, as well as industrial S. cerevisiae strains carrying the chromosome aberrations VIII-t-XVI, XV-t-XVI, and inv-XVI. The presence of these genome alterations was characterized using PCR and specific primers flanking the corresponding breakpoints. In addition, to explore the potential connection between SO 3 H - and SeO 3 = tolerance, we conducted laboratory-based adaptive evolution experiments, under separate selective pressures of SO 3 H - and SeO 3 = . These studies were performed using the diploid oenological strain SB growing on defined YPD media with increasing concentrations (i.e., 15% every 24 h) of each of these compounds. Surprisingly, we found that after 100 generations, individual SO 2 -tolerant colonies isolated on YPD plates having concentrations of SO 3 H - were unable to re-growth under the same selective conditions. Thus, the developed SO 3 H - tolerance appears to be a transient trait. Explanations to this phenomenon are being explored. Similar laboratory-based adaptive evolution experiments are being conducted using SeO 3 = . Finally, in these studies we are using standard allele replacement (i.e., Kan R cassette) and gene fusion strategies to construct a *S. cerevisiae* null SSU1 mutant (Δ*ssu1*) and a chimeric SSU1-GFP gene, respectively. These tools would contribute to our current studies aimed to understand the physiological connection between SO 3 H - and SeO 3 = tolerance in *S. cerevisiae*.

Keywords: yeast, sulfite, selenite
Methods: Evolución adaptativa, PCR

CB-34

A gene reporter strategy to explore molecular triggers of endogenous DUX4 gene expression

Simonetti, Camila R.; *Pagnoni, Sabrina M.*; *Rosa, Alberto L.*
IFEC-CONICET-Departamento de Farmacología Otto Orsingher- Facultad de Ciencias Químicas-Universidad Nacional de Córdoba
camila.simonetti@hotmail.com

The human subtelomeric region 4q35 contains a polymorphic tandem of 11-100 units of 3.3 Kb each, called D4Z4. Our laboratory demonstrated that the DUX4 nuclear transcription factor, encoded within each D4Z4 unit, is toxic to muscle cells and could underly the pathogenesis of facioscapulohumeral muscular dystrophy (FSHD), a relevant autosomal dominant myopathy. Current developmental therapies for FSHD aim to block DUX4 activity in muscle cells. Surprisingly, the mechanisms underlying DUX4 gene expression, as well as the normal role of DUX4 in the few tissues where it is normally expressed, remain unknown. In order to characterize the molecular events triggering DUX4 gene expression, we are using HEK293 cells transfected with the reporter vector DRE-GFP, carrying a DUX4 responsive element (DRE) fused to the gene encoding GFP. This construct contains six binding sites for the DUX4 protein (5'-AGATAATTGAATCATGGGGTAATCCAATCATGGAGTAATTTAATCAGCCGTTAATTGAATCATGGGTAATCCAATCATGGAGTAATTTAATCAGCCG-3') and a minimal TATA promoter, fused to the nuclear turbo-GFP gene. The construct has been validated as a tool to detect the cellular presence of DUX4 [i.e., DUX4(+) cells], when this protein is either transiently expressed in transfection studies, or when expressed from transgenes in stable cell lines or mice. In our preliminary assays, we are studying cells co-transfected with this reporter and various plasmids expressing wild type and mutant versions of DUX4. An alternative reporter, DRE-Renilla-p2A-Mscarlet, is also being characterized in our studies. This reporter would allow us to recognize the expression of the endogenous DUX4 gene using a Renilla assay and/or using fluorescent microscope detection of the Mscarlet protein. Our studies would contribute to the understanding of the molecular mechanisms underlying the normal and pathological expression of the DUX4 gene.

Keywords: Neuromuscular, FSHD, DUX4, Genome damage, Double Strand Breaks

Methods: Cotransfection, Luciferasse assay, cell culture, pcr

CB-35

Involvement of SUMO conjugation in small nuclear RNA biogenesis

Bragado, Laureano; Magalnik, Melina, Pozzi, Berta; Srebrow, Anabella
Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Fisiología, Biología Molecular y Celular, Buenos Aires, Argentina. 2CONICET-Universidad de Buenos Aires, Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE)
laureanobragado@gmail.com

Small nuclear RNAs (snRNAS) are non-coding RNA molecules, which associate with a large set of proteins to form small nuclear ribonucleoprotein particles known as “snRNPs”. While the function of snRNAs and the assembly of snRNPs are well characterized, the regulation of snRNA gene expression is still poorly understood. Most snRNAs are transcribed by RNAPII, including the major spliceosomal components U1, U2, U4 and U5. Unlike pre-mRNAs, snRNAs are intronless and non-polyadenylated. RNAPII transcribes snRNA genes in close proximity to Cajal bodies (CBs). Taking into account the relevance of snRNAs for spliceosome composition and function and consequently for the splicing process, that the regulation of the expression of these non-coding RNAs is still poorly understood, together with previous work from our laboratory that demonstrated the importance of SUMO conjugation (or "SUMOylation") for spliceosome assembly and catalytic activity, we decided to explore a possible connection between the SUMO machinery and the biogenesis of snRNAs, in particular those transcribed by RNA polymerase II. We have identified a variety of proteins that are part of the regulatory complexes of the different stages of snRNA biogenesis as bonafide SUMO conjugation targets. We focused on SNAPC1, a specific snRNA transcription factor that belongs to the SNAPc complex. We have observed that mutating lysine residues 245 and 333 drastically reduced SUMO conjugation to this protein. This obtained SUMOylation-deficient mutant (SNAPC1 K245/333R) is unable to activate the transcription of some snRNA genes, despite the fact it is still recruited to the corresponding promoters. This could indicate that SUMOylation of SNAPC1 is required for SNAPc complex assembly or for this complex to recruit general transcription factors by this complex. Surprisingly, we have observed that in certain mammalian cell lines, the overexpression of this mutant leads to cell death. This could be due to a differential sensitivity to alterations in snRNA levels.

Keywords: Posttranslational Modifications, SUMO conjugation, transcription, snRNAs, splicing

Methods: RT-qPCR, ChIP, confocal microscopy, nickel affinity chromatography, western blot

CB-36

Unveiling the impact of oleic acid on alpha-synuclein aggregation in a *Caenorhabditis elegans* model of Parkinson's Disease.

Delprato, Carla[1,3]; Vranych, Cecilia[1]; Binolfi, Andrés[1,2]; de Mendoza, Diego[1].
[1]Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET-UNR), [2]Plataforma Argentina de Biología Estructural y Metabolómica (PLABEM), [3]Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario (UNR).
delprato@ibr-conicet.gov.ar

Parkinson's disease (PD) is a highly prevalent and incurable neurodegenerative disorder. *Caenorhabditis elegans* shows unique promise in evaluating PD phenotypes. The misfolding and aggregation of the protein alpha-synuclein (aSyn) into filamentous inclusions called Lewy bodies in dopaminergic neurons is a pathological hallmark of PD. Numerous reports indicate that aSyn interacts with lipids as part of its normal functioning; however, it has been shown that perturbations in lipid metabolism influence disease progression. In particular, the oleic acid (OA), an unsaturated fatty acid (UFA), increases aSyn inclusion formation and toxicity in yeast and mammalian cell models and the inhibition of stearoyl-CoA-desaturase enzymes (SCD) reverses those features.

We worked with an established *C. elegans* strain overexpressing human aSyn-YFP construct in muscle cells to characterize the in vivo aggregation of aSyn upon altering UFAs homeostasis, by microscopic assessment. We performed RNAi assays to block the expression of desaturase enzymes involved in the polyunsaturated fatty acids (PUFAs) synthesis pathway. To test the effect of desaturase knockdown on worms' lipid content, we analysed the fatty acid composition by NMR spectroscopy and GC-MS.

We found that blocking the expression of either isoform of the redundant SCDs (Fat-6 or Fat-7) catalysing the first committed step of PUFA synthesis largely decreases aSyn aggregation. This effect was not observed when we blocked the expression of other desaturases that act downstream Fat-6/Fat-7, in later steps of PUFA synthesis. The exogenous addition of the SCD product (OA, a monounsaturated fatty acid), but not the addition of other UFAs, restored aSyn aggregate formation. According to NMR and GC-MS analysis of lipid extracts, worms with interference in fat-6 or fat-7 displayed an increase in saturated fatty acids and a decrease in UFAs, particularly OA, which is consistent with the knockdown of both SCDs.

These results reveal that the perturbation of UFA levels modifies aSyn aggregation in live worms. Moreover, they suggest the role of UFAs in aSyn aggregation is mediated by an early intermediate of PUFA synthesis, most likely OA.

Keywords: Alpha-synuclein, Unsaturated Fatty Acids, Oleic Acid.

Methods: Confocal Microscopy, NMR, GC-MS.

CB-37

Characterization of the pro-inflammatory and antiviral activity of the cellular protein RBM10 in response to dengue virus infection

Magalnik, Melina; Pozzi, Berta; Srebrow Anabella
IFIBYNE-UBA-CONICET; Departamento de Fisiología, Biología Molecular y Celular, FCEyN, UBA
melina.magalnik@gmail.com

In the context of a mammalian cell infection by dengue virus, multiple mechanisms are activated as part of what is referred to as the innate cellular immune response. Among these mechanisms, activation of the cytoplasmic RNA receptor RIG-I triggers a signaling cascade that leads to the expression of pro-inflammatory cytokines and interferons. When secreted, these act in an autocrine or paracrine manner, leading to a strong induction of genes known as Interferon Stimulated Genes. One of these genes is the spermidine/spermine acetyltransferase "SAT1", whose pre-mRNA is regulated by alternative splicing, resulting in an antiviral coding mRNA isoform and a non-coding isoform that is degraded by Non-sense Mediated Decay. This splicing event is regulated by the RNA-binding protein and auxiliary factor RBM10. We recently reported that high levels of RBM10 dampen DENV replication, induce the expression of innate immune response genes, and increase the proportion of the antiviral isoform of SAT1 pre-mRNA. Furthermore, we found that RBM10 interacts with viral RNA and RIG-I and stimulates its non-degradative ubiquitination. In the same line, we observed an increase in the cytoplasmic localization of RBM10 in the context of infection, as well as upon treatment of cultured cells with interferon. When analyzing an RBM10 mutant that displays a more cytoplasmic localization, we found that while its splicing activity decreases in comparison to the wild-type protein, it does not lose its interaction with RIG-I nor its ability to regulate the ubiquitination of this protein. We propose that RBM10 exerts its antiviral action not only by regulating alternative splicing within the cellular nucleus, but also through its cytoplasmic interaction with RIG-I. In the context of dengue virus infection, we plan to analyze RBM10 interactome as well as a plethora of post-translational modifications that may regulate its subcellular localization, its interaction with RIG-I and viral RNA, its splicing factor activity, and its ability to induce the expression of immune response genes.

Keywords: Innate immunity, viral infection, splicing auxiliary factors, post-translational modifications

Methods: Western Blot, RT-qPCR, confocal microscopy, co-immunoprecipitation, Nickel affinity chromatography

CB-38

Decoding the Function of the High Mobility Group B Protein (TcHMGB) in Gene Transcription of *Trypanosoma cruzi*.

de Hernández, Azul[1]; Vargas-Trinidad, Andrea[1]; Smircich, Pablo3; Cribb, Pamela[1,2]
[1]Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET-UNR); [2]Facultad de Cs. Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario (FBIOyF, UNR); [3]Department of Genomics, Instituto de Investigaciones Biológicas Clemente Estable. Montevideo, Uruguay.
dehernandez@ibr-conicet.gov.ar

Trypanosoma cruzi, the etiological agent of Chagas disease, presents unique genome organization and gene expression control mechanisms. Its genes are arranged in tandems transcribed policistronically without canonical promoters and transcription factors, which makes epigenetic regulation mediated by chromatin remodelers and architectural proteins of upmost importance. High Mobility Group Bs (HMGBs) are abundant non-histone chromatin proteins that contribute to chromatin organization and function impacting various cellular processes including transcription, DNA replication, repair and recombination. Through interactions with DNA and other proteins HMGBs can influence the accessibility of DNA sequence and the assembly of transcriptional complexes. *Trypanosoma cruzi* HMGB (TcHMGB) is expressed in the nucleus in all the parasite life stages and can alter chromatin structure like its mammalian orthologs. Given the unique characteristics of transcription in trypanosomatids, our hypothesis is that TcHMGB may have a key role in gene expression control. With the aim of investigating TcHMGB functions, we first constructed transgenic parasites capable of overexpressing the protein under tetracycline induction, which showed that TcHMGB can alter nuclear structure, reducing the nucleolus, the heterochromatin:euchromatin ratio and making chromatin more sensible to micrococcal nuclease treatment. TcHMGB overexpression also affected the parasite fitness, causing a decrease in epimastigote- and amastigote-proliferation, presumably impairing cell division and lowered trypomastigote infectivity in vitro. Then, we used CRISPR/Cas9 to generate knockout (KO) mutants. We verified gene edition by PCR and lower TcHMGB expression by qRT-PCR and western blot in transient transfectants. However, despite multiple attempts, we were unable to generate stable TcHMGB KOs, which suggests TcHMGB gene is essential or crucial for the parasite´s normal functioning. Finally, in order to assess TcHMGB role on transcription, we analysed gene expression by qRT-PCR and RNA-seq on TcHMGB-overexpressing epimastigotes. Our results showed differential expression for a limited number of genes in overexpressing parasites. However, overall results suggest that TcHMGB affects gene trancription in a global manner, consistent with epigenetic control and architectural properties of HMGBs family.

Keywords: Trypanosoma cruzi, HMGB, chromatin, epigenetics, transcription
Methods: RNA-seq, RT-qPCR, CRISPR/Cas9 gene editing, cell culture

CB-39

Inducible overexpression of four different versions of α-tubulin in *Trypanosoma cruzi*

Martinez Peralta, Gonzalo[1, 3]; Alonso, Victoria Lucía[2, 3]
[1]Área Biología, Facultad de Ciencias Bioquímicas y Farmacéuticas. [2]Área Parasitología, Facultad de Ciencias Bioquímicas y Farmacéuticas. [3]IBR-CONICET
gonzalomp88@gmail.com

Trypanosoma cruzi, the causative agent of Chagas disease, has a precisely organized cytoskeleton primarily comprised of stable microtubules. Acetylation on K40 of α-tubulin, conserved from lower eukaryotes to mammals, is associated with microtubule stability and is the most abundant isoform in this protozoa. It is present in its subpellicular corset (responsible for the parasite's shape), the flagellar axoneme, and the mitotic spindle. Despite evidence supporting the role of K40 acetylation in microtubule stability, its biological function in vivo is still under debate. The subpellicular corset in *T. cruzi* appears to be more resistant to depolymerization than mammalian microtubules and its stability is hypothesized to be related to the acetylation levels of α-tubulin.

To study the role of K40 acetylation of α-tubulin in *T. cruzi* we constructed over-expressing lines of different mutants using an inducible system in epimastigotes. The constructions were made incorporating a hemagglutinin (HA) tag at the C-terminal end of the coding sequence for *T. cruzi* α-tubulin. The three mutant versions were generated by substituting K40 with arginine (K40R), which maintains the positive charge but cannot be acetylated; glutamine (K40Q), which mimics the acetylated lysine and alanine (K40A), a neutral amino acid. In other eukaryotes, it has been reported that tagging α-tubulin does not work in many cases. So, the system was first set up with a construction that allowed us to over-express wild-type α-tubulin with an HA tag. Once we successfully obtained this line with a correct localization of the tagged protein to the subpellicular corset and flagellar axoneme we continued with the mutant versions. These constructions were used to transfect *T. cruzi* epimastigotes and thus successfully obtain all the stable lines that overexpress exogenous α-tubulin in its different versions, as well as the wild type, which we were able to verify through western blot and immunofluorescence assays with anti-HA. Finally, we analyzed the growth rates, the acetylation levels using flow cytometry and morphology of these lines using confocal expansion and electron microscopy. Growth alternations and defects in the cytoskeletal structure point to the essentiality of the posttranslational modification in the stability of *T. cruzi* cytoskeleton.

Keywords: Trypanosoma cruzi, tubulin, acetylation

Methods: cell culture, fluorescence microscopy, parasite transfection,

CB-40

Development of a *Caenorhabditis elegans* dauer reporter strain as a strategy for screening compounds involved in intracellular cholesterol mobilization.

Sambrailo, Agustina[1]; de Mendoza, Diego[1]; Mansilla, María Cecilia[1,2]
[1]Instituto de Biología Molecular y Celular de Rosario; [2]Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR)
sambrailo@ibr-conicet.gov.ar

Proper cholesterol transport is crucial for cell functionality; in fact, impaired cholesterol homeostasis is responsible for several human diseases, such as Niemann-Pick type C (NPC) disease and Alzheimer's disease. The nematode *Caenorhabditis elegans* is a valuable model for studying this process because it requires exogenous cholesterol to survive, and its depletion leads to developmental arrest. This arrest has been shown to occur as a result of decreased production of cholesterol-derived hormones called dafachronic acids. These important hormones integrate cues from various signalling pathways, including the transforming growth factor (TGF)-β-like pathway (directed by the TGF-β homologue DAF-7), the insulin-like pathway, and the cyclic GMP pathway. Thus, tight regulation of cholesterol storage and distribution within the organism is critical for its reproductive development. Previous research in our group demonstrated that endocannabinoids (eCBs) play an important role in *C. elegans* by modulating sterol mobilization and that the insulin-like pathway is involved in this process. However, the exact mechanism by which eCBs control cholesterol trafficking remains unknown.
Currently, we are trying to elucidate the components of the pathway by which eCBs abolish larval arrest of *C. elegans* induced by cholesterol depletion. To assess the role of a protein in this process, whether using mutants, RNAi knockdown, or specific inhibitors, our standard procedure involves evaluating if 2-AG, an eCB, can reverse the larval arrest observed in the second generation of worms grown in sterol-depleted medium. This procedure has certain disadvantages when assessing multiple targets. In this work, we introduce a strategic approach that will simplify initial screenings by employing a strain that combines: (I) the use of the daf-7 genetic background, enabling rescue phenotypes to be observed in the first generation, (II) the addition of a reporter fusion -a dauer decision fluorescent marker- that substantially facilitates the visualization of arrested larvae. To obtain it, a genetic cross was performed between hermaphrodites of the CB1372 strain (daf-7) and males of the PS8438 strain (col-183p::mCherry, odr-1p::gfp), and worms that simultaneously exhibited constitutive dauer phenotype at 25°C and GFP marker expression were selected. The constructed strain is hypersensitive to cholesterol depletion and forms about 50% dauer larvae in the absence of external cholesterol at 20°C, despite internally stored sterols. Furthermore, adult and arrested worms are easily distinguishable because the reporter fusion guides mCherry expression specifically to the hypodermis of dauer-committed worms. As a result, if a protein function is connected with intracellular cholesterol

transport, its knockdown or inhibition has a clear impact on worm's dauer phenotype, and cannabinoid treatment effect on this phenotype can be assessed, as we will present in our preliminary results.

Keywords: Cholesterol homeostasis, Cannabinoids, *C. elegans*

Methods: Fluorescent Microscopy, Genetic Crosses, Dauer Formation Assays

CB-41

TcBDF6: A central Bromodomain in the infectivity and development of amastigotes in *Trypanosoma cruzi*.

Boselli, Victoria[1]; *Perdomo Virginia*[2]; *Cribb Pamela*[1]; *Serra Esteban*[1]
[1]*IBR-CONICET;* [2]*UNR – FCByF, Área Parasitología.*
boselli@ibr-conicet.gov.ar

The structure of chromatin, essential for nuclear functions, is established by multiprotein complexes that can both modify histones and exchange canonical histones with variant histones in the nucleosomes. Many of these complexes possess proteins with bromodomains, a module with a bundle of four alpha helices each separated by loop regions of variable lengths that form a hydrophobic pocket that recognizes histone acetylated lysines. For one of the bromodomains in *T. cruzi*, using the CRISPR/Cas9 system, we generated two mutant strains, Dm28cBDF6-/+ and Dm28cBDF6-/-, which showed slower growth in epimastigotes compared to the control strain. The Dm28cBDF6-/- trypomastigotes showed a deficiency in the ability to infect Vero cells and nearly negligible replication of intracellular amastigotes. We performed a complementation by transforming the mutant strain with a pTEXbdf6 plasmid. Epimastigotes and amastigotes showed a partially reversed phenotype. One of our hypotheses is that TcBDF6 might be involved in some DNA repair process (UV damage) or a response related to oxidative stress (H2O2, Benznidazole, and Nifurtimox). To investigate this, we conducted various stress assays and analyzed the results of exposing the BDF6-/- mutant strain. We calculated survival percentages as the ratio between the number of parasites at 72 hours after treatment and the number of parasites in untreated cultures at the same time. However, the Dm28cBDF6-/- strain did not show increased sensitivity to peroxide or UV. This suggests that TcBDF6 may not be involved in DNA repair, at least in the context of the damages caused by the lesions we tested. Concurrently, we conducted an RNAseq analysis and observed that TcBDF6 affects the expression of a limited number of genes, which are decreased. Among these genes is the coding sequence for nitroreductase (responsible for reducing the trypanocidal drugs BZL and NFX), which shows a decrease in mRNA levels. Indeed, Dm28cBDF6-/- displays significant resistance to these drugs compared to the control strain, and this effect is partially reversed when complementing the mutant strain with TcBDF6. The RNAseq results showed that almost

90% of the decreased genes are concentrated in four specific regions of the genome. These results suggest that the function of TcBDF6 could be very important, even essential, in amastigotes, in contrast to what happens in epimastigotes, where growth is almost normal.

Keywords: Bromodomain, trypanosome, cruzi, epigenetics

Methods: Cell Culture, CRISPR/Cas9, oxidative stress, RNAseq

CB-42

Analyses of the subcellular distribution of non-activated and activated α and β estrogen nuclear receptors in the presence of the DUX4 protein

Pagnoni, Sabrina Marcela[1,2]; Rosa, Alberto Luis[1,2]

[1]Departamento de Farmacología Otto Orsingher, IFEC-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina; [2]Fundación Allende-CONICET

sabrina.pagnoni@unc.edu.ar

DUX4 is nuclear transcription factor encoded within a tandem of repeated D4Z4 units, at the human chromosomal region 4q35. Our laboratory demonstrated that DUX4 is a cytotoxic protein, and proposed that underlies the pathogenesis of facioscapulohumeral muscular dystrophy (FSHD), a relevant autosomal dominant inherited myopathy. Additional studies in our laboratory have recently demonstrated that DUX4 is a corepressor of the progesterone and glucocorticoid nuclear receptors. In this work, we extended our analyses of the potential endocrine role of DUX4 to study its cellular interaction and potential corepressor activity on α and β estrogen nuclear receptors (ERα and ERβ). In these studies, we used full-length isoforms of ERα and ERβ genes fused to the green fluorescent protein (GFP). Their subcellular distribution, both in the presence or absence of β-estradiol (E2), was explored in cultured HepG2 cells co-transfected with plasmids expressing either wild-type or mutant versions of DUX4. These studies would provide a rationale to the protective effect of E2 in cultured cells exposed to DUX4. Other research groups have suggested that treatment of cultured cells with estrogens could change the subcellular location of DUX4. We hypothesize that physical interaction between DUX4 and either ERα or ERβ could be responsible of the protective effect of estrogens against the cytotoxic effect of DUX4. These studies would contribute to the understanding of the potential endocrine role of DUX4, as well as to unveil the connection between the pathological expression of DUX4 and two cryptic clinical endocrine and/or hormone-related phenotypes observed in FSHD: gender differences and muscle inflammation.

Keywords: DUX4, estrogen receptors, corepressor, endocrine role, FSHD.

Methods: culture cell, transfection, inmunofluoresce microscopy

CB-43

A high-throughput measurement of core promoter effects on transcriptional output patterns

Iungman, Martin[1,2]; Uriostegui-Arcos, Maritere[3]; Fiszbein, Ana[3]; Schor, Ignacio E.[1,2]
[1]Departamento de Fisiología, Biología molecular y celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina. [2]Instituto de Fisiologia, Biologia Molecular y Neurociencias, UBA-CONICET, Argentina. [3]Biology Department, Boston University, Boston, 02215 USA
martin.iungman@gmail.com

While traditionally the molecular mechanisms that govern gene expression have been studied in populations of cells that are analyzed as a whole, the development of the single cell biology field has revealed the relevance of cell-to-cell variability in transcription. The study of its sources reflects that the RNA produced by each gene in a specific cell is influenced by an intrinsic component of transcriptional noise, in addition to extrinsic factors that may affect each cell differently. Gene expression variability is strongly associated with heritable genetic components, and thus subjected to evolutionary processes, implying that the study of its genetic and mechanistic bases can be highly relevant regarding the function and evolution of transcriptional units.The importance of gene promoters is highlighted as a key determinant of the intrinsic transcriptional noise. While this feature is associated with the relevance of robustness in multiple biological processes, particularly along embryonic development, mutations in promoters affecting it might lead to diseases.

To study the association between core promoter sequence and transcriptional noise in human cells we made variations over a reporter assay by the Fiszbein lab. We commenced by cloning a library of 24000 sequences of human core promoters into a plasmid upstream of the gene of EGFP. The constructions were then transfected in HEK293T containing LoxP sites for a unique and specific insertion in the genome, granting that just one promoter of the library is present in each cell. Finally, cells were sorted according to its EGFP expression and then sequenced the promoters present in each gate. Therefore, we are able to reproduce the enrichment along gates for each one of the promoters: its dispersion is considered a relative value of transcription noise.

With these measurements we expect to analyze how the architectural features of the core promoters (i.e. TATA-box, CRE-elements, transcription factor binding sites, among others) associate with the

transcriptional output in human cells. These measurements of transcriptional noise in human promoters will give us tools to understand its role in promoter evolution, in the robustness of biological processes and in the association with other transcriptional traits.

Keywords: Gene Expression Regulation, Transcriptional noise, promoter sequence, single-cell biology

Methods: Flow Cytometry, Library preparation, Amplicon-Seq

CB-44

Sensitivity of droplet digital PCR for the quantification of 5tiRNA-Gly in seminal plasma and spermatozoa

Grosso, Julieta Belen[1]; Velasquez, Sabrina[1]; Calvo, Karina[2]; Spinelli, Silvana Virginia[1].
[1]Instituto de Inmunología Clínica y Experimental de Rosario (IDICER), [2]Centro Médico Proar
julietagrosso4@gmail.com

Fertility issues impact approximately 15% of couples globally. Traditional semen analysis offers limited insights into male fertility. In our laboratory, we explored the potential of tRNA-derived fragments, specifically 5 tiRNA-GLY produced by angiogenin ribonuclease (ANG), as markers for assessing semen quality in assisted reproduction treatments (ART). These molecules play a crucial role in regulating various physiological and pathological processes, significantly influencing reproductive functions. Our study primarily aimed to quantify 5 tiRNA-GLY in spermatozoa (SPZ) and seminal plasma samples using droplet digital PCR (ddPCR) to enhance the detection of this small RNA. While quantitative PCR (qPCR) has become the conventional method for measuring cDNA levels, it often yields highly variable, artifactual, and non-reproducible data. The root cause of this data quality issue typically lies in inadequate dilution of residual proteins and chemical contaminants, which can variably inhibit Taq polymerase and primer annealing. ddPCR, on the other hand, offers absolute quantification and exhibits a high tolerance to inhibitors, making it a preferred method to overcome the limitations of qPCR. In our study, we aimed to evaluate the diagnostic performance of ddPCR compared to qPCR. We conducted a prospective study involving couples who underwent ART with donated oocytes at the PROAR Medical Center between 2017 and 2021. Samples were collected from normozoospermic men, and we quantified 5 tiRNA-GLY using both qPCR and ddPCR. The results clearly demonstrated that ddPCR outperformed qPCR in terms of higher detection rates and sensitivity for tiRNA, showcasing its potential as a superior tool in this context

Keywords: tRNA-derived fragments, male infertility, ICSI, sperm biochemistry, biomarker
Methods: quantitative PCR, droplet digital PCR

CB-45

Identification of abundant small RNAs in human breast milk

Claus, Clara[1,2]; Ibañez, Mauro[4]; Grosso, Julieta B.[1,3]; Chorostecki, Uciel[5]; Borini Etichetti, Carla[1,2]; and Spinelli, Silvana V.[1,3].

[1]Institute of Clinical and Experimental Immunology of Rosario, IDICER, CONICET-UNR, Rosario, Argentina. [2]Faculty of Biochemical and Pharmaceutical Sciences (UNR), Rosario, Argentina. [3]Faculty of Medicine (UNR), Rosario, Argentina. [4]Faculty of Engineering (UNER), Entre Rios, Argentina. [5]Institute for Research in Biomedicine, Barcelona, Spain

claritaclaus97@gmail.com

Breast milk is one of the most highly complex and heterogeneous biological fluid, rich in macro- and micro-nutrients but also bioactive compounds. Additionally, it plays a key role in facilitating vertical information transfer. Compared to other biofluids, human breast milk contains high quantities of RNA, such as long-coding RNAs (lcRNAs) and small RNAs (sRNAs). However, only a fraction of these RNA species has undergone comprehensive investigation and characterization. To profile small extracellular RNAs in human breast milk and initiate the characterization of their physiological role, we collected breast milk samples from eight donors. For comparison, we also collected five bovine milk and three human serum samples. We conducted an in-depth analysis using sRNA-seq techniques that revealed that a substantial portion of the sequenced fragments originated from tRNA, rRNA, and yRNAs. The results showed that human and bovine milk samples are characterized by a large percentage of rRNA fragments (rRFs) and fragments derived from tRNAs (tRF), while serum is characterized by the presence of fragments derived from yRNA (yRFs). When comparing breast and bovine milk samples we observed similar results, with high similarity between the abundant sRNA sequences preserved in each biofluid. To validate these findings, we conducted both stem-loop quantitative real-time PCR (qRT-PCR) and northern blotting. Our results also demonstrated higher levels of sRNA fragments in milk compared to serum, highlighting their increased stability in this biofluid. In sum, our findings provide preliminary data regarding the unique composition of breast milk in terms of its RNA cargo.

Keywords: small RNAs, Breast Milk, sRNA-seq

Methods: sRNAseq, Stem loop qPCR, Northern Blot.

CB-46

The Effects of Hyaluronan (HA) derivates on the Redox State and HA Receptor Expression in Monocytes/Macrophages

Rosales, Paolo[1] DelSouc, María Belén2 Vitale, Daiana[1], Icardi, Antonella[1], Sevic, Ina[1], Casais, Marilina[2], Alaniz, Laura [1]

[1]Laboratorio de Microambiente Tumoral – Centro de Investigaciones Básicas y Aplicadas (CIBA) – Centro de Investigaciones y Transferencia del Noroeste de la provincia de Buenos Aires (UNNOBA-UNSAdA-CONICET). [2]Laboratorio de Biología de la Reproducción - Instituto Multidisciplinario De Investigaciones biológicas De San Luis (IMIBIO-SL) – Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) – Universidad Nacional de San Luis (UNSL).

paolorosales14@gmail.com

Hyaluronan (HA) is a glycosaminoglycan that is found in the extracellular matrix. It is involved in many bodily processes, including clearing away harmful reactive oxygen and nitrogen molecules. This process involves breaking down HA into small fragments that can trigger inflammation through the CD44-TLR4 receptor cluster. However, the effects of HA and its derivatives on inflammation when administered externally have not been well-studied. This study aimed to assess how different types of HA affect the redox state and HA receptor expression in the immune cells monocytes/macrophages (Mo/MØ). Mo were purified from peripheral blood of healthy donors (n=3) by Ficoll gradient centrifugation and subsequent positive selection using magnetic beads. Mo were cultured under standard culture conditions in multiwell plates overnight. Subsequently, they were treated for 24 hours with low molecular weight HA (LMW HA), partially sulfated HA (sHA 1), and fully sulfated HA (sHA 3), at a concentration of 100 µg/mL, and IFN-γ and IL-4 at a concentration of 10 ng/mL, as differentiation controls. At the end of the treatments, we determined (a) the viability of Mo/MØ by MTS assay at different times (4, 8, 24, 48, 72 h), (b) the concentration of nitrites, in culture media and protein lysate, by Griess assay, and the activity of the antioxidant enzyme catalase by Aebi assay and (c) the expression levels of HA receptors (CD44, RHAMM, HARE, TLR2, TLR4). HA derivates species increased the viability of Mo/MØ and modified the redox state and HA receptor expression compared to the control without treatment. These results highlight the importance of HA and derivatives as modulators of oxidative stress mediated by Mo/MØ. However, further studies are required to understand the mechanisms that trigger this process and its implications in disease contexts and therapeutic use.

Keywords: Sulfated Hyaluronan, Monocytes/Macrophages, Oxidative Stress

Methods: MTS assay, Griess assay, Aebi assay, q-RTPCR

CB-47

Zn2+ sensing through a metallochaperone of the Cation Diffusion Facilitator (CDF) YiiP of *Pseudomonas aeruginosa*

Mihelj, Paula[1]; Carrizo, María Elena[2]; Raimunda, Daniel[1]

[1]Instituto de Investigaciones Médicas Mercedes y Martín Ferreyra, INIMEC. [2]Centro de Investigaciones en Química Biológica de Córdoba CIQUIBIC. 1, 2CONICET-Universidad Nacional de Córdoba. Argentina. pmihelj@immf.uncor.edu

Zinc (Zn2+) is fundamental in almost all living organisms participating as enzyme co-factor and signaling pathways. Thus, cells require a fine tuning of the metal allocation. Membrane transition metal (TM) transporters assisted by metallochaperones are key players in this process, and in gram-negative bacteria, YiiP, a member of the Cation Diffusion Facilitator (CDF) family exports Zn2+ from the cytosol to the periplasm. However, there is yet no evidence of a partnering metallochaperone. Here we provide evidences that in *Pseudomonas aeruginosa*, YiiP/PA3963 participates in a Zn-dependent signaling pathways assisted by a metallochaperone encoded by the PA3962 locus. Bioinformatic studies showed that PA3962 displays a 3D structural similarity with both CopZ, a Cu+ metallochaperone from Bacillus subtilis, and the N-terminal soluble domain of Zn2+-PIB-ATPases. A BLAST analysis shows that this protein is unique to the clade of Pseudomonadales. Guided by multiple sequence analysis and the 3D model predicted by AlphaFold we identify the conserved amino acids candidates for divalent TM coordination. In order to assess the functional role of PA3962 we performed TM (Co2+, Fe2+, Mn2+, Ni2+, Zn2+) binding assay using the PAR (4-(2-Pyridylazo) resorcinol) colorimetric assay and observed a preference for Zn2+. The mutants obtained for the candidate amino acids also were tested, observing a different binding stoichiometry with respect to the WT protein. Due to the role of the periplasmic Zn2+ -sensing system CzcS-CzcR in imipenem resistance associated to OprD transcriptional regulation, we tested the sensitivity of the insertional mutant PA3962::Tn5 strain to this antibiotic and observed a decreased MIC, similar to the previously described for yiiP::Tn5. This sensitivity decreased in yiiP::Tn5 in the presence of Zn2+ while PA3962::Tn5 seemed insensitive to the TM. In light of these results, we propose that PA3962, hereafter periplasmic metallochaperone of YiiP (PmcY), facilitates Zn2+ sensing in the periplasm. We present a model which circumscribe our hypothesis under Zn2+ starvation where the roles of YiiP and PmcY would allow cells to strive under this condition.

Keywords: Transporters, bacteria, metallochaperone, transition metals

Methods: Protein purification, bioinformatic analysis, spectrophotometry

CB-48

Efficiency analysis of commercial enzymes for an TaqMan multiplex detection system for Salmonella sp. by qPCR

*Reyes S. I 1,2; Gonza I. A 1,2; Cristóbal H.A 1,2, **

1 Laboratorio de Investigación y Diagnóstico Genético (LIDGen), Instituto de Investigaciones para la Industria Química (INIQUI)-CONICET-UNSa, 2 Facultad de Ciencias Naturales-UNSa

hacristobal@gmail.com

The application of new diagnostic methodologies for human pathogens detection allows for a rapid, sensitive, and efficient diagnosis. This assay requires a thorough validation of following parameters: selectivity, calibration model, precision, accuracy, uncertainty of measurement, and analytical interference, before they are incorporated into the laboratory. Absolute quantification systems employ a calibration curve where known quantities of the target analyte are amplified in a parallel group of reactions run under identical conditions to that of the unknown samples. Standard molecules, such as genomic DNA or plasmid, can be utilized to make the standard curve. This method is highly reproducible when the specified conditions are maintained. The aim of this study was to determine efficiency and sensitivity of different commercial enzymes using a TaqMan qPCR multiplex system validated in the LIDGen laboratory, where this technology was developed. A unifactorial assay was performed using the calibration curve, obtained for eight increasing dilutions of Salmonella ATCC 14028 strains. Commercial Taq-Man enzyme mix were evaluated; Biolabs (BL), Productos Biologicos (PB), and three versions of INBIO Highway (IHG1, IHG2, and IGH3) and Bioline (BIOL). The qPCR reaction mixture was performed at QuantSatudio5 with a final volume of 10 µl, primer and probe concentrations were kept constant, and the assays were conducted by triplicate. The analysis of the TaqMan qPCR multiplex system efficiency was based on the slope of the standard curve. Amplification reaction efficiency (E) was determined using the equation: (%)E=(10[-1/slope]-1)*100. The coefficient of determination (R2) obtained through linear regression was used to assess the linearity by qPCR assay.

The results shown significant differences in amplification efficiency for enzyme each; that influences the three molecular markers in the multiplex system. The IHG2 and BIOL enzymes shown an comparable results, exhibiting a lineal range with 8 orders of magnitude for three molecular markers. The cut-off values were > 43.67 (Ct) for the three genes. The sensitivity of systems was 7x102 Cg/µL. Both PB and IHG1 enzymes demonstrated similar efficiencies resulting in a shorter linear range (<6 vs >8 orders of magnitude, respectively). The sensitivity of systems was 7x104 Cg/µL in both cases. In all instances, the coefficient of determination was R2>0.98. However, it was observed that the BL master mix enzyme exhibited statistically significant differences from all other enzymes at all points along the curve, amplifying two molecular markers of the triplex system. We conclude, the importance of optimization studies in the components by qPCR, in order to obtain the greatest efficiency in the detection of the pathogen. We have standardized a new prototype for molecular detection for Salmonella spp using nationally produced enzymes and molecular markers.

Keywords: Molecular pathogen diagnosis, Enzyme efficiency comparison, standardization of detection method

Methods: Microbiology culture, DNA extraction, Molecular Biology method, Real time PCR reaction

Plants

PT-01

Characterization of the metabolic network of bioactive phenolic compounds in potato (Solanum tuberosum L.)

Lanteri, María Luciana1; Villarreal, Fernando1; ten Have, Arjen1; Boutet, Stéphanie2; Solis-Gozar, Deyvis-Dante2; Perreau, François2; Andreu, Adriana Balbina1
1) Instituto de Investigaciones Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, CONICET, 7600, Mar del Plata, Argentina. 2) Université Paris-Saclay, INRAE, AgroParisTech, Institut Jean-Pierre Bourgin (IJPB), 78000, Versailles, France
lanteri@gmail.com

Potato is the main non-cereal food crop worldwide and a significant contributor to daily dietary antioxidants. We have demonstrated that polyphenol extracts from Andean potato (Solanum tuberosum L. ssp. andigena) tubers have antioxidant, bactericidal and cytotoxic activities in vitro. In order to determine the compounds responsible for those activities, we analyzed the composition of skin and flesh tubers from three Andean potato cultivars (Santa María, Waicha and Moradita) by HPLC-ESI-MS/MS. We confirmed the annotation of 30 compounds by comparison with databases, literature and authentic standards. We propose the identification of two miscellaneous (sinapine, salicylic acid glucoside, one remains unknown), four polyamines (caffeoyl putrescine, feruloyl putrescine, bis dihydrocaffeoyl spermidine, tris dihydrocaffeoyl spermine), four HCCs (3-CGA, 4-CGA, 5-CGA, cis-5-CGA), ten anthocyanins (four peonidin derivatives, one cyanidin derivative, five pelargonidin derivatives, plus two pelargonidin hexoses without identification), two non-anthocyanin flavonoids (eriodictyol hexose, rutin, plus three putative flavonoids with phenolic acids), and two glycoalkaloids (solanine, chaconine). Among families, we observed that HCCs, polyamines and glycoalkaloids were found in higher concentrations than flavonoids in potato tubers. In general, HCCs presented higher concentrations in the skin than in the flesh. Among polyamines, caffeoyl and feruloyl

putrescine levels were similar between skin and flesh. On the contrary, bis dihydrocaffeoyl spermidine was more abundant in the skin than in the flesh, and tris dihydrocaffeoyl spermine was only found in skin. Miscellaneous metabolites were generally more abundant in flesh than skin. Among anthocyanins, peonidin and pelargonidin derivatives were found in both skin and flesh of Santa María, and in skin of Waicha and Moradita. Non-anthocyanin flavonoids were principally found in Santa María skin and flesh. Among glycoalkaloids, solanine and chaconine levels were more abundant in the skin than flesh, and absent in Waicha flesh. We then aimed to identify the isoforms of the genes involved in the biosynthesis of those bioactive compounds. To study the tuber transcriptome, transcript profiles are being generated by high-throughput RNA sequencing services in tuber flesh and skin. We will establish correlations between transcripts and metabolites. This systemic approach contributes to the development of a model of metabolic regulation of bioactive phenolic compounds and to the identification of novel targets for the breeding of valuable potato cultivars.

Keywords: food, metabolomic, phenolic compound, transcriptomic, Solanum tuberosum

Methods: HPLC-ESI-MS/MS, RNA sequencing

PT-02

Exploring the connection between the MMR system and water stress in *Arabidopsis thaliana*

Ramos, Rocio Soledad; Arce, Lucia Paula; Spampinato, Claudia Patricia

CEFOBI-CONICET

ramos.rocio12@gmail.com

As sessile organisms, plants are continuously exposed to a variety of adverse environmental factors, both biotic and abiotic, which can lead to damage in several biomolecules, such as DNA. Fortunately, all living organisms, including plants, possess a variety of mechanisms for detecting and repairing DNA damage in order to maintain the integrity of the genome. One of them is the DNA mismatch repair (MMR) system. MMR proteins are implicated in sensing and correcting DNA-replication-associated errors and other nucleotide lesions induced by different stresses. Although extensive research has individually examined plant responses to osmotic stress and DNA damage, their potential interconnection remains relatively unexplored. Water stress is known to trigger the production of

reactive oxygen species that can cause DNA damage. Therefore, this study aims to investigate the role of MMR proteins in the plant response to water stress, particularly in Arabidopsis thaliana plants. Our previous data showed that plants lacking the MutS homolog 6 (MSH6) exhibited lower susceptibility to biotic stress caused by the bacterial pathogen *Pseudomonas syringae* pv. tomato strain DC3000 compared to WT plants. This phenotype may be attributed to the observed reduction in stomatal opening in *msh6* mutant plants. Additionally, under control conditions, increased levels of both DNA double-strand breaks and hydrogen peroxide were observed in the *msh6* mutant plants when compared to WT plants. This study extends our investigation to explore the effects of water stress induced by drought, mannitol, and flooding conditions. Our findings indicate that *msh6* mutant plants demonstrate an enhanced drought tolerance while exhibiting a decreased flooding tolerance when compared to WT plants. Additionally, *msh6* mutant plants show a slightly higher tolerance to mannitol-induced water stress. These observations suggest a potential correlation between MMR proteins and water stress in plants. Further investigations are needed to examine how these responses are connected.

Keywords: MSH6, drought stress, flooding stress

Methods: In vitro plant culture, histochemical staining, water stress treatments, qPCR

PT-03

Changes in intracellular distribution of SnRK1.1 in *Arabidopsis thaliana*

Brugnara, Candela; Gianatarra, Gianina; Bultri, Julián; Hita, Francisco Javier; Panozzo Zenere, Esteban; Blanco, Nicolás.
Centro de Estudios Fotosintéticos y Bioquímicos (UNR-CONICET)
brugnara@cefobi-conicet.gov.ar

The energy homeostasis is one of the most important process in all organisms. In the nature, different mechanisms exist to maintain this balance correctly. For instance, when energy levels decrease due to environmental stresses, such as drought, extended darkness, and/or nutrient deficiency, a conserved kinase protein complex named AMPK/SNF1/SnRK1 has been identified as central component to restore energy balance in eukaryotes. In plants, controlling energy homeostasis presents a significant challenge during adaptation to unfavourable growth conditions. Therefore, the SnRK1 complex serves as the central integrator of energy imbalance signals and coordinator of the responses. The catalytic subunit complex of SnRK1, known as SnRK1.1, exhibits dual and dynamic intracellular localization, being

present in the nucleus and endoplasmic reticulum (ER). Studies on the intracellular localization of SnRK1 suggest that a fraction associated with the ER plays a crucial in sensing energy imbalances (Blanco et al., 2019). Based on this, we propose that changes in intracellular distribution of SnRK1.1 are linked to the sense to energy status and orchestration of the energy restoration. We developed a protocol that allow us to quantify the intracellular distribution of SnRK1.1 in nucleus and ER. Using laser scanning confocal microscopy (LSCM), we took images along z-axis of leaves under study and conducted an analysis with ImageJ-Fiji to calculate de intensity fluorescence in different regions of interest (ROI), including one for nucleus and another for ER. The obtained ratio between these values provide us a parameter or index of SnRK1.1 intracellular distribution. To validate this approach and highlight the effect of energy imbalance on the SnRK1.1, we tested 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and Metformin (MET), two compounds that perturb cell energy homeostasis. DCMU is known as a photosynthesis inhibitor that blocks the plastoquinone binding site of photosystem II, decoupling the electron flow from light absorption. MET is a first-line treatment for type 2 Diabetes (T2D) and activates AMPK (mammalian orthologue of SnRK1 (Li et al., 2018)). Treatments were conducted in A. thaliana lines expressing SnRK1.1-fused to GFP and a second fluorescent organelle marker. SnRK1.1 intracellular distribution was analysed at different time points. In line with previous results, significative changes were observed between the intracellular SnRK1.1 fraction, with an augment in the population of this kinase localized in the nucleus. Moreover, the compounds exhibited different response times and magnitudes. These results, along with the physiological aspects of the treatments on lines with different SnRK1.1 content, are discussed in the presentation.
Based on our findings, we propose changes in the catalytic subunit distribution of the AMPK/SNF1/SnRK1 complexes, which are common in the signalling mechanisms involved in cell energy homeostasis.

