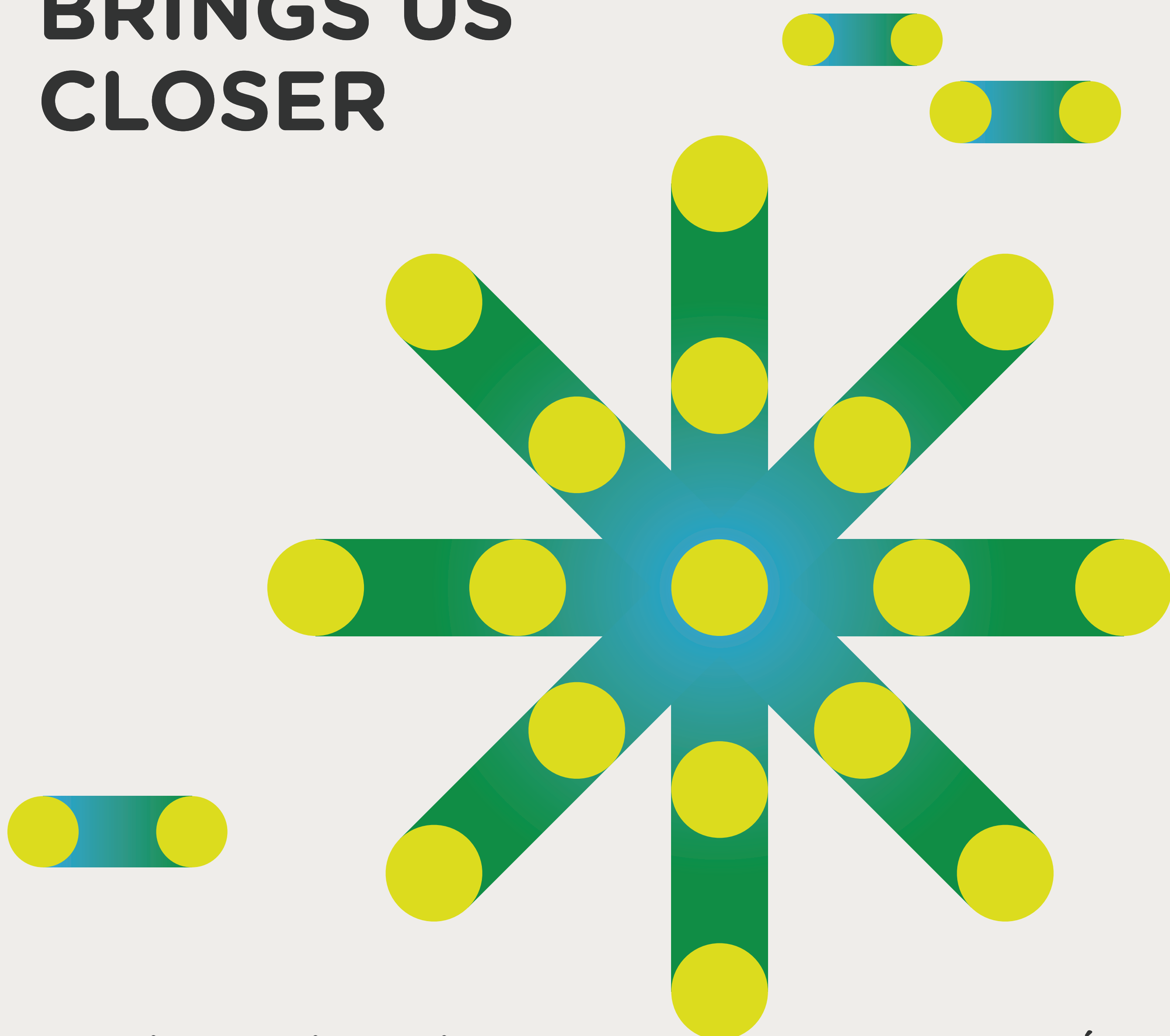
SAIB Sociedad Argentina de Investigaciones en Bioquímica y Biología Molecular

CONGRESO SAIB 2025

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

SCIENCE BRINGS US CLOSER



October 27th–30th, 2025
Pabellón Argentina | U.N.C.
Córdoba, Argentina.