Keywords: SnRK1.1, Homeostasis, Energy, Intracellular, Distribution

Methods: Images analysis Confocal Microscopy, Confocal microscopy, Plant genetics

PT-04

Study of ATPNP-R1, ATPNP-A and XACPNP in the activation of defense responses against stress

Di Paolo, Valeria; Garavaglia, Betiana; Gottig, Natalia; Ottado, Jorgelina
Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET) FCByF, UNR.
dipaolo@ibr-conicet.gov.ar

The innate immunity of plants is composed of pattern-triggered immunity (PTI) and effector-triggered immunity (ETI). In the first, recognition of molecular patterns associated with pathogens (PAMPs) occurs through pattern recognition receptors (PRRs) located on the cell surface, with the aim of preventing pathogen invasion and maintaining plant homeostasis. PRRs can be divided into receptor-like kinases (RLKs) and receptor-like proteins (RLPs). Their extracellular portions contain leucine-rich repeats, which perceive microbe- or plant-derived ligands and are differentiated by their intracellular domains.
AtPNP-R1 is a plasma membrane-associated receptor, containing an extracellular leucine-rich repeat domain, and it binds the natriuretic peptide AtPNP-A. On the other hand, the hemibiotrophic bacterium *Xanthomonas citri* subsp. citri encodes a PNP-type protein (XacPNP), which is similar to AtPNP-A. We determined that the relative expression of AtPNP-A and AtPNP-R1 increases after infection with *Pseudomonas syringae* pv. tomato in *Arabidopsis thaliana* leaves; and that the damaged area is smaller when XacPNP and AtPNP-R1 are present.
We performed a characterization of different mutants and overexpressing lines of the proteins under study against biotic stress. The absence of the receptor under study, AtPNP-R1, leads to greater damage in the infected tissue, suggesting that this receptor plays an important role in activating defense responses in the plant. Additionally, it was observed that this damage is not compensated by overexpression of natriuretic peptides, whether of plant (AtPNP-A) or bacterial (XacPNP) origin. On the other hand, when AtPNP-A is absent, no significant differences were observed compared to the control, and overexpression of the receptor AtPNP-R1 in this parent shows no differences. This illustrates the requirement of the presence of the AtPNP-A - AtPNP-R1 pair for the activation of defense responses against pathogenic attack.

Keywords: plant natriuretic peptide, Pseudomonas syringae pv. tomato, AtPNP-R1, biotic stress, plant immunity

Methods: ion leakage measurements, chlorophyll measurements, bacterial inoculation, RT-qPCR, cell damage measurements

PT-05

Tailocins produced by *Pseudomonas fluorescens* SF4c: biocontrol of bacterial spot disease in tomato

López-Ramírez, Viviana; Barrionuevo, Laura; Gambetta, Carolina; Asconapé, Jorge; Fischer, Sonia
Instituto de Investigaciones Agrobiotecnológicas (INIAB), Universidad Nacional de Río Cuarto (UNRC)—CONICET
vlopezramirez@exa.unrc.edu.ar

Plants are continually exposed to external factors (both biotic and abiotic) throughout their life cycle, which can affect their development. The use of microorganisms, or their metabolites, to protect plants from potential pathogen attacks is an area of growing interest. *Xanthomonas vesicatoria* Xcv Bv5-4a is the causal agent of bacterial spot disease in tomatoes and peppers. The symptoms of this disease are small, irregular, dark, watery lesions on the leaves and early flower drop. Implementing good cultivation practices is essential to reduce the risk of diseases and subsequent economic losses. Therefore, having high-quality seeds is crucial for initiating successful production. Tailocins are antimicrobial agents capable of inhibiting the growth of other microorganisms as a competitive mechanism. *P. fluorescens* SF4c produces two types of tailocins, R1 and R2. Previously in our laboratory, we constructed mutants producing a single tailocin (R1 or R2). R2-tailocin showed antimicrobial activity against strain Xcv Bv5-4a. The aim of this study was to evaluate the effect of SF4c-tailocins on tomato seeds and their impact on strain Xcv Bv5-4a infection. First, tomato seeds were disinfected. Then, the following treatments were applied on seeds: R1/R2 tailocins, R1 tailocin, R2 tailocin and TN50 buffer (control). The seeds were allowed to dry and incubated in seedbeds until germination. At 30 days, plants with different treatments were divided into two groups, one of them was sprayed on the leaves with the pathogen Xcv Bv5-4a and the other group was not infected. Plants were harvested 15 days post-infection. The severity, incidence, and dry weights of all treatments were measured and statistically analyzed through analysis of variance. Results showed that infected plants and treated with SF4c-tailocins exhibited higher shoot and root dry weights compared to other treatments. There were no statistically significant differences when the infected plants were treated with a single tailocin (R1 or R2), compared to the control. Regarding disease severity, a lower infection percentage (less severity) was observed in plants whose seeds were treated with tailocins, whether R1/R2, R1, or R2. Furthermore, a considerable decrease in disease incidence was observed when tailocins were applied to seeds. Reductions exceeding 20% in disease severity were achieved in the experiments, demonstrating the promising potential of applying SF4c-tailocins for controlling bacterial spot disease in tomatoes. This finding is significant for seed treatment as it may indicate that tailocins induce a systemic plant defense response.

Keywords: Tailocins, Pseudomonas, biocontrol, tomato, bacterial spot disease

Methods: Seed priming, pathogen infection, analysis of variance

PT-06

Exploring the role of OXR proteins in growth and stress response in *Arabidopsis thaliana*

Becerra-Agudelo, Evelyn; Eusebí, Delfina; Welchen, Elina.
Instituto de Agrobiotecnología del Litoral (CONICET-UNL). Cátedra de Biología Celular y Molecular, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, 3000, Argentina
evelyneba2301@hotmail.com

The OXR family protein, characterized by the TLDc domain of unknown functions, has been identified in an effort o identified keys factor for maintaining the balance and control of the damaging effects of ROS in eukaryotic cells. Arabidopsis has five OXR protein members that are conserved in plants. AtOXR2, the first characterized mitochondrial protein, produces plants with increased biomass and resistance to oxidative stress when overexpressed. We are currently analyzing other family members and observed that AtOXR4 and AtOXR5 are strongly expressed in cotyledons, hypocotyls, and roots of 6-day-old seedlings, as analyzed by their promoter regions fused to the GUS reporter gene. Phenotypic characteristics and growth-stress responses of single (*atoxr4* and *atoxr5*) and double mutants (*atoxr4* x *atoxr5*) were evaluated. The results showed that at 6 days after germination (DAG) under normal growth conditions, single mutants exhibited a root phenotype comparable to wild-type (WT) plants. In contrast, the *atoxr4* x *atoxr5* plants displayed reduced root length compared to WT, indicating that the absence of these OXR proteins could impact on plant growth. This evidence should be explored over a more extended time. We also evaluated the growth responses to salt stress, Methyl-viologen (MV) exposition, and UV-B irradiation on seedlings at 6 DAG. All mutant lines studied showed a significant reduction in root length compared to WT when subjected to MV stress. For both UV-B and salt stress, only the *atoxr5* mutant and the *atoxr4* x *atoxr5* plants exhibited a significant reduction in root length compared to WT. These results suggest that AtOXR4 and AtOXR5 could not be redundant, and their role under stress may be related to how each stressor is triggered in the cell. Further studies are expected to investigate physiological and molecular changes related to these proteins to understand the differential role of these proteins on plant responses to stress.

Keywords: OXR Proteins, Stress Response, Plant growth

Methods: Gus, salt stress, Methyl-viologen (MV) exposition, UV-B irradiation

PT-07

Cytochrome c acts as a long-distance signal in plant growth and development

Coronel, Florencia P.; Roldan, Facundo; Canal, M. Victoria; Gras, Diana E.; Racca, Sofía; Welchen, Elina; Gonzalez, Daniel H.
Instituto de Agrobiotecnología del Litoral (CONICET-UNL). Cátedra de Biología Celular y Molecular, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, 3000, Argentina
florcoronel1994@gmail.com

Cytochrome c (CYTC) is a soluble heme-protein that serves as an electron carrier in the electron transport chain located in mitochondria. These organelles are capable of sensing and signaling the cellular energy state, coordinating development through interaction with different growth regulatory pathways. We previously studied the role of the two genes encoding CYTC (CYTC-1 and CYTC-2) in plant growth and development. Mutants in CYTC genes show altered shoot and root growth, with smaller rosettes, lower leaf number, delayed flowering, and shorter primary roots. Here, we show that tissue-specific expression of CYTC in mutant plants can rescue these alterations, increasing rosette size and recovering flowering time to wild-type levels. Shoot-directed expression of CYTC also induces root growth and increases root CYTC protein levels, suggesting that this protein may travel from shoot to root to restore mitochondrial function. Root growth recovery and increased root CYTC protein levels were also observed after grafting experiments with CYTC mutant and wild-type lines. In conclusion, CYTC seems to act as a shoot-to-root long-distance signal for plant growth and development.

Keywords: Cytochrome c, long-distance signal, growth

Methods: Grafting, Wester Blot, RTq-PCR

PT-08

Nitrogen Cycling and Gene Expression Dynamics in Algae: Identification of stable reference genes in Ostreococcus under nitrogen deprivation

Bienaime Ignacio1, Fernández Maria Belen1, Calo Gonzalo2, Nejamkin Andres1, Del Castello Fiorella1, Correa-Aragunde Natalia1, Martínez Noel Gisellel2 and Foresi Noelia1.
1 Fisiologia Molecular e Integrativa, Instituto de Investigaciones Biológicas-CONICET, Universidad Nacional de Mar del Plata, Mar del Plata, Argentina . 2 INBIOTEC and FIBA, Mar del Plata, Argentina
npforesi@mdp.edu.ar

Nitrogen (N) is an essential constituent of all living organisms and the main limiting macronutrient. Even though dinitrogen gas is the most abundant form of N, it can only be used by fixing bacteria, but is inaccessible to most organisms, including algae. Algae preferentially use ammonium (NH4+) and nitrate (NO3-) for their growth, and the reactions for their conversion into amino acids (N assimilation) constitute an important part of the N cycle by primary producers. Recently, it was claimed that algae are also involved in denitrification, due to the production of nitric oxide (NO), a signal molecule, which is also a substrate for NO reductases to produce nitrous oxide (N2O), a potent greenhouse gas. We characterized the O. tauri Nitric Oxide Synthase (NOS) enzyme (OtNOS) that oxidizes L-Arg producing NO and citrulline. O. tauri can grow on L-arg as the only N source, likely by OtNOS-mediated L-Arg metabolism. We analyzed the effect of different sources of N on the growth of O. tauri. N deficiency caused an inhibition of culture growth and a decrease in the overall RNA content per cell. In this work, we screened seven genes, Glyceraldehyde 3- phosphate dehydrogenase (GAPDH), Elongation factor (EF-1), Thioredoxin (Trx R), Actin (ACT), Histone (H), Ubiquitin (UBQ), Tubulin α (TUB α), as potential reference genes (RGs) in control and N-deficient O. tauri cultures by RT-qPCR. Their stability was evaluated using four statistical algorithms: NormFinder, geNorm, Bestkeeper and DeltaCT. We demonstrated from the comprehensive evaluation using CT values that the genes analyzed were stable. Validation of RGs was performed analyzing transcript levels of the Nitrate reductase (NR), Nitrite reductase (Nir), Nitrate transporter (Ntr) which are known to be upregulated during 24 h of N starvation and NOS, which is constitutive. The identification of genes with stable expression in N deficiency is complex, given that important metabolic changes occur in this nutritional condition. Finally, this is the first report regarding suitable RGs selection for accurate normalization of gene expression in *O. tauri* cultures exposed to limiting N conditions.

Keywords: Nitric Oxide, Nitrogen, Ostreococcus Tauri, Nitric Oxide Syntase

Methods: Real time PCR, statistical algorithms: NormFinder, geNorm, Bestkeeper and DeltaCT

PT-09

Natural tomatoes as functional food

Defarge, Nicolás1; Menoyo, Inés2; Madrid Daza, Romario3; Garrocq, Francisco3; Burgos Germán3; Gonzalez, Fabián3; Chapo, Gustavo3; Rodriguez, Gustavo1; Zanor, María Inés4
1Instituto de Investigaciones en Ciencias Agrarias de Rosario (IICAR), 2Facultad de Ciencias Médicas, 3Centro de Investigación y Producción de Reactivos Biológicos (CIPReB), 4Instituto de Biología Molecular y Celular de Rosario (IBR)
zanor@ibr-conicet.gov.ar

The tomato (*Solanum lycopersicum*) is one of the most important horticultural crops worldwide and a valuable source of nutrients. Due to its central role in human nutrition, it is also a target crop for the development of functional foods with better nutritional aspects, e.g. as a source of healthy metabolites. Various breeding strategies have successfully used the biodiversity present in wild tomatoes as a source to enrich the genetic basis of cultivated tomatoes, including content of metabolites beneficial to human health. Initial work showed that one metabolite that has been linked to the reduction of adverse effects in metabolic syndromes is much more abundant in the wild tomato *S. pimpinellifolium* LA0722 compared to the cultivated tomato variety Caimanta. In addition, the introduction of distinct genomic regions of *S. pimpinellifolium* LA0722 in the Caimanta cultivar led to several lines offspring showing an increased in the metabolite levels. Currently, food manufacturing companies have embarked on the development of functional foods. In this type of food there are components that have positive effects on health which in general are related to the mitigation of the appearance of certain pathologies or their long-term consequences. The prevalence of obesity has increased rapidly worldwide and it is estimated that by 2030 there will be more than 1 billion people affected. This condition increases the risk of developing other diseases such as insulin resistance, type 2 diabetes, dyslipidemias and hypertension. The objective of this work was to evaluate the morphological and biochemical parameters in rats, the line which develop obesity and hypertriglyceridemia, treated with and without weekly doses of tomato with high or low content of the nutraceutical metabolite. The results of this work suggest that changes in the measured parameters are related to a lower accumulation of abdominal fat in rats fed with high metabolite content and therefore a protective effect of the metabolite can be postulated.

Keywords: tomato, nutraceutical compound, obesity

Methods: molecular assisted markers, 1H-NMR metabolomics, rat feeding studies, blood parameters measurements, morphological measurements

PT-10

Characterization of a *Chlamydomonas reinhardtii* mutant strain with increased growth rate and carbon reserve accumulation

De Marco María Agustina, Guerra Rocío, Curatti Leonardo and Martínez-Noël Giselle
INBIOTEC and FIBA, Vieytes 3103, Mar del Plata, Argentina.
giselleastrid@gmail.com

The SnRK (Snf1-Related protein Kinase) gene family plays a central role in energy sensing and stress-adaptive responses in plants. Orthologs of the plant SnRK protein exist in the model green algae *Chlamydomonas reinhardtii,* but their functions are still unknown. In this study, we characterized the *snrk2.1* strain, which is a null mutant of the SnRK2 protein, that cannot grow under S-deprivation conditions. We showed that, under mixotrophic growth conditions, the snrk2.1 mutant achieved higher cell density (OD750), dry weight, chlorophyll, lipids and starch content than the wild-type (WT) strain. Qualitatively similar results were observed under photosynthetic growth conditions, although growth of all strains was slower. To gain insight into the molecular mechanisms behind these observations, we performed RT-qPCR experiments to analyze the expression of key genes encoding the growth regulator Target Of Rapamycin (TOR), enzymes that regulate the cell-cycle, the transcription factor involved in the P-deficient response PSR1 and an important enzyme in the starch synthesis pathway (PGM1, phosphoglucomutase 1). These assays indicated that the transcript levels of tor, pgm1, and cell-cycle genes were induced in the *snrk2.1* mutant, while psr1 decreased. In conclusion, this preliminary characterization of a *C. reinhardtii snrk2.1* mutant strain suggested potential for biotechnological applications towards production of biofuels and/or other products obtained by fermentation of non-expensive sugars. Additionally, it may open up new venues for unravelling the regulation of algal growth and production of carbon reserves at the molecular level.

Keywords: carbon reserves, Chlamydomonas, growth regulation, SnRKs, stress

Methods: algae growth, starch and lipid quantification, RT-qPCR

PT-11

Interplay between TOR and SnRK1 in the accumulation of proline under salt stress

Lando, Ana Paula1; Barbieri, Giuliano2,3; Martínez-Noël, Giselle1; Rodriguez, Marianela 2,3
1)Instituto de Investigaciones en Biodiversidad y Biotecnología (INBIOTEC-CONICET). Fundación para Investigaciones Biológicas Aplicadas (FIBA). Vieytes 3103, Mar del Plata, Argentina. 2) Unidad de Estudios Agropecuarios (UDEA), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Córdoba, Argentina. 3) Instituto de Fisiología y Recursos Genéticos (IFRGV), Centro de Investigaciones Agropecuarias (CIAP), Instituto Nacional de Tecnología Agropecuaria (INTA), Córdoba, Argentina. ana_lando_8@hotmail.com

Plants are subjected to a diverse array of abiotic stressors and respond to metabolic changes that negatively impact their growth and development. Thus, the kinases TOR and SnRK1 form the core of a complex and intricate regulatory network to coordinate metabolic activities. However, the interplay of TOR and SnRK1 signaling on the accumulation of stress-regulated metabolites is poorly understood. Thus, we studied proline (Pro) which is a multifunctional amino acid that also functions as a protector compound and participates in cell redox regulation. Besides, Pro acts as a metabolic signal to many cellular responses under various stress conditions. The aim of this work was to evaluate the role of TOR and SnRK1 in the accumulation of Pro in Arabidopsis plants with different subcellular localization of SnRK1α-subunit (nuclear or cytosolic) under salt stress. We evaluated Pro accumulation in transgenic plants with forced SnRK1α-subunit localization (NLS- nuclear localization or MYR- cytosolic localization) and Wild-Type (Col-0) subjected to inhibition of TOR activity (AZD) under NaCl treatment. We also analyzed the expression of genes that encode key enzymes of Pro metabolism (P5CS and ProDH). Results showed that under salt stress conditions with TOR inhibition, the cellular localization of SnRK1 influenced Pro accumulation. MYR-plants accumulated significantly less Pro relative to Col-0 and NLS plants. This was reflected in the levels of transcripts related to Pro catabolism which significantly increased in MYR-plants. Overall, our results suggest that TOR negatively regulates ProDH1 transcription under salt stress conditions, especially when SnRK1 is found in cytosol.

Keywords: proline, salt stress response, SnRK1, TOR, Arabidopsis

Methods: Arabidopsis growth, proline quantification, qRT-PCR

PT-12

Optimized protocol, phylogeny and first crystal structure of a malolactic enzyme of Streptococcus mutans

Tronconi, Marcos1; Parisi, Rocio1; Lisa, María Natalia2; Alvarez, Clarisa1.
1) Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI- CONICET), FBIOyF, UNR, 2000 Rosario. 2) Instituto de Biología Molecular y Celular de Rosario (IBR, CONICET-UNR), Ocampo y Esmeralda, Rosario S2002LRK, Argentina
alvarez@cefobi-conicet.gov.ar

Most lactic acid bacteria (LAB) decarboxylate L-malate to L-lactate by an NAD+- and Mn2+-dependent malolactic enzyme (MLE). This enzyme is involved in malolactic fermentation (MLF), a secondary fermentation that occurs when L-malate and acidity are available. As a result of MLF, the bacteria prevent acidification of the cytosol and establish a pH gradient across the cell membrane that drives ATP synthesis. MLF is used in the industry during the production of wines, ciders, cheeses and yogurts, improving the nutritional and organoleptic properties of the final products. In addition, MLF has an impact on human health by being part of the physiology of Streptococcus mutans, the bacterium responsible for dental caries, where MLF is considered a virulence factor. Currently, MLF is a spontaneous and uncontrollable phenomenon mainly due to the lack of information on the biochemical properties of MLE. The identification of the conditions and/or molecular components that modify the activity of MLE could make this fermentation a controlled process. Here, we present an optimized protocol carried out to obtain MLE of S. mutants (SmMLE) without co-purification with other similar proteins, such as the endogenous malic enzyme that could interfere with its characterization. A codon-optimized SmMLE coding sequence cloned into the pET-22 vector was used to express the recombinant protein in E. coli. The pET-22 vector carries a signal peptide that allows the export of the recombinant SmMLE to the periplasm, separating the protein to be purified from the host cytoplasmic proteins. With this protein we managed to obtain the first crystal structure of SmMLE in quaternary complex with NAD+, Mn+2 and pyruvate showing a dimeric conformation where Mn+2 is present at two different sites: the active site and the dimeric interface. We also performed a phylogenetic analysis that showed that MLE diverges from the malic enzyme lineage to gain specialized activity. These results are a good starting point for understanding the key residues involved in this MLE specialization and for the development of ligand binding-based strategies to modulate enzyme activity

Keywords: malolactic, phylogeny, novel expression protocol

Methods: phylogeny, crystallography, protein expression

PT-13

Use of SF4c-tailocins to stimulate corn plants growth under water stress conditions

Gambetta, C; Asconapé, J; López-Ramírez, V; Barrionuevo, L; Travaglia, C; Fischer, S
Instituto de Investigaciones Agrobiotecnológicas (INIAB), Universidad Nacional de Río Cuarto (UNRC)—CONICET
sfischer@exa.unrc.edu.ar

Corn (*Zea mays* L.) is the most predominant cereal in Argentina, being Córdoba province the principal national producer. This crop is significant for economic activity, playing a fundamental role in crop rotation and as an input for several agro-food chains. Water deficit is one of the main problems that affect agriculture nowadays. The intense droughts recorded in the last years have caused a significant decrease in the production of summer crops. In this context, studies that tend to mitigate the effect of drought will mainly benefit the corn. The use of bacteriocins as a bioinput for application in agriculture is a topic that has begun to gain popularity in the last decade, especially for the biocontrol of phytopathogenic bacteria that cause economic losses in crops. New advances have allowed discover new property of bacteriocins, which is the ability to promote plant growth. However, in the world, only one bacteriocin has been evaluated as biostimulant of plants. Tailocins (phage tail-like bacteriocins) are high-molecular-mass protein complexes that resemble and are evolutionarily related to bacteriophage tails. The rhizospheric strain Pseudomonas fluorescens SF4c, belonging to our laboratory collection, produces tailocins with antimicrobial activity against phytopathogenic strains. This study aimed to evaluate SF4c-tailocins as a corn growth biostimulants under irrigation and water stress conditions. Corn seeds (SYN 897 Viptera 3-NK) were surface-sterilized and treated with 2 different doses of SF4c-tailocins: 102 and 103 arbitrary units per ml (AU ml-1). Then, the seeds were sown into pots containing peat: perlite (1:1) and kept in a greenhouse with controlled conditions (16h light, 28° C, 70% humidity, and 8h darkness, 18° C, 80% humidity). After 10 days, a differentiated irrigation regime was applied: 100% of the field capacity for optimal irrigation and 10% of the field capacity for water stress. At 45 days, plants were harvested. Dry biomass, stem diameter, and V8 leaf area were measured. For the statistical evaluation of the results, a univariate analysis (ANOVA) was performed for each parameter, with a posteriori Fisher LSD test, with a significance level of $p < 0.05$. Under optimal irrigation conditions, plants treated with tailocins (102 AU ml-1) had greater stem diameter (16%), V8 leaf area (27%), root dry weight (68%), and shoot dry weight (82%), compared with untreated plants. Under water stress conditions, SF4c-tailocins were able to alleviate the adverse effects of droughts. These results indicate that the application of SF4c-tailocins on seeds has a biostimulant effect on corn.

Keywords: Bacteriocins, Pseudomonas, corn, biostimulant, drought

Methods: Seed priming, Measurement of plant growth

PT-14

First assessment of the expression and biochemical analysis of the whole thioredoxin maize family

Tonetti, Tomás2, Drincovich, María F1; Lisa, Figueroa, Carlos M2; Alvarez, Clarisa1.
1) Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI- CONICET), FBIOyF, UNR, 2000 Rosario. 2) Instituto de Agrobiotecnología del Litoral (UNL-CONICET), Santa Fe.
alvarez@cefobi-conicet.gov.ar

Thioredoxins (Trxs) are small ubiquitous proteins containing an extremely reactive site with a highly conserved sequence, participating in various reactions requiring the reduction of disulfide bonds in selected target proteins. Trxs are encoded by a multigene family and are usually classified according to their function, structure and subcellular localization. Plants possess the largest Trx family across kingdoms; however, little is known about their function in plants when compared with other organisms. In this work we classified all the ten putative Trxs from maize, which is the largest family of Trxs known so far, using the Trx family from Arabidopsis thaliana as template. In maize, Trxs cluster into seven groups related to their presumed evolutionary origin. We then measured the relative abundance of all the putative Trx transcripts in the base, middle, and top leaf sections and found that, in general, accumulation was higher in the middle section. As maize leaves have two specialized cells, we further analyzed Trx transcripts in fractions enriched in bundle-sheath cells (BSC) and mesophyll cells (MC). Our results showed that certain Trx transcripts are preferentially accumulated in BSC (ZmTrx-BSC) or MC (ZmTrx-MC). In silico analysis of the promoters of the genes encoding ZmTRX-MC and ZmTRX-BSC indicate that they would be responsive to ethylene and light, respectively, which is consistent with the putative involvement of ZmTrx-BSC in the photosynthetic process. Based on these results, we focused our attention on the biochemical and kinetic properties of these Trxs. To better understand their function in vivo, we designed, obtained and expressed site-directed mutants in the resolutive cysteine of each Trx isoform, ZmTrx-BSC-MutCys2 and ZmTrx-MC-MutCys2, and performed different binding protocols to find their specific protein targets. These results are the starting point to uncover the pathways in which maize Trxs isoforms are involved.

Keywords: thioredoxin, protein expression, maize, cell expression

Methods: qRT-PCR, protein expression

PT-15

Morpho-physiological and biochemical traits related to drought stress in cotton as a selection tool in a breeding program

Cereijo, Antonela E.1; Winkler, H. Martín1; Muchut, Robertino J.1; Dileo, Pablo N.1; Scarpín, Gonzalo J.1; Roeschlin, Roxana A.1,2; Sartor, Gonzalo J.1; Zanel, Walter1; Lorenzini, Fernando G.1,2; Paytas, Marcelo J.1.
1) Equipo de Investigación en Algodón - Estación Experimental Agropecuaria - INTA Reconquista, Santa Fe, Argentina; 2) Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)
cereijo.antonela@inta.gob.ar

Cotton is the most natural and textile fiber widely produced in the world, being approximately 95% produced by Gossypium hirsutum L. species. It is an essential economic crop since it not only produces raw materials for the textile industry, but it also generates valued raw material for food oils for human consumption and high protein feed for livestock. Although cotton cultivation in Argentina is regionally important due to the value it adds, there are currently few cotton genotypes adapted to the various agroecological conditions of the country. This constantly puts this crop at a disadvantage and highlights the importance of developing local research that involves new adapted genetic materials.
Drought and salinity stresses negatively affect cotton crop, leading to alterations in its physiology, morphology and metabolism that together result in reduced productivity. In response to these unfavorable conditions, plants adapt their defense system to cope with the stress impacts. These adaptations include changes in membrane structure, activation of antioxidant defense systems, and the production of osmotically compatible solutes to maintain cell osmotic balance. The use of physiological and biochemical traits as indicators for selecting genotypes with better adaptation to various adverse environmental conditions has been previously reported in other crops. In this context, the present work aims to contribute to the cotton breeding program by establishing key morpho-physiological and biochemical traits related to water stress tolerance that may be useful in a selection process. Thus, six different cotton genotypes were sown under greenhouse conditions, in 5-liters pots. Two different severity stress levels (50 % and 30 % field capacity) were applied to plants at vegetative stage (4 leaves completely expanded), compared to well-watered control plants. Then, morpho-physiological, and biochemical characteristics such as relative water content (RWC), SPAD, proline, foliar temperature, membrane stability, K+/Na+ ratio and chlorophyl content were determined.

Additionally, antioxidant enzymes activity was measured to establish a relationship between them and the drought severity in cotton plants. The obtained results show significant differences between treatments and compared to control plants for SPAD, foliar temperature and RWC. Besides, there were significant differences among treatments for the chlorophyll content and it was also able to establish a correlation between chlorophyll and SPAD measurements. On the other hand, proline content was significatively different not only between treatments, but also among genotypes. This study contributes with valuable information regarding the cotton plants behavior under different drought stress severity. Besides, these results and their associations would be useful tools for the selection of cotton materials from a breeding program, with improved plasticity, according to differential environmental conditions.

Keywords: Antioxidant system, oxidative stress, Gossypium hirsutum

Methods: Enzymatic activity, SPAD, Chlorophyll and proline quantification, relative water content

PT-16

Genetic analysis of resistance to bacterial blight in cotton germplasm from Argentina

Roeschlin, RA1,2; Lorenzini, F1,2; Winkler, HM1; Cereijo, AE1; Dileo, PN1; Muchut, RJ1; Scarpin, GJ1; Sartor, G1; Zanel, W1; Paytas, MJ1

(1) Equipo de Investigación en Algodón – Estación Experimental Agropecuaria - INTA Reconquista, Santa Fe, Argentina. (2) Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET)
roeschlin.roxana@inta.gob.ar

It is well known that there are variations in resistance to bacterial blight infections, caused by *Xanthomonas citri* subsp. malvacearum (Xcm), among cotton cultivars and that identification of resistant germplasm that are adapted to the local environment is very important in breeding for disease resistance. Genotyping elite lines or germplasm accesesions using known markers that flank resistance genes is a useful strategy to identify putative resistant lines. Molecular markers, in particular the SSR marker CIR246, has been proven as a useful indicator for selecting resistant plants to bacterial blight and subsequently confirming the same with artificial inoculation. In Argentina, only 7 current variaties are available. This suggests a risk for national cotton production, seems it assumes a system with low stability. The cotton germoplasm of our country harbours up to 300 accessions of *Gossypium hirsutum* that include old and advanced cotton genotypes from Argentina and other countries. The

main objetive of this study was to determine by molecular markers and pathogenicity assays the behaivour to bacterial blight disease of 75 cotton accessions from cotton germoplasm. Tissues for DNA isolation were obtained from young leaves of three independent plants grown in greenhouse at the age of three weeks after germination. Sampling was done by punching three discs of the same leaf and the samples were analyzed by PCR using primers for CIR246 molecular marker. The amplicons were then separated by SDS-PAGE, revealed by silver staining, and analyzed for resistance or susceptibility alleles presence. Approximately, a 35 % of the cotton accesions were susceptible to bacterial blight. To confirm these results, the same plants were evaluated by pathogenicity tests and bacterial population growth using two local isolates of Xcm strains. The characterization of cotton germoplasm from Argentina would be useful for the selection of cotton materials for breeding programs.

Keywords: Gossypium hirsutum, Xanthomonas, molecular markers, disease resistance, cotton breeding

Methods: PCR, SDS-PAGE, pathogenicity assay

PT-17

Proteomics analysis of two peach cultivars with different susceptibility to chilling injury

Ferreyra Laura1, Gabilondo Julieta2, Budde Claudio2, Müller Gabriela1, Drincovich Fabiana1, Bustamante Claudia1.
1Centro de Estudios Fotosintéticos y Bioquímicos. Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario 2 INTA San Pedro
lauraferreyra85@gmail.com

Peach is a perishable fruit that deteriorates rapidly once harvested at room temperature. Storage at 0ºC is employed to prevent fruit decay and extend shelf life. However, this practice can induce physiological disorders known as 'chilling injury' (CI), which results in a loss of quality. The objective of this study was to analyze and compare the proteome of two peach cultivars, one resistant to CI (Red Globe, RG), and another susceptible to CI (Flordaking, FD), exposed to various post-harvest treatments: in harvested fruits (H), the stage where it reached physiological maturity; in fruits kept in a chamber at 20◦C for 5 days (organoleptic characteristics suitable for consumption), called R (room temperature-storage); in fruits stored at 0◦C for 21 days (CS21, cold storage for 21 days); and in fruits stored at 0◦C for 21 days followed by 5 days at 20◦C (CS21+R). Proteins were extracted from mesocarp

tissue and runned on polyacrylamide gels before sending them to Proteomics Core Facility CEQUIBIEM for Mass Spectrometry analysis. Proteins containing a fold-change ≥2 and p ≤0.05 were considered for the analysis. In the comparison between CS21 and C, RG cultivar shows a high number of upregulated proteins associated with stress resistance, with respect to FD, including Pru p 1.0301, Pru p 1.0201, Stress-response A/B barrel domain-containing proteins, and Dehydrins, which play a role in cold acclimation. In the comparative analysis CS21+R vs R, an increase in the expression of cell wall proteins is observed in RG cultivar, consistent with the progressive loss of firmness during ripening. Conversely, in the case of FD, no significant changes related to these enzymes were evident, which could be indicative of damage to cell wall integrity. Furthermore, the comparison of R vs. C in FD exhibits an increase in these proteins, in accordance to the natural processes of ripening. Finally, in the RG cultivar, it is observed an increased expression of cell wall degradation enzymes in R vs. C and CS21+R vs. R comparisons, indicating a proper fruit maturation process following refrigeration. Previous results have shown that the RG cultivar exhibits an increase in raffinose and galactinol after prolonged cold treatment. As these sugars act as osmoprotectants, they could be linked to the resistance displayed by RG, along with the elevated levels of stress resistance proteins observed in the CS21 vs. C comparison.

Keywords: peach, chilling injury, proteomic

Methods: Mass Spectrometry, SDS-PAGE

PT-18

Overexpression of AtAPA1 (*A. thaliana* aspartic proteases 1) improves plant recovery after drought.

Berli Anabela, Silva Johana Nair, Villegas Maximiliano Matías, Muñoz Fernando Felipe, Tonón Claudia Virginia, Fiol Diego Fernando, Guevara María Gabriela
Instituto de Investigaciones Biológicas (IIB)-UNMdP
anabelaberli3@gmail.com

Drought is the most severe environmental factor that decreases photosynthesis and plant productivity. The plant response to drought stress includes several mechanisms known as drought escape, drought avoidance, and drought tolerance. We previously reported that the typical aspartic protease APA1 (At1g11910) confers tolerance to mild water deficit when it is overexpressed in A. thaliana. We demonstrate that APA1 is an intermediate in the ABA-induced stomatal closure. Specifically,

ABA-induced APA1 expression could increase, directly or indirectly, the expression of ABA-related genes and, or interact with proteins associated with stomata closure and density. The aim of this work was to evaluate the response of plants overexpressing APA1 (OE-APA1) and plants with null expression of APA (apa1) to severe drought stress treatment. The drought stress was imposed by withholding watering on plants for ten days and then plants were re-watered for three days. We observed that apa1 plants wilted more after 5 days of drought stress, compared with WT and OE-APA1 plants. 10 days of drought stress produced comparable high decreases in relative water content (RWC) in all lines tested. However, after re-watered treatment, OE-APA1 plants recovered RWC values significantly higher than WT plants (80% of recovery vs 60% of recovery), while apa1 plants reached lower RWC values (30% of recovery). Chlorophyll content (CC) was similar in WT, apa1, and OE-APA1 plants under control conditions. After 10 days of drought treatment, a comparable decrease in CC values was recorded in all plant lines. After the re-watering treatment, CC increased in similar ways in WT and OE-APA1 plants, but remained at lower levels in apa1 plants. In addition, we performed the quantification of phenols and anthocyanins levels in the plants treated. After 10 days of drought treatment, both metabolites presented higher values in apa1 and OE-APA1 plants compared to WT plants, which is associated with a greater antioxidant capacity. After re-watering, phenols and anthocyanins levels decreased in WT and OE-APA1, but were even higher in apa1 plants, suggesting that these plants remained stressed. The involvement of APA1 in the response to drought stress is also supported by transcriptional data accessible in the eFP browser and by in silico analysis of cis-acting elements in the promoter region of APA1, which showed the presence of cis-acting elements related to water deficit, drought, and osmotic stress. Altogether, our results show a positive correlation between APA1 expression, the relative plant water content, photosynthetically active area, and the recovery of re-watered A. thaliana plants after drought. This data improves the knowledge of the relationship between APs and plant water stress.

Keywords: Abiotic stress, drought stress, Arabidopsis thaliana, Aspartic proteases, drought tolerance

Methods: Spectrophotometry, in silico analysis of cis-acting elements, expression profile

PT-19

Differential expression patterns of aspartic proteases in two Solanum tuberosum cultivars with variable tolerance to drought stress

Villegas, Maximiliano Matías1; Norero, Natalia Sigrid 2; Tonón, Claudia Virginia1; Feingold, Sergio Enrique2; Décima Oneto, Cecilia Andrea2; Guevara, María Gabriela1.
(1) Instituto de Investigaciones Biológicas, Universidad de Mar del Plata (IIB-UNMdP), Mar del Plata B7600, Argentina. (2) Laboratorio de Agrobiotecnología, IPADS (INTA-CONICET), Balcarce B7620, Argentina.
maximilianovillegas@mdp.edu.ar

Climatic change has significantly altered weather patterns, leading to extreme temperatures, drought, frost events, and increased snowfall in high-altitude regions. These conditions have had a detrimental impact on crop growth, survival, and yield. Among the crops most severely affected is the potato (Solanum tuberosum L.), one of the three most essential crops globally, providing sustenance for over 1 billion people worldwide There are reports indicating a potential decline of approximately 30% in global potato production between 2040 and 2069 due to various stressors associated with climate change. Aspartic proteases are proteolytic enzymes widely distributed in living organisms and viruses, with reported functions in drought tolerance, development processes, resistance to pathogens, and protection against UV light in many plant species. In our laboratory, we previously described 62 genes codifying for aspartic proteases in the potato genome, and the RNA-seq data reported in ePlant potato shows that 21 of these genes modulate their expression in drought or salinity conditions. We selected 4 genes: 2 that up-regulate (StAP1.6 and StAP11.1), and 2 that down-regulate (StAP1.4 and StAP8.7), based on the expression levels in basal conditions, the magnitude of the change between control and osmotic-stress conditions reported and the representation of different types of aspartic proteases. All these genes have presented several cis-acting elements related to water, drought, salt, and osmotic stresses (AT-rich element, AT-rich sequence, DRE, DRE1, as-1, MBS, Myb, MYB recognition site, MyC, STRE). Also, both the genes that up-regulate have the cis-acting sensitive site to ABA (ABRE). To explore the potential roles of these genes in osmotic-stress tolerance, we assessed the expression levels of these four genes under both control and osmotic-stress conditions in two potato cultivars: Spunta (sensitive) and Kennebec (tolerant). For all the genes under investigation, expression levels exhibited temporal variations (at 0 hours, 24 hours, 48 hours, 72 hours, and 7 days), consistently aligning with RNA-seq data. Additionally, variations in expression levels were observed between cultivars, indicating that Kennebec cv. displayed reduced expression levels in both basal and stressed conditions for all genes analyzed when compared to Spunta cv. These results demonstrate that Kennebec's heightened tolerance to osmotic stress may be attributed to the low expression levels of specific genes, suggesting their potential suitability as targets for CRISPR-mediated knockdown in the future.

Keywords: Aspartic proteases, abiotic stress, cis-acting regulatory elements, osmotic stress, solanum tuberosum

Methods: Real Time PCR, Expression Profile, Analysis of Cis-acting elements

PT-20

Characterization of a novel lncRNA potentially involved in the control of flowering time in *Arabidopsis thaliana*

Belén, Federico; Vegetti, Abelardo; Dotto, Marcela
Laboratorio de Biología Evolutiva y Molecular de Plantas, Instituto de Ciencias Agropecuarias del Litoral, Facultad de Ciencias Agrarias, Universidad Nacional del Litoral.
dottomarcela@gmail.com

Previous studies from our group established that Arabidopsis mir394a mir394b double mutant plants exhibit an early flowering phenotype, which correlates with altered expression of flowering genes, including the floral integrators FLOWERING LOCUS T (FT) and SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1). The double mutant plants harbor insertional mutations in each of the two MIR394 genes present in the Arabidopsis genome, resulting in plants with lower accumulation of mature miR394. The insertion present in MIR394B is located in the promoter region of this gene, where it overlaps with the 3' region of an uncharacterized lncRNA. This type of non-coding RNAs are defined as transcripts with more than 200 bp, which do not present protein coding capacity and have been shown to participate in several aspects of plant biology, including gene silencing, flowering time control, root organogenesis, photomorphogenesis, abiotic stress responses and reproduction. Here we present the initial characterization of this novel lncRNA, which is a 310 bp transcript encoded in the opposite strand, partially overlapping MIR394B gene. Through the integrated study of the miR394 pathway and this lncRNA we set out to elucidate its role in Arabidopsis development. Here we present initial bioinformatic structural analysis and expression using RT-PCR and publicly available RNA-seq datasets from Arabidopsis, as well as a general phenotypic analysis of plants overexpressing this lncRNA. Our results indicate this lncRNA is not expressed in leaves and presents a very low expression in most analyzed samples, but is highly expressed in flowers, petioles and stem internodes. Moreover, we established that the insertional mutation affects mature miR394 accumulation, suggesting a possible relationship with the miR394 regulatory pathway and the control of flowering time.

Keywords: Arabidopsis, flowering time, long non-coding RNA, miR394
Methods: transgenesis, RT-PCR, bioinformatics, plant phenotyping

PT-21

New insights into the use of rosemary extract to prime crop plants against pathogens

Feuli,Catalina1; Plačková, Klára1; Martín, Ana Paula1; Garcia, Lucila1,2; Marano, María Rosa1,2.
1.Instituto de Biología Molecular y Celular de Rosario , 2.Facultad de Ciencias Bioquímicas y Farmacéuticas
garcia@ibr-conicet.gov.ar

In the last decades, the identification of novel genes and mechanisms related to microbial invasion and plant defense has greatly advanced contributing with modern breeding programs and the development of disease-resistant plant varieties. Notwithstanding, breeding strategies cannot be applied to all crops and genetic resistance could be broken due to selection pressure on the pathogen by monoculture cropping systems. Consequently, chemical pesticides have become the most predominant approach to managing plant diseases. Plant bioactive compounds offer a promising alternative approach to disease control. Extracts derived from rosemary (*Salvia rosmarinus*) have gained attention due to their notable pharmacological properties, including antimicrobial and antioxidant effects, mainly attributed phenolic compounds such as rosmarinic acid (RA), carnosic acid and carnosol. Nevertheless, the effects of these extracts on plant diseases have remained unexplored. In this study, we demonstrate the bioprotective effect of aqueous rosemary extract (ARE) against a range of pathogens, including viruses, bacteria and fungi, when applied to different crop species, including tobacco (Nicotiana tabacum), lemon (Citrus limon), and soybean (Glycine max). Importantly, the protective effect of ARE on these crops does not arise from its direct antimicrobial activity. Instead, it appears to enhance (prime) the plant defense response. This priming effect was observed in tobacco plants treated with ARE, which showed a reduction in the number of local necrotic lesions induced by tobacco necrosis virus strain A (TNVA). This reduction was associated with a significant decrease in virus replication. Additionally, ARE treatment enhanced the plant systemic defense response, as evidenced by a reduction in systemic TNVA symptoms. Moreover, ARE treatment not only amplifying the defense response triggered by TNVA but also induces jasmonic acid-dependent genes. This suggest that ARE may regulate different defense signaling pathways. ARE treatment increases H2O2 accumulation after TNVA inoculation, further contributing to the plant defense response. Accordingly, the expression of CATALASE was reduced in ARE-treated plants inoculated with TNVA, indicating that ARE might alleviate the plant scavenger system, impairing virus replication. Interestingly, the treatment with rosmarinic acid (RA), the most abundant compound in ARE, shows a similar effect that ARE in TNVA-infected tobacco plants. This suggests that ARE activity may be associated with the presence of RA in the extract. However, subtle differences were observed between ARE and RA treatments, suggesting that other unidentified bioactive compounds in ARE contribute to its priming effect. These findings offer exciting prospects of the potential use of ARE as a bioprotective agent in plant disease management.

Keywords: Bioprotectant, Plant-Pathogen, Priming, Rosemary, Rosmarinic acid
Methods: HPLC, RNA isolation, qPCR, antimicrobial activity, pathogenicity assays, ROS detection.

PT-22

Expression of flavodoxin in chloroplasts of mesophyll maize cells confers increased drought tolerance

Demarchi, María[1]; Arce, Rocío C[1]; Campi, Mabel [2]; Pierella Karlusich, Juan J. [3], Hajirezaei, Mohammad-R.[4], Melzer, M.[4], Chan, Raquel L[2]; Carrillo, Néstor [1]; Lodeyro, Anabella F[1]

[1] Instituto de Biología Molecular y Celular de Rosario (IBR-UNR/CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario (UNR), 2000 Rosario, Argentina. [2] Instituto de Agrobiotecnología del Litoral (IAL-UNL/CONICET), Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral (UNL), 3000 Santa Fe, Argentina. [3] FAS Division of Science, Harvard University, Cambridge, MA, USA. [4] Leibniz Institute of Plant Genetics and Crop Plant Research, OT Gatersleben, Corrensstrasse, D-06466, Stadt Seeland, Germany.

"

lodeyro@ibr-conicet.gov.ar

The cyanobacterial electron shuttle flavodoxin confers increased tolerance to multiple environmental stresses when introduced in chloroplasts from C3 plants, by relieving excess of excitation energy from the photosynthetic electron transport chain (PETC). Flavodoxin effects on stress tolerance of C4 plants displaying different photosynthetic pathways in mesophyll (Ms) and bundle sheath (BS) cells (mostly lineal and cyclic, respectively), remain unknown. The use of tissue-specific transformation in maize has been sparse, especially for photosynthetic studies. We expressed plastid-targeted flavodoxin differentially in maize Ms and BS cells by using tissue-specific promoters and transit peptides. We show that Ms lines exhibited increased tolerance to drought and oxidative stress, whereas BS plants displayed wild-type levels of damage, suggesting that flavodoxin interaction with the lineal PETC of Ms chloroplasts was critical for its protective effect, while interaction with the cyclic pathway of BS plastids was inconsequential. Our report identifies the type of intervention required to improve stress tolerance in maize plants using the flavodoxin approach, thus providing a more effective rationale to engineer this most important crop for better growth and yield in sub-optimal environments. Moreover, combination of tissue-specific expression with alternative electron shuttles such as flavodoxin provides a research tool to investigate the complex relationship between environmental hardships and C4 photosynthesis.

Keywords: maize, drought, mesophyll cells, bundle sheath cells, photosynthesis,
Methods: tissue-specific transformation, photosynthetic parameters determination, inmunoblot, PCR

PT-23

In silico characterization of new molecular actors involved in the tolerance to chilling injury in fruits.

Aimaro, N.; Gismondi, M.; Müller, G; Drincovich, M.F.; Bustamante C.
CEFOBI-UNR. Facultad de Ciencias Bioquímicas y Farmacéuticas (FBioyF). UNR
nicolasaimaro@gmail.com

Cold-induced damage (CI, chilling injury) is a problem arising from fruit refrigeration (CS, cold storage), significantly affecting its commercialization. Multiple strategies aimed at generating CI tolerance have proven to be efficient; one of them is heat treatment (HT). The molecular basis of CI, CS, HT, and other post-harvest conditions have been investigated at the molecular level through several experimental designs and omics approaches, yielding a wide array of molecules as potential protectors against CI. However, the lack of detailed knowledge regarding fruit genomes, their molecular products, and their functions suggests that further studies are needed to select genes of importance and varieties with desirable traits. The catalogue constructed in this work was compiled utilizing a wide variety of tools and available data in Solanum lycopersicum, including the evaluation of genomic data, transcriptional expression or transcriptomic data (including RNAseq and sequence-specific signal data), functional annotations, exon-intron architecture, gene ontology, physical interactions, as well as the presence or absence of cold-associated promoter regions. This compilation serves as a solid starting point for selecting genes potentially involved in the protection against CI in tomato fruit for further investigation, aiming to cover all possibilities, thereby enhancing the likelihood of getting interesting results. GO term searches yielded surprising results, demonstrating the lack of gene annotation related to this agronomically relevant fruit. Additionally, there is less annotation of low-temperature biological processes in this species compared to the model organism *Arabidopsis thaliana*. Conversely, the dataset and certain criteria related to future experiments allowed us to explore different approaches and apply effective filters that aid in the desired selection, prioritizing accurate ortholog assignment, simplicity in UTR, exon and intron annotations, and validation with RNAseq data, few or only one transcriptional variant, the *A. thaliana-S. lycopersicum* conservation of cold-induced transcriptional expression, among others. Based on our catalogue, we selected three genes for further experiments, REIL 1 and REIL2, both of them encoding zinc finger proteins, and HD2C, which encodes a histone deacetylase. This selection encourages the implementation of wet lab experiments to test the conservation of the response of these genes to cold stimulus in our system of interest, the tomato fruit. Our approach has not only yielded genes potentially involved in the tolerance to CI, but also systematically ordered strategies for other purposes.

Keywords: Chilling injury, dataset, Arabidopsis thaliana, Solanum lycopersicum
Methods: CisAnalyzer, Biomart, JBrowse, StringDB

PT-24

Unraveling the functional role of tobacco necrosis Virus A proteins in plant defense

Depetris, D1; Martinez, MF1; Lodeyro, AF1; Marano, MR1,2; García, L1,2.
1. Instituto de Biología Molecular y Celular de Rosario; 2. Facultad de Ciencias Bioquímicas y Farmacéuticas, Area Virología
garcia@ibr-conicet.gov.ar

Plant viruses are intracellular pathogens that depend on a living host cell for replication and movement. The host plant responds to viral infection through two main mechanisms: the biogenesis of small-interfering viral RNAs (vsiRNAs) and the induction of pathogen-associated-molecular-patterns (PAMPs)-triggered immunity (PTI). A second level of response, known as effector-triggered immunity (ETI), is associated with hypersensitive cell death response (HR), which confines the pathogen at the infection site. Tobacco necrosis virus strain A (TNVA), belonging to the Alphanecrovirus genus in the Tombusviridae family, causes necrotic lesions on inoculated leaves in a wide host range. We have recently characterized the mechanisms underlying the local cell death triggered by TNVA in *Nicotiana tabacum*, showing that it shares several components with the HR mediated by resistance proteins. TNVA perception triggers the accumulation of local vsiRNAs and the regulation of biological processes related to PTI. Interestingly, TNVA-vsiRNA accumulated in the inoculated leaves but did not appear to spread systemically, suggesting the suppression of antiviral silencing amplification by TNVA infection. TNVA activated PTI processes such as hydrogen peroxide accumulation, cell wall reinforcement, the activation of unfolded protein response and the induction of SA and SA-dependent pathways. However, these antiviral defences do not prevent either local virus multiplication nor systemic movement, leading TNVA-disease development. Our findings suggest that TNVA induces an impaired plant defence response by modulating host factors, allowing it to persist at low levels in distal tissues. In this work, three TNVA proteins, a movement protein (p6), the capsid protein (CP), and ORF1, were cloned and transiently expressed in tobacco plants. The transient expression of each of these proteins did not induce necrotic lesions in tobacco or N. benthamiana leaves (another host). However, CP and ORF1, displayed suppressor activity against the antiviral silencing response, as evidenced by GFP silencing suppression in *N. benthamiana* expressing GFP plants. The suppressor activity of these proteins could explain the viral movement to non-inoculated tissue and the generation of systemic symptoms, including chlorosis and dwarfism Regarding these symptoms, we investigated their connection to an imbalance in cellular redox status using transgenic plants that accumulate lower chloroplastic and cytoplasmic reactive oxygen species (ROS) when exposed to different stresses. Interestingly, the number of local necrotic lesions remained unchanged in transgenic plants compared to wild-type (control) plants. However, transgenic plants displayed enhanced resistance to TNVA compared to control plants, resulting in reduced systemic symptoms. Moreover, these plants exhibit lower virus replication levels compare to control plants. Upcoming studies will attempt to determine if the lesions

are induced by viral PAMPs and evaluate whether TNVA may modulate the expression of proteins associated with ROS generation

Keywords: alphanecrovirus, local necrotic lesion, PAMPs, viral suppresor, ROS
Methods: virus inoculation, phenotyping, image análisis, qPCR, confocal microscopy, ros quantification.

PT-25

Resistance genes discovery in citrus plant by TALE PthA4AT engineering

Villalobos-Piña, Liara1; Uviedo, Facundo1; Roeschlin, Roxana1; García, Lucila1; Gadea, José2; Marano, María Rosa1.
1. Instituto de Biología Molecular y Celular de Rosario(IBR).CONICET-Universidad Nacional de Rosario(UNR). Rosario, Argentina; 2. Instituto de Biología Molecular y Celular de Plantas (IBMCP), Universidad Politécnica de Valencia-CSIC. Valencia, España
liaravipi@gmail.com

Xanthomonas citri subsp. *citri* (*X. citri*) is a gram-negative bacterium that causes citrus canker, a widespread disease in Argentina's citrus-growing regions. The main virulence factor of this bacterium, the transcription activator-like effector (TALE) PthA4, recognizes specific DNA sequences in the host promoter through its 17.5 amino acid repeats, activating susceptibility genes in citrus. We recently identified and characterized a natural variant of X. citri (X. citri AT) that triggers a hypersensitive response (HR), inhibiting biofilm development and bacterial growth. X. citri AT expresses an unusually short TALE, PthA4AT, which is necessary and sufficient for HR induction and canker suppression, suggesting its role in activating citrus resistance (R) genes. PthA4AT harbors only 7.5 repeats in its DNA binding domine, defining a short 9 bp target sequence. In addition, it has two repeats with ambiguous recognition for two or more bases. This versatility suggests potential interactions with numerous plant genes. Here, we present a novel strategy exploiting TALE modularity to reduce target sites and identify HR and canker suppression-related genes. Our findings demonstrated that the cell death mediated by PthA4AT is sequence-specific. This insight has allowed us to enhance the identification of R genes that PthA4AT targets within plant cells. Futhermore, we uncovered additional R genes that mediate cell death in *Citrus sinensis*. Interestingly, PthA4AT also triggers HR in non-host species for X. citri AT, such as *Nicotiana benthamiana* and *Solanum lycopersicum*. Our approach has identified new genes involved in programmed cell death in *N. benthamiana*. Notably, tomato exhibits HR in both the cytoplasm and nucleus, suggesting the involvement of R genes capable of recognizing different TAL effectors, regardless of their sequence specificity or topology. This study validates genome screening for cell death inducers and other phenotypes, offering the potential to enhance the plant immune system for managing disease in different cultivars.

Keywords: Synthetic Effectors, PthaA4AT, TALE, Hypersensitive Response, Canker, R Genes

Methods: Inoculation assays, Southern hybridization analysis, Cloning and sequencing of TALE genes, Immunoblot analysis, Protein binding microarrays

PT-26

Iron metabolism in *Arabidopsis thaliana* anthocyanin-deficient mutants

Vizcaino, Miguel A.; Gomez-Casati, Diego F.; Pagani, María Ayelén
Centro de Estudios Fotosintéticos y Bioquímicos (UNR-CONICET)
pagani@cefobi-conicet.gov.ar

Iron (Fe) is a crucial micronutrient for plant development. Despite the abundance of Fe in soils, its low bioavailability, especially in alkaline environments, becomes the main restricting factor for plant growth, limiting crop productivity and nutritional quality of the edible parts. Anthocyanins serve multiple functions in plants, such as pollinator attraction and ultraviolet radiation protection, as well as defence against pathogens and herbivores. Previous results have shown that Arabidopsis mutants in anthocyanin biosynthesis exhibited altered expression of a set of genes related to the Fe deficiency response. Specifically the *tt4* and *3gt* mutants showed induction of bHLH38, bHLH39, IRT1, FEP2, BTSL1, and BHLH100 genes, while the 5gt and tt19 lines exhibited exactly the opposite pattern of repression of those genes, in anthocyanin inductive conditions (liquid culture and constant light). Anthocyanins or other metabolites from the flavonoid pathway have not been related to Fe homeostasis before. The objectives of this work were then to investigate the impact of anthocyanins on Fe metabolism. For this, the study of different variables related to iron metabolism was initiated in the anthocyanin deficient mutants tt4, 3gt, 5gt and tt19 grown under regular plant culture conditions (22 ºC, Fe sufficient MS agar medium, light:dark cycles 16:8). Root morphology was determined in plants grown on vertical agar plates. Iron accumulation was observed by Perls staining, and to accurately quantify Fe in roots, shoots and seeds, ICP-MS technique was used. FRO2 activity, the Fe absorption limiting step, was determined in roots by Fe (III) reduction and colorimetric detection. Results obtained confirm the previous findings, and indicate that anthocyanin-deficient mutants present alterations in parameters and variables related to Fe metabolism. Lines tended to show similar results between the tt4 and 3gt group on the one hand, and 5gt and tt19 on the other. 5gt and tt19 lines accumulated more Fe in shoots and seeds than the Col-0 line. However, tt19 roots showed a Fe deficient phenotype, increasing the number and length of secondary roots. tt4 and 3gt lines showed normal Fe concentrations in roots, shoots and seeds but

the metal must have been poorly available, as demonstrated by the poor coloration observed in the Perls staining of tt4 lines. FRO2 activity also supports this hypothesis, as it was increased in this line. Altogether, these results point to a role for anthocyanins in Fe homeostatic mechanisms.

Keywords: *Arabidopsis thaliana*, iron, anthocyanins, FRO2

Methods: ICP-MS, Perls staining, FRO2 activity, root morphology

PT-27

Characterization of a maize UDP-glucosyltransferase involved in flavonoid biosynthesis

Petrich, Julieta1; Casati, Paula1; Grotewold, Erich2; Falcone Ferreyra, María Lorena1
1CEFOBI (CONICET-UNR), 2Dpt Biochem Mol Biol, Michigan State University
petrich@cefobi-conicet.gov.ar

Flavonoids are specialized metabolites widely distributed in plants. These compounds not only have physiological functions during growth and development, but also have protective roles against biotic and abiotic stressful conditions, such as pathogen infections, UV B radiation, high white light, drought, cold and salinity. One important step in the flavonoids biosynthesis is their glycosylation, which enhances the stability and solubility of these compounds. As a result, plants typically accumulate flavonoids as C- and O- mono or di-glycosylated derivatives. Maize is a major crop used for human and animal consumption, however, some enzymes involved in the flavonoid biosynthetic pathway has not been elucidated. Our aim is to functionally characterize a novel UDP-sugar glycosyltransferase, named ZmUGT2, which catalyzes the O-glucosylation of flavonoids in maize plants. Previous studies showed that ZmUGT2 expression is induced by Pericarp Color1, the transcription factor involved in the regulation of flavonoid biosynthesis. Phylogenetic analysis suggests that ZmUGT2 belongs to family 1 glycosyltransferases, which includes other UGTs involved in flavonoid O glycosylation. Here, the recombinant protein ZmUGT2 was purified and kinetically characterized. HPLC and LC-MS analysis showed that ZmUGT2 is able to accept different flavonoids as substrate acceptors (flavanones, flavone and flavonols), and notably, both mono and di-glucoside derivatives are formed. To analyze ZmUGT2 functionality in planta, we generated Arabidopsis transgenic plants expressing ZmUGT2 from the 35S promoter in background WT and mutant in genes encoding enzymes of flavonoid biosynthesis. Now, we are evaluating the accumulation pattern profiles of glycosylated flavonoids in transgenic plants in comparison to WT in different plant tissues and growth conditions.

Keywords: *Flavonoids, maize, UDP-glycosyltransferase*

Methods: purification of recombinant protein, RT-qPCR, HPLC, LC-MS

Signal Transduction

ST-01

Integrated Analysis of Methylation, Copy Number Variations, RNA Expression, and Mutations Data in Prostate Adenocarcinoma using Random Forest Highlights CYP27B1 Methylation as a Precise Fine-Tuning Mechanism

Martinelli Romina P , Esteban Luis
Cátedra de Quimica Biológica , Facultad de Ciencias Médicas. Universidad Nacional de Rosario
lesteban@unr.edu.ar

Prostate cancer is a highly variable disease with diverse molecular characteristics and outcomes, and it is a common cause of male cancer-related deaths. Understanding the regulatory mechanisms of CYP27B1 is crucial for utilizing vitamin D as an adjunct in neoplasia therapy. In this study, we employed a machine learning algorithm to predict CYP27B1 levels in prostate cancer cells and to investigate the key variables involved in its regulation.To achieve this goal, we classified patient samples from the PRAD project (Prostate Adenocarcinoma TCGA, Cancer Genome Atlas) into Low (L), Medium (M), and High (H) groups based on their CYP27B1 gene expression levels. Additionally, we used a list of genes functionally associated with CYP27B1 activity in prostate cancer, derived from a systems biology regulatory model (SAIB 2022). Of them we downloaded data sets, including Copy Number Variations, RNA expression values, Point Mutations, and Methylation information, for both High and Low groups from cBioPortal (https://www.cbioportal.org).We identified Random Forest as the algorithm with the besnetwork Inference, module detectiont performance and constructed a classifier using the Random Forest R package. For validation, we divided the discovery cohort into a training set (66.6%) and a test set (33.3%). ROC curve analysis yielded an outstanding value of 0.98, suggesting that the selected genes are informative regarding CYP27B1 regulation.Custom R scripts were developed to create graphical representations. Mean Decrease in Accuracy and Mean Decrease Gini were utilized to assess the most important variables. RNA expression levels and the Methylation status of various genes emerged as significant factors.Notably, CYP27B1 methylation stood out as the most significant factor. Upon exploring

methylation within the PRAD population using Ualcan (https://ualcan.path.uab.edu/), we observed hypomethylation in the promoter region compared to normal samples. CYP27B1 expression exhibited a negative correlation with methylation. By analyzing data from the 450K Illumina platform available in the UCSC Xena browser (https://xenabrowser.net), we identified several differentially regulated CYP27B1-related CpG sites.

Most of these sites were found to be hypomethylated (alpha < 0.5), although some were hypermethylated, providing a distinctive landscape in this context. The six CpG loci with differential methylation displayed a reduced level in the High group.Furthermore, we placed particular importance on the annotation from ChipSeq experiments(Remap). Specifically, cg25452172 and cg20372759 mapped to the first exon of the neighboring METTL1 gene upstream of the Transcription Start Site. The remaining loci mapped to introns or exons of CYP27B1. Of these, cg01182309, cg23101118, cg07060721, and cg04321714 exhibited associations with EZH2 in prostate cancer cell lines or prostate cancer, providing support for the hypothesis of a finely tuned negative regulation mechanism of CYP27B1 in prostate cancer.

Keywords: CYP27B1, prostate adenocarcinoma, DNA methylation, Randon Forest

Methods: DNA methylation analysis, machine learning

ST-02

Targeting histone acetylation specifically to SMN2 intron 6 using a CRISPR-dead Cas9 strategy cooperates with a nusinersen-like ASO to promote exon 7 inclusion

Jose Stigliano 1, Luciano Marasco 2 and Alberto Kornblihtt 1
1. Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE-CONICET), Universidad de Buenos Aires (UBA), Argentina. 2. Sir William Dunn School of Pathology, Oxford, UK.
jnstigliano@gmail.com

SMA is a motor-neuron disease caused by mutations of the SMN1 gene. The human paralog SMN2, whose exon 7 (E7) is predominantly skipped, cannot compensate for the lack of SMN1. Nusinersen is an antisense oligonucleotide (ASO) that upregulates E7 inclusion and SMN protein levels by displacing the splicing repressors hnRNPA1/A2 from their target site in intron 7. We recently showed (1) that by promoting transcriptional elongation, the histone deacetylase inhibitor VPA cooperates with a nusinersen-like ASO to promote E7 inclusion. VPA acts by removing a roadblock to transcriptional elongation created by the ASO itself, resulting in higher E7 inclusion, without large pleiotropic effects. Combined administration of the nusinersen-like ASO and VPA in SMA mice strongly synergized in SMN expression, growth, survival, and neuromuscular function.

An important question arises from our recent results: Can we replace the effect of VPA, that may affect expression of other genes, by a strategy that promotes histone acetylation specifically at the SMN2 gene? To this end, we used a CRISPR/Cas9-mediated method in which the nuclease Cas9 is mutated to be catalytically inactive and is fused to the herpesvirus transcriptional activator VP64 that acts by recruiting endogenous histone acetylating enzymes. We have designed different guide RNAs to direct the dead-Cas9-VP64 fusion to different positions along SMN2. This strategy was expected to locally affect RNA polymerase II elongation and therefore increase basal and ASO1-stimulated E7 inclusion. Experiments performed in HEK293 cells in culture revealed that when a vector expressing a guide RNA targeting a sequence in intron 6 is cotransfected with the plasmid expressing the dead-Cas9-VP64 fusion, no changes are observed in the levels of E7 inclusion. However, targeting the dead-Cas9-VP64 fusion to intron 6 greatly enhances the upregulation of E7 inclusion caused by the nusinersen-like ASO. Preliminary results indicate that directing VP64 to other sequences located on intron 7 has no effect neither on basal nor on nusinersen-stimulated E7 inclusion.

Our results suggest that, unlike VPA that promotes E7 inclusion per se in HEH293 cells, CRISPR-mediated local acetylation alone has no effect on E7 inclusion but helps to improve nusinersen activity. We intend to develop a novel combinatory strategy for SMA based on ASO1 and chromatin modifications. Even though classical CRISPR-Cas9 therapies may be questionable for the fact that they may introduce off target mutations, it should be noted that the CRISPR-dCas9 strategy does not alter the genetic

information of the targeted gene nor of the putative off targets. By affecting only the epigenome it is predicted that any putative undesired effects will be reversible.

(1) Marasco et al. Counteracting chromatin effects of a splicing-correcting antisense oligonucleotide improves its therapeutic efficacy in spinal muscular atrophy. Cell 185, 2057-2070 (2022). Cover of the issue.

Keywords: alternative splicing, spinal muscular atrophy, CRISPR-dCas9, SMN

Methods: CRISPR-dCas9, RT-PCR, Western Blotting, ChIP-qPCR

ST-03

ROLE OF THE PRENYLATION PATHWAY IN TUMOR AGGRESSIVENESS: LIPOPHILIC MODIFICATION OF SALIRASIB AS A THERAPEUTIC STRATEGY

Arel Zalazar, Evelyn E.1; Borini Etichetti, Carla M.2; Ballari, Maria Sol 3; Porta, Exequiel3; Cocordano, Nabila1; Labadie, Guillermo R.3; Girardini, Javier E.1
1Instituto de Inmunología Clínica y Experimental de Rosario (UNR-IDICER-CONICET). 2Instituto de Fisiología Experimental de Rosario (UNR-IFISE-CONICET). 3Instituto de Química Rosario (UNR-IQUIR-CONICET).
girardini@idicer-conicet.gob.ar

ICMT (Isoprenylcysteine Carboxymethyl Transferase) catalyzes the last step in the Prenylation Pathway. This posttranslational modification process, starts with the addition of an isoprenoid to a cysteine near the C-terminus in target proteins, followed by the cleavage of terminal amino acids. Then, ICMT catalyzes the methylation of the newly generated C-terminus on the cysteine. This modification, regulates critical functional aspects of substrate proteins among which there are several members of the Ras and Rho GTPases families. ICMT has emerged as an interesting target for novel anti-cancer therapies. We have previously shown that ICMT enhances aggressive tumor phenotypes and that its expression is repressed by the p53 tumor suppressor. To further characterize this effect, we studied the role of ICMT on migration and invasion in vitro. Since ICMT regulates proteins involved in actin cytoskeleton dynamics, we also analyzed invasive structures, such as invadopodia. In order to identify novel ICMT inhibitors we generated derivatives of Farnesylthiosalisilic acid (FTS), commercially known as Salirasib. This molecule was reported to inhibit ICMT and recently has reached clinical trials for the treatment of Non Small Cel Lung Carcinoma (NSCLC). We analyzed the effects of a collection of 27

compounds on cancer cell viability. Through bioreduction-based assays we identified four compounds showing significant antiproliferative activity.
Structure-Activity Relationship (SAR) analysis showed that longer lipophilic substituents exerted a stronger effect. In agreement, docking analysis predict that these compounds may be better ICMT interactors, and therefore candidates for specific inhibitors. Interestingly, nutrient deprivation dramatically enhanced antiproliferative activity, suggesting that cells with enhanced nutrient requirements, such as cancer cells, may be more affected. Among compounds which did not affect proliferation we analyzed the ability to reduce metastasis associated phenotypes such as migration and invasion. We performed wound healing assays on the H1299 (NSCLC) cell line. Our results showed that three compounds significantly inhibited cell migration. We also found that two of them significantly reduced invasion in transwell invasion assays using Matrigel-coated filters. In order to gain insight in the molecular mechanisms involved we studied if these compounds may alter actin cytoskeleton through fluorescence microscopy analysis upon phaloidin staining. We also found that some compounds altered Rac1 subcellular localization, which is a key regulator of actin cytoskeleton dynamics. In summary, we showed that ICMT overexpression promotes invasion through alteration of the actin cytoskeleton and we identified novel Salirasib derivatives that reduced cancer-associated phenotypes in vitro and are interesting candidates for leading molecules in cancer therapy.

Keywords: cancer, protein prenylation, nutrient deprivation, metastasis, actin cytoskeleton

Methods: confocal microscopy, in vitro migration/invasion, proliferation, molecular docking,

ST-04

Exploring Calcium Dynamics in Yeast Cells During Pheromone Response: A Single-Cell Perspective

Tarkowski, Nahuel1; Ponce Dawson, Silvina2; Aguilar, Pablo S.3
1 Centro de Investigaciones en Bionanociencias (CIBION), CONICET, Buenos Aires, Argentina;2 Departamento de Física y IFIBA , CONICET, FCEyN-UBA, Buenos Aires, Argentina; 3 Laboratorio de Biología Celular de Membranas, IFIBYNE (UBA-CONICET), Buenos Aires, Argentina
ntarkowski@gmail.com

Yeast cells, Saccharomyces cerevisiae, represent an ideal system for investigating cellular signaling mechanisms. In particular, haploid cells of S. cerevisiae offer a context of special interest, as they

respond specifically to the sexual pheromone released by cells of the opposite sexual type, triggering a sequence of events that can be observed with the naked eye. Among these events, the cell cycle is halted, and polarized growth toward the pheromone-emitting cell begins, ultimately leading to cell fusion and the formation of a zygote. Previous research had confirmed the importance of calcium incorporation during the response to the pheromone, as it allows for the coordination of genes involved in signal transduction and cell survival. Here, we have addressed the study of calcium dynamics during the pheromone response by observing individual cells. To achieve this, we introduced, developed, and validated the fluorescent calcium sensor GCaMP6f in S. cerevisiae. Through in vivo fluorescence microscopy experiments, we identified and tracked individual cells, observing transient increases in the form of short bursts. Moreover, the presence of the pheromone appears to increase the frequency of these calcium bursts, suggesting that the information transmitted by calcium is encoded in the temporal distribution of these bursts. With the aim of understanding how different calcium flow pathways affect the dynamics of cytosolic calcium during the pheromone response, we constructed and analyzed a set of mutant strains lacking all known calcium transporters in S. cerevisiae. Our results suggest that the Fig1 protein, which is involved in calcium uptake from the extracellular medium, plays a central role in calcium dynamics during the pheromone response, and surprisingly, we observed that Yvc1, the vacuolar calcium exporter, also plays a significant role in this process. We also observed that even in the mutant strain lacking all cytosolic calcium elevation pathways (fig1Δ mid1Δ yvc1Δ) and treated with pheromone, there is still an increase in the frequency of calcium bursts compared to the wild-type strain in a low-calcium environment. These results suggest the existence of a third calcium import mechanism.By utilizing models and simulations of cytosolic calcium and vacuolar dynamics, we were able to assign specific characteristics to the different calcium pathways, contributing to the explanation of some of the results obtained in our study.

Keywords: Saccharomyces, calcium, yeast

Methods: Fluorescence microscopy

ST-05

Role of lipids in the activation of the Unfolded Protein Response

Porrini, Lucía; Bortolotti, Ana; Cybulski Larisa Estefanía
Laboratorio de Interacciones Lípido-Proteína en la Señalización Celular. Departamento de Microbiología. Facultad de Cs. Bioq. y Farmacéuticas.
luciaporrini14@gmail.com

Accumulation of unfolded proteins in the endoplasmic reticulum (ER) triggers the so-called unfolded protein response (UPR). This signaling pathway is at least partly controlled by the ER-located transmembrane protein IRE1 (inositol-requiring enzime1) that drives the transcription of genes such as chaperones and folding enzymes.
The IRE1-regulated pathway is conserved from yeast to humans. In yeast, the accumulation of misfolded proteins results in the dimerization of Ire1, a process that activates its cytosolic endoribonuclease function. The substrate for Ire1 endonuclease activity is the transcript of Hac1 transcription factor that binds to promoter UPR elements (UPRE).
Recently, it became evident that aberrant lipid compositions of the ER membrane, referred to as lipid bilayer stress (LBS), are equally potent in activating the UPR. The underlying molecular mechanism, however, remains unclear. We first tried to confirm the implication of the LBS in the adaptive response of yeast cells to ER stress. To analyze the Induction of the UPR in S. cerevisiae cells we use a reporter gene (LacZ), encoding β galactosidase, under the control of UPRE. In this context, induction of β-galactosidase activity reflects the cellular amounts of functional Hac1.
We examined the expression of UPR in the presence of β-mercaptoethanol, a reducing agent that induces ER stress by generating accumulation of unfolded proteins. We found that the activation of Ire1 is modulated by the growth stage of the cells. We also analyzed the effect of inhibitors of fatty acid (FA) biosynthesis and performed GC-mass to analyze the resulting FA profile and co-relate with the expression of UPRE

Keywords: Unfolded Protein Response, IRE1, Lipid Bilayer Stress, Endoplasmic Reticulum

Methods: β-galactosidase assay, cell viability, GC-mass

ST-06

In vitro study of the interaction between the protein kinase PDK1 and two kinase substrates

Gross, Lissy Z. F. 1; Galceran, Facundo 1; Sacerdoti, Mariana 1; Rinaldi, Jimena 3; Klinke, Sebastián 3; Otero, Lisandro H. 4,5; Leroux, Alejandro E. 1; Biondi, Ricardo M. 1,2
1 Instituto de Investigación en Biomedicina de Buenos Aires (IBioBA) – CONICET – Partner Institute of the Max Planck Society, Argentina; 2 Department of Internal Medicine I, Universitätsklinikum Frankfurt, Germany; 3 Fundación Instituto Leloir, IIBBA-CONICET, Argentina; 4 CRIP-IIB-UNSAM, Universidad Nacional de San Martín, Argentina; 5 INBIAS, Universidad Nacional de Río Cuarto, Argentina
facugalce@gmail.com

Protein kinases serve as crucial cellular switches, governing essential ON-OFF functions within cells. The improper regulation of these kinases frequently plays a role in the development of diseases like cancer and diabetes. Consequently, stringent and precise control mechanisms are in place to regulate their activities.

Phosphoinositide-dependent protein kinase 1 (PDK1) plays a pivotal role as a master AGC kinase in the PI3K signaling pathway. It phosphorylates the activation loop of over 23 other AGC kinases, including Akt/PKB, S6K, SGK, PKC, PRK, and more. This phosphorylation is important because it is required for the activity of all these protein kinases. We demonstrated an allosteric regulation within the protein kinase domain, from a regulatory site termed PIF-pocket to the active site, as well as the existence of the reverse process. This bidirectional allosteric mechanism of regulation between both pockets can be modulated by small molecules that bind to the active orthosteric site and either enhance or inhibit the interaction at the PIF-pocket allosteric regulatory site. As a probe directed to the PIF-pocket, we employed the polypeptide PIFtide, which corresponds to the last 23 residues of the protein kinase PRK2. We showed in the past that the metabolite adenosine can enhance the interaction of PDK1 with PIFtide, while the ortosteric inhibitor GSK2334470 displaces it. However, detailed information regarding the regulation and structure of a complex between PDK1 and its substrate kinases is still missing. We will present the initial studies on the in vitro interaction of PDK1 with two protein kinase substrates, S6K and PRK2 using AlphaScreen technology. PDK1 interacted with high affinity with both kinases and these interactions were inhibited by PIFtide. Using this assay, we demonstrate how small compounds and metabolites can modulate these protein-protein interactions, exerting orthosteric as well as allosteric effects on the protein complex. It was curious that the already phosphorylated PRK2 interacted with high affinity with PDK1, because our standard model suggested that the active form would not have the PIF-sequence exposed. Interestingly, the interaction of PRK2 with PDK1 mediated by the C-terminal PIF-sequence was compatible with PRK2 activity. Finally, we will provide preliminary information on the PDK1-substrate complex using transmission electron microscopy (negative-stain TEM). Our study increases our understanding of the PDK1 regulation and specificity, based on the regulated docking interaction with substrates.

Keywords: protein kinase, protein-protein interaction, protein complex, protein structure, protein-substrate interaction

Methods: AlphaScreen (protein-protein interaction assay), transmission electron microscopy, nonradioactive kinase activity assay, SEC-SLS

ST-07

Implication of APP phosphorylation at threonina668 induced by Aß oligomers in amyloidogenic processing of APP: Role of GßɣɣGßɣɣ/p38MAPK signaling.

Almirón, Romina Soledad; Antonino, Magdalena; Lorenzo, Alfredo; Bignante, Elena Anahí
Instituto de investigación médica Mercedes y Martin Ferreyra-INIMEC-CONICET
ralmiron@immf.uncor.edu

Protein phosphorylation is a fundamental post-translational modification, with serine, threonine and tyrosine residues generally being covalently linked to phosphate groups. Protein phosphorylation is closely involved in a variety of cellular events, indicating their crucial role in physiological and pathological conditions. Pathologic hallmarks of AD include amyloid-beta (AB) plaques, neurofibrillary tangles mainly composed of abnormally phosphorylated tau, dystrophic neurites surrounding Ab plaques, and overt loss of synapses and neurons. The study of phosphorylation signaling pathways in AD focuses mainly on tau protein, due to their abundant phosphorylation sites and kinases, while much less is known about APP phosphorylation and its impact on this disease. The phosphorylation of APP at threonine 668 (APP-P-T668) of the cytoplasmic domain has been correlated with an increase in the proteolysis of APP by beta-secretase, due to the activation of kinases such as GSK-3, JNK. It has been shown that APP-P-T668 is prevalent in AD and is increased in the hippocampus of AD patients. Furthermore, APP-P-T668 colocalizes with BACE1 in enlarged endosomes in hippocampal neurons. Previous studies from our laboratory demonstrated that p38MAPK kinase is a downstream effector of the APP/Go/Gβγ pathway, mediating tau phosphorylation and Aβ fiber-induced dendritic dystrophy. Based on this evidence, we propose to study the implication of APP phosphorylation at T668 induced by the Go/Gβγ/p38MAPK pathway as a possible triggering event for the amyloidogenic processing of APP. Our experiments were performed in cultures of cortical neurons from 10 DIV rat embryos, which were treated with preparations enriched in oligomeric AB (ABo,1uM), gallein, a beta-gamma subunit blocker (5uM),SB203580, inhibitor of p38MAPK,(20uM) or vehicle. Subsequently, western blot assays were performed to quantify the levels of endogenous phosphorylated APP in the different conditions, observing a significant increase in the same in neurons treated with ABo, which was prevented in those neurons that previously received gallein. Immunofluorescence studies show an increase in the APP-P-T668 label in both soma and dendrites of neurons treated with ABo and a decrease in the synaptophysin label, the latter being an indicator of synapse loss. These effects were avoided with pretreatment of the cultures with gallein or SB203580. In conclusion, ABo induces an increase in APP-P-T668 which correlates with an increase in its amyloidogenic processing. This effect is mediated by the Go/Gβɣ/ p38MAPK pathway since it was inhibited by treatments with gallein and SB203580. Clarifying the mechanisms by which the cycle of toxicity induced by AB is generated and its

consequences on neurons is important to be able to find early alterations of the pathology as well as to develop therapeutic strategies that allow a more selective intervention in the treatment of AD.

Keywords: phosphorilated APP; p38MAPK, amyloid Beta peptide, Alzheimer disease

Methods: immunofluorescence, western blot, neuronal culture

ST-08

EXPLORING ALLOSTERY IN ACE2: CAN SMALL MOLECULES AFFECT ENZYME ACTIVITY AND PROTEIN-PROTEIN INTERACTIONS?

Acebedo Martinez, Macarena 1; Sacerdoti, Mariana 1; Gross, Lissy Zoe Florence 1; Di Lella, Santiago 2; Gironacci, Mariela 3; Biondi, Ricardo Miguel 1; Leroux, Alejandro Ezequiel 1
1: 1Instituto de Investigación en Biomedicina de Buenos Aires (IBioBA) - CONICET - MPSP; 2: 2Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN) - UBA - CONICET; 3: Instituto de Química y Fisicoquímica Biológicas (IQUIFIB) - UBA - CONICET
macarena.acebedo@gmail.com

Allostery refers to the process in which an interaction at one specific site on a protein (allosteric site) causes a conformational change that impacts the function of a distant site. This phenomenon is bidirectional, meaning that binding at the distant site can also induce a conformational change at the regulatory site. Protein-protein interactions are very difficult to disrupt with small molecules, and therefore an enormous challenge for drug discovery. In previous work conducted in the lab on protein kinases we showed that protein-protein interactions can be enhanced or potently disrupted allosterically (Trends Biochem Sci 45(1):27-41, 2020). We then aimed to investigate the possibility to expand our chemical biology studies on allostery to other important biomedical proteins. The protein ACE2 catalyses the cleavage of Angiotensin II into Angiotensin (1-7). In addition, ACE2 is the receptor that is used for infection by coronaviruses such as SARS-CoV2. We analyzed the published information on ACE2 and concluded that it could be an allosteric protein; therefore small molecules could potentially allosterically disrupt interactions with Spike and could potentially serve as anti-infectives (ChemMedChem. 15(18):1682-1690, 2020).
In the meantime, a group at the NIH performed high-throughput screening using AlphaScreen and deposited the raw data of the screening online. To date, the NIH group and others have identified small compounds that disrupt the ACE2-Spike interaction. However, the mechanism of action of the

compounds have not been investigated. Here, we describe our initial studies on the modulation of ACE2 by small compounds. Firstly, we independently analyzed the NIH screening data and investigated the effect of hits and structural analogs of interest in our own AlphaScreen interaction assay and on the effect on enzyme activity. We analyzed the enzyme activity using a fluorogenic assay. Notably, we identified compounds that produced a 3-4 fold increase in the enzymatic activity of ACE2 suggesting an allosteric mechanism. Those molecules identified as activators or inhibitors were further evaluated using a radioactive assay with the endogenous substrate (HPLC). Additionally, we setted-up a differential scanning fluorimetry assay to indirectly validate compound binding. The hit compound 53, an activator, generates a destabilization of ACE2 in our thermal stability assay, confirming its binding to the protein. Lastly, docking results using peptidase domain of ACE2 allowed us to identify a possible binding site for the activator compound 39. Our ongoing work supports the model where ACE2 is an allosteric protein. Most notably, our results indicate the existence of an allosteric regulation of the enzymatic activity of ACE2. This finding suggests that ACE2 activity could be physiologically regulated. Moreover, the findings open the possibility that ACE2 activity could be modulated pharmacologically, for example, as an anti-hypertensive treatment.

Keywords: Allostery, Drug discovery, Angiotensin converting enzyme II (ACE2), SARS-Cov2

Methods: AlphaScreen, High-throughput screening of compound libraries, Differential scanning fluorometry, Molecular Dynamics, Enzymatic activity fluorogenic assay

ST-09

Xrn1 and Rpb4 are mRNA coordinators that regulate cAMP-PKA specificity upon heat stress

Ortolá Martínez, María Clara; Galello, Fiorella; Portela, Paula and Rossi, Silvia.
Universidad de Buenos Aires. Facultad de Ciencias Exactas y Naturales. Departamento de Química Biológica. Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales-Consejo Nacional de Investigaciones Científicas y Técnicas (IQUIBICEN-CONICET). Buenos Aires, Argentina.
marita.ortola@hotmail.com

Signal transduction pathways control transcriptome and proteome remodeling in response to different environmental conditions and stressors. The cAMP-PKA pathway plays a critical role in regulating several cellular processes and is conserved across species. Considering PKA's multiple functions, an

important question is how signaling specificity in response to different stimuli is achieved. In S. cerevisiae, PKA is composed of two catalytic (Tpk1, Tpk2 or Tpk3) and two regulatory subunits (Bcy1). Diverse mechanisms determine specificity of PKA signaling. One of them is the regulation of PKA subunits expression, which is reflected in changes in the isoform composition of the holoenzyme. We have previously demonstrated that the expression of each PKA subunit is different in response to stress. Tpk1 is the only subunit upregulated during mild heat shock. To further understand the molecular basis of cAMP-PKA specificity, we evaluated the mechanisms involved in PKA subunits expression during thermal stress adaptation or thermotolerance, where cells exposed to a mild heat stress dose (37ºC for 30 minutes) survive a subsequent severe heat stress dose (45ºC for 10 minutes). We observed that only TPK1 promoter activity and mRNA levels increase during thermotolerance. Gene expression implies an important crosstalk between mRNA transcription, decay and translation. This is possible since key factors involved in one stage of gene expression may play non-canonical roles in other stages. These factors were referred to as "mRNA coordinators" and act through co-transcriptional imprinting of mRNAs. Rpb4/7 subunits of RNA Pol II and mRNA decay factors such as the 5'-3' exonuclease Xrn1 or Ccr4-NOT were described as mRNA coordinators. We evaluated the role of Rpb4 and Xrn1 in cAMP-PKA pathway specificity. First, we investigated their effect on PKA activity in vivo through the analysis of physiological PKA readouts (stress resistance and glycogen accumulation). Both xrn1Δ and rpb4Δ mutant strains are more resistant to heat shock than a WT strain. A xrn1Δ strain shows higher glycogen accumulation than a WT strain, consistent with a lower PKA activity. However, a rpb4Δ strain shows no significant differences in comparison with a WT strain. We also evaluated the roles of Xrn1 and Rpb4 in TPK1 gene expression. TPK1 mRNA half-life increases in a xrn1Δ strain under optimal growth conditions. Moreover, TPK1 promoter activity and mRNA levels are upregulated upon thermotolerance in an Xrn1-dependent manner. Accordingly, Xrn1 is recruited to the TPK1 promoter upon heat stress, consistent with its role in TPK1 transcription regulation. Although during thermotolerance TPK1 mRNA levels are strikingly upregulated in a xrn1Δ strain, Tpk1 protein levels severely decrease, indicating that Xrn1 also regulates Tpk1 translation. Noteworthy, Xrn1 does not regulate other PKA subunits expression. Regarding the role of Rpb4 in TPK1 expression, TPK1 mRNA levels are higher in a rpb4Δ strain than in a WT strain, but do not change in response to stress. Tpk1 protein levels are also higher in a rpb4Δ than in a WT strain in all conditions. Bcy1 protein levels are highly upregulated in rpb4Δ, explaining the lack of differences in glycogen accumulation in comparison to WT strain. Our results support the idea that Xrn1 and Rpb4 are central elements that coordinate TPK1 transcription in the nucleus with posttranscriptional cytoplasmic processes upon heat stress.

Keywords: PKA, S. cerevisiae, Xrn1, Rpb4, expression regulation

Methods: Reporter genes, ChIP, Northern blot, Western blot, RT-qPCR

ST-10

Role of Mip6 in TPK1 expression levels

Gareis, Daniela Belén; Jaraslasky, Iván; Ortolá Martínez, María Clara and Galello, Fiorella
Universidad de Buenos Aires. Facultad de Ciencias Exactas y Naturales. Departamento de Química Biológica. Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales-Consejo Nacional de Investigaciones Científicas y Técnicas (IQUIBICEN-CONICET). Buenos Aires, Argentina.
fiorellagalello@gmail.com

In eukaryotic cells, mRNA export from the nucleus is crucial to ensure the appropriate protein output. This process is connected with downstream and upstream events that regulate the mRNA fate and requires RNA-binding proteins (RBPs) participation. It has been demonstrated that the RBP Mip6 plays a major role in the nuclear export and expression regulation of Msn2/4 dependent mRNAs under non-stress conditions. Mip6 interacts with different factors implicated in several steps of mRNA homeostasis, such as the exonuclease Xrn1. In Saccharomyces cerevisiae, cAMP-PKA signaling pathway is involved in the coordination of different cellular functions and biological processes in response to environmental fluctuations. Transcriptional and post-transcriptional regulation, as well as PKA subunits expression levels, are crucial to maintain the signaling specificity. We have demonstrated that TPK1 promoter is upregulated under heat stress in an Msn2/4 dependent manner. TPK1 mRNA levels are also increased under heat stress; however, protein levels show no change. On the other hand, we have also demonstrated that Xrn1 regulates TPK1 mRNA levels during heat stress. In order to shed light on the different factors involved in the specificity of cAMP-PKA pathway, we proposed to evaluate the role of the RBP Mip6 on TPK1 expression under stress and non-stress conditions. Using qRT-PCR we evaluated TPK1 mRNA levels in wild type and mip6Δ strains. TPK1 mRNA levels increase in a mip6Δ strain both in non-stress and thermal stress conditions. Moreover, Tpk1 protein levels are lower in a mip6Δ strain than in a wild type strain, both under non-stress and thermal stress conditions. This results indicate that Mip6 may play a role both in regulation of TPK1 mRNA levels and mRNA export. Since RBPs are known to participate in several steps of gene expression, we wondered whether Mip6 is recruited to TPK1 promoter. Our Chromatin immunoprecipitation assay (ChIP) revealed that Mip6 is recruited to TPK1 promoter under non-stress and heat stress conditions. Overall, we conclude that Mip6 is involved in TPK1 expression regulation both under non-stress and stress conditions.

Keywords: Expression regulation, RBPs, PKA, Saccharomyces cerevisiae

Methods: ChIP, qRT-PCR, Western blot

ST-11

Inositol polyphosphate pathway regulates TPK1 expression during heat stress

Jaraslasky, Iván; Gareis, Daniela Belén; Ortolá Martínez, María Clara; Rossi, Silvia and Galello, Fiorella
Universidad de Buenos Aires. Facultad de Ciencias Exactas y Naturales. Departamento de Química Biológica. Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales-Consejo Nacional de Investigaciones Científicas y Técnicas (IQUIBICEN-CONICET). Buenos Aires, Argentina.
fiorellagalello@gmail.com

Yeasts are constantly challenged by environmental conditions and stressors. Saccharomyces cerevisiae uses different cellular mechanisms to adapt rapidly to these changes. One of them is the reorganization of expression patterns, where chromatin remodeling is crucial. It has been demonstrated that the inositol polyphosphate pathway plays a major role in the stress response and modulates the functions of several chromatin remodeling complexes. Protein kinase A (PKA) from S. cerevisiae controls a wide variety of biological processes and cellular functions in response to environmental changes. Yeast PKA is composed by two catalytic subunits encoded by TPK1, TPK2 and TPK3 genes, and a dimer of regulatory subunits encoded by BCY1 gene. Antecedents from our laboratory indicate that upon heat stress only TPK1 promoter activity is upregulated and TPK1 mRNA levels increase. TPK1 promoter has three positioned nucleosomes that are evicted during stress. This chromatin remodelling is SWI/SNF dependent. The purpose of this work is to obtain further knowledge in regards to the role of the inositol polyphosphate pathway on the expression regulation of yeast PKA subunits. Firstly, using β-galactosidase assays and qRT-PCR, we evaluated TPKs and BCY1 promoter activities and mRNA levels in null mutant strains for the inositol polyphosphate pathway. TPK1 promoter activity and mRNA levels decrease in the plc1Δ, ipk2Δ and kcs1Δ strains. The TPK2, TPK3 and BCY1 promoters activities present no changes in the null mutant strains evaluated, indicating that the effect of the inositol polyphosphate pathway is specific of TPK1 promoter. MNase protection assay shows that while plc1Δ and ipk2Δ strains exhibit alterations in chromatin remodelling of the TPK1 promoter during heat stress, the ipk1Δ strain does not. Lastly, we investigated the recruitment of SWI/SNF complex to TPK1 promoter in the SNF2-TAP ipk2Δ strain using ChIP assay. The absence of Ipk2 impairs the recruitment of SNF2-TAP to TPK1 promoter under heat stress conditions. Taken together, these results indicate that the second messenger PP-IP4, whose synthesis depends on enzymes Plc1, Ipk2 and Kcs1, is involved in the transcriptional regulation TPK1 during heat stress.

Keywords: Chromatin remodeling, inositol phosphates, transcriptional regulation, PKA, Saccharomyces cerevisiae

Methods: Reporter genes, MNase assay, ChIP, qRT-PCR

Microbiology

MI-01

IDENTIFICATION AND CHARACTERIZATION OF *Stemphylium* SPECIES THAT CAUSE GRAY SPOT ON TOMATO LEAVES IN URUGUAY

González, Ana Clara[1]; Rubio, Leticia [2]; Mena, Eilyn[1]; Delgado-Cerrone, Leonardo [1]; Arruabarrena, Ana [2]; Gonzáles-Arcos, Matías [2]; Ponce de León, Inés[1]

.

[1]Departamento de Biología Molecular, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay; [2]Instituto Nacional de Investigación Agropecuaria- INIA Salto Grande, Uruguay anaclgonzalez92@gmail.com

Fungi of the genus *Stemphylium* cause gray leaf spot of tomato, a destructive disease responsible for large production losses in susceptible varieties. Based on this, as an initial objective we propose to identify the *Stemphylium* species associated with the gray spot of the tomato leaf in Uruguay and to evaluate the genetic diversity and aggressiveness of the isolates. We currently have 36 isolates from tomato leaves with symptoms from different departments of the country, including Salto, Artigas, Montevideo and Canelones. By amplifying the spacer region of the internal transcript (ITS) and glyceraldehyde-3-phosphate dehydrogenase (*gpd*) and subsequent sequencing and comparison with sequences from different *Stemphylium* deposited at the NCBI, we were able to determine that 3 isolates correspond to *S. vesicarium* and the remaining 33 to the species *S. lycopercisi*, turning out to be the predominant one in the Uruguayan territory. In turn, we performed amplifications with ISSRs (inter simple sequence repeat) markers to assess the genetic diversity of the 33 *S. lycopercisi* isolates, obtaining a dendrogram that separated the isolates into 3 groups.

A morphological characterization of the isolates was carried out and the growth rate was analyzed, obtaining a great diversity within *S. lycopercisi* with different colorations of the colonies ranging from black or dark gray to yellow or white. The 3 isolates of *S. vesicarium* were very similar in appearance, presenting an olive gray color with velvety mycelium. Differences were also found in the growth rate of the isolates, from 2.11 cm to 3.6 cm of growth halo after 7 days. Through a microscopic analysis of the conidia produced by each isolate, we observed a morphological difference between both species, with the conidia of *S. lycopercisi* being thinner and more elongated, while those of *S. vesicarium* more compact.