EDICIÓN 61

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DELEGATES OF SCIENTIFIC SECTIONS

Dr. Mauricio Martin
Cell Biology
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Dr. Ariel Quiroga
Lipids
IFISE-CONICET. National University of Rosario

Dra. Julieta Fernández
Microbiology
IBBM-CONICET. National University of La Plata

Dra. Georgina Fabro
Plants
CIQUIBIC-CONICET. National University of Córdoba

Dr. Andrés Dekanty
Signal Transduction
IAL-CONICET. National University of Litoral

PROGRAM AT A GLANCE

Monday 27/10

10.30 h - 12.15 h. Registration

12:15 h - 14:30 h Break

14.30 h - 14.45 h. Opening Ceremony (Sala de las Américas)

14.45 h - 15.30 h. "IUBMB" Plenary Lecture
Dr. Francisco Quintana (Sala de las Américas)

15.30 h - 17.30 h. Young Investigators Simposia I (Sala de las Américas)

17.30 h - 18.00 h. Coffee-Break

18.00 h - 19.15 h. "Héctor Torres" Plenary Lecture
Dra. Raquel Chan (Sala de las Américas)

19.30 h - 21.00 h. Welcome Cocktail (Hall Central)

Tuesday 28/10

08.30 h - 10.30 h. Oral Communications
Microbiology (Sala de las Américas)
Lipids (Salón de Actos)

10.30 h - 11.00 h. Coffee-Break

11.00 h - 12.15 h. "PABMB" Plenary Lecture
Dr. **Holger Sondermann** (Sala de las Américas)

12:15 h - 13:15 h Methodological advances (Sala de las Américas)

12.15 h - 14.30 h. Lunch Time

14.30 h - 16.30 h. Symposia
Microbiology (Sala de las Américas)
Lipids (Salón de Actos)

16.30 h - 17.00 h. Coffee-Break

17.00 h - 18.15 h. "Alberto Sols" Plenary Lecture
Dr. **Jesús Perez Gil** (Sala de las Américas)

18.15 h - 20.00 h. POSTERS

Wednesday 29/10

08.30 h - 10.30 h. Oral Communications
Plants (Sala de las Américas)
Signal Transduction/Neurosciences/Biotechnology/Enzymes (Salón de Actos)

10.30 h - 11.00 h. Coffee-Break

11.00 h - 12.15 h. Plenary Lecture
Dr. Pablo Manavella (Sala de las Américas)

12.15 h - 14.30 h. Lunch Time

14.30 h - 16.30 h. Symposia
Plants (Sala de las Américas)
Signal Transduction (Salón de Actos)

16.30 h - 17.00 h. Coffee-Break

17.00 h - 18.15 h. Plenary Lecture
Dr. Francisco García Portillo (Sala de las Américas)

18.15 h - 20.00 h. POSTERS
19.00 h – 22.00 h. SAIB Assembly (Sala de las Américas)

Thursday 30/10

08.30 h - 10.30 h. Oral Communications
Cell Biology (Sala de las Américas)
Plants/Microbiology/Biotechnology (Salón de Actos)

10.30 h - 11.00 h. Coffee-Break

11.00 h - 12.45 h. POSTERS

12.45 h - 14.30 h. Lunch Time

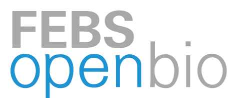
14.30 h - 16.30 h. Symposia
Cell Biology (Sala de las Américas)
Young Investigators II (Salón de Actos)

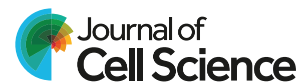
16.30 h - 17.00 h. Coffee-Break

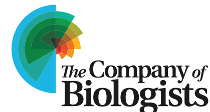
17.00 h - 18.15 h. “Ranwel Caputto” Plenary Lecture
Dra. Marisa Colombo (Sala de las Américas)

18.15 h-20.00 h. Awards and Closing Ceremony Room (Sala de las Américas)

This meeting was supported by:







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CONGRESO SAIB 2025

PROGRAM

Monday, October 27, 2025

10:30-12:15. Registration

12:15-14:30. Lunch Time

14:30-14:45. Opening Ceremony
Sala de las Américas

14:45-15:30. "IUBMB" Plenary Lecture
Sala de las Américas
Dr. Francisco J. Quintana
Ann Romney Center for Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA. Gene Lay Institute for Inflammation and Immunology, Harvard Medical School, Boston, MA, USA.Broad Institute of MIT and Harvard, Cambridge, MA, USA.
"Regulation of the Immune Response in the Central Nervous System by Astrocytes"
Chairperson: Dr. Mario Guido

15:30-17:30. Young Investigators Symposia I
Sala de las Américas
Chairpersons: Dr. Laura Delgui –Dr. Claudio Fader Kaiser