At the same time, the infection tests were set up both in the plant and in the detached leaf. In the tests on detached leaves of susceptible tomato plants, the area with symptoms was calculated using representative fungi of the three groups obtained in the dendrogram. The results showed that *S. lycopercisi* isolates UYSLS28, UYSLS29 and UYSLS32 are highly virulent, whereas UYSLS20 is significantly

less virulent. The highly virulent isolates and the less virulent isolate belong to different groups and are far apart on the dendrogram. In addition, the most virulent isolates turned out to be those with the fastest growth in PDA medium. From the infection tests, leaf samples were taken at different times (days post inoculation; dpi), which were stained with solofenil to see the spores and hyphae of the fungus, and with propidium iodide, in order to see the cellular structure of the fungus. With these images, we were able to observe the germination of the spores at 1 dpi, the growth of the initial hyphae at 2 dpi, and their entry through stomata at 3 dpi. We were even able to observe at 5 dpi the thickening of the cell wall, characteristic of the defense response of the plant. These trials showed that the mycelium of the UYSLS32 fungus colonizes the plant tissue much more than that of the UYSLS20 fungus, which correlates with its level of virulence.
Finally, the expression of *S. lycopersici* genes related to pathogenicity will be evaluated during the infection of tomato leaves by means of qRT-PCR. This will allow us to generate information on the molecular basis of virulence mechanisms in different isolates.

Keywords: *Stemphylium*; tomato; Uruguay

Methods: infection assays, calculation of infection area, phylogenetic tree, ISSR analysis, confocal microscopy

MI-02

XER SITE-SPECIFIC RECOMBINATION AND IS26 MEDIATED PLASMID REARRANGEMENTS IN *Acinetobacter baumannii*: IMPLICATIONS FOR RESISTANCE GENE ACQUISITION AND EVOLUTION

Giacone, Lucía; Viale, Alejandro; Moran-Barrio, Jorgelina
Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR), Rosario.
giacone@ibr-conicet.gov.ar

Acinetobacter baumannii (Ab) represents a major cause of healthcare-associated infections generally affecting immunocompromised and severely injured patients, with the global spread of a number of epidemic clonal complexes (CC) displaying multidrug-resistance (MDR) phenotypes including resistance to last-resort carbapenems (carbR). The most frequent cause of carbR in Ab relies on acquired OXA-type carbapenemases, with the respective *blaOXA* genes carried by plasmids. Thus, a detailed characterization of MDR Ab plasmids is essential for understanding the evolution and dissemination of these resistance structures.
We characterized a number of epidemiologically-related MDR Ab strains belonging to the CC15 prevalent in our geographical region. Two carbR strains (Ab242 and Ab825) housed different iteron plasmids carrying a *blaOXA-58* and *TnaphA6*-containing resistance module (RM) conferring resistance to carbapenems and aminoglycosides, respectively. This RM is bordered by 28-bp sequences recognized by the XerC and XerD tyrosine recombinases (pXerC/D-like sites), suggesting functions of

this site-specific recombination (SSR) system in their horizontal mobilization. In Ab825, the RM is present in a 36-kbp multireplicon (pAb825_36) composed of a 27 kbp bireplicon domain (pAb825_27) and a 9-kbp monoreplicon domain (pAb825_9). Interestingly, we found a 7 kbp plasmid (pAb244_7) sharing homology to pAb825_9 in a local carbS strain, Ab244, isolated two years earlier than Ab242 and Ab825[1,2]. Here, we propose an evolutionary pathway for the genesis of Ab resistance plasmids that involves both Xer-dependent SSR and IS26-mediated mechanisms based on bioinformatics analysis and experimental evidence.
BlastN searches of the GenBank database using Ab825 plasmid queries retrieved a series of pAb825_9-like plasmids carried by CC15 Ab strains isolated in Argentina and Chile. These plasmids, including pAb244_7, shared with pAb825_9 the replication and mobilization backbone[2]. We found in Ab825_9-like plasmids a DNA region containing genes for a toxin-antitoxin system, bordered by directly oriented pXerC/D sites. The identification of a SSR hybrid site in pAb244_7 indicates that a XerC/D-mediated event contributed to the acquisition of this stability module, and our experimental evidence supports this hypothesis. In addition, pAb244_7 contained an IS26 element, which was also part of a composite transposon carrying blaTEM and *aacC2* resistance genes present in some pAb825_9-like plasmids. Our results underscore a pivotal role for Xer-SSR and IS26-mediated recombinatorial events in Ab plasmid evolution, aiding in the dissemination of resistance genes and a rapid adaptation of the bacterial host(s) to highly dynamic environments.
[1]Giacone *et al.* 2023 Front. Microbiol., 9(14). doi:10.3389/fmicb.2023.1057608
[2]Cameranesi *et al.* 2020 Microb. Genom., 6(9). doi:10.1099/mgen.0.000360

Keywords: Carbapenem resistance, Recombination, Plasmid evolution, *Acinetobacter baumannii*

Methods: PCR, DNA Sequencing, Bioinformatics, Microbiology analysis

MI-03

CHLAMYDIA TRACHOMATIS POLYMORPHIC MEMBRANE PROTEIN C PARTICIPATES IN DEVELOPMENTAL TRANSITIONS DURING BACTERIAL RECOVERY FROM INTERFERON GAMMA AND PENICILLIN-INDUCED PERSISTENCE

Anna, Ailen Nair; Panzetta, María Emilia; Bettucci Ferrero, Gloria Nazarena; Veraguas, Sofía; Saka, Héctor Alex.
Departamento de Bioquímica Clínica, CIBICI-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina.
nazarena.bettucci@unc.edu.ar

Chlamydia trachomatis (CT) is the most prevalent etiologic agent of bacterial sexually transmitted infections worldwide. CT infection is a frequent cause of asymptomatic and persistent infections, especially in young women, leading to severe complications like pelvic inflammatory disease, infertility, and ectopic pregnancy. CT is an obligate intracellular pathogen with a unique lifestyle involving two developmental forms, the infectious elementary body (EB) and the replicative but non-infectious

reticulate body (RB). EBs first adhere to epithelial cells and then get confined within an intracellular vacuole or "inclusion", to rapidly transition into replicative RBs. Around mid-cycle, RBs transition back to EBs and bacteria are released to the extracellular space to reinitiate the infectious cycle. When exposed to stressors such as gamma-interferon (INFγ) or penicillin antibiotics, CT halts replication and enters a viable but non-cultivable state called chlamydial persistence. Upon removal of stressors, CT resumes replication. Previous results from our group identified that members of a *Chlamydia*-specific family of autotransporter proteins called Polymorphic Membrane Proteins (Pmps), are implicated in persistence. A *pmpC*-null mutant (L2 *pmpC*::*GII*) confirmed that PmpC is required for CT recovery from INFγ- and penicillin-induced persistence. To assess the mechanistic basis of impaired recovery, we carried out electron microscopy analysis of CT-infected cells and evaluated the content of developmental forms. No differences were found between L2 *pmpC*::*GII* compared to L2 wild type strain in the untreated condition. However, during recovery from both, IFNγ- and penicillin-induced persistence, L2 *pmpC*::*GII* inclusions show fewer EBs and a reduced EB/RB ratio. This indicates that lack of PmpC negatively affects RB to EB transition, which in turn explains impaired generation of infectious progeny upon recovery. To further investigate the role of PmpC in CT persistence and pathogenesis, two different PmpC fragments were expressed in *Escherichia coli*, tag-purified, and used to generate PmpC polyclonal antibodies, which were then successfully used in immunoblots and immunofluorescence assays. PmpC was found to be expressed throughout mid to late stages of the CT life cycle. Interestingly, PmpC-specific fluorescent signal was found partially not co-localizing with the bacteria body both in untreated and recovery conditions. Moreover, fractionation assays of CT-infected HeLa cells carried out to separate bacterial (pellet) and host cells (supernatant) showed that PmpC was detected in both fractions. Finally, by using the two previously mentioned anti-PmpC antibodies, we found evidence of post-translational cleavage between Ser-564 and Gly-596. Overall, these findings indicate that PmpC is proteolytically processed, secreted to the lumen of inclusions and required for efficient RB to EB transition during recovery from INFγ- and penicillin-induced persistence.

Keywords: *Chlamydia trachomatis*, *Chlamydia* persistence, Interferon-gamma, penicillin, bacterial pathogenesis

Methods: Cell culture, Bacterial culture, electron microscopy, immunofluorescence microscopy, immunoblotting

MI-04

INTEGRITY OF THE PREDICTED PROTEASE MOTIF OF CTL0175/PTR IS REQUIRED FOR *Chlamydia trachomatis* RECOVERY FROM IFNγ-INDUCED PERSISTENCE

Bettucci Ferrero, Gloria Nazarena; Panzetta, María Emilia; Veraguas Herran, Sofía; Anna, Ailen Nair; Saka, Héctor Alex
Departamento de Bioquímica Clínica, CIBICI-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina.
nazarena.bettucci@unc.edu.ar

Chlamydia trachomatis (CT) is the most frequent sexually transmitted bacterial pathogen and a common cause of asymptomatic, persistent infections leading to serious complications and negative outcomes in young women's reproductive health. CT displays an obligate intracellular lifestyle involving the infectious elementary body (EB) and the replicative and non-infectious reticulate body (RB). Infection begins when EBs attach to epithelial cells and get internalized within a membrane-bound intracellular vacuole or "inclusion". At early times post-infection, EBs transition into metabolically active and dividing RBs. At mid-cycle, upon impending release of the bacteria to the extracellular environment, RBs asynchronously transition back to EBs. If during replication CT is exposed to antimicrobial stimuli such as those triggered by the host cytokine gamma-interferon (IFNγ), these bacteria undergo a transient interruption in its replication cycle and enter into a viable but non-cultivable, "persistent" state, which can last for long periods of time. Upon removal of the stressor, CT is able to resume normal replication and developmental transitions to complete the interrupted cycle. The ability to undergo persistence is considered critical for CT pathogenesis, however, little is known about the molecular basis of this phenomenon. Previous findings from our laboratory demonstrated that the uncharacterized locus *ctl0175/ptr*, is required for RB to EB transition and recovery from IFNγ-induced persistence. Bioinformatic analysis indicates that *ctl0175/ptr* encodes a 108 KDa protein showing 23% homology with *Escherichia coli* zinc-dependent protease Ptr, whose protease activity has been linked to the presence of the conserved motif HXXEH in the N-terminal region of the predicted protein. Interestingly, CT Ptr contains the protease conserved motif HX(F)X(T)EH. To assess if Ptr protease activity may be involved in CT recovery from IFNγ-induced persistence, we generated an E108Q point mutant version of Ptr (*ptr*E108Q) by site-directed PCR mutagenesis on a CT expression plasmid encoding full length wild type ptr (*ptr*FLAG). Next, we transformed these plasmids into a *ctl0175/ptr* null CT serovar LGV-L2 background (L2 *ptr::GII*), obtaining L2 *ptr::GII*-PtrFLAG and L2 *ptr::GII*-PtrE108Q complemented strains. Using a self-generated anti-Ptr antibody, we verified that L2 wild type and complemented strains were able to express Ptr. Then, we carried out IFNγ-induced persistence experiments using the mentioned strains and quantified the generation of infectious progeny upon removal of IFNγ (recovery assay) by means of inclusion-forming units quantification. Interestingly, we found that while *ptrFLAG* rescued L2 *ptr::GII* defective recovery, *ptr*E108Q did not. Overall, our findings suggest that protease activity of Ptr mediates rapid recovery of CT upon IFNγ-induced persistence and contribute to elucidate Ptr's role in CT persistence and pathogenesis.

Keywords: *Chlamydia trachomatis*, *Chlamydia* persistence, Interferon gamma, *Chlamydia trachomatis* pathogenesis

Methods: molecular cloning, site directed mutagenesis, cell and bacterial culture, immunofluorescence microscopy, bacterial transformation

MI-05

BIOPHYSICAL AND FUNCTIONAL STUDIES OF MICROCIN V DOMAINS USING SUICIDE PROBES

Pintanel, J; Ríos Colombo, N. S; Bellomio, A; Chalón, M.C.
Instituto Superior de Investigaciones Biológicas (INSIBIO, CONICET-UNT) e Instituto de Química Biológica "Dr. Bernabé Bloj," Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Chacabuco 461, San Miguel de Tucumán T4000ILI, Argentina.
milichalon053@gmail.com

Bacteriocins are small peptides of ribosomal synthesis, which have antimicrobial activity against phylogenetically related bacteria. Microcin V (MccV) is a linear peptide of 88 amino acids; it kills sensitive cells by acting at the level of the internal membrane through the dissipation of the membrane potential. Suicide probes consist of the fusion of a single-pass membrane protein called EtpM with a bacteriocin at the C-terminus. They have a toxic effect on the bacteria that express them. It is proposed to construct truncated versions of the EtpM-MccV suicide probes that include the different domains of the peptide, to elucidate *in vivo* the role of each of them in the mechanism of MccV toxicity. By using computer software and genetic engineering techniques, truncated suicide probes were constructed from the chimeric protein. Viability tests were performed to evaluate their toxicity once they were expressed in *Escherichia coli* strains by adding arabinose. Fluorescence spectroscopy using DiSC3(5) probe were studied to determine changes in membrane potential. Two truncated suicide probes were constructed and through viability assays we observed that, under the conditions used, the proteins turned out to be non-toxic for the *E. coli* strains that expressed them. On the other hand, when subjecting the cells to the study with the fluorescent molecule, we saw that one of the constructions dissipates the membrane potential (the protein that has a transmembrane portion) but the other (which does not have this portion) does not. Our results confirmed that using suicide probes it is possible to detect changes in membrane permeability even though the effect is not sufficient to produce cell death.

Keywords: suicides probes, bacteriocin, ETPM

Methods: cloning, fluorescence, viability, bioinformatics

MI-06

SlpE METALLOPROTEASE OF *Serratia marcescens* IS REGULATED BY IRON AND IS EXPRESSED WITHIN EPITHELIAL CELLS

Cura Michelino, David A.; García Véscovi, Eleonora; Mariscotti, Javier F.
Instituto de Biología Molecular y Celular de Rosario – CONICET-UNR.
mariscotti@ibr-conicet.gov.ar

Serratia marcescens is an opportunistic human pathogen and is widely distributed in the environment and a broad range of host organisms. In humans, it causes a variety of infections with increasing incidence, primarily due to the acquisition of antibiotic resistance mechanisms, the ability to survive for months on inanimate surfaces, and resistance to conventional disinfection procedures. Despite its clinical prevalence, the factors and mechanisms contributing to *Serratia* pathogenicity remain unclear. *S. marcescens* ability to adapt and survive in hostile or changing environments is related to the bacterial capacity to express a wide range of secreted enzymes, including chitinases, phospholipase, hemolysin, nuclease, and proteases, including the metalloprotease SlpE produced in our clinical isolate. Recent studies showed that SlpE is secreted by the LipBCD transporter, highlighting this enzyme as an important virulence factor in cell line cultures. SlpE is found in our clinical isolate RM66262 and in most clinical isolates, but it is absent in most non-clinical isolates, including the reference strain Db11 of *S. marcescens*. However, little is known about the environmental signals and regulatory factors that modulate its production. In this study, we evaluated the regulation of the metalloprotease SlpE using a GFP-containing reporter plasmid. The results showed that SlpE expression is induced during the stationary growth phase, although its expression levels are five times lower than the major protease PrtA at 30ºC. One defense mechanism of vertebrate hosts against bacterial infection is nutrient deprivation to prevent bacterial growth, a process known as nutritional immunity. The most significant form of nutritional immunity is iron sequestration. We found that under iron-depleted conditions, P*slpE-gfp* transcription levels are five times higher than in iron-rich medium, reaching levels equivalent to *prtA* expression at 30ºC. Furthermore, our results suggest that this increase in SlpE expression levels depends on the ferric uptake regulator (Fur). Here, we also analyzed the expression of reporter plasmids through immunofluorescence and flow cytometry, demonstrating that *S. marcescens* induces the intracellular expression of SlpE during the invasion of epithelial cells. Additionally, we observed that this induction is partially reversed upon supplementing the medium of the epithelial cells with iron. These findings suggest that *S. marcescens* SlpE is involved in proteolytic activity under iron-limiting conditions within the intracellular host niche.

Keywords: *Serratia marcescens*, metalloprotease, iron, epithelial cells

Methods: reporter plasmid, Quick Change, immunofluorescence, flow cytometry

MI-07

FUNCTIONAL CHARACTERIZATION OF aphA6 GENE FROM A LOCAL *Acinetobacter bereziniae* CLINICAL ISOLATE

Morales Flores, Ailén; Limansky, Adriana; Marchiaro, Patricia; Morán-Barrio, Jorgelina
Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR), Rosario
moralesflores@ibr-conicet.gov.ar

Acinetobacter is an aerobic Gram-negative genus ubiquitous in nature. Some species are opportunistic human pathogens, being *A. baumannii* (*Ab*) the most commonly isolated from clinical specimens. However, other species have been frequently associated with nosocomial infections including *A. nosocomialis*, *A. lwoffii*, *A. pittii* and less frequently, *A. bereziniae*[1]. The carbapenem resistant *A. bereziniae* HPC229 local isolate characterized in our group[1,2] carries pNDM229 plasmid harboring bla_{NDM-1} and *aphA6* resistance genes, responsible of carbapenem (e.g. imipenem, IMP) and aminoglycosides (AG) resistance, respectively. In turn, HPC229 showed resistance towards amikacin (AKNR) but sensitivity towards gentamicin (GENS). The ISA*ba14*-*aphA6*-Tn*125* structure observed in pHPC2291 is widely distributed in carbapenem resistant *Acinetobacter* strains, and is mostly associated to AKNS due to a lack of promoter upstream *aphA6*[3]. However, it has been reported that the recombinant production of *aphA6* from its native promoter resulted in 4-fold increases in MIC against AKN in *A. baumannii* strains[4].
The aim of this work is to characterize the potential functionality of the *aphA6* gene in the *A. bereziniae* HPC229 local strain, taking into account their activity against AG, through the mobilization of this gene from HPC229 to different *Acinetobacter* strains by horizontal gene transfer (HGT). Thus, *A. nosocomialis* M2 (An M2) and Ab ATCC 17978 sensitive strains were transformed employing the total HPC229 purified plasmids (pHPC229), and further selected for AKNR. Also, the genetic context of *aphA6* was analyzed by PCR and sequencing.
These assays resulted in An M2/pHPC229 and Ab17978/pHPC229 transformant strains displaying AKNR, GENR and IMPS by disc diffusion method. The methods used confirmed the presence of the ISA*ba14*-*aphA6* region in M2/pHPC229 strain, which encompasses a 746-bp DNA sequence including 550 bp from the 3' end of the ISA*ba14*, 65 bp from the 5' end of the *aphA6*, and 41 bp of intergenic region. This region showed 99% nucleotide identity with the sequence described in the above mentioned structure. In turn, this fragment was not detected in Ab17978/pHPC229 strain, suggesting a different genetic context of *aphA6* and/or other gene(s) responsible for AG resistance among other hypothesis. Overall results reveal not only the mobilization of this resistance gene, but also its functionality. Notably, the GENR phenotype observed in both transformants uncovers a broader substrate spectrum of the corresponding protein, APH(3´), in the new hosts. Alternatively, the resistance to both AG could be due to the transfer of other unidentified resistance gene(s) in HPC229 by ResFINDER. Altogether, our results show that the AG resistance exhibited by HPC229 is susceptible to HGT dissemination confirming the plasmid localization of the corresponding gene(s). In addition, the gene(s) transferred by HGT not only confer resistance to AKN but also GEN resistance in An M2 and Ab 17978 transformant strains, suggesting a differential display of the AG resistance in different hosts. Further characterization of M2/pHPC229 and Ab17978/pHPC229 plasmids will allow us to underscore the AG resistance regulation in clinical *Acinetobacter* strains.

[1]Brovedan M et al. (2015) doi: 10.1128/AAC.00367-15
[2]Brovedan M et al. (2019) doi.org/10.1371/journal.pone.0220584

[3]Hu H et al. (2012) doi: 10.1128/AAC.06199-11
[4]Yoon E et al. (2014) doi:10.1128/mBio.01972-14

Keywords: Carbapenemes, *Acinetobacter*, aminoglycoside, dissemination

Methods: PCR, sequencing, microbiological testing

MI-08

EVALUATING MinION SEQUENCING FOR GENOTYPING *Trypanosoma cruzi* THROUGH AMPLIFICATION OF THE MINICIRCLE HYPERVARIABLE REGIONS

Díaz, Anahí Guadalupe; Rusman, Fanny; Floridia-Yapur, Noelia; Ponce, Tatiana; Acuña, Leonardo; Diosque, Patricio; Tomasini, Nicolás
Instituto de Patología Experimental
anahi1990gd@gmail.com

Trypanosoma cruzi, the causative agent responsible for Chagas disease, shows notable genetic diversity. Such diversity is classified into six Discrete Typing Units (DTUs), TcI to TcVI. The mitochondrial DNA in this parasite comprises thousands of minicircles each with four minicircle Hypervariable regions (mHVR). The mHVRs have been employed for *T. cruzi* detection and for strain and DTU identification. Although massive amplicon sequencing of mHVR using Illumina sequencers has been previously suggested, their limited accessibility in Argentine endemic areas makes alternatives desirable. Oxford Nanopore Technologies (ONT), particularly the MinION device, have emerged as an attractive platform for laboratories due to its low cost, accessibility and rapid turnaround time despite the higher base-calling error rate. In this study, we evaluated the usefulness of MinION sequencing for typing *T. cruzi* DTUs. To achieve this, we built mHVR libraries for strains previously studied using Illumina platform and sequenced them on a MinION Mk1c device. Sequencing procedures followed ONT instructions, and a custom script was developed for data analysis in the Google Colab platform. Sequencing by MinION successfully genotyped the studied strains. Moreover, each strain best correlated on mHVR cluster abundances with the same strain studied using Illumina sequencing. The results demonstrate the feasibility of typing *T. cruzi* strain using MinION and suggest the potential usefulness of this scheme in biological samples.

Keywords: Chagas disease, *Trypanosoma cruzi*, mHVR, Illumina, MinION

Methods: Illumin MiSeq sequencing, nanopore sequencing

MI-09

EXPLORING THE ROLE OF *Bacillus subtilis* NCIB 3610 LIPID COMPOSITION IN BIOFILM FORMATION

Magliotti, Antonela; Serra Diego O; Altabe, Silvia G.
Instituto de Biología Molecular y Celular de Rosario (IBR), UNR-CONICET
altabe@ibr-conicet.gov.ar

Bacterial biofilms are widespread in natural environments and play crucial roles in various clinical, industrial, and ecological contexts. While we have extensive knowledge about biofilm formation in model bacteria like *Bacillus subtilis*, there remains a significant gap in our understanding regarding the role of lipids in this intricate developmental process. As membrane lipid adaptation is a vital aspect of bacterial response when cells are subjected to harsh or unstable conditions, we investigated how the lipid composition is adjusted when *B. subtilis* NCIB 3610 grows in biofilms compared to its planktonic mode of growth. We established optimal conditions to cultivate robust biofilms at the air-liquid interface (pellicle) as well as to obtain biomass available for fatty acid (FA) analyses. Cells were grown in six-well plates in a defined medium (SSgg) at 25°C for three days, pellicles were removed (biofilm), and the cell suspension in the well was collected (planktonic cells). FAs from samples of both pellicle and planktonic cells were extracted, converted to methyl esters (FAME), and analyzed by gas chromatography/mass spectrometry (GC-MS). Membrane FAs of *B. subtilis* are characterized by a high quantity of iso and anteiso-branched chain fatty acids (BCFA) with 15 and 17 carbon atoms, a lower proportion of straight chain of saturated FA (SSFA) with 16 and 18 carbon atoms, and a small proportion of unsaturated FAs (UFAs). One of the most notable differences we have identified is a more than ten-fold increase in the percentage of SSFA, accompanied by a significant decrease in anteiso-BCFA, in the planktonic cells. These changes in FA compositions might indicate a more rigid membrane structure in planktonic cells compared to biofilm cells. However, although UFAs are present in low proportions in both forms, they are elevated in the planktonic cells, probably reflecting an adaptive change to restore the membrane fluidity. Furthermore, we conducted a comparative analysis at both macro- and microscopic levels to examine the morphology of agar-grown microcolony biofilms of *B. subtilis* and its derivative *ugtp* mutant, which is deficient in the UDP-diacylglycerol transferase and therefore impaired in the glucolipid biosynthesis. This analysis revealed that macrocolonies of the *ugtp* mutant had a smaller diameter but a thicker cross-section and exhibited significantly fewer wrinkles than wild-type macrocolonies. Since wrinkles serve as clear indicators of structural consolidation in biofilms, these results suggest that changes in glucolipid composition strongly affect biofilm morphogenesis. This may be attributed to changes in the secretion of extracellular matrix components, which could also account for the reduced horizontal expansion of the colony. In summary, these findings underscore the critical connection between membrane lipid composition and biofilm formation, providing valuable insights for potential advancements in biofilm control strategies.

Keywords: *Bacillus subtilis*, biofilms, fatty acids, lipids

Methods: Lipid analysis, Gas chromatography-mass spectrometry, microscopy, mutagenesis

MI-10

Schinus areira ESSENTIAL OIL: FROM ANTIBACTERIAL CHARACTERIZATION TO THE DEVELOPMENT OF NANOFORMULATIONS

Cutró Andrea C.[1,2], Ferreyra Maillard Anike P.V.[1], Bordon Anahí[1], Coria M. Sumampa[3,4], Rodriguez Sergio A.[3]; Hollmann Axel [1,5]*

[1]Laboratorio de Compuestos Bioactivos, CIBAAL CONICET –UNSE; [2]Facultad de Ciencias Médicas UNSE. [3]Facultad de Agronomía y Agroindustrias UNSE; [4]INBIONATEC-CONICET-UNSE; [5]Laboratorio de Microbiología Molecular, UNQ

cutro.andrea@gmail.com

Essential oil (EO) obtained from *Schinus areira* leaves has been shown to have antibacterial activity against *S. aureus* in our previous research. This activity was accompanied by significant changes in the electrical potential of the cell surface and bacterial membrane model. The main goal of the present research is to get a deeper understanding of the mechanisms underlying the antibacterial effects previously observed. In this sense, through fluorescence microscopy, the integrity of *S. aureus* cells was evaluated as a function of EO concentration and incubation time. It could be noticed a significant increase in cell permeability, which showed dose and time dependence. Additionally, by fluorescence spectroscopy and dynamic light scattering studies using DMPC:DMPG (5:1) liposomes, it was possible to evaluate the alterations that EO induces in the lipid membranes. According to these studies, the EO caused changes in the lipid membrane packing, improving fluidity, reducing cohesive interactions between phospholipids, and making it easier for water to access the interior of the lipid bilayer. All these alterations result in an increase on the permeability of the liposomes in good agreement with *S. aureus* experiments. Afterwards, potential intracellular targets for the EO, as genomic DNA and oxidative metabolism were investigated. These experiments showed that *S. areira* EO partially degraded DNA and brought on oxidative stress. Finally, in order to achieve practical applications such as hydrosoluble nanoformulations, green silver nanoparticles were synthesized by using the EO as a biocomposite for reduction and stabilization. The nanoparticles obtained showed antimicrobial activity. All these findings highlight the multifaceted antibacterial action of this EO and its potential applications as an antimicrobial obtained from a locally significant natural resource.

Keywords: *Schinus areira*, antibacterial, mechanisms, nanoformulations.

Methods: Electrophoresis, fluorescence microscopy, fluorescen espectroscopy, Dinamic Light Scattering, Microbiology techniques

MI-11

OBTAINING AND ANTIMICROBIAL CHARACTERIZATION OF EXTRACTS FROM TUSCA OR *Vachellia aroma* LOCATED IN SANTIAGO DEL ESTERO

Castelli M.V.[1], Carreras R.[2], Paez K.3, Depompa S.[2], Hollmann A.[4], López S.N.[1], Cutro A.C.[3,4]
[1]Facultad de Ciencias Bioquímicas y Farmacéuticas UNR, CONICET, Rosario, Argentina; [2]Facultad de Ciencias Forestales UNSE, Santiago del Estero, Argentina; [3]Facultad de Ciencias Médicas UNSE, Santiago del Estero, Argentina; [4]Centro de Investigación en Biofísica Aplicada y Alimentos (CIBAAL)UNSE-CONICET, Santiago del Estero CP 4200, Argentina.
cutro.andrea@gmail.com

Medicinal plants are still used in traditional and alternative medicine. They contain bioactive secondary metabolites such as alkaloids, flavonoids, steroids, terpenes, sesquiterpenes, diterpenes, phenolics and saponins. These substances are crucial in helping plants adjust to their changing environment and biotic and abiotic stressors. In this sense, plants represent a potential natural source of chemicals with pharmacological value, including potential antimicrobial agents. In the latter situation, they represent an alternative to traditional antibiotics for the treatment of clinical infections with pathogenic bacteria, resistant bacteria, multi-resistant bacteria and resistant forms such as biofilms that represent a serious health problem worldwide. In this context, *Vachellia aroma* (former name *Acacia aroma* Gill ex Hook. & Arn.) also known as Tusca, is a small and robust tree with alternate leaves, yellowish flowers whose fruit is made up of a legume found in the northwest, Cuyo and northeast regions of Argentina. The infusions and juices of this plant were reportedly used as an antiseptic, among other things. In this sense, previous *in vitro* studies have reported the antibacterial activity of its extracts. In this framework, ethanolic extracts were obtained from the leaves and flowers of different specimens located in Santiago del Estero, with a yield of 5–10%. Chemical profiles were determined by thin layer chromatography; as well as its antibacterial activity against Gram positive and Gram negative model bacteria. Bioautography assays of said flower and leaf extracts have shown chromatographic bands with antibacterial activity against *Staphylococcus aureus* and not against *Escherichia coli*. In addition, the minimum inhibitory and bactericidal concentrations (MIC and MBC) against *S. aureus* were determined. Most of the leaf extracts showed a MIC value of 2 mg/mL; except for one, which showed an MIC of 0.5 mg/mL and a MBC of 2 mg/mL. The latter was also able to inhibit the development of *S. aureus* biofilm from 72 h of formation at a sub-MIC concentration (0.25 mg/mL). However, the chemical profile of the leaf extracts were very similar to each other under the conditions evaluated. This would suggest that the variation in activity observed is probably due to different relative abundances of the main metabolites present. On the other hand, all of the flower extracts obtained from the different specimens have been shown to have a MIC value of 1 mg/mL. The findings suggest that the extracts obtained from Tusca or *V. aroma* located in Santiago del Estero represent a potential source of antimicrobial compounds, and further research into this matter is required.

Keywords: Tusca, *Vachellia aroma*, biofilm, antibacterial, extracts
Methods: bioathography, tlc chromatography, microbiology techniques, absorbance spectroscopy, solvent plant extraction

MI-12

SOLVING THE PUZZLE OF COPPER METABOLISM IN *Trypanosoma cruzi*

Merli Marcelo L., Mediavilla María G., Acosta-Oviedo Héctor, Cricco Julia A.
Instituto de Biología Molecular y Celular de Rosario (IBR), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)— Universidad Nacional de Rosario (UNR), Rosario, Argentina.
cricco@ibr-conicet.gov.ar

Trypanosoma cruzi is the Chagas disease agent, the most prevalent parasitic disease in many countries of America. This parasite has a complex life cycle, which involves a mammalian host and a triatomine insect vector. Along its life cycle, it acquires copper from the different hosts it encounters through the action of many importers and chaperons, first at the plasma membrane, then within the cytosol and finally inside the organelles, especially in the mitochondria. Copper is an essential ion for organisms of all kingdoms, especially for aerobic organisms, but it is also a toxic ion. Free Cu ion can cause oxidative damage via the Fenton-like reaction, so its metabolism must be regulated. Despite its importance, Cu metabolism has not been studied in *T. cruzi*. In this work, we characterized the effect of changes in Cu ion availability along the life cycle of *T. cruzi*: at epimastigote, trypomastigote and intracellular amastigote stages. Stress conditions were obtained by the addition of copper sulfate or copper chelators, and intracellular copper content was verified by ICP-OES. Our results show that Cu is an essential ion for epimastigotes proliferation and metacyclogenesis process. In contrast, Cu caused a negative effect on intracellular amastigote replication. In this sense, Cu ions are part of defense mechanisms against intracellular pathogens used by mammalian cells. By bioinformatic methods, we identified several proteins that could be involved in Cu reduction, importation, and distribution across the organelles. Even so, we did not identify cytosolic chaperones for intracellular distribution. The expression of these genes was studied across the life cycle stages as well as their response to Cu concentration. We determined that these genes are overexpressed in the infective stages of the parasites and that some of them respond to copper availability. In conclusion, *T. cruzi* needs copper as a cofactor along its life cycle and at the same time needs to tolerate copper stress. With the data obtained, for these functions we propose that the transmembrane importer *Tc*IT at the plasmatic membrane and the transmembrane transporter *Tc*CuATPAse at Golgi complex are involved in copper uptake and distribution, and we present a model for Cu transportation and intracellular regulation.

Keywords: *Trypanosoma cruzi*, Chagas disease, copper, cupro-proteins, copper transport

Methods: qRT-PCR, cell culture, ICP-OES, Oxygen consumption

MI-13

*Tc*HRG PROTEIN CONTROLS HEME UPTAKE IN *Trypanosoma cruzi*

Tevere, Evelyn[1]; Chasen, Nathan Michael[2]; Etheridge, Ronald Drew[2] and Cricco, Julia Alejandra[1]
[1]Instituto de Biología Molecular y Celular de Rosario (IBR), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)- Universidad Nacional de Rosario (UNR), Rosario, Argentina.
[2]Department of Cellular Biology, Center for Tropical and Emerging Global Diseases (CTEGD), University of Georgia, Athens, Georgia, USA.
cricco@ibr-conicet.gov.ar

Trypanosoma cruzi is the causative agent of Chagas disease, which is a widespread parasitic disease in the American continent, mainly Latin America. This parasite undergoes a complex life cycle, which involves a mammalian host and a triatomine insect vector. *T. cruzi*, like other trypanosomatids that cause neglected diseases in humans such as *T. brucei* and *Leishmania* spp., are heme auxotroph and they must take up this essential cofactor from their hosts or vectors. In the triatomine midgut, hemoglobin (Hb) derived from the bloodmeal is subjected to proteolysis, leading to the release of the heme moiety. Therefore, *T. cruzi* epimastigotes in their natural habitat encounter both Hb-bound and free heme. It has been established that trans-membrane proteins belonging to the Heme Responsive Gene (HRG) family are involved in the transport of heme from the environment in trypanosomatids, among which is *Tc*HRG (*T. cruzi* HRG, previously named *Tc*HTE) in *T. cruzi*.

We have demonstrated that *T. cruzi* can control intracellular heme content (IHC) by modulating *Tc*HRG expression when a free heme source is added to axenic culture. On the other hand, Hb uptake via receptor-mediated endocytosis at the flagellar pocket (FP) was demonstrated in *T. brucei* and *Leishmania* spp., but endocytic phenomena through the FP have not been observed in *T. cruzi*. Instead, *T. cruzi* has a specialized organelle called cytostome-cytopharinx complex (SPC), which is involved in nutrient acquisition. In the epimastigote stage, endocytosed nutrients enter the cell through the cytostome, are transported via endolysosomal vesicles through the cytopharynx and are finally stored in reservosomes at the posterior end of the cell. The SPC and the reservosomes are absent in *T. brucei* and *Leishmania* spp.

We explored here the utilization of Hb as a heme source in epimastigotes and examined the role of *Tc*HRG in Hb-derived heme homeostasis by analyzing endogenous TcHRG expression (by qRT-PCR and Western blot) and intracellular localization (super resolution microscopy), the effect of the overexpression of *Tc*HRG, and the abolition of endocytosis when cultured in Hb-supplemented medium (growth profile, IHC).

We show that endogenous *Tc*HRG responded similarly to Hb as it did to free heme (added as hemin) at both the mRNA and protein level. Also, the intracellular heme content was increased in epimastigotes that overexpress recombinant *Tc*HRG and was not affected in endocytic-null parasites when Hb was used as a heme source. Besides, endogenous *Tc*HRG was localized in the flagellar pocket region and in the cytoplasm of cells partially overlapping with the mitochondrion, thus validating its role in heme homeostasis. Our results support an extended model for heme homeostasis in *T. cruzi* epimastigotes that includes both heme sources. We postulate that free heme obtained after extracellular Hb degradation is the main pathway for Hb-derived heme uptake in epimastigotes and it is enhanced and controlled by *Tc*HRG.

Keywords: *Trypanosoma cruzi*, Chagas disease, heme, hemoglobin, heme transport

Methods: Western blot, qTR-PCR, super resolution confocal microscopy

MI-14

CHARACTERIZATION OF c-di-GMP-MEDIATED REGULATION IN *Bordetella bronchiseptica* MOTILITY AND BIOFILM FORMATION

María Florencia Sechi, Valentina Arenas Gil, Malena Tapia, Valentina García, Valentina Giaccone, Federico Sisti, Julieta Fernández.
Instituto de Biotecnología y Biología Molecular CCT La Plata CONICET. Dto. Cs. Biológicas. Fac. Cs. Exactas. UNLP.
julieta@biol.unlp.edu.ar

Bordetella bronchiseptica is a Gram-negative pathogen responsible for the development of atrophic rhinitis in pigs. Among the different phenotypes contributing to pathogenesis and persistence in the host, motility and biofilm formation have been identified as crucial. We previously reported that both phenotypes are regulated by the second messenger c-di-GMP. The synthesis and degradation of c-di-GMP are controlled by diguanylate cyclases (DGCs) and phosphodiesterases (PDEs), respectively. Like most bacteria, the *B. bronchiseptica* genome exhibits diversity in potential DGCs and PDEs.
In this study, our aim was to characterize DGCs and the PDE PdeD of *B. bronchiseptica* 9.73H+ regarding their possible roles in regulating both phenotypes. Clean deletions were made in genes to generate the respective mutants. These mutants were evaluated for their ability to swim in agar plates and to form biofilm.
The overexpression of *pdeD* increased the motility of *B. bronchiseptica* as expected for an active PDE. The absence of the gene did not alter motility or biofilm formation. This suggests that PdeD may not participate in the regulation of these phenotypes under the tested conditions or that it does so redundantly with other PDEs, making it difficult to observe the effect by deleting *pdeD* alone.
PdeD has a putative periplasmic CSS domain, like the one described by Hengge´s group in 2018. CSS domain can respond to redox stress, and consequently activate the phosphodiesterase domain. We evaluated biofilm formation in the presence of dithiothreitol (DTT) to determine if PdeD activity was enhanced. We observed a reduction in biofilm formation in the presence of DTT, indicating an increase in PDE activity.
On the other hand, we evaluated BdcE, BdcG, and BdcJ as potential DGCs. We previously described that overexpression of these DGCs from a plasmid inhibited motility and increased biofilm formation, indicating its active DGC function. Deletion of these DGCs did not affect motility or biofilm formation. Considering that we had previously described that another DGC, BdcA, could also redundantly regulate both phenotypes with others DGCs, we constructed double mutant combinations. Combined *bdcA-bdcE* mutant exhibited reduced biofilm-forming capacity, demonstrating redundant regulatory activity by both DGCs. Surprisingly, absence of *bdcA* and *bdcE* reduced motility 10% compared to wild type.

In conclusion, we have made progress in characterizing the intricate regulatory network mediated by the second messenger c-di-GMP in *Bordetella*, impacting both motility and biofilm formation phenotypes.

Keywords: *Bordetella*, biofilm, motility, c-di-GMP

Methods: Biofilm crystal violet, soft agar motility.

MI-15

CHARACTERIZATION OF *Pseudomonas fluorescens* SF4c MUTANTS AFFECTED IN BACTERIOCIN PRODUCTION

Asconapé, J; Gambetta, C; López-Ramírez, V; Barrionuevo, L; Travaglia, C; Fischer, S
Instituto de Investigaciones Agrobiotecnológicas (INIAB), Universidad Nacional de Río Cuarto (UNRC)-CONICET
jasconape@exa.unrc.edu.ar

Bacteriocins are proteinaceous antimicrobials that exhibit high specificity and kill closely related competitors to the producing bacteria. Currently, the biotechnological purpose of these antimicrobials is focused on their application in agriculture, food and pharmaceutical industry. *Pseudomonas fluorescens* SF4c is a native strain that produces tailocins (phage tail-like bacteriocins). SF4c-tailocins inhibit phytopathogenic bacteria of the genera *Pseudomonas* and *Xanthomonas*.
The objective of this study was to obtain and characterize mutants affected in the regulation of bacteriocin synthesis in *P. fluorescens* SF4c. Previously, we constructed a transcriptional fusion of the SF4c-tailocin promoter to the *gfp* reporter gene (pPROBE::Ptail). The recombinant plasmid was mobilized to *P. fluorescens* SF4c. When promoter is activated, fluorescent colonies are observed on LB medium under blue light. A random mutagenesis with mini-Tn5 Km1 was performed by triparental mating with *P. fluorescens* SF4c (pPROBE::Ptail) as recipient, *E. coli* CC118λpir (pUT mini-Tn5 Km1) as donor and *E. coli* HB101 (pRK600) as helper. The transconjugants were selected on minimal medium supplemented with Kanamycin (25 µg·ml-1) and Tetracycline (10 µg·ml-1). The clones were screened on LB medium under blue light for expression of GFP. Mutants with increased or decreased fluorescence were chosen. Then, promoter activity was monitored with a spectrofluorometer and bacteriocin production analyzed. The presence of the mini-Tn5 Km1 transposon in the genome of mutants was confirmed by PCR amplification of the Kanamycin-resistance gene, specific to the transposon. Mutants SF4c-209 and SF4c-210 showed basal levels of fluorescence, indicating that there is no expression of the tailocin promoter. Mutant SF4c-233 and SF4c-253 presented a statistically significant increase in the fluorescence. Therefore, the promoter is active. Mutant SF4c-233 was selected for the next studies. In addition, the bacteriocin production was evaluated on LB medium with different inducer and on

minimal medium with different carbon and nitrogen sources. Promoter activity and bacteriocin production was higher when ciprofloxacin and mitomycin C were used as inducers. Best carbon and nitrogen source was citrate and ammonium chloride, respectively. These findings provide valuable information for future research in bacteriocins production and scaling to higher volumes, which could improve efficiency and reduce costs.

Keywords: Bacteriocin, *Pseudomonas*, Tailocin, GFP protein

Methods: PCR, Random mutagenesis, fluorescence spectroscopy

MI-16

SEARCHING FOR NOVEL INHIBITORS OF THE *Staphylococcus aureus* VraTSR SYSTEM USING COMPOUNDS PRODUCED BY *Streptomyces* STRAINS

Brovedan, Marco A.[1]; Antinori, Melisa B.[1]; Rodríguez, Eduardo J.[1,3]; Testero, Sebastián[2,3]; Llarrull, Leticia I.[1,3]
[1]Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET-UNR, Rosario, Argentina. [2]Instituto de Química Rosario (IQUIR), CONICET-UNR, Rosario, Argentina. [3]Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario (UNR), Rosario, Argentina.
brovedan@ibr-conicet.gov.ar

Infections caused by antibiotic-resistant strains of *Staphylococcus aureus* have reached epidemic proportions globally. The overall burden of staphylococcal disease, particularly that caused by methicillin resistant *S. aureus* strains (MRSA), is increasing in many countries. In 2017, The World Health Organization developed a global priority pathogens list of antibiotic-resistant bacteria to help in prioritizing the research and development of new antibiotic treatments. MRSA was one of the highest ranked Gram-positive bacteria (high priority). In this context, the rapid spread of virulent community-acquired MRSA underscores the increasing need to understand and control resistance in this pathogen. The main mechanism of resistance to β-lactam antibiotics in *S. aureus* is due to the inducible expression of the serine-β-lactamase PC1 (coded by the *blaZ* gene in the *bla* operon), and of the transpeptidase PBP2a (coded by the *mecA* gene in the *mec* operon), with reduced affinity for these antibiotics. Some strains also have decreased susceptibility to glycopeptides, antibiotics of last resort for the treatment of MRSA infections. This effect is due to the action of the VraTSR system, which quickly detects cell wall damage and coordinates a comprehensive response, allowing the bacteria to survive. The objective of this work is to search for inhibitory compounds of the VraTSR and bla systems of *S. aureus*.
In the laboratory, we have reporter strains of *S. aureus* in which the *gfp* gene is regulated by the VraTSR or bla systems. When faced with increasing concentrations of an inducing antibiotic, these systems are activated, resulting in the production of GFP and, consequently, an increase in the intensity of emitted fluorescence. By monitoring the intensity of GFP fluorescence and the optical density, we selected

culture supernatant containing compounds that prevent the activation of the VraTSR and/or bla systems without influencing the growth of the reporter strains. In addition, the bioactive products present in the Actinomycetes cultures were isolated by silica chromatography. The purified compounds were employed for agar diffusion assays using clinical strains with intermediate resistance to vancomycin (VISA and hVISA) and growth curves, assessing the potential of these compounds in combination with currently available antibiotics to restore the effectiveness of antimicrobials.
In conclusion, we have constructed reporter strains for the bla and VraTSR systems useful for the identification of activating or inhibitory compounds of these systems. We have identified Streptomyces strains that produce compounds capable of inhibiting the systems under study and that restore the effectiveness of β-lactam antibiotics when tested with resistant strains of *S. aureus*.
Acknowledgments: Agencia I+D+I for grant PICT-2018-3362 and postdoctoral Fellowship to M.A.B. CONICET for grant PIP 22920160100039CO. E.J.R., S.A.T. and L.I.L. are staff members from CONICET.

Keywords: Actinomycetes, methicillin resistant, β-lactam antibiotics

Methods: Fluorescence, TLC, Silica chromatography, Agar diffusion assays

MI-17

ScsD, A PERIPLASMIC PROTEIN IMPLICATED IN COPPER/REDOX HOMEOSTASIS IN THE *Salmonella enterica* ENVELOPE

Méndez, Andrea AE[1]; Bertonati, Bianca[1]; Zhao, Zhenzhen[2], Argüello, José M[2], Soncini, Fernando C[1]; Checa, Susana K[1]
[1]IIBR-CONICET, U.N.R., Rosario, Santa Fe, Argentina [2]WPI, Worcester, USA
andrea.emendez@gmail.com

Salmonella enterica is a species that includes a large group of food-borne and facultative intracellular pathogens causing infections that ranges from self-limited gastroenteritis to severe invasive illness in susceptible hosts. During its infective cycle, *Salmonella* adapts to and survive in different conditions, including the menacing host environment. Once inside the *Salmonella*-containing vacuole (SCV) within infected phagocytic cells, copper, a highly reactive and toxic metal, actively accumulates to contain the multiplication and dissemination of the pathogen. These ions exacerbate redox stress, primarily in the bacterial cell envelope, where many of the Cu distribution proteins and cuproproteins reside. ScsABCD, a putative thioredoxin system in *S. enterica*, absent in *Escherichia coli* but present in other enteropathogens, contributes to both Cu and redox stress tolerance. Our previous work showed that this operon is induced by Cu. ScsB, ScsC and ScsD carry putative Cu-binding motifs in their periplasmic thioredoxin-like domains. ScsB and ScsC form a redox pair resembling described IM-bound reductases and periplasmic oxidase/isomerase partners, such as DsbD/DsbC, present in *Salmonella* but not involved in Cu tolerance. Regarding *Salmonella* ScsD, its AlphaFold 2 structure resembles that of the membrane-linked thiol:disulfide interchange proteins such as DsbE/CcmG and TlpA, involved in cytochrome c and aa3 biogenesis. Considering that we have determined the function of Salmonella

ScsC and ScsD under Cu^{+} stress depend on ScsB and that DsbE is a DsbD partner in *E. coli*, we propose ScsD as another ScsB redox partner. In this work, we focused our studies on *Salmonella* ScsD. We tagged the *scsD* chromosomal gene with a 3xFLAG epitope to determine its intracellular localization and expression. We confirmed that it accumulates in the membrane fraction under conditions of copper treatment, both in the presence or absence of the CpxR/A two component system that controls *scsABCD* transcription. This suggests the presence of an alternative mechanism of Scs function regulation which responds to Cu but is independent of CpxR/A. ScsD periplasmic soluble domain containing the putative copper binding motif was cloned and expressed as an MBP fusion protein in *E. coli* to perform in vitro studies. Its secondary structure was analyzed by circular dichroism, supporting the *in silico* structural analysis. To evaluate ScsD Cu(I) binding and its ability to form a redox pair with ScsB, a mutant of the ScsD Cu-binding motif, C71xxS74, was generated using Quick-change PCR. As expected, the domain bounds Cu(I) ion and the mutation of this signature Cys in thioredoxins prevented Cu(I) binding. Moreover, the redox trap assay performed between both ScsB and ScsD Cu-binding motif mutants is an indicative of them forming a redox pair. These results shed light on the role of the ScsABCD system and particularly the ScsD protein in the metal/redox homeostasis of the *S. enterica* envelope.

Keywords: *Salmonella enterica*, envelope, copper, redox stress

Methods: Western Blot, protein expression and purification, circular dichroism, quick-change PCR

MI-18

COMPLEX LIPIDS BIOSYNTHESIS: ROLE OF ACYL-AMP LIGASES

Galvan, Virginia; Gramajo, Hugo; Arabolaza, Ana
Instituto de Biología Molecular y Celular de Rosario (IBR) - CONICET
virginiagalvan13@gmail.com

Adenylation represents a fundamental process for the activation of a broad collection of metabolites, which can then be incorporated in more complex structures. This mechanism activates carboxylates by condensation with ATP, giving adenylate intermediates. Fatty acyl AMP ligases (FAAL) activate fatty acids (FA) as acyl-AMP in a first step, and form a thioester bond during second step, usually with acyl carrier proteins (ACP) as acyl acceptors. Natural products biosynthesis is often assisted by adenylation. Several examples of molecules with a wide set of functions, such as antibacterial, antifungal, and cytotoxic activities have been described, in which the presence of the acyl chain seems to have a critical role. The immense biosynthetic potential of Actinomycetota phylum, might explain the strikingly high abundance of FAAL coding genes in their genomes. *Mycobacterium tuberculosis* (Mtb), the etiologic agent of tuberculosis, encodes for 12 FAAL homologs. It has been suggested that this is due to specialization of different homologs for the synthesis of structurally diverse compounds from the pathogen. Mycolic acids, sulfolipids, and other cell wall lipids, are examples of such compounds. Some of them are essential for growth or virulence. FAAL34 from Mtb is encoded in a genetic cluster including

a type II ACP coding gene, and a putative α-oxoamino synthase (AOS) with an extra domain. We aimed to study the physiological role of FAAL34 and its genetic cluster, considering a possible involvement in unknown mycobacterial lipids production. We found the cluster is strictly conserved in Mtb complex, whereas a partial version is present in slowly growing mycobacteria, and rapidly growing species lack of orthologues. Acylation reactions followed by conformational urea-PAGE electrophoresis allowed to confirm FAAL34 is capable of acylating its ACP partner in the presence of ATP, displaying preference for short/medium chain FA. In order to investigate the destination of the activated FA, we first performed bioinformatic analysis on the AOS component of the cluster. Sequence and structure alignments with members of the AOS family reveled it presents conserved residues reported as relevant for cofactor binding, as well as, a high overall structural similarity. Phylogenetic analysis including varied classes of AOS, showed clustering with serine palmitoyl transferases (SPT), which perform the synthesis of sphingosine skeleton present in sphingolipids by condensing serine and palmitoyl-CoA. SPTs have been extensively studied in eukaryotes, although its prevalence and physiological role in prokaryotes had not been explored until last years. *In vitro* activity essays of this novel AOS exhibited difficulties due to limitations regarding protein solubilization and product identification. New strain constructions in mycobacteria are being developed to get insights into the possible function of the AOS through an *in vivo* approach.

Keywords: Complex lipids, biosynthesis, activation, fatty acids

Methods: Bioinformatic analysis, *in vitro* activity essays, urea-PAGE electrophoresis, TLC

MI-19

A WHOLE-CELL-BACTERIAL BIOSENSOR FOR PHOSPHONATES ALLOWS QUANTIFICATION OF GLYPHOSATE-BASED HERBICIDES REMOVAL BY THE HIGHLY EFFICIENT GLYPHOSATE DEGRADER *Achromobacter xylosoxidans*

Masotti, Fiorella[1]; Muñoz, Ana[1]; Krink, Nicolas[2]; Lencina, Nicolas[1]; Gottig, Natalia[3]; Garavaglia, Betiana[1]; Nikel, Pablo[2]; Ottado, Jorgelina[1]

[1]Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET-UNR), Rosario, Santa Fe, Argentina. [2]The Novo Nordisk Foundation Center for Biosustainability (DTU Biosustain), Technical University of Denmark, Lyngby, Denmark. [3]Instituto de Procesos Biotecnológicos y Químicos Rosario (IPROByQ-CONICET-UNR) Rosario, Santa Fe, Argentina

ottado@ibr-conicet.gov.ar

Glyphosate-based herbicides (GBH) are widely distributed in most of the economically productive lands in which crop production is mainly based on glyphosate-resistant genetically modified plants. Our country possesses one of the most fertile and favorable agricultural land in the world and its crop management is based on these genetically modified crops resistant to glyphosate. Nevertheless, the use of glyphosate has been considered less harmful compared to other pesticides and as consequence of its extensive use, its presence is widespread in different environments. Therefore, it is

essential to ensure the detection of GBH in environmental samples to identify polluted hotspots where bioremediation processes might be required. For this purpose, synthetic biology tools were applied in order to obtain a simple, economic and reliable tool as an alternative to the traditional ones based on spectroscopy and chromatography methods and was directly applied to detect remnant glyphosate residues in laboratory samples treated with environmental bacterial isolates. For doing so, previously we have isolated an *Agrobacterium tumefaciens* CHLDO with ability to degrade glyphosate and that bears a C-P lyase complex codified in a *phn* cluster, involved in the hydrolysis of the C-P bond present in phosphonates. The transcriptional analysis of *phn* genes revealed that glyphosate could be performed by means of this C-P lyase pathway. Then, we have constructed a whole-cell-bacterial biosensor using the promoter of the C-P lyase complex from *A. tumefaciens* CHLDO (PhnG) fused to the fluorescent protein Scarlet in the environmental *A. tumefaciens* CHLDO chassis. Further, the biosensor was optimized by including an extra copy of *phnF*, coding for the transcriptional repressor of the cluster. By this Methods, biosensor leakage in the absence of inductor was diminished and the dynamic range, which reveals the fold change between the induced and non-induced state, obtained was acceptable. Here, we used this biosensor to monitor the degradation ability of a previously isolated bacterial strain from soils belonging to fields with repetitious applications of GBH. This strain, *Achromobacter xylosoxidans* SOS3, belongs to a genus that has been characterized as an excellent glyphosate degrader since it has been described that an *Achromobacter* sp. remediated the herbicide in soil samples 2–3 folds faster than endogenous microorganisms. By means of the biosensor we could measure glyphosate degradation in cultures of *A. xylosoxidans* SOS3 grown in minimal medium lacking phosphates and in the presence of 1.5 mM glyphosate. At 3 days of culture, *A. xylosoxidans* SOS3 was able to degrade all the glyphosate present, highlighting the importance of studying bacterial molecular mechanisms that can be translated to develop useful tools for the analysis of pollutants.

Keywords: phosphonates, glyphosate-based herbicides, whole-cell-bacterial biosensor

Methods: Synthetic biology to construct an optimized vector, measurement of bacterial growth and fluorescence

MI-20

CHARACTERIZATION OF CYANOBACTERIAL BLOOMS USING METAGENOMIC ANALYSIS AND MICROBIAL ECOLOGY

*Fainstein, Lola[*1]; Alvarez, Florencia[*2,3]; Alba Posse, Ezequiel[1,2]; Macchiaroli, Natalia[1,2]; Kamenetzky, Laura[1,2]; Lozano, Verónica[3]; Figuerola, Eva[*1,2]; Gasulla, Javier[*1,2]*

[1]iB3- FCEN-UBA- CABA- Argentina 2- CONICET- CABA- Argentina 2-Universidad Nacional de Salta- Salta- Argentina

jvr.gasulla@gmail.com

Cyanobacterial blooms constitute a global problem, affecting the water quality and producing economic losses, along with concerns about their impact on public and environmental health. Their

frequency is increasing, boosted by high nutrient load provided by agricultural, industrial and domestic pollution coupled with global warming. Cyanobacteria are photosynthetic organisms naturally present in surface water. However, the massive growth affects the whole community of micro and macroorganisms. Additionally, on cyanobacterial harmful blooms (CyanoHABs), a wide variety of cyanotoxins are produced by several genera of cyanobacteria, entailing a risk for human and animal life, although not all the blooms are toxin-producers. Abiotic factors conditioning CyanoHABs have been widely studied, but biotic factors, like biotic interactions, and ecosystem diversity and stability, have received less attention. This is despite the fact that both diversity and interspecific interactions are considered to be determining factors of ecosystem stability and that the toxicity primarily depends on their composition of toxic and non-toxic genotypes. Hepatotoxins of the microcystins family are by far the most reported cyanotoxins found in CyanoHABs of our country. Nevertheless, recent studies indicated the presence of cyanobacteria capable of producing neurotoxins (saxitoxin and anatoxin), and cytotoxins (cylindrospermopsin).

In this project, we are applying a metagenomic approach focused on two objectives: 1. The construction of co-occurrence networks for a set of freshwater bodies affected by CyanoHABs. To this end, we started a year-long campaign last fall on 7 water bodies; including rivers, lagoons, and dams from 5 provinces. To date, we have collected 30 water samples from 12 sampling points and their metadata. The samples were filtered and processed to obtain high quality DNA. The structure of these networks will give us insights about biotic interactions, and relationships with physico-chemical factors and stability. 2. Identify toxin biosynthesis gene clusters that could guide toxin monitoring programs. We have performed long read (ONT) sequencing of the metagenome of a sample from El Limón dam (Salta), which provides water supplies to the city of Tartagal. The sequencing produced 7.3M of reads, accounting for 6.1 Gb with an average quality of 18.9. Preliminary analysis showed the presence of *Microcystis*, *Raphidiopsis raciborskii* and *Planktothrix* in accordance with microscopy observation. Also, the presence of *mycD*, critical step in the biosynthesis of microcystins.

The information obtained to date indicates that the proposed metagenomic approach is useful for the detection of toxigenic strains and the identification of the cyanotoxin biosynthesis genes. The analysis of the complete set of samples will give valuable insights to the understanding of the biotic mechanisms that regulate cyanoHABs and will help the development of monitoring programs.

Keywords: Cyanobacteria, metagenomics, ONT sequencing, cyanotoxins

Methods: ONT sequencing, environmental sampling, bioinformatic, microcystin detection

MI-21

THE IS6770 INSERTION SEQUENCE MODIFIES THE TRANSCRIPTION PATTERN OF THE *kup* GENE, ENCODING THE POTASSIUM SYMPORTER, IN *Enterococcus faecalis* JH2-2 UNDER LOW pH CONDITIONS

Taborra, Maria Eugenia 1-2 ; Acciarri, Giuliana 1-2; Blancato, Víctor S. 1-2; Magni, Christian 1-2
[1]Laboratorio de Fisiología y Genética de Bacterias Lácticas. Instituto de Biología Molecular y Celular de Rosario (IBR), Consejo Nacional de Ciencia y Tecnología (CONICET). [2]Facultad de Ciencias Bioquímicas y Farmacéuticas (FBioyF), Universidad Nacional de Rosario (UNR).
taborra@ibr-conicet.gov.ar

Enterococcus faecalis is a phylogenetically and industrially relevant microorganism associated with Lactic Acid Bacteria. Some strains of this bacterium are employed as probiotics in commercial applications, while others serve as the principal component in starter cultures for regional cheese production. However, over the last decade, this species has emerged as an opportunistic multiresistant pathogen, raising concerns about its impact on human health. *E. faecalis* is adapted to tolerate multiple and different environmental stress conditions. The coordination protons, sodium and potassium of the homeostasis not only allows the generation of metabolic energy that guarantees the transport of nutrients but also is essential for the response to stress. Recently, we identified multiple potassium transporter systems in *E. faecalis*, including the Ktr systems (KtrAB and KtrAD), Kup, KimA and Kdp complex (KdpFABC). In this study, we observed a modification in the promoter region of the *kup* gene, which code for the Kup protein in the JH2-2 strain. This chance occurred because of a complete copy of an IS30 family insertion sequence, specifically IS6770, was inserted. To analyze the influence of IS6770 on the expression of the *kup* gene, we conducted a mapping of the promoter region of this gene in the *E. faecalis* JH2-2 and a V19 strain (V583-derived clinical strain), employing fluorescence gene reporters. In addition, a transcriptional analysis of the *kup* gene was executed in the *E. faecalis* V583-derived strain (a strain free of IS30-related insertion element), to determine the transcriptional start site of this gene. Finally, the expression of the *kup* gene was assessed through RT-qPCR under different pH-stress conditions. A strong induction of the *kup* gene was observed at initial pH 5.0 in the *E. faecalis* V583-derived strain. Nonetheless, in the *E. faecalis* JH2-2 strain, this activation was not observed, as the presence of IS6770 impeded the transcription process. In conclusion, the identification of alterations in the *kup* gene promoter region in *E. faecalis* JH2-2 due to the presence of IS6770 highlights the potential implications for gene expression regulation, which may be linked to the observed differences in behavior between strains.

Keywords: Lactic Acid Bacteria, Potassium transporter, *Enterococcus faecalis*, Opportunistic pathogen, Insertion sequence

Methods: Detection and quantification of Fluorescence gene reporters, Quantitative real-time PCR (RT-qPCR), Rapid Amplification of cDNA ends by polymerase chain reaction (5′ RACE PCR)

MI-22

COMPREHENSIVE INSIGHTS INTO THE *Bacillus subtilis* Des PATHWAY: VISUALIZING COMPONENTS BY SINGLE-MOLECULE SUPER-RESOLUTION MICROSCOPY AND ADVANCING STRUCTURAL STUDIES WITH ANTI-DesK NANOBODIES

Pereyra, Julián I.[1]; Alzogaray, Vanina[2]; Escalante, Gonzalo[3]; López, Lucía[3]; Mechaly, Ariel[4]; Stefani, Fernando[3]; Albanesi, Daniela[1]

[1]Instituto de Biología Molecular y Celular de Rosario, IBR-CONICET-UNR, Rosario, Argentina. [2]Fundación Instituto Leloir, IIBBA-CONICET, Ciudad de Buenos Aires, Argentina. [3]Centro de Investigaciones en Bionanociencias, CIBION, Buenos Aires, Argentina. [4]Instituto Pasteur, Universidad de la ciudad de París, Plataforma de Cristalografía, París, Francia

albanesi@ibr-conicet.gov.ar

The Des signaling pathway of *Bacillus subtilis* represents a paradigm for studying sensors that regulate membrane fluidity in both unicellular and multicellular eukaryotic organisms. This pathway comprises an integral membrane desaturase, Δ5-Des, encoded by the *des* gene, and the two-component system DesK/DesR, which controls its expression. DesK is a transmembrane multifunctional histidine kinase with kinase, phosphotransferase, and phosphatase activities. When the temperature drops below 30°C, accompanied by a decrease in membrane fluidity, DesK acts as a kinase and autophosphorylates. The phosphoryl group is then transferred to DesR. Phosphorylated DesR (DesR-P) activates *des* expression. Once synthesized, Δ5-Des desaturates the acyl chains of the membrane phospholipids, restoring their fluidity. Upon an increase in membrane fluidity, DesK adopts its phosphatase state, dephosphorylating DesR-P to turn off the response. Structural biology, biochemical and molecular biology studies with the soluble cytoplasmic domain of DesK (DesKC) allowed us to elucidate the molecular mechanism for the catalytic regulation of this sensor protein. However, the mechanism of signal detection and transmission by DesK's TMSs remains a mystery. Moreover, at present, there is limited information about how signaling pathways, such as the Des pathway, are regulated within the cell in terms of the localization and dynamics of the proteins involved in the adaptive response. *In vitro* studies have revealed differential interactions between DesK and DesR depending on the functional status of DesK. Additionally, it has been determined that both exogenous and endogenous unsaturated fatty acids induce the phosphatase state of DesK, suggesting a potential interaction or co-localization with Δ5-Des to efficiently halt the adaptive response. These findings have led us to hypothesize that changes in membrane fluidity could impact the distribution and interaction of the components of the Des pathway within the cell. To test this hypothesis, we aim to precisely localize and co-localize the components of the Des pathway (DesK, DesR, Δ5-Des) using single-molecule super-resolution fluorescence microscopy techniques under various membrane fluidity conditions. To this end, we have developed specific anti-DesK nanobodies fused to a docking DNA oligomers by "click chemistry" or to an HA (Hemagglutinin) tag at the C-terminus that were successfully used, respectively, in DNA-PAINT (Point Accumulation for Imaging in Nanoscale Topography) and STORM (Stochastic Optical Reconstruction Microscopy) super-resolution microscopy experiments. Moreover, we have initiated Cryo-EM (Cryo-Electron Microscopy) studies with the anti-DesK nanobodies-DesK complexes. This work is a first step towards understanding the functioning of the Des pathway from a structural and cellular point of view.

Keywords: Des pathway, *Bacillus subtilis*, Nanobody, Super-resolution microscopy, structural biology

Methods: molecular biology, biochemistry, click chemistry, DNA-PAINT, STORM

MI-23

IDENTIFICATION OF A VIRULENCE FACTOR IN *Brucella abortus* THAT MODULATES HOST CELL INVASION AND INTRACELLULAR SURVIVAL

Ortiz, Ezequiel A.; Del Giudice, Mariela; Gonzalez Wusener, Ana; Arregui, Carlos O.; Ugalde, Juan E.; Czibener, Cecilia
Instituto de Investigaciones Biotecnológicas, Universidad Nacional de San Martín (UNSAM)-Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Escuela de Bio y Nanotecnologías (EByN), Universidad Nacional de San Martín, San Martín, Buenos Aires, Argentina
eortiz@estudiantes.unsam.edu.ar

Brucellosis is one of the most widespread distributed zoonosis worldwide and is caused by species of the genus *Brucella*: gram-negative intracellular facultative pathogens with the capacity to modulate the biology of the host cells. Among the activities that this bacterium modulates is the adhesion/invasion mediated endocytosis and phagocytosis that ultimately impacts the capacity to evade phagolysosome killing and establishing a successful intracellular replication niche. In this presentation we present evidence that the protein encoded in the *bab1_1611* gene of *Brucella abortus* is involved in the early invasion events of the bacterium modulating the dynamics of the cytoskeleton and the plasma membrane. Our results show that a deletion mutant in *bab1_1611* has a defect in the intracellular replication and that the resulting strain is rough (lacks the complete assembled LPS). We present data showing the subcellular localization of the protein and propose a mechanism for its secretion and interaction with the host cell. The link between the synthesis of LPS, subcellular localization, secretion and actin cytoskeletal modulation is discussed.

Keywords: *Brucella*, invasion, actin

Methods: Western Blot, Conjugation, Infection, Microscopy, cloning

MI-24

UNRAVELING THE COORDINATION OF CELL MEMBRANE AND CELL WALL BIOSYNTHESIS IN GRAM-POSITIVE BACTERIA

Benatti, Maria de los Angeles[1]; Fernández, Pilar[1,2]; Albanesi, Daniela[1,2]
[1]IBR-CONICET-UNR; [2]Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario
angelesbenatti@gmail.com

Gram-positive bacteria are characterized by a unique cell envelope composed of a plasma membrane (PM) and a peptidoglycan (PG) cell wall. Maintaining the integrity of this cell envelope during cell expansion requires synchronized synthesis of both constituents. While the individual pathways for PM and PG biosynthesis are well characterized, the mechanisms underlying the coordination of these biosynthetic processes remain unclear. Previous microarray analysis revealed a potential molecular cross-talk between lipid and PG biosynthetic pathways. Specifically, we observed that inhibiting lipid biosynthesis with the antibiotic cerulenin led to the induction of the *yocH* gene, which encodes a PG autolysin. The expression of *yocH* is regulated by the essential two-component system (TCS) WalR (response regulator)- WalK (histidine kinase). WalR is phosphorylated at D53 by WalK. Recent research has also identified an alternative phosphorylation site, T101, mediated by the PrkC Ser/Thr kinase. To investigate the role of these signaling systems in lipid starvation-mediated *yocH* induction, we introduced a P_{yocH}-*lacZ* reporter fusion into *B. subtilis* strains expressing *walR* punctual mutants. Our results demonstrate that both kinases, WalK and PrkC, are essential for *yocH* induction during lipid biosynthesis inhibition. Additionally, we are currently conducting radiolabeling assays to assess the impact of lipid starvation on PG biosynthesis rates. This work represents a first step toward elucidating the molecular mechanisms underlying the assembly of the Gram-positive envelope. Given the importance of this aspect for the survival of Gram-positive microorganisms, we anticipate that the findings from this project could pave the way for identifying potential targets in the development of new antibiotics.

Keywords: Plasma Membrane, Peptidoglycan, Lipid Biosynthesis, Cell Envelope Biogenesis, Coordination

Methods: Molecular Biology, Bacterial Genetics, Bacterial Physiology, Radiolabeling

MI-25

UNVEILING THE MECHANISM AND SUBSTRATES OF A BACTERIAL PROTEASE: INSIGHTS FROM HIGH RESOLUTION MASS SPECTROMETRY

Aguilar Lucero, Dianela; Bianchimano, Luciana; Ceccarelli, Eduardo; Rosano, Germán
Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET.
aguilarlucero@ibr-conicet.gov.ar

Protein removal is essential for maintaining cellular proteostasis. Protein quality control is carried out by multi-component proteolytic machinery. Bacteria possess a significant arsenal of molecular chaperones, including the ClpA chaperone, proteases such as ClpP, and regulatory proteins like ClpS, which conform the ClpAPS proteolytic complex and play vital roles in processes of protein elimination, rescue from aggregation, or refolding. In particular, the *Escherichia coli* ClpS protein is responsible for substrate recognition via the N-degron pathway of protein degradation. This rule dictates that the half-life of a protein is determined by the nature of the residue at its N-terminus. Research has shown that the amino acids tryptophan, leucine, phenylalanine, and tyrosine, located at the N-terminal position of a polypeptide, determine its fate by making it a substrate of the ClpAP proteolytic system, where a catalytic amino acid triad hydrolyzes polypeptide bonds.
In this study, we analyzed the peptides generated by ClpAPS by the *in vitro* degradation of FR-GFP. For these assays, both the fluorescent substrates FR-GFP and ClpAPS were obtained in recombinant form. The experiment involved incubating the ClpAP complex and FR-GFP while monitoring fluorescence over time. We measured the degradation of the fluorescent substrate, as its N-terminal end contains an N-degron recognized by ClpS. The generated peptides were separated by gel filtration chromatography and subsequently analyzed by mass spectrometry. We found that the ClpP protease cuts proteins every 7 or 8 residues indiscriminately. Furthermore, we applied proximity labeling followed by mass spectrometry (PL-MS) to comprehensively identify novel ClpS substrates. We attached a labeling enzyme to ClpS, enabling the biotinylation of interacting molecules within a 10 nm radius. We developed the necessary molecular tools for PL-MS using the biotin ligase TurboID as the labeling enzyme. In contrast, TurboID ClpS-AA served as a control, exhibiting very low affinity for N-degrons and lacking the ability to support the degradation of N-terminal rule proteins *in vivo*. Biotin-tagged proteins were then purified using streptavidin-coated beads and identified through mass spectrometry. This innovative strategy aims to fully unveil ClpAPS complex substrates, capturing even transient and weak interactions that conventional methods often miss.

Keywords: N-degron pathway, Proteolysis, Adaptor protein, *Escherichia coli*

Methods: *in vitro* degradation, mass spectrometry, Proximity labeling

MI-26

IN VIVO MITIGATION OF SCOPOLAMINE-INDUCED CHOLINERGIC DYSFUNCTION AND OXIDATIVE STRESS BY SELECTED *Lactobacillus* STRAINS

Bulacios, GA[1]; Naja, J[1]; Cataldo, PG[2]; Martínez Urquiza, P[2]; Elean, MD[2]; Posse de Chaves, E[3]; Taranto, MP[2]; Beauquis, J[4]; Hebert, EM[2] and Saavedra, L[1]
Genetics and Molecular Biology Laboratory, (CERELA-CONICET), Tucumán- Argentina. [2]Technology and Development Laboratory, (CERELA-CONICET), Tucumán- Argentina. [3]Department of Pharmacology, Faculty of Medicine and Dentistry, University of Alberta, Canada. [4]Institute of Biology and Experimental Medicine (IBYME-CONICET), Buenos Aires- Argentina"gaby_bulacios92@hotmail.com

Life expectancy has increased exponentially over the last 50 years. This extended lifespan has led to the emergence of age-related disorders, with dementia taking center stage. Among the various forms of dementia, Alzheimer's disease (AD) stands out as the most common, accounting for 60-70% of cases. Currently, there is no definitive treatment for this condition, and all drugs and interventions focusing solely on symptom alleviation, primarily through cholinesterase inhibitors and memantine. In this context, scientists have embarked on a quest to uncover agents or combination of them capable of forestalling or enhancing the quality of life for those affected by this condition. Recent research has spotlighted nutritional interventions and the burgeoning field of probiotics, which hold promise for mental health. In the present study, we evaluated the effect of daily oral administration of two probiotic strains: *Lactobacillus delbrueckii* subsp. *lactis* CRL 581 ($1x10^8$), an *in vitro* acetylcholinesterase (AChE) inhibitor, and *Levilactobacillus brevis* CRL 2013 ($1x10^9$), an efficient gamma-aminobutyric acid (GABA) producing strain. This administration spanned a period of 30 days and aimed to investigate their impact on oxidative stress and cholinergic dysfunction within a scopolamine-induced mice model. Scopolamine, acting as a cholinergic receptor antagonist, induced memory loss, cognitive impairment, and elevated AChE activity, thereby replicating some pathological alterations observed in AD. AChE activity exhibited a significant increase in brain homogenates of mice treated with scopolamine compared to the control group. Both probiotic strains demonstrated the ability to decrease AChE activity, with CRL 581 showing particularly noteworthy efficacy. Furthermore, CRL 2013 administration increased catalase activity in mice brains. In females, solely treatments with CRL 581 strain showed a decrease in MDA levels, an end-product of lipid peroxidation, whereas in males, both strains displayed the capacity to reduce it. No significant differences were observed in GSH activity across all groups. Data from the shotgun proteomic of scopolamine- and psychobiotics- treated brain homogenates revealed distinctly and unique differential expression patterns in each group. These findings strongly support the potential development of a functional supplement using t, offering a promising non-pharmacological intervention for individuals affected by Alzheimer's disease.

Keywords: Probiotics, Cholinergic dysfunction, Oxidative stress, Age-related disorders

Methods: Animal experimentation, neurobiochemical assays, shot-gun proteomics

MI-27

EXPLORING THE ROLE OF Maf IN MYCOBACTERIAL LIPID METABOLISM AND CELL DIVISION

Rollan, Lara; Gramajo, Hugo; Diacovich, Lautaro
Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas (FBIOyF), Universidad Nacional de Rosario (UNR)
larirollan@gmail.com

Mycobacterium tuberculosis (Mtb) is the etiological agent of tuberculosis (TB), one of the leading infectious diseases causing death worldwide. This has been exacerbated since the breach of extensive

antibiotics treatments and the ongoing emergence of multi-resistant (MDR) and extensively resistant strains (XDR). In addition, the HIV and SARS-CoV2 outbreaks, along with the deterioration of public health systems in undeveloped countries have been contributing to TB propagation. Consequently, there is an urgent need for the discovery and development of new antitubercular agents that target new biochemical pathways and treat drug resistant forms of the disease.

Mycobacteria possess an unusually lipid-laden cellular envelope, including mycolic and methyl-branched acids. Some of them are essential for bacteria viability and its pathogenicity. Thus, the biosynthetic pathways that generate these compounds offer an attractive target for the development of new antimycobacterial agents. In Mtb, the enzymatic complexes acyl-CoA carboxylases (ACCase) catalyze essentials steps in lipid biosynthesis. They generate malonyl-, methyl-malonyl-CoA, and carboxylated long-chain acyl-CoA as substrates for the biosynthetic pathways. In our laboratory, three different Mtb ACCase complexes were characterized at biochemical, genetic and structural level. However, less is known about the modulation of its activity. Recently, we have identified the Rv3282 gene, coding for a protein denominated Maf, and it is found adjacent to a cluster of genes involved in the production of the ACCase subunits. Maf protein has aminoacidic sequence similarity to inhibitors of septum formation, but the function of Mtb Maf is unknown. In this study we overexpress Maf protein in *E. coli* and then purified it using Ni-affinity columns. The activity of one Mtb ACCase complex was reconstructed *in vitro*, and the presence of Maf protein resulted in a dramatic increase of the activity. However, the Maf-induced ACCase activation was not sustained over time, indicating either protein instability or a need for a co-factor. Using *Mycobacterium smegmatis* as a model, we constructed a deletion mutant strain on *maf* in order to physiologically characterize the role of this protein and analyze whether there are growth differences. This strain also allows us to measure ACCase activity in the absence of the endogenous Maf. To further characterize Maf, we have constructed vectors to complement the mutant strain and overexpress the protein to evidence a distinguishable phenotype, as has been observed in other microorganisms. The pJB vectors were used for constructing a Maf-GFP fusion, which will allows the analysis of the particular localization of the protein by confocal and fluorescence microscopy. The information of these studies provides new insights into the regulatory mechanism for the biosynthesis of lipids in Mtb and opens the opportunity to identify molecules that could work as antimycobacterial compounds.

Keywords: *Mycobacterium tuberculosis*, acyl-CoA complexes, Maf protein, inhibitor of septum formation

Methods: Cloning and genetic modification of bacteria, protein purification, size exclusion chromatography, radioactive and coupled essays to determine enzymatic activity

MI-28

THE ROLE OF THE GLUTATHIONE METABOLISM ON THE OXIDATIVE STRESS RESPONSE OF *Streptococcus pneumoniae* IN DIFFERENT HOST CELL ENVIRONMENTS

Zappia, Victoria; Hernandez-Morfa, Mirelys; Olivero, Nadia B.; Cortes, Paulo; Human, Candela; Echenique, José

CIBICI-CONICET, Dpto. Bioquímica Clínica, Facultad de Cs. Químicas, Universidad Nacional de Córdoba

victoria.zappia@unc.edu.ar

Streptococcus pneumoniae (Spn), a main human pathogen, is the causal agent of otitis, sinusitis, pneumonia, and meningitis. During the course of infection, Spn encounters O_2 and its reactive forms that must eliminated for its survival.

To better understand its oxidative stress response (OSR), we investigated the role of genes involved in the ROS detoxification through glutathione, such as *gshT*, which encodes for a known glutathione transporter that is essential because Spn is unable to synthetize glutathione. It has also been reported that the relevance of glutathione peroxidase GpoA in bacterial OSR is significant. We performed a bioinformatic analysis of the pneumococcal genome, and we found the *gpoA* gene that probably encodes for a glutathione peroxidase. In addition, we also found the *ahpD* gene, which encodes for a putative alkyl hydroperoxidase, a key enzyme in the OSR of other bacteria.

To test the roles of these genes on the OSR, we constructed the Δ*gshT*, Δ*gpoA* and Δ*ahpD* mutants by targeted mutagenesis. The three mutants showed an impaired ability to survive under H_2O_2-induced oxidative stress conditions in bacterial cultures in relation with the wild-type strain, suggesting that these genes are involved in the OSR.

Previously, we have demonstrated the ability of Spn to survive within host cells despite the presence of intracellular oxidative stress. To validate our findings, we examined the survival capacity of these mutants in different types of host cells with varying levels of ROS production. Specifically, we utilized A549 pneumocytes, RAW 264.7 macrophages, and PLB-985 neutrophils, as well as PLB-985 nox2-KO cells lacking NADPH oxidase 2 and exhibiting lower ROS production compared to PLB-985 cells. To assess survival, we employed protection assays using gentamicin to eliminate non-endocytosed or non-phagocytosed pneumococci, with an appropriate multiplicity of infection values for each cell type. Our results revealed a significant decrease in survival among the three mutants in all cell types compared to the wild-type strain. This decrease was particularly pronounced in RAW 264.7 and PBL-985 cells, which can be attributed to the higher levels of ROS in macrophages and neutrophils compared to pneumocytes. Notably, when A549 and RAW 264.7 cells were pre-treated with N-acetylcysteine, a ROS inhibitor, the survival of the three mutants was significantly improved. However, it is important to note that this increase in survival did not reach the level observed in the wild-type strain within the intracellular environment.

Our findings highlight the significant contributions of *ahpD*, *gshT*, and *gpoA* in the OSR of Spn during its survival within host cells. Specifically, the glutathione and AhpD ROS-scavenging pathways are of particular importance. This research enhances our comprehension of the strategies employed by the pneumococcus to mitigate the oxidative stress induced by host cells.

Keywords: pneumococcus, oxidative stress, host cells

Methods: mutagenesis, PCR, culture cells

MI-29

EVOLUTIVE AND PHYLOGENETIC ANALYSIS OF THE SARS-COV-2 OMICRON VARIANT IN THE CITY OF CÓRDOBA

Zappia, Victoria E.[1]; Olivero, Nadia B.[1]; Gargantini, Pablo[2]; Human González, Candela[1]; Márquez, Judith[2]; Hernández-Morfa, Mirelys[1]; Cortes, Paulo[1]; Ceschin, Danilo[3]; Ortiz, Lucia[4]; Pérez, Daniel[4]; Echenique, José[1]
[1]CIBICI-CONICET, Dpto. Bioquímica Clínica, Facultad de Cs. Químicas, Universidad Nacional de Córdoba. [2]Clínica Universitaria Reina Fabiola, Universidad Católica de Córdoba. [3]Instituto Universitario de Ciencias Biomédicas de Córdoba (IUCBC), Centro de Investigación en Medicina Traslacional "Severo R. Amuchástegui" (CIMETSA); [4]Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, United States.
victoria.zappia@unc.edu.ar

Since the emergence of the SARS-CoV-2 Omicron variant, it has raised significant concerns worldwide due to its increased transmissibility and potential impact on the COVID-19 pandemic. Comprehensive genomic and phylogenetic analysis is crucial to understand the variant's spread and evolution within specific regions. This study focuses on the genomic characteristics and evolutionary dynamics of the SARS-CoV-2 variants in Córdoba City during the period December 2021-October 2022. We collected nasopharyngeal swab samples from 98 COVID-19 patients who attended the Clinica Universitaria Reina Fabiola (Universidad Católica de Córdoba), Córdoba City, Argentina. The viral RNA of these samples was purified by columns and the presence of SARS-CoV-2 was confirmed by amplification of ORF1 and N genes by qPCR. The viral genomes were sequenced by Nanopore technology according to the Artic Network V4 protocol. Bioinformatic analysis of the raw genomic data revealed that the 70% of the SARS-CoV-2 samples belonged to Omicron, and we focused our study on this variant. We performed comparative genomics using global reference sequences from the NCBI, NextStrain and GISAID databases. Phylogenetic analysis was conducted to assess the Omicron variant's evolutionary relationships and divergence within the local population. In this sense, we constructed a comprehensive phylogenetic tree to elucidate the evolutionary relationships among Omicron variants within the clinic. Furthermore, a haplotype network was created to illustrate the genetic distances among the 70 Omicron genomes, and those belonging to the same lineage were clustered together. To analyze the spread and diversification of the Omicron variant in Córdoba during the same time period, we compared the SARS-CoV-2 genomes identified in this region (available at GISAID), with the genome sequences obtained by Nanopore technology in our lab. On the other hand, we identified the most frequent mutations within the Omicron variant genomes, with a particular focus on amino acid substitutions in the Spike protein. We highlight the presence of the A67V, G142D, and R346K mutations, which have been associated with an increased capacity of Omicron to evade immunity from neutralizing antibodies, whether produced by natural infection and/or vaccination. Remarkably, we have identified three mutations that were not yet reported in Argentina, such as R3756K in the ORF1a gene, and Y145D and L212I in the S gene, which are poorly disseminated worldwide.

This study provides critical insights into the genomic and phylogenetic attributes of the SARS-CoV-2 Omicron variant in Córdoba City, contributing to a broader understanding of its regional impact. The use of Nanopore sequencing underscores its utility in rapidly characterizing emerging variants in real-time. Our findings emphasize the importance of genomic surveillance to guide public health measures, vaccination strategies, and global efforts to combat the COVID-19 pandemic.

Keywords: sars-cov-2, viral evolution, epidemiology, genomic analysis

Methods: nanopore sequencing, RNA purification, qPCR

MI-30

DRAMATIC CHANGES IN THE SECONDARY STRUCTURE OF PROTEINS Rqc2 AND HRK1, DEPENDENT ON POLYMORPHIC AMINO ACID TRACT LENGTHS, DO NOT CONFER A NULL ALLELE PHENOTYPE

Raymond Eder, María Laura; Rosa, Alberto Luis
Laboratorio de Genética y Biología Celular y Molecular, Departamento de Farmacología Otto Orsingher, IFEC-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina.
mlraymondeder@unc.edu.ar

Yeast microsatellite loci consist of short tandem-repeated DNA motifs (TRMs) of variable length useful for strain differentiation, population genetics, and evolutionary biology. We have previously shown that allelic variants for some microsatellite loci of the wine yeast species *Starmerella bacillaris*, *Hanseniaspora uvarum*, *Saccharomyces cerevisiae*, *Saccharomyces uvarum*, *Torulaspora delbrueckii*, *Brettanomyces bruxellensis*, and *Meyerozyma guilliermondii* were dependent on their polymorphic TRMs, as well as SNPs and/or indels flanking the corresponding TRMs. We further explored the presence of microsatellite sequences within protein coding regions, and confirmed that some microsatellites from *H. uvarum*, *S. cerevisiae*, *T. delbrueckii*, *B. bruxellensis*, and *M. guilliermondii* localize into coding sequences. In this work we studied the predicted protein secondary structures and the phenotypic impact of alternative alleles of *S. cerevisiae* microsatellites YPL009C and SCYOR267C, whose TRMs are part of the open reading frames of proteins Rqc2 and HRK1, respectively. The TRM of locus YPL009C (Rqc2) consist on a polymorphic number of the trinucleotide GAA, which encodes glutamic acid, while locus SCYOR267C (HRK1) encodes a polyglutamine tract. Previous studies have shown that: i) the deletion of the Rqc2 gene confers increased sensitivity to cycloheximide, and ii) the deletion of the HRK1 gene increased tolerance to hygromycin B. Based on these observations, we hypothesized that length variations at the polymorphic amino acid tracts of proteins Rqc2 and HRK1 could disturb the normal function of these proteins, leading to yeast strains displaying different levels of drug tolerance. Predictions of secondary protein structures showed that the analyzed repetitive amino acid tracts are located into disordered regions of the molecules and dramatically modify the predicted structures. Surprisingly, phenotypic analyses of cycloheximide and hygromycin B tolerances, in *S. cerevisiae* strains carrying alternative alleles of loci YPL009C and SCYOR267C, revealed that no direct relationship exists

between drug tolerance and the predicted alterations of the protein structures. We propose that allele variants of Rqc2 and HRK1 conserve their wild type function due to a remarkable structural plasticity of these proteins.

Keywords: yeast, microsatellite, coding regions, protein

Methods: PCR, microfermentation, protein modeling

MI-31

ANALYSIS OF MICROBIAL COMMUNITIES OF COMPOST USING SEQUENCES SHOTGUN

Cerioli MF1, Moliva M1, Sambuceti N2, Raviolo J2, Sánchez-Pérez3, Caminati F1, Rosenblueth Laguette MT3, Reinoso EB1

[1]Facultad de Ciencias Exactas, Fco. Quimicas y Naturales de la Universidad Nacional de Rio Cuarto, Córdoba-Argentina. [2]Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México. florenciacerioli@gmail.com

In Argentina, dairy cow barns with compost bedding, especially in the province of Córdoba, are becoming increasingly relevant. These systems are sustainable, beneficial for peri-urban areas, and enhance animal welfare, which impacts the quality of dairy products and consumer perception of the dairy industry. Additionally, they offer advantages such as lower effluent levels and water consumption compared to other methods, while ensuring animal health and profitability for producers. As these systems intensify, there is greater control over the animals and the environment, but it also requires strict surveillance of health and diseases associated with increased animal density. Animal housing influences overall health, including mammary and hoof health. Investigating the compost microbiota and its potential relationship with infectious diseases is crucial. Our study focused on applying metagenomic techniques to identify pathogens in compost samples that may cause infections in cows. We sampled four different points, including enclosure and pre-calving areas for both cows and heifers. Subsequently, we extracted DNA from the samples and processed it using the Illumina Novaseq 6000 platform, analyzing the obtained reads for taxonomic assignment. Through bioinformatic analysis, we identified species-level reads, including *Bacillus pumilus* (1% of the *Bacillus* genus), *Corynebacterium stationis* (13% of the *Corynebacterium* genus), *Pseudomonas stutzeri* (27% of the *Pseudomonas* genus), and *Sphyngomonas* (2% of the total DNA reads in compost beds). *Saccharomonospora viridis*, a representative of the *Saccharomonospora* genus (Pseudonocardiaceae family), was present in all samples (1-5% of total reads). Bioinformatic analysis in this study, applied to shotgun metagenomics, has been crucial for understanding bacterial communities in compost beds, which are often challenging to identify. Furthermore, this research has revealed the presence of pathogens in these samples, providing valuable insights into their potential contribution to disease occurrence.

Keywords: compost bedding, cows, infections, metagenomic

Methods: Extracted DNA, metagenomic techniques, Illumina Novaseq, bioinformatic analysis

MI-32

STUDIES ON THE FUNCTIONAL ROLES OF THE Fe/Co TRANSPORTER (AitP) AND A POTENTIAL ASSOCIATED METALLOCHAPERONA (AitQ) IN *Pseudomonas aeruginosa*

Moreyra, Tomas1; Carrizo María Elena2; Raimunda, Daniel Cesar1.
1: Instituto de investigaciones médicas Mercedes y Martin Ferreyra. INIMEC 2: Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC) 1 y 2: CONICET-Universidad Nacional de Córdoba (UNC). Argentina.
tmoreyra@immf.uncor.edu

All forms of life require small concentrations of transition metals (Mn^{2+}, Ni^{2+}, $Cu^{+/2+}$, Co^{2+}, Zn^{2+}, $Fe^{2+/3+}$) to carry out various cellular physiological processes. These micronutrients are relevant at the host-pathogen interface of bacterial infectious processes. In the opportunistic pathogen *P. aeruginosa*, members of the Cation Diffusion Facilitator (CDF) family export transition metals from the cytosol to the periplasm, enabling the pathogen to cope with conditions of both high and low metal availability. Our laboratory has characterized the AitP transporter of the CDF family in this microorganism as responsible for exporting intracellular Fe^{2+} and Co^{2+}. Our working hypothesis suggests that AitP actively participates in the homeostasis of these metals, preventing damage to cellular structures caused by their accumulation. This, in turn, prevents the formation of free radicals through the Fenton reaction by Fe^{2+} and the attack on exposed iron-sulfur centers (Fe-S clusters) by Co^{2+}. In the rhizobacteria, *Sinorhizobium meliloti*, we showed that hemin mitigates Co^{2+} sensitivity, likely complementing intracellular Fe loss mediated by AitP. We have recently confirmed a similar scenario in *P. aeruginosa*. In this organism, the adjacent operon to AitP consist of PA1300, PA1301, and PA1302, which form an ABC transporter-type hemin uptake system, HxuARI. Fur, a transcriptional factor, appears to regulate this system. Between this operon and AitP, the locus PA1299 encodes a protein of unknown function (DUF3109). There is evidence that this protein is overexpressed in response to Co^{2+} along with AitP, that it is cytosolic and contains cysteine-rich domains. Recently, we expressed and purified the $His_{(6)}$-tagged version of the locus PA1299 product, which we named AitQ. Preliminary results from metal binding assays using the colorimetric method with the PAR indicator (monosodium salt of 4-(2-pyridylazo) resorcinol) showed that the protein is loaded with a divalent transition metal. We also show here, the generation of a scarless AitQ deletion mutant using CRISPR/Cas9 gene editing system. Future studies

using these tools will enable us to assign a functional role for this protein in heme or Fe/Co transport, and explore its potential role in metal-induced oxidative stress responses.

Keywords: Fe-S clusters, *Pseudomonas aeruginosa*, heme transport, iron transport, metal resistance, transition metals

Methods: MIC determinations, PCR, Gene editing by CRISPR/Cas9, metal binding assay by colorimetric method with the PAR indicator (monosodium salt of 4-(2- pyridylazo) resorcinol), heterologous expression and protein purification, SDS-PAGE and Western Blot.

MI-33

STUDY OF THE CUPROPROTEINS *Tc*SCO-A AND THEIR CONTRIBUTION TO THE FUNCTIONING OF THE CYTOCHROME C OXIDASE OF *Trypanosoma cruzi*

Acosta-Oviedo, Héctor; Merli, Marcelo Luciano; Mediavilla, María Gabriela; Cricco, Julia Alejandra.
Instituto de Biología Molecular y Celular de Rosario. CONICET - UNR. Rosario, Santa Fe.
mediavilla@ibr-conicet.gov.ar

In *Trypanosoma cruzi*, the etiologic agent of Chagas disease, the presence of a branched respiratory chain with cytochrome c oxidase (COX) type *aa*3 as the main terminal oxidase has been described[1-3]. Recently, the importance of COX was demonstrated since the impairment of the enzyme activity compromised parasite survival, proliferation, and infectivity[4].