> **15:30-16:00**
> Dr. Jimena Leyria
> *CIBICI, Córdoba.*
> **"Neuroendocrine control of reproduction in *Rhodnius prolixus*, model organism and Chagas disease vector: insights into juvenile hormone modulation"**
>
> **16:00-16:30**
> Dr. Rocío Tognacca
> *IFIBYNE, CABA.*
> **"Splicing the way to germination: *RS31* and *DAG1* shape light responses in *Arabidopsis thaliana* seeds"**
>
> **16:30-17:00**
> Dr. Matías Capella
> *IAL, Santa Fe.*
> **"The segregase CDC48 integrates blue light and hormonal cues to regulate plant Development"**

17:00-17:30
Dr. Andrés Cardozo
CIMETSA, Córdoba.
“Super-Resolution Insights into Chromatin Architecture in Neuronal Development”

17:30-18:00. Break

18:00-19:15. "Héctor Torres" Plenary Lecture
Sala de las Américas
Dra. Raquel Chan
Instituto de Agrobiotecnología del Litoral (IAL-CONICET-FBCB - UNL). **“Bridging the gap: overcoming challenges in applying transcription factor research from model systems to crop improvement in field environments”**
Chairperson: Dr. María Elena Alvarez

19:30-22:00. Welcome Cocktail
Hall Central

Tuesday, October 28, 2025

08:30-10:30. Oral Communications
Microbiology. Sala de las Américas
Lipids. Salón de Actos

10:30-11:00. Coffee-Break

11:00-12:15. “PABMB” Plenary Lecture
Sala de las Américas
Dr. Holger Sondermann.
CSSB Centre for Structural Systems Biology, Hamburg, Germany.Deutsches Elektronen-Synchrotron DESY, Hamburg, Germany.Christian-Albrechts University zu Kiel, Kiel, Germany.
“Dinucleases at the crossroads of bacterial signaling and RNA degradation”
Chairperson: Dr. Federico Sisti

12:15-13:15. Methodological advances Symposium
Sala de las Américas
Chairperson: Dr. Paula Casati

12:15-12:35
Daniela González
ETC Interncional
“Seeing Biology in 5D. Element’s AVITI 24TM: Next Level Solution for NGS and Multiomics Applications”

12:35-12:55
Ricardo Fechio
Bioesanco

“Good pipetting practices”

12:55-13:15
María Fernanda Sosa
OneLab Solutions SA
“The Green revolution in science: sustainable and innovation in molecular biology laboratories”

12:15-14:30. Lunch Time

14:30-16:30. Microbiology Symposium
Sala de las Américas
Chairpersons: Dr. Julieta Fernández-Dr. Alex Saka

14:30-15:00
Dra. Natalia Gottig
Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET).
“Decoding C-Di-GMP control in Mn(II) Oxidation in Pseudomonas resinovorans for Grownwater Bioremediation”

15:00-15:30
Dr. Andrés Garriz
Instituto Tecnológico de Chascomús.
“Polyamines as Regulators of Oxidative Stress Response in Phytopathogenic Bacteria”

15:30-16:00
Dra. Daniela Albanesi
Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET).
“**Membrane and Cell Wall Biogenesis in Gram-positive Bacteria: Two Independent or Coordinated Processes?”**

16:00-16:30
Dra. Ana LauraVillasuso
Instituto de Biotecnologia Ambiental y de la Salud (INBIAS-CONICET).
Universidad Nacional de Rio Cuarto.
“Harnessing the microbiome of vermi-compost to control fungal disease”

14:30-16:30. Lipids Symposium
Salón de Actos
Chairpersons: Dr. Martin Oresti – Dr. Cecilia Casali

14:30-15:00
Dra. Natalia Alza
Instituto de Investigaciones Bioquímicas de Bahía Blanca – UNS.
“Exploring natural products to overcome ferroptosis, a form of cell death driven by lipid peroxidation”

15:00-15:30
Dr. Agustín Mangiarotti

Centro de Investigaciones en Química Biológica de Córdoba – UNC.
“When membrane-bound meet membrane-less: wetting, remodeling, and damage stabilization”

15:30-16:00
Dr. Lucas Sosa Alderete
Instituto de Biotecnología Ambiental y Salud - UNRC.
“Influence of xenobiotic exposure on the daily changes of glycerophospholipid turnover: insights from tobacco hairy roots as a model system”