COX is a multiprotein complex in whose assembly various proteins participate[5]. Among them are the SCO (synthesis of cytochrome c oxidase) metallochaperones, belonging to the superfamily of thioredoxins and whose function has been shown to be essential not only for their participation in the supply of copper for the assembly of the catalytic nucleus of COX, but also in copper homeostasis and cellular redox homeostasis in various organisms[6-9].

In our laboratory we have identified, through bioinformatic analysis, 3 genes that would encode for an electron transport protein SCO1/SCO2 like (BCY84_03573) that we named *Tc*SCO-A and two cytochrome c oxidase assembly factors (BCY84_19910) and (BCY84_01670) named as *Tc*SCO-B and *Tc*SCO-C respectively. The three proteins present a characteristic CXXXC motif of cuproproteins and thioredoxins, despite presenting a low percentage of identity between them: 43.8% (*Tc*SCO-A/*Tc*SCO-B), 32.4% (*Tc*SCO-A/*Tc*SCO-C), 30.4% (*Tc*SCO-B/*Tc*SCO-C) and not showing a phylogenetic relationship with other eukaryotes, including its two hosts: arthropod and chordata.

The overexpression of *Tc*SCO-A and *Tc*SCO-B in Dm28c epimastigotes transfected with p*Tc*INDEX/*Tc*SCO-A and p*Tc*INDEX/*Tc*SCO-B produced a delay in growth which was increased in the presence of 250 µM Cu(II) and the copper chelator Neocuproine. Incubations of epimastigotes overexpressing *Tc*SCO-A failed to reverse the effect of elesclomol, an anticancer drug recognized to be

an inducer of oxidative stress and producer of cuproptosis in cancer cells[10]. These preliminary results position *Tc*SCO-A as an interesting target for the development of future drugs with trypanocidal activity.
References: [1]doi: 10.1017/S1462399409001252; [2]Rev Inst Med Trop Sao Paulo. 1964;6:93-100.; [3]J Parasitol. 1960;46:529-39.; [4]doi: 10.1007/978-1-4899-1651-8_7; [5]doi: 10.1126/science.1110289; [6]doi: 10.1016/0305-0491(86)90016-7; [7]doi: 10.1016/j.pt.2006.08.007; [8]doi: 10.1007/s10863-011-9369-0; [9]doi: 10.1042/BCJ20170084; [10]doi: 10.1186/s13046-022-02485-0.

Keywords: *Trypanosoma cruzi*, Chagas disease, metallochaperones, SCO, cuproproteins

Methods: Polymerase chain amplification, Cloning, transfection of parasites, bioinformatic and *in silico* analysis, drug effects on cell growth

MI-34

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF SALMONELLA SPP. ISOLATED FROM CLINICAL SAMPLES IN SALTA, ARGENTINA

*Said-Adamo, María 1; Arancibia, Cecilia1; Alvarez, Roque1; Silva Viviana 2; Mollo Valeria 2; Cristóbal, Héctor 1**

1 Laboratorio de Investigación y Diagnóstico Genético (LIDGen) - Instituto de Investigaciones para la Industria Química (INIQUI-CONICET)- Universidad Nacional de Salta 2 Hospital San Bernardo, Salta
hacristobal@gmail.com

In the last 5 years, more than 6,000 cases of salmonellosis were reported in Salta province, mainly affecting children under 15 years of age. Currently, outbreaks of Salmonella spp. represent an important problem for the public health of the province. To make any decision for infection treatment, it is necessary to have knowledge about pathogen type characteristics that circulates in the population through for an accurate diagnosis. The objective of this study was to determine the morphological and biochemical characteristics of Salmonella spp. strains isolated from clinical patients. We analyzed 50 Salmonella spp. isolated from clinical patients that suffered symptoms infection. Samples were provided from San Bernardo's Hospital in Salta, within the framework of an agreement with the University and with the corresponding bioethics endorsements approved. Several morphological studies of all samples were performed by colony growth in Salmonella-Shigella agar (SS agar), Gram staining and scanning electron microscopy. Additionally, biochemical tests were evaluated, including glucose fermentation, indole motility, urea hydrolysis, lysine decarboxylation, citrate, methyl red and Voges Proskauer tests. Gram-negative bacilli were confirmed and colorless colonies with black center grew in SS agar, like characteristic phenotype of Salmonella spp. colonies. The results of scanning electron microscopy showed considerable variation in bacilli length within the same sample and between the samples analyzed (range from 1,3 to 3,8 µm). Biochemical tests did not show differences between evaluated samples, they all followed the pattern: positive glucose fermentation, negative indole motility, negative urea hydrolysis, positive lysine decarboxylation, positive citrate, positive methyl red and negative Voges Porkauer test. In conclusion, biochemical characteristic evaluated, confirmed

the presence of Salmonella spp. isolated from clinical patients but no differences were observed between strains analyzed. However, we found morphological variation in the size of the bacteria that could infer the presence of different strains of Salmonella. This study should be continued with molecular DNA sequencing analyzes to determine if these are indeed genetically different strains.

Keywords: 16S rDNA, Salmonella Paratyphi B, restriction analysis (RFLP), Phylogenetic analysis, PCR

Methods: Microbiology method, Molecular study (PCR reaction, Sequencing analysis, PCR-RFLP)

MI-35

Molecular Characterization of Clinical and Environmental Salmonella sp. Strains Causing Infectious Diseases in the Salta city

*Gonza Ivana 1,2; Reyes Sarita 1; Álvarez Nicolás1; Davalos Florencia 3, Ramos Ana 3; Cristóbal Héctor 1,2**
1 Laboratorio de Investigación y Diagnóstico Genético. INIQUI – CONICET – Universidad Nacional de Salta – Argentina. 2 Facultad Ciencias Naturales - Universidad Nacional de Salta – Argentina. 3 Hospital San Bernardo, Salta
hacristobal@gmail.com

In Salta, Salmonella Parathyphi B represents the main cause of Salmonellosis in the population, affecting children and seriously causing threat to public health due to the high number of reported cases. Each year, approximately 1 of 10 people contract the disease, and records from the last 5 years report more than 7,000 cases, accumulating a total of 679 cases by 2023. The phylogenetic relationships between pathogens can be inferred from comparisons of their 16S ribosomal (16S rDNA) sequences. This has had a huge impact on bacterial taxonomy and rapid and accurate identification. Microbiology restriction analysis (RFLP) represents a great molecular tool that allows strains grouping and identification through comparative techniques from known strain patterns. The aim of this study was to compare the 16S rDNA genetic profiles from enzymatic restriction with HaeIII, and additionally establish the phylogenetic relationship and distance between clinical and environmental isolates.
A total of 83 clinical Salmonella spp. isolates from clinical studies collected between 2022 and 2023 from hospital San Bernardo of Salta, as well as one environmental isolate from superficial water cultures were studied. Non-Salmonella strains (17 samples) from the LIDGen laboratory were used as negative control. The 16S DNAr was amplified using endpoint PCR with universal primers (63f and 1367r). Genomic material was extracted using phenol-chloroform-isoamyl alcohol, and the eluates were used for PCR reactions employing primers for approximately 1400 pb. PCR products were visualized through 1% (w/v) agarose gel electrophoresis. The genetic profile of each strain was assessed using the HaeIII enzyme, and the cleavage was visualized on a 2% agarose gel at 60 V for 4 hours until bands were obtained. In addition, an in silico analysis of restriction enzymes was performed and compared with in vitro results. Furthermore, this profile was employed to group strains based on their enzymatic profiles. Additionally,

16S DNAr for six strains were sequenced and compared with reference sequences from the NCBI database, and a phylogenetic relationship tree was constructed.
This report describes the application of a PCR-RFLP for molecular categorization strains of Salmonella sp. from clinical samples. Genetic profiling revealed discernible size patterns distinguishing between Salmonella and non-Salmonella strains. Within the former group, a 1400 bp fragment was amplified, and enzymatic cleavage further confirmed that all identified Salmonella strains exhibited a consistent banding pattern, distinct from the negative strains. This Salmonella group yielded four distinct fragments, measuring 325, 275, 225, and 175 base pairs, respectively.Conversely, the non-Salmonella group produced a different enzymatic profile. Additionally, it is the first time that the phylogenetic relationship of sequences from clinical and environmental samples from Salta. A phylogenetic analysis revealed that the sequenced strains belong to Salmonella Paratyphi B, and were clustered within a well-supported independence to non-Salmonella group. This study provides valuable information about their genetic profiles from clinical strains, and phylogenetic analysis that revealed a distinctive clustering pattern, shedding light on their relationships among samples from different origins.

Keywords: Salta city, Salmonella, Microscope, Morphology, Disease

Methods: Microbiology method, Biochemical analyses, Electron and Optical Microscopy study

MI-36

DEVELOPMENT OF ACTIVE BIVALENT VACCINES AGAINST ENTEROPATHOGENIC STRAINS.

Ballesteros, MF; López, FE; Pescaretti, MM; and Delgado, MA.
INSIBIO (CONICET-UNT) and Instituto de Química Biológica "Dr. Bernabé Bloj"
monicadelgado868@gmail.com

Foodborne bacterial diseases are among the first five causes of death in children under 5 years old and immunosuppressed patients. In previously studies, we demonstrated that, in the NOA region, *Shigella* is the prevalent pathogen, being *Shigella flexneri* the most frequently isolated, mainly in the population from 3 to 5 years old. The study of the antibiotic resistance profile (ARP) revealed that the majority of clinical isolates (CI) were presented as Multidrug Resistant (MDR). Therefore, the development of bivalent vaccines with genetically modified microorganisms represents an excellent strategy to combat these diseases, since they can induce an effective immune response not only against pathogens of the same genus and species (attenuated vaccines) but also against phylogenetically related pathogens (recombinant vaccines). The results obtained in our laboratory on the study of *Salmonella* Typhimurium strains attenuated in virulence, indicated that these mutants can be the basis for the bivalent vaccines development. For this purpose, antigen genes selected from analyzed Shigella' CI were cloned in specialized vectors, containing elements of the Salmonella' SPI2 to control its expression and secretion. The obtained constructions were introduced into the attenuated strain, and the correct expression and secretion of the recombinant antigens was studied. In addition, new attenuated strains derived from the previously studied were obtained, and them the protection conferred against virulent CI of different

Salmonella serovars was evaluated. Our results demonstrated that the *S.* Typhimurium and Enteritidis CI established the infection on BALB/C mice (orally inoculated) during the first week postinfection (p.i.). Meanwhile, mice infected with the attenuated strains survived up to 15 days p.i., even after receiving a second challenge with the both virulent CI. Additionally, the immune response produced in challenged mice was studied. Our results indicated that the attenuated strains induce host immunity against *S.* Typhimurium and Enteritidis serovars

Keywords: vaccines, Salmonella, Shigella, pathogens, NOA

Methods: Molecular cloning, protein purification, Elisa, animal infection, chromosomal mutations

Lipids

LI-01

CANNABIDIOL (CBD) IS ABLE TO RELEASE ABNORMAL CHOLESTEROL ACCUMULATION IN AGED ASTROCYTES

Allende, Leandro G.; Martin, Mauricio G.
Laboratorio de Neurobiología, Instituto de Investigación Médica Mercedes y Martín Ferreyra (INIMEC-CONICET- UNC), Universidad Nacional de Córdoba, Córdoba, Argentina.
lallende@immf.uncor.edu

The brain stands out as the body's most cholesterol-abundant organ, accounting for approximately 25% of the total cholesterol present in the body. Within neurons, cholesterol's significance is underscored by its pivotal role in facilitating neurite growth, synaptogenesis, and the optimal functioning of both pre and post-synaptic compartments. As a result, precise regulation of cholesterol homeostasis within the brain is imperative to avoid potential imbalances that could significantly impair brain performance.
Prior findings from our research group have strongly suggested a connection between the decline in neuronal cholesterol levels during the aging process and the emergence of cognitive impairments. Given the impermeability of the blood-brain barrier to cholesterol, the maintenance of brain cholesterol equilibrium relies heavily on de novo synthesis, primarily orchestrated by glial cells. Fully developed neurons predominantly rely on cholesterol synthesized by astrocytes, which is subsequently transported in the form of ApoE-Cholesterol complexes. In the scope of this study, we present evidence indicating that the aging process triggers an increase in *miR33* levels. This, in turn, instigates a Niemann-Pick

phenotype within aging astrocytes, leading to the accumulation of cholesterol within lysosomal compartments. Moreover, utilizing astrocyte-neuron co-cultures, we have ascertained that the transfer of cholesterol from astrocytes to neurons becomes compromised in vitro-aged astrocytes. By immunofluorescence and TIRF assays we show that the buildup of cholesterol within aged astrocytes can be mitigated by cannabinoid treatment through a mechanism independent of the CB1 receptor. Accordingly, we found that the cholesterol transport from aged astrocytes to neurons could be improved by cannabinoids.
It is our belief that comprehending these underlying mechanisms will pave the way for the discovery of novel therapeutic targets or preventive strategies against central nervous system disorders associated with the aging process.

Keywords: Aging, Cholesterol, Cannabidiol, miR33, NPC1
Methods: Co-culture, Immunofluorescence, Confocal Microscopy, qPCR, Western Blot

LI-02

THE IMPORTANCE OF GLYCOSPHINGOLIPID SYNTHESIS IN MDCK 3D EPITHELIAL MORPHOGENESIS AND PROPER PRIMARY CILIUM DEVELOPMENT

Labrousse, Mariano Eliseo; Mosca, Jazmín María, Krivocapich, Clara; Pescio, Lucila Gisele
Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. IQUIFIB – CONICET
pesciolucila@gmail.com

Madin-Darby canine kidney (MDCK) cells are a well-established cell line model for studying epithelial morphogenesis. MDCK cells can be cultured in 2D and 3D conditions, rendering, in both cases, apico-basolateral polarity. However, evidence shows that 3D culture is a better model to study morphogenesis, forming cysts with a central lumen surrounded by a single layer of polarized cells. Instead, 2D cultures form monolayers of polarized cells, without lumenogenesis. Previous results from our laboratory showed that glycosphingolipids have a central role in MDCK cell differentiation in 2D cultures. In this study, we describe a method to obtain MDCK cell cysts on a collagen matrix and analyze the effect of glycosphingolipid depletion in epithelial morphogenesis. We monitor the 3D cystogenesis by bright-field microscopy. The time course showed three stages: lumen establishing, lumen enlarging, and lumen maintenance. We hypothesize that inhibition of glycosphingolipid synthesis affects epithelial morphogenesis and primary cilium development. Since lumen generation occurs on day 4, we treated 3D cultures with D-PDMP, an inhibitor of glucosylceramide synthase, on day 3 and performed immunofluorescence assays on day 9. Labeling of the actin cytoskeleton with phalloidin-FITC showed cysts with normal lumen: a monolayer of polarized cells surrounding an open central lumen, but multiluminal, atypical lumen, no lumen, and small cysts were also found. Most of the cysts presented a

normal lumen both in control and treated cells, but D-PDMP-treated cells showed a higher percentage of small cysts. An important stage in the polarization process is the formation of primary cilia from the apical membrane projecting into the lumen. Immunofluorescence for acetylated tubulin allowed primary cilia detection and length analysis when observed by confocal scanning microscopy. Mostly of the cysts with normal lumen showed primary cilia extending towards the lumen, but D-PDMP-treated cells displayed longer cilia than control cells. Previous studies indicated that non–fully differentiated cells have longer primary cilium when compared to fully differentiated cells, proposing that cilia length reflects the cell status. Our findings suggest that glycosphingolipids are essential for MDCK 3D epithelial morphogenesis and proper primary cilium development.

Keywords: SPHINGOLIPIDS, 3D CULTURES, MDCK CYSTS, PRIMARY CILIUM, EPITHELIAL MORPHOGENESIS
Methods: 3D Cell culture, Immunofluorescence, Confocal microscopy

LI-03

SPHINGOSINE KINASE 2 (SK2) EXPRESSION AND LOCALIZATION IS ESSENTIAL IN THE EPITHELIAL-MESENCHIMAL TRANSITION OF RENAL EPITHELIAL CELLS

Palavecino, Agustín; Loiácono Germán; Romero, Daniela; Pescio, Lucila G; Favale, Nicolas O
Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, IQUIFIB-CONICET, Argentina.
nofavale@gmail.com

Sphingosine-1-phosphate (S1P) is a bioactive lipid formed by the action of sphingosine kinases 1 and 2 (SK1 and SK2), which can modulate physiological processes such as cellular differentiation and de-differentiation. The de-differentiation process of epithelial cells is known as epithelial-mesenchymal transition (EMT). 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 showed that fully differentiated Madin-Darby canine kidney (MDCK) cells at the wound edge can undergo EMT during wound healing to acquire their migratory profile by the activation of the S1P specific receptor 2 (S1PR2)/ERK1/2 pathway. However, less is known about the SK isoform involved in S1P production and EMT activation. Therefore, we used our study model to establish which SK isoforms participate in the de-differentiation and migration process. We found that the pharmacological inhibition of SK2 prevents changes in EMT markers, such as actin cytoskeleton rearrangement and adherent junction (AJ) disassembly (E-cadherin/beta and alpha catenin plasma membrane co-distribution). Then we evaluated SK2 expression and subcellular localization. We found an increase in SK2 expression in cells adjacent to the wound edge (N zone cells - first ten rows of cells adjacent to the wound) as compared to cells away from the wound edge (F zone cells – ten to fifteen rows of cells adjacent to the N zone). We also observed that SK2 was localized in the

Golgi apparatus in the F zone but acquired a vesicle-like distribution compatible with lipid droplets in the N zone. We further evaluated the SK1 effect on EMT and found that SK1 inhibition also prevented changes in EMT markers, as observed for SK2 inhibition. Interestingly, we found that SK1 inhibition blocked SK2 redistribution, which suggests SK1 involvement in SK2 mobilization. Similar results were observed when S1PR2 was inhibited. These results suggest that the relocalization of SK2 is a central event in EMT and depends on previous S1PR2 activation by S1P synthesis by SK1. These findings highlight the versatility and complexity of sphingolipids in cellular fate determination.

Keywords: Epithelial-mesenchymal transition, Sphingosine-1-phosphate, Sphingosine kinase 2, Epithelial renal cells
Methods: Fluorescent microscopy, Cell culture, Fluorescent proteins, Western Blot

LI-04

EXPRESSION OF GENES INVOLVED IN TESTOSTERONE AND 17β-ESTRADIOL PRODUCTION IN EX VIVO CULTURED PREPUBERAL MOUSE TESTES

Tajes Ardanaz, Oliverio Julián; Luquez, Jessica Mariela; Sanchez Chaves, Manuela; Oresti, Gerardo Martín.
INIBIBB, CONICET-UNS y Dpto. Biología, Bioquímica y Farmacia, UNS, Bahía Blanca, Argentina.
gmoresti@criba.edu.ar

A highly efficient testicular culture system involving a gas-liquid interface has emerged. This method has demonstrated the ability, although a low rate, to differentiate male germ cells from neonatal spermatogonia to haploid cells. However, the model requires thorough examination to facilitate forthcoming experimental approaches in physiological and toxicological reproductive studies. Sequential male germ cell proliferation and differentiation requires the production and the action of steroid hormones. Consequently, our aims were to record production levels of testosterone (Tes) and 17β-estradiol (E2) and to examine the expression of genes encoding proteins that are involved in such hormone production in *ex vivo* cultured testes. The mRNA expression patterns of 17β-Hydroxysteroid dehydrogenase 1 (*17β-Hsd1*), 3β-Hydroxysteroid dehydrogenase (*3β-Hsd1*), Sex hormone binding globulin (*Shbg*) and Cytochrome P450-Aromatase (*Cyp19a1*) were followed in 6.5 days old C57BL/6J mouse testis explants cultured during 44 days and were compared to those recorded during in vivo

development. Notoriously, *17β-Hsd1* and *3β-Hsd1* had a similar expression pattern *ex vivo vs. in vivo*, but the levels of Tes decreased from 5 to 20 days in culture and thereafter remained low. Concomitantly, the mRNA expression of *Shbg* was up-regulated with time in the explants, linked to the low Tes production. Finally, as *in vivo*, *Cyp19a1* expression tended to increase from days 10 to 30, while the E2 levels recorded from 15 to 20 days were the highest. The latter was associated with the appearance of haploid spermatids. Interestingly, when retinoic acid was added to the media, the levels of Tes were increased and mRNA expression levels of *17β-Hsd1* and *3β-Hsd1* were up-regulated, while those of *Shbg* were down-regulated. Our results demonstrated active production of steroid hormones in the explants and suggest that modulation of these hormones could enhance the *ex vivo* spermatogenesis process. Supported by SGCyT UNS-PGI-UNS [24/B272 to GMO], FONCyT, [PICT2020- 02056 to GMO] and SGCyT UNS-PGI 24/B341 to GMO and JML.

Keywords: Steroid hormone, testosterone, spermatogenesis, estrogen
Methods: qPCR, Microscopy, histology, Eclia

LI-05

DEVELOPING AN ORTHOGONAL SYSTEM FOR PRODUCING TARGETED LIPID COMPOUNDS IN MICROORGANISMS

Jirgl, Lucas; Tripaldi, Matias; Gramajo, Hugo; Arabolaza, Ana.
Instituto de biología molecular y celular de Rosario (IBR-CONICET). Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR)
Jirgl49@gmail.com

Oleochemicals are described as a category of aliphatic compounds industrially derived from oils and waxes, of vegetable or animal origin. These products are classified according to the length of the carbon chain, their reduction state (aldehyde, ester, alcohol, alkane) and their modifications (unsaturation, hydroxylation). These chemical characteristics dictate the value and uses of each oleochemical, which range from biofuels, detergents, lubricants, industrial surfactants, to medicines and personal care products. This diversity of applications, and particularly the production of biodiesel, is driving the continued growth of the oleochemical industry. In particular, the majority of fatty acids (FA) present in commercial vegetable oils are classified as linear and long-chain (≥C16), whereas natural sources of medium-chain lipids (C8-C12) are less common (with the exception of some oils such as coconut and palm kernel oil). In this context, the "low structural diversity" of the FA constituents of common commercial oils gives metabolic engineering an important role to play, generating and providing concrete solutions, not only in terms of the performance of the compounds in their various applications, but also in relation to their renewable and food-independent origin. Industrial

fermentation of microorganisms using sustainable substrates is therefore a viable and attractive alternative. Specifically, with regard to the production of oleochemicals in microorganisms, progress has been made mainly in the discovery of metabolic pathways involved in lipid synthesis, in the elucidation of key regulatory points controlling FA biosynthesis in model hosts, and in the successful demonstration of metabolic engineering strategies to produce, for example, alkanes, methyl ketones and FAMEs (fatty acid methyl esters).
At present, we are focusing on generating a partially orthogonal pathway capable of producing MCFA using selected heterologous enzymes that work in parallel with the fatty acid synthase pathway from the bacterium's central metabolism. Initial results have been very promising, showing that these enzymes have an extraordinary ability to produce significant amounts of fatty acids with variations in chain length of 8, 10, 12 and 14 carbon atoms.
Specifically, we have achieved a cumulative production of hydroxylated fatty acids (HFA) of 477.79 mg/L from a total fatty acid pool of 821.63 mg/L, representing 58.15% of the FA content.
These results represent a significant advancement in our research efforts and provide valuable insight into the potential for acquiring specific fatty acids through the implementation of this novel strategy.

Keywords: THIOESTERASE; LIPIDS; OLEOCHEMICALS; MEDIUM CHAIN FATTY ACIDS
Methods: Lipid extraction and visualization by TLC (thin-layer chromatography); Preparation of lipidic samples for GCMS (Gas chromatography coupled to mass spectrometry); Protein expression and visualization by SDS-Page; Cloning techniques using restriction enzymes;

LI-06

EFFECTS OF RETINOIC ACID ON TESTICULAR LIPIDS WITH POLYUNSATURATED FATTY ACID DURING *EX VIVO* TISSUE MAINTENANCE

Sanchez Chaves, Manuela; Luquez, Jessica Mariela; Tajes Ardanaz, Oliverio Julián; Oresti, Gerardo Martín.
INIBIBB, CONICET-UNS y Dpto. Biología, Bioquímica y Farmacia, UNS, Bahía Blanca, Argentina.
gmoresti@criba.edu.ar

In vitro spermatogenesis has been successfully achieved by utilizing a gas-liquid interphase culture system. Within this experimental framework, monitoring of lipid metabolism provides a deeper understanding of its significance within the spermatogenic process, thereby yielding valuable insights that could be applied to *ex vivo* spermatogenesis biotechnology. It is known that retinoic acid (RA) is required for spermatogonia differentiation, to complete the meiotic divisions in spermatocytes and to support the full development of spermatogenic cells into elongated spermatids. The molecular mechanism by which RA enhances the spermatogenesis process remains in part unknown. Here, we

evaluated the effects of RA supplementation on testicular lipids with long-chain (C18-C22) polyunsaturated fatty acids (PUFA) during *ex vivo* spermatogenesis progression in prepuberal mouse testicular explants. An analysis of spermatogenic cell types in testis explants from 6-day-old mice cultured for 22 days revealed progress in the differentiation of spermatogonial stem cells to haploid round spermatids. After 44 days in culture, some sperm were detected. Notably, the incidence of spermatozoa per unit of tissue was higher in the presence of RA than in its absence. In addition, the explants exposed to RA had lower amounts of neutral lipids, especially triacylglycerols, than those cultured in basal medium. Moreover, the addition of RA led to a decrease in the proportion of uncommon PUFAs (20:3n-9 and 22:4n-9), which we had previously observed as accumulating in neutral lipids from explants cultured under basal conditions. Simultaneously, similar to what occurs *in vivo*, membrane glycerophospholipids and cholesterol esters increased their proportion of C20-C22 n-6 PUFA. These lipids changes were linked to an augmentation in testosterone production and an increase in the haploid cell in the explants supplemented with RA. The similarity between these lipid changes and those observed in vivo during normal testicular posnatal development underscores the pivotal role of RA in optimizing *ex vivo* spermatogenesis. Supported by SGCyT UNS-PGI-UNS [24/B272 to GMO], CONICET [PIP11220210100420CO to GMO] FONCyT, [PICT2020- 02056 to GMO] and SGCyT UNS-PGI 24/B341 to GMO and JML.

Keywords: PUFA, phospholipids, neutral lipids, retinoic acid, spermatogenesis
Methods: Gas chromatography, TLC, histology, Eclia, Tissue culture

LI-07

EFFECT OF POLYUNSATURATED FATTY ACIDS ON THE EXPRESSION OF TRP53 IN THE DEVELOPMENT OF TONGUE TUMORIGENESIS IN MICE

Don, Julieta [1,3]; Ferrero, Victoria [1,2]; Mazo, Tamara [1,2]; Bernárdez, Sol [3]; Diaz, Verónica [1,2]; Baroto, Nelso [2]; Brunotto, Mabel [1,3]; Pasqualini, María Eugenia [1,2].
[1] Instituto de Investigaciones en Ciencias de la Salud (INICSA-CONICET) - Facultad de Ciencias Médicas, Universidad Nacional de Córdoba. [2] Cátedra de Biología Celular, Histología y Embriología, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba. [3] Departamento de Biología Bucal, Facultad de Odontología, Universidad Nacional de Córdoba.
julieta.don@unc.edu.ar

Oral cancers have a low survival rate and an important impact on the life quality of patients and are associated with sociocultural health determinants such as diet. The objective was to evaluate the modulating effect of polyunsaturated fatty acids (PUFAs) (ω-3 and ω-6) on the *TRP53* expression of a tongue squamous cell carcinoma development, induced by a carcinogenic agent using murine experimental model. The generation of this knowledge may constitute a tool that can improve

prevention strategies and early diagnosis of this disease. Balb-c mice (n=72) were fed with a semisynthetic diet enriched with corn oil (Control, CM, high ω-6), or with fish oil (CP, high ω-3) or Control or Fish oil diets with DMBA (carcinogen). (Four groups were established: Control (CM)(n=12), fish control (CP) (n=12), CM + DMBA (DM)(n=24), and CP+ DMBA (DP (n=24). Tumor induction was performed by tongue topication with DMBA/formaldehyde (initiator/promoter), three times/week and at different periods: 30, 60, and 90 days. Animals were sacrificed and tongues were collected. We analyzed: histopathology (H/E stained), membrane cell lipid profile (by Gas Chromatography), DNA isolation and *TRP53* codon 270 identification by PCR allele-specific. Experiments were repeated at least three times and analyzed by ANOVA, p-value<0.05 will be established for statistical significance.
Higher degrees of hyperkeratosis, hyperplasia, and mitotic figures, especially in sample tongues derived from CM and CM + DMBA groups were observed in comparison to CP and CP + DMBA groups at 60 and 90 days. Cell membrane lipid profile from (CP) and (CP+ DMBA) groups presented significantly increment in ω-3 (EPA,20:5) at 60 days (p=0.0375) and 90 days p=0.0386); (DPA, 22:5) at 30 days (p=0.0384) and 90 days (p=0.0347) and (DHA, 22:6) at 30 days (p=0.0361) and 90 days (p= 0.0080)). The omega-6 PUFAs (LA, 18:2) and (AA, 20:4), were increased in CM and CM + DMBA with respect to CP and CP +DMBA groups. The percentage of *Trp53R270H* mutated variant was higher (25%) in CM + DMBA in relation to the CP+DMBA (16.67%) group.
The ω-3 PUFAs could prevent the DMBA mutagenic effect on the *TRP53* in this tongue squamous cell carcinoma experimental model.

Keywords: PUFAs, TRP53, tongue tumorigenesis
Methods: Gas Chromatography, DNA isolation, PCR alelle specific

LI-08

DURING POSTNATAL RENAL DEVELOPMENT, SPHINGOSINE 1-PHOSPHATE ACTS EITHER INTRACELLULARLY OR EXTRACELLULARLY IN COLLECTING DUCT CELLS DEPENDING ON THEIR DIFFERENTIATION STATE

Guaytima, Edith del Valle (1); Favale, Nicolás Octavio (2); Sirerol Rosales, Facundo Marcelo (1); Romero, Juan Victor (1); Carrizo, Julieta Malen (1); Sterin-Speziale, Norma Beatriz (2, 3); Márquez, María Gabriela (1)
(1) Instituto de Investigaciones en Ciencias de la Salud Humana, Universidad Nacional de La Rioja, Argentina. (2) Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, IQUIFIB-CONICET, Argentina. (3) Laboratorio Nacional de Investigación y Servicios de Péptidos y Proteínas - Espectrometría de Masa (LANAIS PROEM).
eguaytima@unlar.edu.ar

Renal development is a complex event that is completed postnatally in mammals. A characteristic of mammalian kidneys is the hypertonicity of the interstitial fluid in the renal papilla, which during postnatal development induces and maintains the differentiation of collecting duct (CD) cells. Previously, our group has demonstrated that regulation of sphingosine kinase (SK) expression and activity leads to sphingosine 1-phosphate (S1P) formation in the neonatal period, consistent with the immature-proliferative stage of neonatal papilla CD cells. S1P can act intracellularly (iS1P) as a second messenger or extracellularly (eS1P) as a ligand of five different G protein-coupled receptors (S1PR1-5). Thus, the aim of our current work is to deepen the understanding regarding the involvement of SK/S1P pathway in CD cell differentiation induced by external hypertonicity (HT), during postnatal development. We performed primary cultures of papillary CD cells isolated from 10-day-old rats with 10% fetal calf serum, which contains S1P. To mimic the physiological condition, cells were subjected to gradual increases of NaCl concentration. Treatment with D,L-threo-dihydrosphingosine (t-DHS), an SK activity inhibitor, and pharmacological inhibition of S1PR1, 2 and 3 were performed. In primary developing CD cell cultures, different populations of cells regarding their differentiation state were found. We observed that, to initiate the differentiation process induced by HT, CD cells need the activity of SK- that is iS1P-, since cells subjected to HT in the presence of t-DHS exhibited impairment in cell-cell adhesion structures. In this experimental condition, cell death was observed in areas where more immature cells were present. When cells were exposed to t-DHS after having been subjected to HT condition, their characteristic differentiated epithelial phenotype was preserved, suggesting that CD cells do not need iS1P once their differentiated state was acquired. On the other hand, cells subjected to HT in the presence of the S1PR1-3 antagonists exhibited a more differentiated epithelial phenotype. Interestingly, the presence of circular cellular structures containing central apoptotic cells were also observed, surrounded by cells that do not express epithelial cell markers such as ZO-1, α- and β-catenin, and E-cadherin. These structures might constitute the initial event of the tubular lumen formation, although it cannot be accomplished in the 2D culture conditions. These last results suggest that CD cells need to desensitize S1PR1-3 to achieve a more differentiated state induced by HT, being eS1P not necessary. Therefore, during the postnatal development S1P could act intracellularly or extracellularly, depending on the differentiation state of CD cells. Altogether, S1P might modulate through several cellular events the formation of the epithelial cell sheet, that finally would give rise to the renal papilla collecting ducts.

Keywords: renal development, collecting duct, hypertonicity, sphingosine 1-phosphate
Methods: primary cell culture, immunofluorescence, confocal microscopy, imaging and data processing.

LI-09

SEARCH FOR NEW INSECTICIDES AND ANTIPARASITICS THROUGH THE INHIBITION OF A SPECIFIC STEROL DESATURASE

Puca, Gervasio; Alonso, Leonardo; Nusblat, Alejandro
Instituto de Nanobiotecnología (UBA-CONICET)
gervasio.puca@gmail.com

The C7 sterol desaturase is a Rieske-type monooxygenase initially described in insects and nematodes. The enzyme is involved in the synthesis of steroid hormones and plays a crucial role in the embryonic and larval development of the mentioned organisms. Orthologs have been found in other animals but not in mammals. Due to its role in the development of insects and nematodes of health and agriculture relevance, and its absence in mammals, C7 sterol desaturase could be used as a target for the development of new, specific insecticides and antiparasitic agents. These compounds would act by inhibiting the enzyme, leading to the arrest of early development and subsequent death of the target organisms. However, the 3D structure and mechanism of action of the enzyme are unknown, making its characterization necessary for the rational development of inhibitors.

The objectives of this study were to express and purify the C7 sterol desaturase for the subsequent determination of its conformational characteristics and to evaluate potential enzyme inhibitors. To achieve this, the ciliated model organism *Tetrahymena thermophila*, which possesses an ortholog of C7 sterol desaturase, was used.

The enzyme was expressed in the ciliate fused to a tag for subsequent purification by affinity chromatography, for which a recombinant strain with this tag was generated. For the expression of the enzyme in *E. coli*, gene constructs were generated, optimizing codon usage frequency. Additionally, the sequence of the Maltose Binding Protein was added to the constructs to facilitate purification via affinity chromatography. As a result, the enzyme was successfully expressed in both systems and obtained with high purity.

Furthermore, to evaluate potential compounds with insecticidal or antiparasitic activity, compounds were selected based on similarity to the substrate and through an updated literature search in various scientific and patent databases. The determination of in vivo inhibitory activity was carried out using *T. thermophila*. Ten compounds with potential inhibitory activity on the enzyme were evaluated, none of which showed a significant difference in C7 sterol desaturase activity compared to the control.

In conclusion, this study laid the groundwork for the development of new insecticides and antiparasitic agents targeting the inhibition of C7 sterol desaturase. An in vivo system for determining enzyme activity and inhibition was developed and optimized, and a recombinant protein production system was generated. This advancements will allow for further testing of potential inhibitors, as well as the determination of the protein's conformation and the prediction of the structure of the enzyme-substrate complex for the rational identification of inhibitors.

Keywords: antiparasitic, insecticide, sterol desaturase, Tetrahymena
Methods: affinity chromatography, cell culture, HPLC, molecular cloning

LI-10

ACYL-COA SYNTHETASE 4 EXPRESSION MODIFIES microRNA EXPRESSION PROFILE IN BREAST CANCER CELL LINE

Quevedo, Luciano [2]; Seminara Cunto, Zoe [1,2]; Bigi, Mercedes [2]; Prada, Jesica [2]; Maloberti, Paula [1,2]; Yang, Seong Wook [3]; Castillo, Ana Fernanda [1,2]

[1]Universidad de Buenos Aires, Facultad de Medicina, Departamento de Bioquímica Humana. Buenos Aires, Argentina. [2]CONICET-Universidad de Buenos Aires, Instituto de Investigaciones Biomédicas (INBIOMED). Buenos Aires, Argentina. [3]Department of Systems Biology, Institute of Life Science and Biotechnology, Yonsei University. Seoul, Korea.

castillofernanda@yahoo.com

Acyl-CoA synthetases are essential enzymes that activate fatty acids by converting them to acyl-CoA esters. Particularly, acyl-CoA synthetase 4 (ACSL4) catalyzes the esterification of long-chain fatty acids, with a preference for arachidonic acid as a substrate. ACSL4 expression is increased in breast, prostate, colon, and hepatocellular carcinomas. Its expression levels correlate with the phenotype of breast and prostate cancer cells. Previous results have shown that an increase in ACSL4 expression leads to the induction of a highly aggressive phenotype of breast and prostate cancer cells and to the growth of breast tumors in vivo.

It is relevant to know which molecular events are triggered by this enzyme to change the normal cellular phenotype to a highly aggressive one. We focused on the study of microRNAs (miRNAs) as mediators of its effects on the tumor phenotype. miRNAs are small non-coding RNAs that carry out the silencing of target genes by targeting mRNAs inducing their transcriptional repression. Their involvement in cancer has been demonstrated, and their expression patterns are associated with tumor type and grade. Using large-scale mRNA sequencing (RNA-Seq) data, we have preliminarily shown that ACSL4 expression alters the expression of several miRNA precursor transcripts. In this study, we investigated the expression of functional and mature miRNAs in response to changes in ACSL4 expression. We performed mature miRNA sequencing (miR-Seq) using an MCF-7 breast cancer cell model in which ACSL4 was stably transfected under the repressible Tet-Off system (MCF-7 Tet-Off/ACSL4). The differential expression profile was analyzed comparing ACSL4-overexpressing cells with control cells using a cutoff of Log2 fold change > |1|. This doxycycline-repressable overexpression system allowed us to adjust the miRNA profile to those miRNAs for which decreasing ACSL4 levels in MCF-7 Tet-Off/ACSL4 resulted in an expression pattern similar to that of control cells. Thus, we selected those miRNAs whose levels were specifically altered by the expression of the enzyme. A total of 56 differentially expressed miRNAs were obtained, of which only 3 miRNAs were found to be upregulated by ACSL4. Based on the miRNA signature obtained, a

predictive analysis was performed for each miRNA regarding its role in biological pathways, tissue expression, physiological conditions or related pathologies in which it might be involved. For this purpose, databases such as KEGG Pathway, WikiPathways and miRPathDB were used. Several miRNAs were selected for further validation of their expression by RT-qPCR, considering not only their differential expression levels, but also the information provided by the databases.
Therefore, we describe and validate the profile of miRNAs whose expression levels are modified by ACSL4. These results suggest that these small RNAs may be involved in mediating the effects of this enzyme on the cellular phenotype of breast cancer cells.

Keywords: acyl.CoA synthetase, microRNA, miR-Seq, expression profile, breast cancer cells
Methods: protein overexpression, miR-Seq, bioinformatic analysis, RT-qPCR

LI-11

Capsaspora owczarzaki SYNTHESIZES ARACHIDONIC ACID BY THE ALTERNATIVE "D8" PATHWAY.

Cassidy, Belkis M; Costa, Joaquín; Altabe, Silvia G; Uttaro, Antonio D.
Instituto de Biología Molecular y Celular de Rosario (IBR)-CONICET, Dpto. Microbiología, FBIOyF, UNR
uttaro@ibr-conicet.gov.ar

Capsaspora owczarzaki belongs to the clade *Filozoa* (*Filasterea, Choanoflagellata* and *Metazoa*) and is one of the closest unicellular relatives of animals. Its life cycle alternates between filopodial (adherent), aggregative, and cystic stages. The analysis of the fatty acid profile of each stage revealed a progressive increase from the filopodial to the cystic stage, in polyunsaturated fatty acids (PUFA) of 18 and 20 carbons in length, mainly arachidonic acid (20:4D5,8,11,14). PUFAs are synthesized by the action of enzymes currently called desaturases and elongases. The canonical pathway for arachidonic acid synthesis involves desaturation of linoleic acid to gamma linolenic acid (18:3D6,9,12) by a D6 desaturase, elongation to 20:3D8,11,14 PUFA, and the action of a D5 desaturase. Few organisms have a variation of the canonical pathway, where linoleic acid is first elongated to 20:2D11,14 and then unsaturated by two consecutive enzymes with D8 and D5 desaturase activities. This alternative route, termed the "D8" or "*Euglena*" pathway, was previously described in *Euglena gracilis* and was suggested to also be present in *Isochrysis sp.* and *Acanthamoeba sp.*, where it was partially characterized. A BLAST search on the *C. owczarzaki* genome revealed the presence of numerous putative desaturases. Two of them were selected based on structural and sequence similarities with enzymes of the canonical pathway. Unexpectedly, CAOG_004984 was characterized as D8 desaturase, after expression in yeast, using 20:2D11,14 as a substrate, while CAOG_006169 had D5 desaturase activity, capable of utilizing

various unsaturated substrates. These findings add *Capsaspora sp.* to the very short list of unrelated microorganisms that have the alternative "*Euglena*" pathway. The presence of this route in a close relative of mammals is discussed in the framework of the suggested existence of D8 desaturases in some human tissues and tumor cells, not yet conclusively determined.

Keywords: Fatty acids, Desaturases, Arachidonic acid, Biosynthesis
Methods: Expression in yeast, Lipid analysis, Gas chromatography-mass spectrometry, Bioinformatics.

Enzymes

EN-01

Functional characterization of a novel O-glycoside phosphorylase in *Nostoc* sp. 7120 involved in sucroglucan metabolism

Giarrocco, Laura E 1; Pérez, Rebeca 2; Salerno, Graciela L 3
1 FIBA and CIC Prov. Buenos Aires; 2 Departamento de Bioquímica e Biologia Molecular, UFPR, Curitiba Brasil; 3 FIBA, Universidad Nacional de Mar del Plata and CONICET
glsalerno@gmail.com

The accumulation of compatible solutes is one of the physiological responses for the adaptation of cyanobacteria to abiotic stress. In filamentous diazotrophic cyanobacteria we have previously described the synthesis of sucrose and sucroglucans (series of glucose polymers derived from sucrose) in response to salt stress. We also demonstrated that the *Nostoc* genes *all1058* and *all1059* are organized into a transcriptional unit and participate in sucroglucan metabolism.
In this study, we first analyzed the phenotype of Nostoc mutants in genes involved in the synthesis of sucrose and sucroglucan (susA-, susB- sense and antisense, and susAB-), which have different levels of sucrose and sucroglucan in response to abiotic stress. The results show a differential role of sucrose and of the oligosaccharides after a salt treatment and under desiccation.
A phylogenetic analysis revealed that *all1058* sequence clusters with GH65, a family of inverting phosphorylases that act on alpha-glucosides to produce beta-glucose 1-phosphate. To biochemical identify the *all1058* protein product, we generate the recombinant protein in *E. coli* which was purified through a Talon metal affinity resin. To assay enzymatic activity, we purified sucroglucan oligosaccharides (degree of polymerization n=1 to 5) from salt-stressed *Nostoc* cells by Bio-Gel P2 chromatography to use them as substrates. Enzyme activity determination was performed incubating 2.5 mM of the tetrasaccharide (n=2,

alpha-D-glucopyranosyl-(1-->2)-[alpha-D-glucopyranosyl-(1-->2)]2-beta-D-fructofuranoside) as substrate, 10 mM MgCl2 and 10 mM inorganic phosphate, pH 7.5, at 30ºC. The products of the reaction are the trisaccharide (n=1) and beta-glucose-1P, which was quantified at OD 340nm, after incubating with auxiliary enzymes (beta- phosphoglucomutase and glucose-6-phosphate dehydrogenase). From our results, we can report on a new enzyme and propose that it be called sucroglucan phosphorylase and that the accumulation of sucroglucan allows for better coping with desiccation than with salt stress. Supported by UNMdP EXA 1142/23, CIC and FIBA.

Keywords: Cyanobacteria, salt stress, sucroglucan metabolism, O-glycoside phosphorylase

Methods: Recombinant protein expression, oligosaccharide purification, cyanobacteria mutation, enzyme activity prediction

EN-02

Physiological implications of substrate/product regulation of ferredoxin-NADP(H) reductase in bacteria.

Don, Carla del Rosario; Petrocelli, Silvana; Ceccarelli, Eduardo A. and Catalano Dupuy, Daniela L.
Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET-UNR.
dcatalano@gmail.com

Ferredoxin-NADP+ reductases (FNRs) are flavoenzymes involved in redox metabolisms. FNRs catalyze the reversible electron transfer between NADP(H) and ferredoxin, flavodoxin, or heme oxygenase. The plastidic FNRs show much higher exchange rates than bacterial enzymes. These differences were attributed to their physiological role. In bacteria, FPR participates in the response to oxidative stress produced by reactive oxygen species. We have obtained evidence that *Escherichia coli* FNR (EcFPR) contains NADP+ tightly bound after isolation. This binding produces inhibition of the catalytic activity that disappears when the nucleotide is released from the enzyme. Three arginines (R144, R174 and R184) are involved in a high affinity and structured site responsible for this binding and they are absent in plastidic FNRs. We obtained EcFPR variants with replacements in these arginine residues and they lost the aforementioned catalytic regulation. Bacteria need to finely regulate the homeostasis of the NADP(H) pool, enabling them to appropriately respond to stress conditions, so we proposed that this tight NADP+ binding could be involved in this NADPH/NADP+ regulation. In this work, we studied the implications of the loss of EcFPR regulation by NADP+, especially in the response to oxidative stress. The functionality of the different previously obtained EcFPR variants in the response to oxidative stress was

tested by cross-complementation of an *E. coli* fpr mutant (RR6A) which exhibits a high susceptibility to the effect of methyl viologen (MV), a superoxide-generating agent. Bacterial resistance to MV was evaluated by the disk diffusion method in both M9 and LB medium. Cell survival to MV treatment in liquid LB cultures was tested by subsequent colony count on LB plates supplemented with MV. Our results showed that RR6A complemented with a recombinant FPR exhibited similar behavior to the wild-type strain with MV treatments, while RR6A complemented with the different EcFPR variants responded like the fpr mutant strain. These observations suggest that the loss of FPR regulation by NADP+ has relevant implications in the response to oxidative stress in bacteria.