16:00-16:30
Dra. Francisca Verónica Bronfman Cáceres
Universidad Nacional Andrés Bello - Santiago de Chile - Chile.
“Uncovering the functional effects and molecular targets of lipids derived from the macroalga *Gracilaria chilensis*”

16:30-17:00. Coffee-Break

17:00-18:15. “Alberto Sols” Plenary Lecture
Sala de las Américas
Dr. Jesús Perez-Gil
Department of Biochemistry and Molecular Biology, Faculty of Biology, and Research Institut “Hospital 12 de Octubre (imas12)”, Madrid, Spain.
“**Molecular and celular mechanisms behind the mechanical and biological protection of the respiratory surface”**

Chairperson: Dr. Gerardo Fidelio

18:15-20:00. Posters Microbiology (MI), Lipids (LI).

Wednesday, October 29, 2025

08:30-10:30. Oral Communications
Plants. Sala de las Américas
Signal Transduction/Biotechnology/Neurosciences/Enzymology. Salón de Actos

10:30-11:00. Coffee-Break

11:00-12:15. Plenary Lecture
Sala de las Américas
Dr. Pablo Manavella
Instituto de Hortofruticultura Subtropical y Mediterránea (IHSM) 'La Mayora', Universidad de Málaga-Consejo Superior de Investigaciones Científicas (UMA-CSIC), 29010 Málaga, Spain.
“Transposon-mediated chromatin folding defines Pol-II transcriptional activity”
Chairperson: Dr. Georgina Fabro

12:15-14:30. Lunch Time

14:30-16:30. Plants Symposium
Sala de las Américas
Chairpersons: Dr. Nicolás Cecchini- Dr. Georgina Fabro

14:30-15:00
Dra. María Eugenia Zanetti
Laboratorio de Biología de Raíz, Instituto de Biotecnología y Biología Molecular (Consejo Nacional de Investigaciones Científicas y Técnicas - Universidad Nacional de La Plata).
"The plant specific histone H3K27 demethylase MtPKDM9B functions in root development and the nitrogen fixing symbiosis in the model legume *Medicago truncatula*"

15:00-15:30
Dr. Santiago Signorelli
Universidad de la República, Uruguay.
"The role of proline biosynthesis in plants: a biochemical perspective"

15:30-16:00
Dr. Elina Welchen
Instituto de Agrobiotecnologia del Litoral, IAL-CONICET.
"**Power Struggles: Growing, Defending, or Both?"**

16:00-16:30
Dr. Ezequiel Petrillo
Instituto De Fisiología, Biología Molecular Y Neurociencias (IFIBYNE, CONICET-UBA).
"Understanding the Whys, Hows, and Consequences of Alternative Splicing Regulation in Plants"

14:30-16:30. Signal Transduction Symposium
Salón de Actos
Chairpersons: Dr. Andres Dekanty – Dr. Graciela Boccaccio

14:30-15:00
Dr. Omar Coso
Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE, CONICET-UBA).
"**Signal transduction in the context of aids related malignancies: Kaposi Sarcoma and beyond"**

15:00-15:30
Dr. Diego Rayes
Instituto de Investigaciones Bioquímicas de Bahía Blanca – UNS.
"**Surviving a Hostile World: Neural Coordination of Stressor-Specific Strategies in *C. elegans*"**

15:30-16:00
Dr. Nara Muraro
Instituto de Investigación en Biomedicina de Buenos Aires (IBioBA)-CONICET.

“**Why do we Sleep? Insights from Drosophila”**

17:00-18:15. Plenary Lecture
Sala de las Américas
Dr. Francisco García del Portillo
Laboratorio de Patógenos Bacterianos Intracelulares. Centro Nacional de Biotecnología (CNB). Consejo Superior de Investigaciones Científicas (CSIC). Madrid. España.
“Diversity of morphogenetic peptidoglycan synthases in the domain bacteria”
Chairperson: Dr. José Echenique

18:15-20:00. Posters Plants (PL), Signal Transduction (ST), Structural Biology (SB), Biotechnology (BT), Enzymology (EN). Neurosciences (NS).

19:00-22:00. SAIB Assembly
Sala de las Américas

Thursday, October 30, 2025

08:30-10:30. Oral Communications
Cell Biology. Sala de las Américas
Biotechnology/Structural Biology/Neurosciences. Salón de Actos

10:30-11:00. Coffee Break

11:00-12:45. Posters Cell Biology (CB).