Keywords: Ferredoxin-NADP(H) reductase, *Escherichia coli*, oxidative stress, catalytic regulation

Methods: Cloning, mutagenesis, bacterial cultures, cell survival, disk diffusion method

EN-03

Structural and kinetic characterization of SdAmyUS100 alpha amylase, a chimeric enzyme from *S. degradans* and *B. stearothermophilus*

Natael M. Wayllace; María V. Busi and Diego F. Gomez-Casati
Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI-CONICET) – Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR)
wayllace@cefobi-conicet.gov.ar

Saccharophagus degradans is a gram-negative marine bacterium. It is the most versatile bacterium in terms of the degradation of complex polymers (CP) found to date. The high rate of degradation of different polysaccharides that this bacterium has makes it a candidate to obtain and investigate the properties of the enzymes that degrade these polymers. In previous studies, we identified and characterized an alpha amylase from *S. degradans* (SdAmy). The enzyme is composed mainly by a N-terminal catalytic domain (CD), a central AamyC domain (AamyC) and a carbohydrate-binding module family 20 at the C-terminal (CBM20). The enzyme showed maximum activity at 40°C and pH 5.0. One of the most widespread uses of alpha amylases is in the food industry. The main commercial application of these enzymes is in the starch industry. Alpha amylases catalyze the liquefaction of starch to produce maltodextrins for use in food processing and many fermentation processes. This process is carried out at temperatures close to 100°C, therefore thermostable alpha amylases are used. One of the most used enzymes is the alpha amylase from *B. stearothermophilus* (US100) with an

optimal temperature of 80ºC and high thermostability at 90ºC. In this work, we have carried out the construction of a chimeric enzyme using the catalytic domain of SdAmy and the C-terminal domain of the US100 amylase from *B. stearothermophilus*. The chimeric enzyme, named SdAmyUS100 was successfully expressed in *Escherichia coli* BL21 DE3 cells. The activity was confirmed by assays with soluble potato starch as a substrate. We expect that this chimeric enzyme will possess interesting kinetic characteristics such as greater thermostability compared to SdAmy.

Keywords: amylases, starch, *Saccharophagus degradans*, *B. stearothermophilus*

Methods: enzyme biochemistry, Protein Purification, structural bioinformatics

EN-04

In situ substrate generation to characterize the maltosil-transferase GlgE from *Rhodococcus jostii* and its promiscuity towards substrates

Iglesias MJ1; Iglesias AA1; Álvarez HM2; Asencion Diez MD1
1 Instituto de Agrobiotecnología del Litoral (UNL-CONICET), Santa Fe, Argentina. 2 Instituto de Biociencias de la Patagonia (CONICET – UNPSJB)
matias.asencion@gmail.com

In recent years, a new maltosil transferase (EC: 2.4.99.16, GlgE) was discovered in Actinobacteria, leading to the hypothesis of a novel pathway for glycogen synthesis. GlgE elongates a preformed glycogen molecules in two glycosidic moieties using maltose-1P as a substrate, contrasting the classic pathway where glycogen is the acceptor of a single glucose subunit from ADP-glucose (ADP-Glc). Maltose-1P may be synthesized by the maltosil-1P synthase (EC: 2.4.1.342, GlgM) that uses Glc-1P and ADP-Glc as substrates. The NDP-sugar is produced from Glc-1P and ATP by ADP-Glc pyrophosphorylase (EC: 2.7.7.27, GlgC). This is the key step in glycogen synthesis as it is regulated by different effectors related to the main carbon pathway(s) in the organism, setting Glc-1P destiny to glycogen storage. So far, this alternative pathway for glycogen synthesis has only been described in Mycobacteria by the characterization of the GlgM and GlgE enzymes. Further studies on this enzymatic route in other microorganisms are lacking in the main literature, although glgE was predicted to be in 14% of known bacterial genomes. Our previous work reports the characterization of GlgC and GlgM from *Rhodococcus jostii* (*Rjo*GlgC and *Rjo*GlgM). Remarkably, both enzymes were able to use glucosamine-1P (GlcN-1P) alternatively to Glc-1P as a substrate. Here, we present the recombinant obtention, purification and

kinetic characterization of GlgE from *R. jostii* (*Rjo*GlgE), the third step in the alternative pathway for glycogen synthesis from Glc-1P. Enzymatic activity determinations of *Rjo*GlgE were carried out by the maltose-1P *in situ* generation, adding *Rjo*GlgM to a coupled assay containing ADP-Glc, Glc-1P and glycogen in the reaction mixture. This strategy facilitates GlgE studies since maltose-1P is a compound with scarce availability. *Rjo*GlgE demonstrated a high specificity towards glycogen, in contrast to its homologue from *Streptomyces coelicolor*, *Sco*GlgE, which uses different oligo and polysaccharides as aglycons. As we hypothesized, *Rjo*GlgE depicted enzymatic activity when Glc-1P was replaced by GlcN-1P in the reaction mixture, suggesting that *Rjo*GlgM produces a Glc-GlcN-1P disaccharide suitable as a *Rjo*GlgE glycosidic donor to elongate glycogen. Results reinforce the idea that GlgM/GlgE is the major biosynthetic pathway in rhodococcal glycogen metabolism. Besides, enzymatic promiscuity towards glucosamine derivatives suggests that the amino sugar could be stored in a glycogen-like molecule yet to be reported.

Keywords: glucosamine-1P, glucose-1P, glycogen, maltose-1P

Methods: recombinant protein production, cloning, enzyme activity

Structural Biology

SB-01

Incorporation of lanthanides in the sensor/transducer MecR1 protein of *Staphylococcus aureus* to monitor conformational changes

Mihovilcevic, Damila(1,2); Suarez, Irina P(1,2);; Tabares, Leandro(3); Llarrull, Leticia I.(1,2)
1. Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET-UNR . 2. Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario (UNR), Rosario, Argentina. 3.Institut de Biologie Intégrative de la Cellule. Gif-sur-Yvette, Francia
mihovilcevic@ibr-conicet.gov.ar

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a pathogen that poses a worldwide threat. MecR1 is an integral membrane metalloprotease that is involved in the resistance to β-lactams in MRSA. It has been proposed that MecR1 and its homologue BlaR1 (34% identity) share a common signal transduction mechanism. Activation would be triggered by covalent binding of the β-lactam to the extracellular sensor domain of MecR1/BlaR1, giving rise to cleavage of the repressor proteins MecI/BlaI, and to expression of the resistance determinants. Cryo-electron microscopy structures of BlaR1 revealed a

domain-swapped dimer. β-lactam binding would shift the equilibrium to a state that is permissive of efficient cleavage of the repressor, but the nature of the required conformational change in metalloprotease domain is still elusive. In this work, we present a spectroscopic strategy to monitor changes in oligomerization of MecR1 and/or β-lactam-induced conformational changes in the cytoplasmic metalloprotease domain of MecR1. We have purified full-length MecR1 (E205A mutant) as a fusion to Mistic in detergent micelles. Size-exclusion chromatography with multi-angle light scattering (SEC-MALS) experiments showed the presence of monomer and dimer. We also checked that both species bind the fluorescent penicillin Bocillin-FL, showing that the sensor domain was folded. We engineered proteins with a single or double lanthanide binding tag (LBT). LBTs are amino-acid sequences rich in carboxylates with high binding affinity for lanthanides. Addition of Tb(III) to the single and double MecR1-LBT constructs resulted in the characteristic Trp-enhanced luminescence of Tb(III):LBT complex and was indicative of the correct folding of the proteins. Moreover, after binding of Bocillin-FL, we were able to detect Luminescence Resonant Energy Transfer (LRET) between the Tb(III) and the Bodipy moiety of Bocillin-FL. Different from Tb(III), Gd(III):LBT does not give a luminescence signal but it can be used as a spin probe in Electron Paramagnetic Resonance (EPR) experiments. Gd(III):LBT-Mistic-MecR1 EPR spectra were consistent with binding of Gd(III) to the LBTs in all the constructs. We concluded that Mistic-MecR1 is in equilibrium between monomer and dimer. We have corroborated that Tb(III) and Gd(III) bind to the LBTs engineered in Mistic-MecR1, and detected LRET in Bocillin-FL-bound Tb(III):LBT variants. The Tb(III):LBT versions of Mistic-MecR1 will allow us to perform distance measurements between cytoplasmic loops and the Bocillin-FL acylated sensor domain. Using EPR, the distance between two Gd(III) can be determined which will allow us to follow structural changes in the cytosolic domain.

Acknowledgments

Agencia I+D+i for grants PICT-2015-2521 and PICT-2020-3773. CNRS-IEA and University of Paris-Saclay for grants to L.T. and L.I.L. CEBEM and ASacTeI for the fellowships to D.M. I.P.S. and L.I.L. are staff members from CONICET. D.M. is a PhD Fellow of CONICET. We benefited from the Biophysics platform of the I2BC supported by FRISBI and IBISA.

Keywords: Staphylococcus aureus; Resistance antibiotics; Membrane proteins; Conformational changes

Methods: advanced Electronic Paramagnetic Resonance Spectroscopy (EPR); Luminescence Resonant Energy Transfer (LRET), Size-exclusion chromatography with multi-angle light scattering

SB-02

Cryo-EM structure the hemocyanin of Pomacea canaliculata provides insights into its role in the innate immune system

Brola1, Tabata Romina; Chiumiento1, Ignacio Rafael ; Ituarte1, Santiago; Heras1.2, Horacio; Otero Lisandro Horacio3.4; Dreon, Marcos Sebastián1

1. Instituto de Investigaciones Bioquímicas de La Plata "Prof. Dr. Rodolfo R. Brenner", INIBIOLP, CONICET-UNLP; 2 Cátedra de Química Biologica, FCNyM, Universidad Nacional de La Plata; 3. Plataforma Argentina de Biología Estructural y Metabolómica (PLABEM); 4. Instituto de Biotecnología Ambiental y Salud, INBIAS. CONICET - Universidad Nacional de Río Cuarto.

tabatabrola@gmail.com

Hemocyanins are large multifunctional soluble proteins found in the hemolymph of most mollusks and some arthropods. Besides their well-known role as an oxygen transport, they may turn into a phenol oxidase-like enzyme (PO) associated with innate immunity functions. To expand the structural knowledge of gastropod hemocyanins and their derived physiological implications, we studied the molecular structure and the intrinsic and proteolytically induced PO activity of the hemocyanin of Pomacea canaliculata (PcH), a worldwide invasive South American freshwater snail. We performed a single-particle cryo-EM analysis of PcH and obtained a 4.4 Å resolution density map. We constructed an ab initio 3D model by homology modeling using its deduced amino acid sequence, the resulting structure was docked and real-space refined in the Cryo-EM density map, retrieving a cylindrical rearrangement assembled by di-decamers related by D5 symmetry. Each decamer is composed of five antiparallel dimers, with each subunit composed of eight paralogue functional units showing conserved structural features among hemocyanins. The density map resolution allowed us to trace the entire subunit backbone, interaction networks of adjacent subunits, contacts between decamers and to visualize densities corresponding to side chains ascribed to six N-glycosylation sites for each subunit.

We also characterized PcH intrinsic PO activity, tested using catechol and dopamine as substrates, and found that PcH only catalyzed catechol with a KM = 50.5 mM and a Kcat = 2.9 min-1. Furthermore, PO activity increased by limited proteolysis using digestive and bacterial proteases such as trypsin, chymotrypsin and subtilisin. Besides, by an in silico analysis, we identified accessible proteolytic cleavage sites that may initiate the structure remodeling and the induction of PO activity in PcH.

The present study provides a 3D model of PcH with a near-atomic resolution level allowing a deeper comprehension of the inducible PcH PO activity, triggered by structural changes triggered by endogenous and/or exogenous proteases. In addition, our results further support the idea that molluscan hemocyanins may participate as PO in the innate immune system.

Keywords: Hemocyanin, Mollusc, Phenol oxidase, Innate immunity, Cryo-EM

Methods: single-particle Cryo-EM, SEC, Model Building, Enzymology, limited proteolysis

SB-03

Structural analysis of the recognition of β-lactam antibiotics by the histidine kinase VbrK of Vibrio parahaemolyticus

Palanca, Ignacio 1,2; Suarez, Irina P. 1; Lacava, Franco 1,2, Méndez, Luciana 2, Testero, Sebastian A. 2,3, Llarrull, Leticia I. 1,2.

1 Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET-UNR, Rosario, Argentina. 2 Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR, Rosario, Argentina. 3 Instituto de Química Rosario (IQUIR), CONICET-UNR, Rosario, Argentina.

palanca@ibr-conicet.gov.ar

Emergence of β-lactam resistant bacteria represents a public health challenge worldwide. Resistant strains of the Vibrio genus (causing agent of acute intestinal infections) have been reported around the world, constituting a threat to human health. A β-lactam antibiotic resistance system has been recently described in Vibrio parahaemolyticus. The resistance is activated by a two-component system consisting of a membrane histidine kinase (VbrK) and a cytoplasmic response regulator (VbrR). In the presence of a β-lactam antibiotic, the system activates the expression of a CARB β-lactamase. Our objective is to understand the molecular mechanism underlying the antibiotic detection and signal transduction resulting in the resistance. VbrK consists of a signal peptide, a putative periplasmic sensor domain, a transmembrane helix, and a cytoplasmic C-terminal catalytic and ATP-binding (CA) domain, also known as HATPase. The signal detected by the kinase is proposed to be the β-lactam molecule, but this still remains controversial. To study the recognition of β-lactams by VbrK we have optimized the expression and purification of its sensor domain (VbrKSD). We have confirmed the predicted cleavage site of the signal peptide by mass spectrometry. Combining Ellman's assay and 1H STD NMR we described the interplay between the oxidation state of the four cysteine residues of VbrKSD and β-lactam binding. Photoaffinity labeling is a useful technique employed to study noncovalent interactions between protein-ligands and protein-protein complexes. A photoaffinity labeling reagent is a molecule that contains a photoreactive group which produces highly reactive intermediates upon photolysis. The most widely used classes of photoactive functionality include benzophenones (Bp), trifluoromethylphenyldiazirines (Dz), and arylazides (Az), which give rise to diradicals, carbenes and nitrenes by UV irradiation, respectively. These generated intermediates can produce, reactions with neighboring biomolecules, giving stable covalent adducts. To identify the binding region, we have synthesized three ampicillin-derived photoprobes, Dz-Amp, Az-Amp and Bp-Amp. We have employed

Dz-Amp and Az-Amp to covalently modify VbrKSD and we identified the modified peptides by LC-MS/MS. To confirm that the photoprobes activate VbrK, we used the ADP-Glo kit (Promega), which allowed us to detect kinase activity in vesicles containing VbrK. Our results corroborated binding of ampicillin to VbrKSD and localized its binding site.

Acknowledgments

I.P. is a Ph.D. Fellow of Agencia I+D+i. I.P.S., S.A.T. and L.I.L. are staff members from CONICET. We acknowledge Agencia I+D+i for grants PICT-2018-3720 and PICT-2020-3773. We acknowledge Fernanda Villalonga, Sergio Tindiglia and Pablo Duche from IQUIR, Rosario; Andrea Coscia and Alejandro Gago from IBR, Rosario; Germán Rosano and Alejo Cantoia from UEM-IBR, Rosario.

Keywords: histidine kinase, Betalactams, conformation,

Methods: NMR, Mass spectrometry, Ellman assay,

SB-04

Apple snail eggs are a Pandora's box: a novel hemoprotein in the animal kingdom

Pasquevich, María Yanina; Pavía, Ignacio; Bauzá, Letizia; Benítez, Guillermo Ignacio; Heras, Horacio;
Instituto de Investigaciones Bioquímicas de La Plata ¨Prof. Dr. Rodolfo R. Brenner ¨ (INIBIOLP, UNLP-CONICET), La Plata, Buenos Aires, Argentina
mypasquevich@med.unlp.edu.ar

Apple snails are freshwater molluscs of the Pomacea genus that include Pomacea canaliculata and P. maculata two invasive snails that are agricultural pests and nematode parasite vectors. They lay calcareous and pigmented eggs out of water containing a perivitelline fluid with multifunctional proteins (perivitelins) that nourish, defend, and protect the embryo from the surrounding environment. Proteomic studies have shown that eggs have more than 30 proteins, >50 % of either unknown and/or uncharacterized. Some perivitellins have been characterized as the carotenoprotein PV1, an anti-nutritive protein that protects the embryo from solar radiation and whose carotenoids provide antioxidants and a warning coloration to the eggs to deter predators. Our group is focused on classifying, identifying, and studying the structure, functions and evolution of these unknown egg proteins. We seek to understand the snail reproductive strategies from a biochemical perspective and, at the same time, to select some of these new proteins with potential as bioactive compounds to exploit

their application in Biomedicine. In this work, we isolated and characterized a yellowish-colored protein found in the PV3 fraction of eggs.
The protein, hereafter named PmaHP, was purified from P. maculata egg masses, combining differential centrifugation, density gradient ultracentrifugation, SEC, and anion exchange HPLC. PmaHP was then studied by UV-VIS spectrophotometry, electrophoresis, and mass spectrometry. Besides, its sequence was compared with homologs from other mollusks, and putative heme binding sites were predicted.
The absorption spectrum is typical for metalloporphyrins, and ICP-MS analysis revealed the metal was iron. The metalloprotein is resolved in acidic electrophoresis under native conditions at pH 4.3, but not in basic electrophoresis, a characteristic of basic proteins. The protein was positive for heme stain with TMB. A MW 18.2 kDa was calculated and its complete amino acid sequence was identified by MS in the UniProt database. Sequence similarity with other homologous proteins of the genus is 48-52% and the sequence contains up to 5 potential binding sites to the heme group.
We describe the first structural data of a new iron-containing hemoprotein of unknown function other than contributing to the coloration of P. maculata eggs. The pigment is conjugated with a protein forming an extracellular soluble hemoprotein that has no sequence homology with any other hemoprotein from the animal kingdom. More work is underway to better understand its role in the survival and reproductive success of a snail species with large economic, ecological, and health importance.

Keywords: Mollusc, eggs, metalloporphyrin, perivitellin, hemoprotein, color, perivitelline fluid

Methods: Liquid chromatography, spectrophotometry, ICP-MS, acid electrophoresis, mass spectrometry; bioinformatics

SB-05

Protein stability modulates the efficiency of post-translational N-glycosylation

Couto, Paula Monserrat (1); Guardia, Carlos Manuel Alberto (2); Couto, Facundo Luis (3); Labriola, Carlos Alberto (1); Caramelo, Julio Javier (1)
(1) Instituto de Investigaciones Bioquímicas de Buenos Aires- Fundación Instituto Leloir. (2) Reproductive and Developmental Biology Laboratory, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC, 27703, USA (3) Instituto de Biociencias, Biotecnología y Biología Traslacional (iB3), Departamento de Fisiología, Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires
pauliscouto@hotmail.com

N-glycosylation is one of the most a frequent and drastic protein modifications, affecting nearly 25 % of the eukaryotic proteome. This process is carried out by the catalytic subunit (STT3) of the oligosaccharyl transferase complex, which adds a high mannose glycan to the lateral chain of Asn residues within the context Asn-X-Ser/Thr (X ≠ Pro), a motif known as N-glycosylation "sequon". In metazoans, OST has two alternative STT3 subunits: STT3A and STT3B. OST with STT3A is associated with the SEC61 translocon and functions co-translationally. Vacant sequons have another opportunity for glycosylation by OST-carrying STT3B. However, the presence of an N-glycosylation sequon does not guarantee its full occupation by the OST. For this reason, a protein may present a mixture of partially occupied sequons, a phenomenon known as N-glycosylation macroheterogeneity. N-glycosylation efficiency depends on many factors, playing the chemical identity of the sequon a major role. Interestingly, identical sequons can display disparate occupancy levels. Here, we investigate the impact of the thermodynamic stability of acceptor proteins on this process. The occupancy of suboptimal sequons improves as the thermodynamic stability of the protein acceptors decreases. This effect is greatly pronounced in cells expressing only STT3B. Less stable proteins exhibit a slower rate of conformational maturation in vivo, which extends the time window for STT3B to operate. These findings can be explained by a kinetic model that distinguishes between local information within sequons and conformational maturation kinetics in vivo.

Keywords: N-glycosylation, STT3B

Methods: Western blot; Live cell imaging

SB-06

A novel mechanism that regulates the interaction of the autophagy receptor p62/SQSTM1 and the autophagosome protein LC3

Ricardo M. Biondi1, Lucia Alcober-Boquet2,&, Tabea Zang2,&, Larissa Pietsch2,3, Evelyn Suess2, Markus Hartmann4, Ewgenij Proscak3,4, Lissy F.Z. Gross1, Mariana Sacerdoti1, Stefan Zeuzem2, Vladimir V. Rogov4,5, Alejandro E. Leroux1, Albrecht Piiper2

1-IBIOBA-CONICET, Buenos Aires, Argentina; 2- Biomedical Research Laboratory, Medizinische Klinik 1, Universitätsklinik Frankfurt, Germany; 3- German translational cancer network (DKTK), site Frankfurt/Mainz, 60438 Frankfurt, Germany; 4- Institut für Pharmazeutische Chemie, Goethe-Universität

Frankfurt, D-60438 Frankfurt am Main, Germany; 5- Structural Genomics Consortium, Buchmann Institute for Molecular Life Sciences, Goethe University, 60438 Frankfurt, Germany
dabiondi@yahoo.co.uk

Autophagy is a highly conserved cellular process that allows degradation of large macromolecules. p62/SQSTM1 is a key adaptor protein that interacts both with material to be degraded and with LC3 at the autophagosome, enabling degradation of cargos such as protein aggregates, lipid droplets and damaged organelles by selective autophagy. Dysregulation of autophagy contributes to the pathogenesis of many diseases. y. In collaboration with our Frankfurt network of colleagues, we previously conducted a compound library screening and identified a small compound that binds to LC3, disrupting its interaction with the polypeptide LIRtide, a sequence derived from the LC3 Interacting Region (LIR) of p62/SQSTM1 (Hartmann et al., J Med Chem. 2021, 64(7):3720-3746. doi: 10.1021/acs.jmedchem.0c01564). In this study we investigated if the interaction of p62/SQSTM1 with LC3b could be regulated. We purified full-length p62/SQSTM1 and established an in vitro assay that measures the interaction with LC3b. We used the assay to determine the role of the different domains of p62/SQSTM1 in the interaction with LC3. We identified a mechanism of regulation of p62 where the ZZ and the PB1 domains regulate the exposure of the LIR-sequence to enable or inhibit the interaction with LC3b. A mutation to mimic the phosphorylation of a site on the ZZ domain leads to increased interaction with LC3b. Also, a small compound that binds to the ZZ domain enhances interaction with LC3b. We conclude that the exposure of the LIR sequence is negatively regulated by the ZZ domain. We arrived at this conclusion through domain deletion experiments, mutagenesis to disrupt ZZ domain folding, mutagenesis to Glu of a ZZ domain Thr known to become phosphorylated, and further verification using a ZZ-domain binding compound that enhanced the interaction with LC3b. Dysregulation of these mechanisms in p62/SQSTM1 could have implications for diseases where autophagy is affected. In conclusion, our study highlights the regulated nature of p62/SQSTM1 and its ability to modulate the interaction with LC3b through a LIR-sequence Accessibility Mechanism (LAM). Furthermore, our findings suggest the potential for pharmacological modulation of the exposure of LIR, paving the way for future therapeutic strategies.

Keywords: autofagia, SQSTM1; LC3, small molecule
Methods: alphascreen (protein-protein interaction); Temperature stability assays;

SB-07

Combinatorial Assembly of Large Bromodomain Containing Complexes of Trypanosmatids

Rodriguez Araya, Elvio; Serra, Esteban
(1) Instituto de Biología Molecular y Celular de Rosario - IBR; (2) Facultad de Ciencias Bioquímicas y Farmacéuticas - UNR

elvio.rodriguez4@gmail.com

Las vías de acetilación en tripanosomátidos, un grupo de organismo de ramificación temprana, son poco conocidas debido al alto grado de divergencia de las proteínas que las mantienen. Además, virtualmente no existe evidencia estructural sobre los complejos involucrados. Recientemente, Staneva et al sistematizaron el análisis de las proteínas presentes en muchos reguladores de la cromatina putativos, utilizando co-inmunoprecipitación seguido de espectrometría de masas para identificar redes de proteínas. En este trabajo, tomamos estos set de datos, extrajimos el subconjunto de redes que involucran factores con bromodominio (BDF), relevantes para reconocer y transducir la señal de lisinas acetiladas; y computamos todas las interacciones proteína-proteína potenciales del subconjunto utilizando AlphaFold2-multimer y computación de alto rendimiento. Luego realizamos otra ronda de predicción estructural de complejos para ensamblar aquellos trímeros, tetrámeros y pentámeros con alta probabilidad de interaccionar. Utilizando estas predicciones como base, realizamos un ensamblaje secuencial utilizando CombFold para ensamblar los complejos de orden superior. Los resultados mostraron 6 complejos distintos que se ensamblan con diferentes grados de subunidades compartidas. Dos de ellos son complejos parcialmente conservados presentes en humanos y levaduras, el complejo NuA4 y el complejo Swr1, ambos involucrados en la remodelación de la cromatina mediada por acetilación. Otro complejo comparte muchas similitudes estructurales con NuA4, conteniendo varias proteínas parálogas que se ensamblan en un complejo completamente nuevo, pero que no parecen compartir subunidades. Uno de los complejos contiene 4 de los BDFs interactuando juntos, potencialmente desempeñando roles funcionales similares a los de las grandes proteínas con bromodominios tándem observadas en otros organismos (poli-bromo), que no están presentes en tripanosomátidos. Otro forma un complejo que contiene una histona deacetilasa que comparte BDF2 como subunidad con Swr1. El último fue el más desafiante debido al tamaño del factor de bromodominio involucrado, BDF7, que es homólogo de ATAD2 humano. Se organiza como un hexámero, formando un anillo con un poro central, similar a lo que se ha observado para este tipo de proteínas. Estos hallazgos arrojan luz sobre las vías de señalización de acetilación en tripanosomátidos, proporcionando información valiosa sobre la organización y estructura de los complejos que contienen factores de bromodominio. La identificación de complejos novedosos y sus posibles roles funcionales amplía nuestra comprensión de los mecanismos de remodelación de la cromatina a través de la acetilación en estos organismos de ramificación temprana y ofrece perspectivas emocionantes para investigaciones adicionales en regulación epigenética.

Keywords: Bromodomains, Trypanosomatids, Epigenetics, Complexes, AlphaFold2
Methods: Protein Structure Prediction, Network Analysis, Combinatorial Assembly

SB-08

Study of epitope mapping of an anti-lysozyme IgM family using homology modeling and docking: the challenge of global study and the selection of the best model

Cauerhff, Ana1, Goldbaum, Fernando A.2
1. Depto de Química Biológica-FCEyN-UBA-CONICET; 2. IBBA-FIL-CONICET
anacauer@gmail.com

Recently, the interest in the use of the IgM molecule as a biotherapeutic has grown, for this reason, we think that it is necessary to investigate the primary humoral immune response towards various antigens (Ags) such as proteins that in turn are enzymes. Fifteen IgM isotype monoclonal antibodies (mAbs) were obtained through two immunization protocols using chicken egg lysozyme (HEL) as a model Ag. Our study includes the variable regions VH, VL sequencing, affinity measurement, and the characterization of recognized epitopes (Ep) by those anti-HEL IgMs. For epitope mapping, in vitro experiments such as ELISA and SPR biosensor competition assays were carried out, it was shown the existence of at least one group of IgM mAbs that recognize an Ep shared by a set of anti-HEL IgG isotype mAbs previously characterized by us and other authors. The reported anti-HEL IgGs mostly recognize three dominant Eps, besides, the mAb IgG D11.15 recognizes an intermediate Ep located between Ep 1 and 3, which could be named Ep 4. The existence of an intermediate Ep recognized by one of those IgGs indicates that the anti-HEL IgMs could be recognizing Eps (perhaps less dominant than the three previously described) that overlap with two, with three dominant Eps, or even with no Ep recognized by the IgGs anti-HEL. Thus, a reliable method to identify the Eps of anti-HEL IgM from a more realistic point of view is needed. The first identified group of anti-HEL IgM that recognizes the same Ep was those formed by NTB8/Ac16-8/Moi69/Moi244/NTB2-3, and it was the best characterized by ELISA and by SPR biosensor competition assays. To identify the structure of the Eps recognized by the anti-HEL IgM, homology modeling and docking experiments were performed using the servers: AbBuilder, SIRB, and HADDOCK, ClusPro, AbAdapt, PyDock and Zdock servers, respectively. The first challenge to solve was the evaluation of a huge number of complex models returned by the docking servers from several IgM Fv models obtained with the antibody homology modeling servers. The selection of the most representative models was carried out by calculating the theoretical paratope (Pt) and the theoretical Ep recognized by each IgM. The sequences of the VH, VL, and CDR regions of the IgM were aligned with their corresponding regions in the published anti-HEL IgGs, through the BLAST server. This theoretical Ep was used to discriminate the numerous models obtained with the antibody-protein docking servers based on the recognition of the three dominant Eps and the intermediate Eps including Ep 4. In conclusion: even for the same IgM group, the recognition of the same dominant Ep does not happen the same way; intermediate Eps have been identified in low-affinity IgM. The population study of the Eps

recognized by IgM combined with the experimental results can be used as a criterion to identify the most realistic model of the IgM-HEL interaction for each docking method.

Keywords: IgM, Anti-protein immune response, Epitope mapping, Homology modeling, Docking

Methods: ELISA, SPR Biosensor competition assay, Homology modeling, Docking, Sequence alignment

SB-09

Biophysical and biochemical characterization of Trypanosoma cruzi protein disulfide isomerases

Treffinger Cienfuegos, María Soledad (1,2); Labriola, Carlos Alberto (1); Caramelo, Julio Javier (1,2)
(1) IIBBA- FIL (2) FCEN-UBA
soledadtreffinger@gmail.com

Approximately one-third of eukaryotic proteins belong to the secretory pathway. They are synthesized by ribosomes attached to the endoplasmic reticulum (ER) membrane and enter the ER lumen and/or integrate into its membrane through the SEC61 translocon. In the ER lumen, proteins undergo various co- and post-translational modifications such as glycosylation and the formation of disulfide bonds. This latter modification is carried out by enzymes called protein disulfide isomerases (PDIs). The PDI systems of Trypanosoma cruzi have not been studied to date. Its genome contains at least four putative PDIs in the ER. We aimed to perform their biophysical and biochemical characterization using spectroscopy and in vivo assays. Oxido-reductase and chaperone activity have been observed in three of them. Additionally, we investigated their interaction with the typical T. cruzi lectin/chaperone, calreticulin (CRT). This lectin is involved in the quality control system for the folding of glycoproteins. Based on this information, we can propose that T. cruzi possesses a minimal system for disulfide bond oxidation and isomerization.

Keywords: Trypanosoma cruzi, Protein Disulfide Isomerase, Calreticulin, Quality Control, ER

Methods: Western Blot, Inmunoprecipitation, IMAC, FPLC, Spectroscopy

SB-10

The use of small molecules to modulate full-length PDK1 kinase conformation and substrate specificity towards Akt

Sacerdoti M 1, Gross LZF 1, Zehnder K 2, Riley AM 3, Ghode A4, Suess E 2, Capellari MV 5, Klinke S 6, Anand GS 4, Aramendia PF 5, Svergun D 7, Gräwert MA 7, Leroux AE 1, Potter BVL 3, Camacho C 8, Biondi RM 1,2
1IBioBA – CONICET – MPSP, 2Universitätsklinikum Frankfurt, 3University of Oxford, 4National University of Singapore, 5CIBION, 6Fundación Instituto Leloir, 7 European Molecular Biology Laboratory (EMBL), 8 University of Pittsburgh
dabiondi@yahoo.co.uk

3-Phosphoinositide-dependent protein kinase 1 (PDK1) is a master kinase of the PI3-kinase signalling pathway that phosphorylates at least 23 other evolutionary related AGC kinases. It has an N-terminal kinase domain, a linker region and a C-terminal PH domain that binds PIP3 and other inositol polyphosphates. Our laboratory has previously used a chemical and structural biology approach to study and characterize the bidirectional allosteric regulation between the ATP-Binding site of PDK1 and the PIF pocket, a regulatory site located on the small lobe of the kinase domain. Phosphorylation by PDK1 is a necessary step to activate several other kinases, such as Akt, SGK, S6K and PKC. The interaction between substrate kinases (except Akt) and PDK1 is mediated through a docking interaction with the PIF pocket of PDK1. Interestingly, the interaction with the PIF pocket of PDK1 is not a requirement for the phosphorylation of PKB/Akt after PI3-kinase activation, but both proteins can colocalize at the cell membrane by binding PIP3 through their PH domains.
We now describe the conformational landscape of full length PDK1, identifying one dimeric conformation and at least two distinct monomeric conformations. What´s more, the conformations appear to have distinct substrate specificity and PIF pocket availability, related to the relative position of the PH domain. Small molecules that bind to different sites on PDK1 were able to disrupt dimers and stabilize specific monomeric conformations with different abilities to phosphorylate Akt. Our methods include a multitude of biochemical, biophysical and bioinformatical assays. Given the relevance of PDK1 in signaling pathways that control cell growth and survival, this research could contribute to the development of compounds to selectively inhibit PDK1 activation of Akt for cancer treatments.

Keywords: kinase, allostery, small molecule,

Methods: AlphaScreen, HDX-MS, SAXS, Thermal Shift, STORM microscopy

Biotechnology

BT-01

Evaluating the ability of three in-vitro biotransformation models to remove selected antibiotics and 17 α-ethinylestradiol and identification of their respective transformation products

Sosa Alderete Lucas G,1; Sauvêtre Andrés 2, 3; Chiron Serge 2;Tadić Đorđe 2.

1 Instituto de Biotecnología Ambiental y Salud, INBIAS-CONICET. Universidad Nacional de Río Cuarto, Argentina. Ruta Nacional 36 Km 601 (CP 5800), Río Cuarto, Córdoba, Argentina; 2 HSM, Univ Montpellier, CNRS, IRD, Montpellier, France; 3 HSM, Univ Montpellier, IMT Mines Ales, CNRS, IRD, Ales, France

lucasaureus@gmail.com

Three in-vitro biotransformation models (i.e., pure enzymes, hairy root and *Trichoderma asperellum* cultures) were used to evaluate the degradation of three antibiotics (sulfamethoxazole, trimethoprim and ofloxacin) and one synthetic hormone (17 α-ethinylestradiol); and investigated the relevance of the formation of transformation products (TPs) in constructed wetlands (CWs) bioaugmented with *T. asperellum* fungus. The identification of TPs was carried by high resolution mass spectrometry, using databases or by interpreting MS/MS spectra. Enzymatic assays with pure enzymes, such as peroxidase, laccase and β-glucosidase were performed to confirm the presence of TPs corresponding to phase I and II from metabolism of xenobiotic compounds (MXC), respectively. Results showed synergies in the transformation mechanisms between these three models. Phase II conjugation reactions and mainly glycosylation reactions were predominating in hairy root cultures while phase I metabolization reactions (e.g., hydroxylation and N-dealkylation) were predominating in *T. asperellum* culture. Following their accumulation/degradation kinetic profiles helped in determining the most relevant TPs. Identified TPs contributed to the overall residual antimicrobial activity because phase I metabolites can be more reactive and glucose-conjugated TPs can be transformed back into parent compounds. Similar to other biological treatments, the formation of TPs in CWs is of concern and deserves to be investigated with simple in-vitro models to avoid the complexity of the studies at field-scale. This work brings new findings on the emerging pollutants metabolic pathways established between *T. asperellum* and model plants, including extracellular secreted enzymes.

Keywords: Emerging pollutants; transformation products; hairy root cultures; Trichoderma; xenobiotic metabolism;

Methods: High resolution mass spectrometry; Spectrophotometry; Enzymatic reaction; maintenance of in vitro cultures

BT-02

Identification of protein binders in eighteenth century Andean paintings

Ricardo Martín Neme Tauil123, German Fernández12, Maria Pía Valacco12, Marta Maier24, Silvia Moreno12
1 Centro de Estudios Químicos y Biológicos por Espectrometría de Masas (CEQUIBIEM), Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires 2 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) 3 Facultad de Filosofía y Letras, Universidad de Buenos Aires 4 Laboratorio de Investigaciones y Análisis de Materiales en Arte y Arqueología (LIAMA), Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires
silviammoreno@gmail.com

In this work we present the study of protein binders in samples of 18th century paintings, two from murals of churches located in the northern region of Chile (Levy et al, 2021) and one located in the current Bolivian territory (Levy et al, 2018), using two complementary mass spectrometry techniques. We introduce a Methods that combines a protein extraction procedure with a typical treatment for analysis as tryptic peptides by mass spectrometry. We applied MALDI-TOF/MS and subsequently LC-ESI/MS (Orbitrap) to achieve higher reliability in identification.

In the first two murals the protein fraction of the samples was extracted with ammonia following a previously optimized Methods and analyzed by LC-MSMS. We identified collagen as well as egg white and yolk proteins according to a tempera painting technique. In addition, we detected for the first time the presence of muscle proteins in two of the microsamples. Collagen and muscle proteins could be attributed to bovine (*Bos taurus*) and llama (*Vicugna pacos*), a domesticated camelid used as a source of food and for goods transport in the Andes since pre-Hispanic times.

In the third church seven microsamples of representative colors were extracted from one of the mural paintings. Previous analysis by gas chromatography (GC) of the amino acid derivatives of the protein fraction extracted from three of the samples suggested the presence of animal glue and egg. We detected several peptides from egg white proteins, in particular ovalbumin, ovotransferrin and lysozyme, and from egg yolk proteins, vitellogenin-2 and apolipoprotein B, with peptides of high confidence. The presence of collagen was determined in all samples.

These findings provide new aspects on the knowledge of the composition of ancient animal glues and painting practices in the Andean region, with reliable information useful for restoration and

conservation work. This is also the first time that protein binders have been unambiguously identified in Andean paintings by mass spectrometry. References
Ivana K. Levy, Ricardo NemeTauil, Maria P. Valacco, Silvia Moreno, Gabriela Siracusano, Marta S. Maier, Investigation of proteins in samples of a mid-18th century colonial mural painting by MALDI-TOF/MS and LC-ESI/MS (Orbitrap), Microchemical Journal, Volumen 143, 2018, pp. 457-466,ISSN 0026-265X, https://doi.org/10.1016/j.microc.2018.07.030
Ivana K. Levy, Ricardo NemeTauil, Ana Rosso, Maria P. Valacco, Silvia Moreno, Fernando Guzmán, Gabriela Siracusano, Marta S. Maier, Finding of muscle proteins in art samples from mid-18th century murals by LC–MSMS, Journal of Cultural Heritage, Volume 48, march 2021, pp. 227 - 2351, ISSN12962074, https://doi.org/10.1016/j.culher.2020.11.005

Keywords: Proteomics, mass spectrometry, painting, mural, Andes

Methods: protein extraction, MALDI-TOF-TOF mass sprectrometry, LC-ESI-Orbitrap mass spectrometry

BT-03

Optimizing Multicomponent Vaccine Strategies: Contribution of *Lactobacillus* to Partial Protection Against *T. cruzi*

Zabala BA, Vázquez ME, Barrientos MC, Acuña L, Pérez Brandán CM
Universidad Nacional de Salta, IPE-CONICET, Argentina.
bren.zabala@gmail.com

The search for an immunoprophylactic formulation capable of preventing *Trypanosoma cruzi* infection is a task that has been ongoing for many years. Despite all the efforts and advances, no vaccines are currently available for human application. In a previous study, our research group assessed the effectiveness of a live dose of *Lactobacillus acidophilus* ATCC 4356 as a potential vaccine against *T. cruzi* infection in a mouse model. Notably, the results obtained indicated partial protection against a lethal virulent challenge, suggesting the induction of mechanisms conferring some degree of cross-protection. This effect may involve trained innate immune responses mediating heterologous immunity against virulent strains of *T. cruzi*. In light of these findings, and with the aim of enhancing protection directed towards the parasite, we next evaluated a novel multicomponent vaccine candidate which combines the primary surface protein SlpA of *L. acidophilus* and the protein Tc52, a well-known antigen of *T. cruzi*, both fused in a chimeric protein. To further elucidate the relative

contribution of live bacteria versus the chimeric antigen alone, we evaluated both a formulation presenting the chimeric protein adhered to the bacterial cell surface as well as the chimeric protein on its own. This experimental design allowed us to assess whether live bacteria facilitated the protective response or if the chimeric antigen alone could induce significant immunity when delivered as a standalone formulation. Insights from these comparisons may inform optimization of vaccine formulation and delivery approach for optimal efficacy. An immunization regimen was designed for C57BL/6 mice to assess the potential efficacy of the formulations via subcutaneous administration. The animals received three inoculations spaced 21 days apart. Blood samples were collected before each dose and 21 days after the final dose. To evaluate the response to *T. cruzi* infection upon immunization, mice were challenged with a lethal dose of *T. cruzi* trypomastigotes. After experimental infection, parasitemia and mortality was checked and recorded twice a week until control groups succumbed to death. To determine whether the vaccine candidates have modulatory effects in the humoral response, serum samples from all experimental groups were evaluated by ELISA for IgG1 and IgG2c measurement. Our results prompt us to continue working in novel biotechnological advances for vaccine approaches with a multicomponent design that could potentiate the immune response against *T. cruzi* infection and potentially other parasitic diseases. Specifically, our findings demonstrated partial protection against a lethal challenge with the chimeric antigen compared to the control group, motivating continued development of this vaccine candidate.

Keywords: Trypanosoma cruzi, Lactobacillus acidophilus, Multicomponent Vaccines, Neglected Diseases.

Methods: Heterologous Expression, In Vivo Assay, ELISA.

BT-04

Reutilization of citrus industry wastewater for the production of fungal laccases

Millán, Esteban Rogelio; Benitez, Silvana Florencia; Chelaliche, Anibal Sebastián; Acosta, Gabriela Alejandra; Zapata, Pedro Dario; Fonseca, Maria Isabel
Universidad Nacional de Misiones. Facultad de Ciencias Exactas Químicas y Naturales. Instituto de Biotecnología Misiones "Dra. María Ebe Reca" (INBIOMIS). Laboratorio de Biotecnología Molecular. Misiones, Argentina.
flopybenitez13@gmail.com

Citrus-processing industries produce large volumes of wastewater (WW) characterized by a high content of organic matter. An alternative for their management is their reuse to obtain value-added products. In this sense, white-rot fungi (WRF) can be applied for enzyme biosynthesis. However, WW can inhibit fungal growth, hence, fungal tolerance and strategies to improve it should be evaluated. The aim of this work was to evaluate *Trametes sanguinea* BAFC 2126 tolerance towards citrus WW, and laccase (Lac) production in solid and liquid media using *Luffa cylindrica* as immobilization substrate. Citrus WW and *L. cylindrica* were kindly provided by Cooperativa Citrícola Agroindustrial de Misiones Ltda. (Leandro N. Alem, Misiones) and Espudela (Jardín América, Misiones), respectively. Tolerance was evaluated in 85 mm ø Petri dishes in triplicate containing 12 mL of MEA (malt extract 12.7 g.L-1, agar 17 g.L-1) supplemented with filtered citrus WW (25, 50, 75 and 100 %); pH was adjusted to 5.7 ± 0.1 with 1 M NaOH. One agar plug (7 mm ø) was inoculated; fungal radial growth was measured daily and analyzed using predictive mycology (Sadañoski et al. 2018) to determine τ (time to attain half the plate) and κ (growth rate) values. Lac production was revealed at the moment the mycelium covered 50 % of the plate by adding 10 mL of 2,6-dimethoxyphenol (DMP) 5 mM in sodium acetate buffer 0.1 M (pH 3.6) and incubating in gentle agitation in darkness for 20 min.

For Lac production in liquid media, experiments were carried out in triplicate in 250 mL Erlenmeyer flasks containing 1 g of L. cylindrica (1 cm3) and 50 mL of liquid media. Culture media consisted in different concentrations of citrus WW (0, 25, 50, 75, and 100 % v.v-1), concentrations were adjusted with distilled water. Aditionally, the effect of CuSO4 (0.5 mM) was evaluated. Initial pH was adjusted to 5.7 ± 0.1 with 1 M NaOH. Autoclave-sterilized flasks (105 °C, 20 min) were inoculated with three agar plugs (~7 mm ⌀) of the strain and incubated for 19 d at 28 ± 1 °C in static or agitation condition (100 rpm), samples were taken periodically. Lac activity was evaluated in spectophotometer using DMP in a sodium acetate buffer 0.1 M (pH 3.6, 30 °C) as substrate.

Tolerance assays showed that the strain BAFC 2126 was able to grow and produce Lac in all the concentrations tested, with an average τ and κ of 3.17 ± 0.08 d and 0.92 ± 0.03 mm.d-1, respectively.

As for Lac production in liquid media, the highest yield was obtained with 100 % v.v-1 citrus WW after 15 d of incubation in agitation without the addition of CuSO4 (2539.18 ± 443,44 U.L-1); followed by cultures with 50 % v.v-1 after 9 d of incubation in agitation with CuSO4 (1272.6 ± 4.16 U.L-1).

These results suggest that citrus WW can be reuse to produce enzymes of biotechnological interest.

Keywords: CITRUS WASTEWATER, ENZYME, LACCASE, WHITE-ROT FUNGI

Methods: PREDICTIVE MICOLOGY, SPECTROPHOTOMETRY

BT-05

CAZyme-Based Strategies to Boost Silage Nutritional Value

Gizzi Fernán, 1,4; Terzariol Marina, 1,4; Acciarri Giuliana, 1,4; Martín Mariana, 2,4; Storani Alem, 3; Iglesias Alberto 3, Guerrero Sergio, 3; Magni Christian,1,4; , Blancato Víctor, 1,4.
1 Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET), 2 Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI-CONICET), 3 Instituto de Agrobiotecnologia del Litoral (IAL-CONICET), 4 Facultad de Ciencias Bioquímicas y Farmacéuticas (FBioyF), Universidad Nacional de Rosario (UNR).
fernangizzi@gmail.com

The capacity of ruminants to transform plant biomass into meat and milk is highly dependent on the digestibility of plant cell walls. These cell walls consist of an intricate matrix of carbohydrates, including cellulose, hemicellulose, and pectin. These carbohydrates are linked together by strong bonds that are difficult for ruminal microorganisms to break down. CAZymes include cellulases which hydrolyze cellulose into glucose, and xylanases which hydrolyze xylan into xylose. The use of CAZymes in silage can improve the digestibility of plant cell walls making the carbohydrates more accessible to ruminal microorganisms. This can lead to increased animal productivity and improved feed efficiency. There are several ways in which CAZymes can improve the digestibility of plant biomass for ruminants. First, they can break down complex carbohydrates into smaller molecules that are more easily digested by ruminal microorganisms. Second, they can release fermentable sugars, which can provide energy for the ruminant animal. Third, they can reduce the viscosity of silage, which can improve the intake and digestion of silage by ruminants. Inoculating silage with concentrated acid lactic bacteria is a common technique that enhances the fermentation of these forages. *Lactococcus lactis* is a generally regarded as safe (GRAS) lactic acid bacterium recognized as an efficient microbial cell factory. Previously we employed this strain to produce *Bacillus subtilis* xylanase Bsxyn11A. In this work, we aimed to produce another enzyme, a cellulase, in *L. lactis* to improve silage quality. To achieve this, the gene was cloned into the expression vector fused with a secretion signal and electroporated to *L. lactis*. The concentration of inducer and growth time of this strain was optimized to increase protein yield. Then, the activity of the enzyme was evaluated against its specific substrate. Next, biomass degradation capabilities were confirmed by incubation with grounded sorghum and posterior TLC analysis. Finally, culture supernatants of *L. lactis* producing the enzymes or purified cellulase and/or xylanase enzymes were combined to enhance biomass degradation and produce a greater release of soluble carbohydrates. The approach followed could provide a cost-effective strategy for optimizing current methods, thereby improving nutritional value of silage. Ultimately, this could lead to increased animal productivity and enhanced feed efficiency.

Keywords: Silage, Cazymes, Lactococcus, Forages, Expresion.

Methods: PCR, SDS-PAGE, CLONING, TLC, Protein purification

BT-06

Biotransformations via de novo and ex novo pathways mediated by *Aspergillus niger* MYA 135: lipid accumulation from industrial residues

Reyes, Dardo Antonio; Ponce, Ruth Araceli; Loto, Flavia del Valle; Baigorí, Mario Domingo; Pera, Licia Maria
PROIMI-CONICET. Av. Belgrano y Pje. Caseros. 4000. Tucumán Argentina
dardoantonioreyes@hotmail.com

Industrial residues such as crude glycerol (CG) and waste cooking oil (WCO) pose important ecological and economic problems. Thus, the synthesis of microbial lipids using biotransformation routes constitute an attractive alternative to employ those residues under the circular economy concept. It is known that oleaginous microorganisms are capable of utilizing both hydrophilic and hydrophobic substrates to accumulate lipids via de novo and ex novo, respectively. Besides, current problems of filamentous fungi fermentations and their further successful developments as microbial cell factories are dependent on control fungal morphology. In submerged fermentation, fungal morphology can vary from compact pellets to dispersed mycelia. Thus, the main objective of this work was to analyze biotransformations yielding lipids mediated by A. niger MYA 135 from CG (100 g/L) or WCO (25 g/L) in the presence of morphological effectors such as talc microparticles (8 g/L), FeCl3 (1 g/L) and MnCl2 (3.6 mg/L). Shake flask fermentation were conducted at 30ºC during 96 h with or without the supplementation of effectors according to a 23 factorial design. Under de novo lipid fermentation, lipid content was not significantly affected by fungal pelletization ($p=0.315$). The maximum lipid content was 32.38 ± 1.35 % of its defatted dry weight (w/w). However, the fatty acid (FA) profile obtaining from pellets (SFA: 19.57 %, PUFA: 36.43 %, MUFA: 44.00 %) was different to that obtaining form dispersed mycelium (SFA: 24.30 %, PUFA: 30.10 %, MUFA: 45.61 %). In addition, a significant interaction between FeCl3 and MnCl2 was detected ($p<0.001$). Under ex novo lipid fermentation, lipid content was significantly affected by the form of fungal growth ($p=0.010$). In this case, pelletization favored lipid accumulation. The maximum lipid content was 87.00 ± 1.41 % of its defatted dry weight (w/w). However, the FA profile obtaining from pellets (SFA: 22.01 %, PUFA: 19.92 %, MUFA: 58.07 %) was almost similar to that obtaining from dispersed mycelium (SFA: 21.19 %, PUFA: 22.82 %, MUFA: 55.99 %) and different to that observed from WCO (SFA: 12.11 %, PUFA: 48.32 %, MUFA: 38.31 %). In addition, the interaction between FeCl3 and MnCl2 was not significant ($p=0.092$). Thus, when the biotransformations swift from de novo to ex novo the FA profiles were changed toward synthesizing higher amounts of MUFA and lower amounts of PUFA.

Interestingly, under both biotransformation pathways the SFA content was similar. Finally, the biodiesel properties of the corresponding FAMEs, FAEEs and FABEs were theoretically estimated by using the FA profiles with the help of derived empirical formulas. Then, the data were compared by international standards such as EN14214 and ASTM D6751. The analysis showed that the oleaginous biomass from A. niger MYA 135 could be used as potential feedstock for biodiesel production. S: Saturated; PU: Poly unsaturated; MU: Mono unsaturated; M: Methyl; E: Ethyl; B: Butyl; Es: esters.

Keywords: Biotransformation, Industrial residues, Microbial lipid, Submerged fermentation, Fungal morphology

Methods: Folch method, Sudan Black staining, Thin layer chromatography, Gas chromatography, Experimental design

BT-07

Sustainable two-step multienzymes-assisted aqueous processing of soybean flour yielding free oil, proteins and carbohydrates: enzymatic cocktail production, characterization and potential reutilization

Ponce, Ruth Araceli; Reyes, Dardo Antonio; Loto, Flavia del Valle; Baigorí, Mario Domingo; Pera, Licia Maria
PROIMI-CONICET
rutharaceliponce@gmail.com

Oil production by enzyme-assisted aqueous extraction (EAAE) may be a viable, safer, and environmentally friendly alternative to the traditionally hexane extraction. Enzymes are necessary to improve oil extraction yields, as they break down the cell wall and protein barriers surrounding the oil bodies. Cellulases, phospholipases, and proteases are widely used to overcome these barriers. However, the use of commercial enzymes makes this process more expensive. Thus, in order to create a more efficient and sustainable system, the main objective of this work was to synthetize and characterize a multi-enzymatic cocktail obtained by submerged fermentation from solid and liquid wastes generated during an EAAE process using *Brevibacillus agri* E12. Firstly, a genome-wide identification of coding sequences corresponding to cellulases, proteases, and phospholipases activities from *B. agri* E12 was done. Then, the microbial culture supernatant obtained after 24 h of incubation from the Luria-Bertani (LB) reference medium was used in a EAAE process at pH 9 and 50 °C. The solid and liquid residues of

this process were employed to formulate the R1 culture medium (liquid fraction: 5 %, v/v; solid fraction: 10 %, p/v). The stability of the culture supernatants obtained from R1 and LB media were assayed under the EAAE conditions (50 °C at different pH values during 24 h). The hydrolytic enzymes showed residual activity above 60 %. Additionally, the optimal temperature and pH for each hydrolytic activity were as follows: for cellulase, 40 °C, pH 5; for phospholipase, 37 °C, pH 7; and for protease, 60°C, pH 9. Finally, as proof of concept, two-step EAAE were carried out using culture supernatants from R1 and LB media. Step 1: solid-liquid ratio of 1:20, at 50 °C, for 24 h, and under different pH values (5, 7 and 9). Step 2: solid-liquid ratio of 1:20, at 50 °C, for 4 h, and at pH 9. The highest yields with respect to soybean flour were obtained at pH 9 (LB: 17.17 ± 0.45 %; R1: 18.09 ± 0.53 %; Hexane: 21.05 ± 1.02 %). Besides, the fatty acid profile of the oil extracted by EAAE using the multienzymes cocktail from R1 medium was similar to that extracted by hexane. The results showed that the multienzymes cocktail recycling could also be viable as after EAAE at pH 9 biocatalysts retained over 78% of their activities. In addition, the liquid fraction obtained after EAAE at pH 9 contained the same concentration of carbohydrates (LB: 0.91 ± 0.07 g/L; R1: 1.04 ± 0.03 g/L) and proteins (LB: 5. 49 ± 0.31 g/L; R1: 4.83 ± 0.25 g/L) (means are not significantly different). The electrophoretic profile of proteins in the liquid fraction showed the presence of several peptides with molecular weights less than 25 KDa when the EAAE was carried out at either pH 7 or 9. Thus, under the circular economy concept, solid and liquid wastes generated from a EAAE process can be revalorized via enzyme production allowing a viable, efficient, and sustainable soybean oil EAAE process.

Keywords: Soybean oil, Aqueous extraction, Hydrolytic enzymes, Waste valorization, Sustainable technologies,

Methods: Oil extraction, Enzymatic determinations, Polyacrylamide gel electrophoresis, Thin layer chromatography, Gas chromatography

BT-08

EXPANDING THE BIOCATALYTIC TOOLBOX WITH A NEW REDUCTASE FROM LEPTOSPIRA BIFLEXA

Zocchi, Sofía B.1; Ceccoli, Romina D.1; Bianchi, Dario A.2; Rial, Daniela V.1
1 Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, y CONICET. 2 Instituto de Química Rosario (IQUIR, CONICET-UNR), Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario.
zocchisofia@gmail.com

The search for ecofriendly ways to synthesize chemical substances has led to the incorporation of biocatalysts, such as pure enzymes, whole microorganisms or plant-derived materials, into synthetic

routes. This usually results in operational benefits due to the high efficiency, exquisite selectivity and specificity of enzymatic reactions. Since most enzymes are active under mild conditions, their compatibility with the environment is an advantage. The main interest of our group is to develop sustainable processes based on the design of biocatalysts with potential applications in the field of chemical synthesis. The Old Yellow Enzymes (OYEs) are flavoproteins capable of catalyzing the asymmetric reduction of C=C bonds activated by an electron-withdrawing group, such as an aldehyde or a ketone. They have been used for the preparation of a wide variety of compounds, many of them of great interest for the pharmaceutical industry (1,2). In order to access OYEs that could significantly increase the toolbox of redox biocatalysts, we searched for sequences coding for this type of enzymes using bioinformatics methods. We identified the sequence of a possible OYE in the genome of *Leptospira biflexa* and cloned it into an expression vector. The recombinant protein was produced in *Escherichia coli* cells transformed with the constructed plasmid and its soluble expression was determined by polyacrylamide gel electrophoresis under denaturing conditions. The ability of the new biocatalyst to reduce activated C=C bonds of α,β-unsaturated ketones was evaluated by in vivo biotransformation assays using resting recombinant whole cells as biocatalyst and a set of α,β-unsaturated ketones as substrate candidates. *E. coli* cells transformed with the expression vector were used as control. Bioconversions were analyzed by gas chromatography coupled to mass spectrometry. Under the conditions tested, this biocatalyst was able to reduce the α,β-unsaturated ketone 1-phenyl-1-hexen-3-one to the corresponding saturated ketone. Therefore, this work provides a new biocatalyst with carbon-carbon double bond reduction activity for potential applications in chemical synthesis.

1) Parmeggiani, F.; Brenna, E.; Colombo, D.; Gatti, F. G.; Tentori, F.; Tessaro, D. ChemBioChem, 2022, 23, e202100445. 2) Shi, Q.; Wang, H.; Liu, J.; Li, S.; Guo, J.; Li, H.; Jia, X.; Huo, H.; Zheng, Z.; You, S.; Qin, B. Appl. Microbiol. Biotechnol., 2020, 104, 8155-8170.

Keywords: α,β-unsaturated ketone, OYE, biocatalysis, biotransformations.

Methods: molecular cloning, recombinant protein expression, gas chromatography.

BT-09

PLOMBOX 3.0: A DEVICE FOR OPEN-SOURCE METROLOGY TO FIGHT LEAD CONTAMINATION IN DRINKING WATER.

*Alvarez Macarena*1,3, Gándola Yamila*1,2, Gasulla Javier1,3 and Nadra Alejandro D.1,3*

1IB3-FBMC-FCEyN-UBA. 2Departamento de Química Biológica, FFyB, UBA, 3CONICET.

jvr.gasulla@gmail.com

Lead is one of ten chemicals that the World Health Organization (WHO) has identified as a major public health concern. Although there is no level of exposure to lead that is known to be without harmful effects, it is recommended to be lower than 10 µg/L (ppb) in drinking water. Currently, lead in water measurements require expensive and specialized equipment to reach relevant sensitivity levels. In developed countries, lead screening tests may be carried out in both public and private water supplies. However, in low -and middle- income countries access lead level analysis is limited and may be prohibitively expensive. A low-cost sensor with the capability to measure a concentration of 10 ppb lead in water is therefore desirable. We show the development of such a sensor based on a genetically modified *Escherichia coli* DH5α strain bearing a lead-sensing genetic construction on a pUC57mini plasmid. Briefly, this construction possesses a lead regulator protein (pbrR) which binds Pb+2, and a regulatory zone attached to the β-galactosidase gene as the reporter gene. In addition, we included a transporter protein (pbrT), to increase Pb+2 intracellular concentration. Expression of both pbrR and pbrT proteins is regulated by the intermediate strength promoter P479; β-galactosidase expression is regulated by a natural lead sensitive promoter/operator in the pLVPK virulence plasmid from *Klebsiella pneumoniae*. Results show that our biosensor can detect lead in drinking water at levels as low as 10 ppb and that could be used in a low-cost, portable and easy to use device for testing household water quality. As part of the validation of the device, household tap water samples were analyzed using our lead biosensor and Atomic Absorption as a reference, obtaining a Pearson correlation coefficient of 0.76 comparing the lead values obtained by both methods. In addition, there is 95% confidence that the PlomBOX can detect lead with a concentration of 10 ppb with a confidence interval of 14.94 +/- 6.70 ppb. The final goal is to make widely-distributed and real-time, crowd-sourced monitoring of lead levels in drinking water by using a custom device, PlomBOX, that acquires,analyzes and shares the data through a cell phone app (PlomApp).

Keywords: lead, biosensor, water quality, open-science

Methods: synthetic biology, molecular biology,

BT-10

Development of a surrogate ELISA neutralization assay for SARS-CoV-2 immunity assessment in Argentina

Perez, Dan Alejandro 1; Rey Serantes, Diego Armando 1; Acuña Intrieri, Maria Eugenia 2; Medrano, Mayra 1; Alvarez, Diego Ezequiel 1; Comerci, Diego Jose 1; Cerutti, Maria Laura 2
1 Instituto de Investigaciones Biotecnológicas (IIBIO-UNSAM-CONICET). 2 Centro de Rediseño e Ingeniería de Proteínas (CRIP-EByN_UNSAM)
dperez@estudiantes.unsam.edu.ar

COVID-19 pandemic, caused by SARS-COV-2 coronavirus, led to an unprecedented development of vaccines and to a worldwide massive immunization campaign. Most vaccines are designed against the viral Spike (S) glycoprotein because antibodies directed toward this protein can neutralize the infection. Specifically, SARS-CoV-2 neutralizing antibodies are directed against the Spike RBD domain, the part of the antigen that interacts with the ACE2 host cell receptor. Although the emergency phase of COVID-19 is over, the virus continues to spread and evolve, constituting a threat, especially to particular high-risk groups like elderly persons, adults with underlying health conditions, pregnant women and frontline health workers. Detection of serum neutralizing antibodies (nAbs) is a key measure of human immunity, and, thus, a critical parameter to evaluate vaccination efficacy and the extent of protection provided to individuals. Gold standard methods for nAbs determinations generally rely on neutralization tests with live virus or pseudovirus particles; nonetheless, these methods are expensive, time-consuming, and not highly processive. To overcome such disadvantages, we here present the development and validation of a surrogate neutralization ELISA assay for SARS-CoV-2 nAbs detection and quantification in sera samples of infected or vaccinated individuals. The assay is based on the competitive binding of serum neutralizing antibodies to the solid-phase immobilized viral RBD domain in the presence of its cognate receptor, ACE2. The ELISA development involved the production of the recombinant RBD and ACE2 proteins in mammalian cells, ACE2 labeling, and the setup of several assay parameters, such as incubation times, reagent concentrations, and controls selection. In addition, the surrogate neutralization ELISA performance was validated against a viral neutralization test with live SARS-CoV-2 particles, exhibiting comparable specificity and sensitivity values. Overall, our surrogate ELISA SARS-CoV-2 neutralization test, the first of its kind developed in our country, constitutes a rapid and cost-effective tool for evaluating national herd immunity and supporting public health decision-making on high-priority groups vaccination. Future assay validations against circulating omicron RBD variants are underway.

Keywords: ELISA, COVID-19, ANTIBODY, NEUTRALIZATION, SARS-CoV-2

Methods: ELISA, Plaque Reduction Neutralization Test

BT-11

Stitched together: *in silico* optimization of a potent neutralizing anti-malarial antibody

Wecera, Martina (1, 2); Dale, Gordon (1); Batista, Facundo (1)
1. Ragon Institute of MGH, MIT and Harvard, 2. Universidad Nacional de La Plata (UNLP)
marwecera@gmail.com

Malaria is a tropical infectious disease caused by the parasite *Plasmodium falciparum*. Globally, malaria is estimated to have a disease burden of 241 million cases and ~627,000 deaths, primarily in children, in 2020. For more than 40 years, work has been conducted on generating an effective and durable vaccine. Despite the recent approval of the first malaria vaccine (RTS,S), lasting protection remains low, with protective antibody titers found for less than 1 year. Thus, complementary approaches are necessary to reduce disease burden.

One such approach is the use of passive immunization. Indeed, clinical trials using the potent neutralizing antibody CIS43 have demonstrated significant protection for up to one year in malaria endemic regions. Although this is a promising strategy, it is limited by cost-effectiveness due to the relatively large quantity of antibody that needs to be transferred. In order to address this, the Batista Lab and colleagues, recently developed a CIS43 variant, D3, that has a significant increase in affinity and protection in preclinical models of malaria infection. This is critical because increases in affinity allow for a decreased dose and cost for equivalent protection, enabling passive immunization to be a viable strategy to prevent malaria infections.

In this work, we sought to apply rational design to further improve this antibody and in doing so achieve two goals: (1) increase the affinity of CIS43 to its cognate antigen, and (2) provide proof of principle for rational in silico antibody design. Using a novel workflow, we designed several CIS43 variants. These variants were cloned, expressed, and purified. Using endpoint ELISAs we screenedwe, screened promising candidates for further interrogation and affinity measurement using biolayer interferometry. As a result of this work, we produced several candidate monoclonal antibodies that have greater affinity than D3, including a new best-in-class antibody, Frankie.

Keywords: Malaria, antibody, in silico design

Methods: in silico design, ELISA, biolayer interferometry, protein expression

BT-12

Coated Bacterial Vaccines: A new approach for non-heterologous antigen surface display on *B. subtilis* and its use in recombinant tetanus vaccine development

Harguindeguy, Inés1; Hiriart, Yanina2; Gorgojo, Juan P.1; Alarcón, Vanina3; Cavalitto, Sebastián F.1; Ortiz, Gastón E.1

1Centro de Investigación y Desarrollo en Fermentaciones Industriales, UNLP-CONICET, Argentina. 2Instituto de Estudios Inmunológicos y Fisiopatológicos, UNLP-CONICET, Argentina. 3Instituto Biológico Dr. Tomás Perón, Ministerio de Salud de la Provincia de Buenos Aires, Argentina.

ineharguin2806@gmail.com

Tetanus is an acute and frequently fatal infectious disease, caused by the tetanus toxin produced by *Clostridium tetani*. The current tetanus vaccine is effective but its production presents several drawbacks, including the need for manipulating toxigenic strains of *C tetani* and the handling of toxin. In this context, it has been demonstrated the effectiveness of the non-toxic C-terminal fragment of tetanus toxin (TTFC) to induce immunity against this toxin. Thus, this fragment has been used for development of vectorized vaccine based on surface antigen presentation, such as Live Bacterial Vaccines (LBVs) and Gram-positive Potentiating Matrix Particles (BLPs). In this way, our group has developed an antigen presentation system called Coated Bacterial Vaccines (CBVs). This system involves the exogenous production of the target vaccine antigen fused to the C-terminal end of S-layer proteins of *Lactobacillus* spp (FasTAG®). This domain exhibits a strong affinity for Gram-positive bacterial membranes, such as *Bacillus subtilis*. Consequently, the chimeric antigen can be produced in heterologous expression systems such as *Escherichia coli*. Subsequently, the intrinsic affinity of this can be used to coat the surface of chemically inactivated Gram-positive bacteria, which act as antigen-presenting carriers. Based on the above, the purpose of this work was to evaluate the functionality of the CBVs system to developdeveloped a recombinant tetanus vaccine based on model antigen TTFC. For this purpose, the recombinant antigen TTFC-FasTAG® was produced in *E. coli* and displayed on the surface of chemical inactivated *B. subtilis* to formulate the CBVs. Next, so as to analyze the vaccination capacity of CBVs, BALB/c mice were immunized intraperitoneally with three doses of 5 ug/dose TTFC. In order to evaluated the response, the anti-TTFC serum antibody levels were determined by ELISA. The results show that vaccinated mice increased the levels of specific anti-TTFC IgG antibody titer in sera to log=4, similar to reported for commercial vaccine immunization protocols. Moreover, to characterize the Th cell response inducedinduce by CBVs, we analyzed the distribution of TTFC-specific IgG isotypes present in sera of immunized mice. Immunization with CBVs induced higher IgG1 antibody titers compared to IgG2a, resulting in a Th2-polarized response, thus inducing a strongly humoral immune response. Furthermore, so as to determine whether the presence of specific antibodies correlated with protection, animals were challenged with 100xLD50 of purified toxin and demonstrated

to raise complete protection against this toxin. Finally, the set of experiments carried out here validate the potential and functionality of this system and showed that it was able to confer active immunity to a murine model. Our results provide a theoretical basis for the development of a safe and effective recombinant tetanus vaccine as a potential substitute for the current toxoid vaccine.

Keywords: Antigen surface display, Tetanus, Vaccine, Bacillus subtilis

Methods: Bacterial fermentation, Affinity chromatography, SDS-PAGE, Immunization, ELISA

BT-13

Antibacterial effect of biogenic silver nanoparticles on resistant bacteria

Dorado, Rita D. 1Pécora, Luciana A. 2 Hollmam, Axel 3 Rodríguez, Mónica 4 Rodríguez, Cecilia 5 Romero, Cintia M. 6 Espeche Turbay, M. Beatriz 1,2

1 Instituto de Bionanotecnología del NOA (Consejo Nacional de Investigaciones Científicas y Técnicas - Universidad Nacional de Santiago del Estero); 2 Facultad de Agronomía y Agroindustrias, Universidad Nacional de Santiago del Estero; 3 Centro de Investigación en Biofísica Aplicada y Alimentos (Consejo Nacional de Investigaciones Científicas y Técnicas - Universidad Nacional de Santiago del Estero); 4 Hospital Independencia de la Provincia de Santiago del Estero; 5 Centro de Referencia para Lactobacilos (Consejo Nacional de Investigaciones Científicas y Técnicas); 6 Planta Piloto de Procesos Industriales Microbiológicos (Consejo Nacional de Investigaciones Científicas y Técnicas) beaespeche@gmail.com

Antimicrobial resistance is currently a topic of global concern, which demands attention at different levels, and one of them is the need for novel antimicrobials effective against multidrug resistant (MDR) strains. Silver nanoparticles (AgNPs) enhances the antimicrobial activity of silver ions and, due to their versatile antimicrobial mechanism, makes them strong candidates for evading resistance mechanisms developed by microorganisms. Our aim was to develop AgNPs through an eco-friendly process and evaluate their antimicrobial activity against MDR strains. AgNPs were synthesized from an extracellular extract of *Bacillus* sp (AgNP@bc) and were characterized by UV-Vis and vibrational spectroscopy (FTIR), surface and transmission electron microscopy (SEM and TEM, respectively), particle size (DLS) and zeta potential (pZ). The antimicrobial effect was evaluated by agar diffusion and minimum inhibitory concentration, in Gram positive and Gram negative control strains, as well as in MDR strains isolated from clinical settings. The effect of AgNP@bc on the cell envelope (pZ of microorganisms) was

determined, and the possible mechanisms of action on the cytoplasmic membrane (non-polar fluorescent probe 1-N-phenylnaphthylamine; NPN), and the ability to produce species reactive oxygen (ROS, nitrotetrazoyl blue reduction technique, NBT).
The results demonstrated that through biogenic synthesis, AgNPs with spherical morphology, with a negative surface charge, were formed by the action of exopolysaccharides present in the extracellular extract. The AgNP@bc exhibited antimicrobial activity against Gram positive and -negative strains whose mechanism of action was strain dependent, in Gram negative strains disrupting the cytoplasmic membrane while in Gram positive strains the effect was associated with the production of ROS. The AgNP@bc were active on Gram-negative clinical strain sensitive to colistin, suggesting a similar possible mechanism. These results demonstrate that AgNP@bc are promising candidates for new antimicrobial therapies against MDR strains. Future studies are needed to evaluate their clinical application.

Keywords: antimicrobial resistance; biogenic synthesis; silver nanoparticles

Methods: Spectroscopy, microscopy, agar diffusion, minimum inhibitory concentration

BT-14

Biosorption, bioaccumulation and bioreduction of hexavalent chromium by *Trichoderma koningiopsis* LBM 253

Tatarin, Ana Silvia 1; Sadañoski, Marcela Alejandra 12; Polti, Marta Alejandra 3; Fonseca, María Isabel 12
1: Universidad Nacional de Misiones. Facultad de Ciencias Exactas, Químicas y Naturales. Instituto de Biotecnología Misiones. Laboratorio de Biotecnología Molecular. CP3300. Posadas, Misiones, Argentina. 2: Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina. 3: Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET), Avenida Belgrano y Pasaje Caseros, 4000, San Miguel de Tucumán, Tucumán, Argentina
anasilvia2705@gmail.com

Chromium (Cr) is one of the most toxic metals that cause the pollution of soil and groundwater. Cr(III) and Cr(VI) are the most stable forms of this metal. The high toxicity of Cr(VI) poses a significant threat to human health due to its potent oxidizing, mutagenic, and carcinogenic properties. Mycoremediation is an eco-friendly and effective technology to clean Cr contaminated sites. Fungal exposure to heavy metals can induce the development of mechanisms that allow them to metabolize and reduce the

concentration of these pollutants through mostly involving biosorption, bioaccumulation and/or bioreduction. Based on this background, the aim of this work was to determine the main mechanisms implicated in Cr mycoremediation. Biosorption, bioaccumulation and bioreduction assays were carried out with 4, 8, and 12 days of fresh fungal mycelia cultured in presence of 200 mg L-1 of Cr(VI). Controls were carried out with fungal mycelia cultured without Cr(VI). Mycelia were fixed, dehydrated, dried, and gold metalized to analyze using a scanning electron microscope coupled with an energy-dispersive X-ray spectrophotometer (SEM-EDX) to biosorption assays. Fresh mycelia were disrupted with liquid nitrogen, and the supernatant was used for Cr(VI) bioaccumulation and intracellular chromate reductase (ChrR) activity determinations. Metal bioaccumulation capacity was calculated based on Cr(VI) intracellular concentration. SEM-EDX analysis confirmed biosorption mechanism through the presence of Cr on the mycelial surface of *T. koningiopsis* LBM 253. EDX analysis did not detect Cr on fungal surfaces from the control assays. The determination of Cr(VI) bioaccumulation revealed values of 3.47 ± 0.28; 4.82 ± 0.14 and 6.63 ± 0.45 µg g-1 at 4, 8, and 12 days of incubation, respectively. These results demonstrate that *T. koningiopsis* LBM 253 was able to bioaccumulate Cr(VI) inside the cell which could be attributed to the high level of tolerance of this fungus. *T. koningiopsis* LBM 253 showed both extracellular and intracellular ChrR activity indicating their involvement in Cr(VI) bioreduction. Cr(VI) presence in culture media induced changes in both intracellular and extracellular ChrR activity. Notably, higher titers of extracellular ChrR activity were observed in absence of Cr(VI), and the peak of ChrR activity was detected at 4 d of incubation. In the presence of Cr(VI), the highest extracellular ChrR titers were detected at 4 and 12 d of incubation. Intracellular fractions of culture medium containing Cr(VI) exhibited the highest titers of ChrR activity at 8 d of incubation. Based on our findings, we propose that *T. koningiopsis* LBM 253 utilizes biosorption, bioaccumulation, as well as extracellular and intracellular bioreduction, which occur simultaneously and dynamically, enhancing the overall remediation process. This response highlights the ability of *T. koningiopsis* LBM 253 to remove Cr(VI) by employing different intracellular and extracellular response mechanisms.

Keywords: Biosorption, Bioaccumulation, Bioreduction, Hexavalent Chromium

Methods: Spectroscopy, Electronic microscopy

Neurosciences

NS-01

Intercellular communication-based approach for Parkinson's disease treatment

Díaz-Reyes, Mercyleidi[1]; Magaquian, Darío[1]; Gatti, Sabrina[1]; Salvador, Gabriela[2]; Banchio, Claudia[1]
[1]Laboratorio de Biología Molecular y Celular de Lípidos, IBR (CONICET-UNR); [2]Instituto de Investigaciones Bioquímicas de Bahía Blanca, INIBIBB (CONICET)
mercy961015@gmail.com

Parkinson´s disease is the second most prevalent neurodegenerative disease in the world. It is caused by death of dopaminergic neurons in the substantia nigra and characterized by the aggregation of the α-Synuclein protein in cytoplasmic inclusions called Lewy bodies, mitochondrial dysfunction, generation of reactive oxygen species and apoptosis. The present study aimed to establish "in vitro" models of Parkinson's disease. For this, SH-SY5Y neuroblastoma cells were transfected with plasmids designed to overexpress the wild type α-Synuclein or the mutant A53T (mutation of adenine by threonine in amino acid 53 that increases protein aggregation), or cells were treated with 6-hydroxydopamine, a drug that induces mitochondrial deficits and stimulates several pro-apoptosis molecular factors. To validate the models, survival and cellular death were analyzed and quantified by biochemical and morphological methods. We also analyzed the effect of different molecules in the survival of neurons in the stablished models of Parkinson's disease

Keywords: Parkinson disease, in vitro models, alfa-synuclein, 6-hidroxidopamine

Methods: confocal microscopy, western blot, MTT, dichlorofluorescein, double staining

NS-02

Relevance of APP-Go signaling for Alzheimer´s pathogenesis

Marmo, Paula1; Moro, Lucía[2]; Antonino, Magdalena[1]; Almirón, Romina[1]; Morales, Carolina[1]; Las Peñas, María Laura[3]; Busciglio, Jorge[4]; Bisbal, Mariano[1]; Bollo, Mariana1; Bignante, Anahí[1]; Lorenzo, Alfredo[1]

[1]Instituto de Investigación Médica Mercedes y Martín Ferreyra - INIMEC-CONICET-UNC.; [2]LIAN-FLENI-CONICET; [3]IMBIV-CCT CORDOBA-CONICET; [4]Department of Neurobiology and Behavior, Institute for Memory Impairments and Neurological Disorders (iMIND), and Center for the Neurobiology of Learning and Memory (CNLM), University of California, Irvine
pmarmo@immf.uncor.edu

Alzheimer's disease (AD) is the most common cause of cognitive impairment and dementia in older adults, for which it is imperative to find effective therapies. Brain accumulation of amyloid β (Aβ) plays a central role in the pathogenesis of AD by promoting neurodegeneration. Aβ is generated as a result of the processing of the amyloid precursor protein (APP). Furthermore, evidence from different laboratories shows that APP would be necessary for cellular responses to Aβ, at least in part by functioning as a receptor capable of signaling through the heterotrimeric protein Go (Go). In vitro data indicate that histidines 657–658 in the intracellular domain of APP are critical residues for Go interaction/activation. Based on these data and the identification of a natural non-pathogenic variant of APP (M-APP), in which histidine 658 is replaced by a proline (APPH658P), we postulate that this mutation is protective. To assess APP-Go interaction in living cells, we used bimolecular fluorescent complementation (BiFC). Transfection of N2a cells with APP:VN (or M-APP:VN) and Goα:VC reconstituted Venus fluorescence (BiFC) and revealed that M-APP does not affect Go interaction. To study Go activation in intact cells, we developed a BiFC biosensor based on the complementation of the peptide KB-1753:VN with activated Goα:VC. Preliminary observations suggest that this biosensor might be useful for addressing the role of APP-Go signaling in response to Aβ. In N2a cells transfected with APPwt, Aβ enhances BiFC, which is abolished by Pertussis toxin (an inhibitor of Go/i activation). To generate a human neuronal model to study the relevance of M-APP for AD, we developed isogenic human-induced pluripotent stem cells (IPSC). Progress in the neuronal differentiation of these cells is presented.

Keywords: Alzheimer's disease, Heterotrimeric Go protein, Amyloid beta, Amyloid precursor protein, Induced pluripotent stem cells

Methods: Förster Resonance Energy Transfer (FRET), Bimolecular Fluorescence Complementation (BiFC), CRISPR/Cas9, Immunofluorescence Microscopy, Transfection

NS-03

Deciphering α-synuclein impact on neurodegeneration in Parkinson's Disease through targeted perturbation and snRNA-seq

Camila D. Arcuschin[1], Alexia Lantheaume[2], Saeede Salehi[2], Abdolhossein Zare[2], Pedro Salaberry[1], Marina Pinkasz[1], Martín Iungman1, Michael Briese2, Philip Tovote[2], María Soledad Espósito3 and Ignacio E. Schor[1,4]
[1]Instituto de Fisiología, Biología Molecular y Neurociencias (UBA-CONICET), Buenos Aires, Argentina; [2]Julius-Maximilians-UniversitätofWürzburg, Würzburg, Germany; [3]Medical Physics Department, Centro AtomicoBariloche, Río Negro, Argentina; [4]Departamento de Fisiología, Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina
arcucamila@gmail.com

α-Synuclein (α-syn) is a distinctive neural protein known for its aggregation within specific brain regions in Parkinson's disease (PD). The uneven manifestation of PD across brain regions has sparked the hypothesis that the disease follows a route of pathogenic aggregates propagation, impacting vulnerable cells within the adult brain. However, these aggregates don't consistently correspond to cell degeneration and their role is still controversial. To better understand the early stages of neurodegeneration and dissect the mechanisms behind neuronal autonomous susceptibility to α-syn, we devise an approach to identify common molecular patterns among sensitive neuronal subgroups that differ from their resistant counterparts. To specifically investigate α-syn-induced effects in vivo, we introduced a pathogenic form of the protein (hα-synA53T) into specific neurons in mice, by combining Cre transgenic mice and stereotactically injected Cre-inducible AAV. We also injected another Cre-dependent AAV coding for a nuclear lamina associated fluorescent protein (GFP-KASH) to allow purification of the target cells by FACS, which was followed by single-nucleus RNA-seq. Our initial focus was on the noradrenergic locus coeruleus (LC), a less explored brain region in comparison with the more extensively studied substantia nigra. Co-injection of AAV-hα-synA53T and AAV-GFP-KASH into LC of adult NET::Cre mice (which expressed Cre on noradrenergic neurons) resulted in a small neuronal loss with signs of neurodegeneration after 2-3 months. We therefore performed snRNA-seq in two different sample sets, each including both control and treated LCs from the same mouse, dissected 1 and 2 months after injection. After sequencing, we pre-processed the data, filtered trustworthy nuclei, integrated the different samples, analyzed the cell populations and compared the expression profiles between treatments. Our preliminary analysis, involving 13000 nuclei altogether, unveiled distinct cell clusters enriched in GFP-KASH and hα-synA53T expression, implying the potential existence of more than one noradrenergic LC cell subpopulation. Also these cells exhibited distinctive pathway enrichment identified through gene set variability and overdispersion analysis. Moreover, some of these clusters exhibited unique gene expression responses to hα-synA53T overexpression compared to the control treatment, as assessed through differential expression and functional enrichment analysis. As a whole, our results suggest the presence of diverse molecular mechanisms contributing to α-syn-induced degeneration within different LC subpopulations. We next plan to further study the biological identity of the different cell subpopulations, as well as to experimentally test the relevance of genes that are consistently regulated in response to hα-synA53T expression.

Keywords: snRNA-seq, Parkinson's disease, α-Synuclein, gene regulatory networks, neurodegeneration

Methods: snRNA-seq, AAV, Cre transgenic mouse, gene expression analysis

NS-06

Chromatin Nanodomains observed by superresolution microscopy during human neuronal differentiation from induced pluripotent stem cells

Cardozo Gizzi, Andres; Remedi, Laura; Gastaldi, Laura; Cáceres, Alfredo
Instituto Universitario de Ciencias Biomédicas de Córdoba (IUCBC), Centro de Investigación en Medicina Traslacional "Severo R. Amuchástegui" (CIMETSA); G.V. al Instituto de Investigación Médica Mercedes y Martín Ferreyra (INIMEC-CONICET-UNC), Córdoba, Argentina.
andres.cardozo@iucbc.edu.ar

During the course of differentiation, neural progenitors must turn on and off a different set of genes to accomplish a change in cellular phenotype. This is believe to occur thanks to changes in the epigenomic landscape of gene promoters and cis regulatory elements. Moreover, there is a functional relationship between the epigenetic modifications on chromatin and its 3D nuclear organization. Here, we used a human model of neural development derived from induced pluripotent stem cells to study how chromatin post-translational modifications change during the course of differentiation. In particular, we investigated the histone marks H3K27me3 (associated to the Polycomb repressive complex), H3K9me3 (constitutive heterochromatin) and H3K4me3 (associated to active promoters). A non-supervised multiparametric analysis, UMAP, was conducted on microscopy images to separate the different populations in the cell culture. It was found that human neurons are characterized by an intense H3K27me3 mark whereas neural progenitors (Sox2 positive) have relative low levels of this post-translational histone modification. Remarkably, Ezh2, the most active enzyme responsible of the covalent deposition of the mark, is considerably down-regulated as cells progresses to neurons. This lead us to question the role of Ezh2 in neurons. By using immunofluorescence combined with superresolution microscopy we were able to see in single-cells at the nanoscale the domain organization of H3K27me3 (associated to the Polycomb repressive complex) and H3K4me3 (associated to active promoters). Both by expansion and STED microscopy, it was found the existence of nanodomains of H3K27me3 whose size and intensity distribution changed according to cell type.
Overall, we found that epigenetic marks intensity and spatial pattern are highly dependent on cell type. By interfering with epigenetic remodeling, i.e. chemical inhibition of activity or knockdown of Ezh1/2 we expect to reveal changes in the expression of key genes in single cells. This initial characterization of the human model lays the ground to study how chromatin spatial organization is required as an additional

layer of gene expression regulation. We propose a novel approach to discern new mechanisms of transcriptional regulation in the context of human neuronal differentiation.

Keywords: epigenetics, chromatin 3D architecture, genome organization, polycomb repressive complex, human stem cells

Methods: STED microscopy, expansion microscopy, automated image analysis, non-supervised multivariate analysis

NS-07

Contribution of the epigenetic repressors REST, CoREST and G9a to the growth and survival of human iPSC-derived neurons in culture

Drube, José; Martínez, Imanol; Serniotti, Lucas; Gastaldi, Laura; Remedi, Mónica; Cáceres, Alfredo; Wilson, Carlos

Instituto Universitario de Ciencias Biomédicas de Córdoba (IUCBC), Centro de Investigación en Medicina Traslacional "Severo R. Amuchástegui" (CIMETSA); G.V. al INIMEC-CONICET-UNC, Córdoba, Argentina. Av. Naciones Unidas 420, Barrio Parque Vélez Sarsfield, X5016KEJ – Córdoba, Argentina

carlos.wilson@iucbc.edu.ar

Epigenetic regulation is critical for many aspects of neuronal life, ranging from development to disease. The prevailing view in the field states that the transcriptional repressor REST must be silenced during neurogenesis, being undetectable in post-mitotic neurons. Consequently, it is considered a master regulator of neuronal differentiation. However, recent reports have tempered these interpretations, since REST has been detected in post-mitotic mouse brains as well as in aging human brains. Intriguingly, REST is repressed in Sporadic Alzheimer's Disease brains, suggesting neuroprotective roles. Therefore, its expression, localization and contribution tightly depend on the physiological context.

Using induced pluripotent stem cells obtained by reprogramming healthy human fibroblasts (hiPSC) and confocal microscopy, we detected that REST accumulates in the nucleus of hiPSC-derived neurons within the first 21 days of differentiation in vitro (DIV). This observation supports the notion that REST is not only expressed in aging human neurons but also during post-mitotic development. Then, we targeted REST by treating neurons with the small molecule X5050, currently used in clinical approaches in Huntington's disease. Consequently, REST-suppressed neurons exhibited a massive depolarization-induced Ca^{2+} influx preceding axonal retraction and death. In addition, we detected the

expression of the transcriptional repressors G9a and CoREST in hiPSC-derived neurons, most likely recruited by REST to the chromatin. However, their expression pattern differs from that reported in murine neurons. Accordingly, our data confirms the expression of CoREST in neurons within the first 21 DIV, although we were unable to detect a strong nuclear signal as reported in rat/mouse neurons. Unexpectedly, we detected CoREST outside the nucleus, particularly in MAP2-positive dendrites, suggesting non-genomic functions. Finally, we also identified nuclear and cytoplasmic G9a expression within this timeframe, promoting axonal growth and neuronal survival.
Collectively, our data unveils the developmental and survival roles of REST in growing hiPSC-derived neurons. Moreover, the behaviors of G9a, CoREST and REST expression stress the notion of human-specific epigenetic mechanisms. Our next steps aim to precise their contribution to the physiology and dysfunction of neurons, identifying regulatory mechanisms and gene targets. Funding: PICT 2021-GRF-TI-00630, PIBA 2022-2023 (to CW) (ANPCyT, MINCyT), PIP 2021-2023 11220200102807CO (to AC, CW) (CONICET)

Keywords: neurons, iPSC, epigenetics, REST, growth

Methods: Neuronal culture, iPSC, confocal microscopy, calcium imaging

NS-08

Role of malonyl CoA in the neuronal control of feeding in *Caenorhabditis elegans*

Garnero, María Paula[1,2]; Mansilla, María Cecilia[1,2]
[1]Instituto de Biología Molecular y Celular de Rosario (CONICET-UNR), Argentina; [2]Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario
garneromariapaula@gmail.com

Obesity is an increasingly serious problem worldwide, and the few drugs available to treat and prevent the disease have severe side effects. Therefore, it is of great interest to elucidate the mechanisms that control appetite and body weight, to develop new therapies.
In mammals, changes in hypothalamic malonyl-CoA concentration have been postulated to contribute to regulation of appetite control. An increase in the levels of this molecule correlates with a concurrent reduction in appetite and consequently, body weight. Malonyl-CoA is a precursor of fatty acid biosynthesis, so the inhibition of Fatty Acid Synthase (FAS) increases its concentration. To determine if a

similar behaviour occurs in worms, we modified malonyl-CoA concentration using the mycotoxin cerulenin. This drug binds covalently to a cysteine residue in the active site of the eukaryotic FAS I ketoacyl synthase domain, blocking the interaction of the enzyme with malonyl-CoA. The feeding behaviour of the worms was evaluated by their rate of food intake, determined by measuring pharyngeal pumping of young adult worms treated with different concentrations of the mycotoxin. In all instances, lower pharyngeal pumping rates were observed in cerulenin-treated worms compared to untreated ones. Additional assays were performed to examine the potential impact of the drug on other relevant aspects of behaviour, such as mobility and egg laying. In these cases, no significant changes were evident in treated worms. Additionally, the malonyl-CoA concentration was modulated by gene expression silencing of fasn-1 using RNA interference (RNAi). To block the neuronal expression of FAS, TU3401 strain was utilized, in which interference is restricted to neurons. These worms exhibited a decrease in pumping rates, whereas the wild-type N2 strain, which underwent interference in all tissues except neurons, did not affect pumping speed following treatment.
Our results suggest that an increase in neuronal malonyl-CoA leads to a reduction in feeding rate, implying that this nematode could serve as a valuable model to contribute to the understanding of analogous processes in mammals.

Keywords: *Caenorhabditis elegans*, malonyl-CoA, appetite control

Methods: Measurement of pharyngeal pumping, egg laying screens, measurement of mobility rates, RNA interference

NS-09

Impact of post-translational incorporation of phenylalanine into tubulin on neuronal development.

Marín, Leandra; Zorgniotti, Agustina; Aranda, M. Florencia; Bisig, Gastón; Ditamo, Yanina
Universidad Nacional de Córdoba, Facultad de Ciencias Químicas, Departamento de Química Biológica Ranwel Caputto, CIQUIBIC- CONICET
leandra.marin@mi.unc.edu.ar

Phenylketonuria (PKU) is an inborn error of metabolism caused in most cases by nonsense mutations in the gene encoding the enzyme phenylalanine hydroxylase (PAH), which catalyzes the hydroxylation of phenylalanine (Phe) to generate tyrosine (Tyr). Absence or malfunctioning of the enzyme results in Phe

accumulation. Elevated plasma Phe levels are associated with several neurodevelopmental alterations such as microcephaly, epilepsy, permanent intellectual disability, developmental delay and motor disorders. These symptoms appear to be related to neuronal cell depletion, dendritic simplification and synaptic density reduction. Tubulin, the main constituent of microtubules, undergoes a process in which the alpha-chain C-terminalTyr residue is cyclically removed and re-incorporated by specific enzymes. There is increasing evidence of the importance of this posttranslational modification in several specific functions of microtubules. We have previously shown, both in vitro and in vivo, that Phe can be incorporated into and released from α-tubulin, showing kinetics very similar to those of Tyr. We also demonstrated that Phe tubulin polymerizes and that the microtubules enriched in Phe present altered dynamics. Now, we have examined the impact of the incorporation of Phe into the C-terminus of α-tubulin in cells of neuronal origin. Our results revealed a significant reduction in the length of the longest process and changes in the number of neurites per cell. These results suggest that the incorporation of Phe into tubulin affects the mechanisms involved in neurites formation. In addition, we analyzed mitochondrial transport and the distribution of these organelles along the cell processes.
Our findings could be relevant in the context of hyperphenylalaninemia observed in PKU where the elevated levels of Phe may lead to post-translational incorporation of this aminoacid into tubulin. The formation of Phe-enriched microtubules might represent one of the initial events contributing to the severe neurodevelopmental defects observed in untreated PKU.

Keywords: microtubules, postranslation modification of tubulin, phenylalanine, phenylketonurya

Methods: confocal microscopy, immunofluorescence, cell culture

NS-10

Huntingtin: a regulator of vesicle trafficking in neurosecretion

Canal, María Pilar Marengo, Fernando Diego, Gallo, Luciana Inés
Instituto de Fisiología, Biología Celular y Neurociencias (IFIBYNE, CONICET-UBA); Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires
pili.canal31@gmail.com

Huntington's disease (HD) is a hereditary neurodegenerative disorder caused by an expanded poly-glutamine stretch in the huntingtin protein. To date, the functions of normal huntingtin (Htt) and the mechanisms by which mutant Htt (mHtt) causes the disease remain unclear. Among the known

cellular processes affected by mHtt, dense-core vesicle (DCV) secretion is decreased in HD animal models, and HD patients feature symptoms associated with altered neuropeptide functions. Additionally, emerging data indicate an important role for Htt in vesicle trafficking. However, how mHtt affects the regulated-secretory pathway in neuronal and neuroendocrine cells is still unknown. Chromaffin cells are a classical experimental model of neurosecretion, where neuropeptides and catecholamines are co-packaged in DCVs. DCVs bud from the Golgi apparatus as immature vesicles which need to mature and transport to the plasma membrane. At the cell periphery, DCVs fuse to the plasma membrane after membrane depolarization and therefore they secrete their cargo by calcium-regulated exocytosis. In this study we analyzed the mobility and distribution of DCVs in chromaffin cells by spatially mapping DCVs inside the cell using confocal imaging and bioimage analysis tools. We report that overexpression of either Htt or mHtt decreased the transport of DCVs to the cell periphery and generated changes in the transport regimes of DCVs. We have preliminary data showing that, in the presence or absence of stimuli, the overexpression of Htt favored the directed movement of DCVs. On the other hand, the expression of mHtt blocked this behavior, and DCVs showed a predominant confined motion in both conditions. Our approach will allow us to study and better understand how Htt regulates trafficking in neurosecretion.

Keywords: Neurosecretion, Huntington´s Disease, Vesicle trafficking

Methods: Confocal microscopy, Live cell imaging