12:45-14:30. Lunch Time

14:30-16:30. Cell Biology Symposium
Sala de las Américas
Chairpersons: Dr. Mariano Bisbal- Dr. Mariana Bollo

14:30-15:00
Dr. Ramiro Rodríguez Virasoro
Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET).
“**Regulation of cell cycles and cell biology processes driving plant organ growth”**

15:00-15:30
Dr. Diego Croci Russo
Instituto de Histología y Embriología de Mendoza.
“**Modified miRNAs Restore Chondrocyte Function Under Inflammatory Stress: Toward a Reliable Disease-Modifying Strategy for Osteoarthritis”**

15:30-16:00
Dr. María Paz Marzolo
Pontificia Universidad Católica (PUC). Santiago de Chile.

“Intracellular trafficking defects underly dysfunctions in reelin signaling: implications for a rare genetic disease affecting the central nervous system”

16:00-16:30
Dr. Graciela Boccaccio
Fundación Instituto Leloir.
“Smaug, Hydra, or Dragon? Condensation of a Post-Transcriptional Regulator into Membraneless Organelles”

14:30-16:30. Young Investigators II Symposia
Salón de Actos
Chairpersons: Dr. Laura Delgui – Dr. Paula Casati

14:30-15:00
Dra. Ana Racca
CIBICI, Córdoba.
“Regulatory Mechanisms at the Maternal–Fetal Interface: Insights from pregnancy pathologies and connection to cáncer”

15:00-15:30
Dr. Noelia Foressi
UNMdP, Mar del Plata.
“Redox homeostasis and tor kinase as central regulators of primary nitrate response in plants”

15:30-16:00
Dr. Damián Cambiagno
UDEA, Córdoba.
“Systemic resistance to pathogens in Arabidopsis requires HASTY-dependent miRNA cell-to-cell movement”

16:00-16:30
Dr. Leandro Lucero
IAL, Santa Fe.
“From Bud to Branch: Gene Expression Regulation at Multiple Levels Dictates Plant Architecture”

16:30-17:00. Coffee-Break

17:00-18:15. “Ranwel Caputto” Plenary Lecture
Sala de las Américas
Dra. María Isabel Colombo
Laboratory of molecular mechanisms involved in vesicular trafficking in the phagocytic and autophagic pathways. IHEM-CONICET- UNCUYO-FCM-Mendoza, Argentina.
“**Intracellular pathogens and autophagy: partners in a tango dance”**
Chairperson: Dr. José Luis Bocco

18:15-20:00. Awards and Closing Ceremony
Sala de las Américas

ORAL COMMUNICATIONS- Tuesday, October 28th

Microbiology. Sala de las Américas
Chairpersons: *Dr. Claudia Sola– Dr. Daniel Raimunda*

8:30 MI-01
β-LACTAMASE SUB-CELLULAR LOCALIZATION
Capodimonte L, Vila AJ

8:45 MI-02
SPATIAL PATTERNS OF SURVIVAL AND REGROWTH OF CELL SUBPOPULATIONS WITHIN *ESCHERICHIA COLI* BIOFILMS FOLLOWING ANTIBIOTIC TREATMENT
Valentinis Rossi FL, Obando MC and Serra DO

9:00 MI-03
INTRACLONAL DIVERSIFICATION THROUGH RECOMBINATION DRIVES THE EMERGENCE OF NOVEL EPIDEMIC CARBAPENEMASE-PRODUCING *KLEBSIELLA PNEUMONIAE* ST258 SUBLINEAGES
Morandini FN, Lipari, FG, Irrazabal MG, Ruiz SE, Cordoba CPE study group, Saka HA

9:15 MI-04
ULTRASTRUCTURAL ANALYSIS OF *STAPHYLOCOCCUS AUREUS* EXPOSED TO *LACTOCOCCUS SPP*., PENICILLIN G, AND THEIR COMBINATION.
Capello MI, Aguirre GE, Zarazaga MP, Isuardi N, Paz MC, Litterio NJ

9:30 MI-05
MICROCINS AS THERAPEUTIC AGENTS AGAINST EPIDEMIOLOGICALLY RELEVANT *SALMONELLA* STRAINS IN SALTA PROVINCE
Sandoval RA, Pioli MA, Occhionero MA, Maresca MM, Slavutsky AM, Acuña L, Corbalán NS

9:45 MI-06
INSIGHTS OF THE RESPONSE MECHANISM TO ALBUMIN AND CALCIUM ON BIOFLM FORMATION IN *BORDETELLA BRONCHISEPTICA*
Mugni SL, Sisti F, Fernández J

10:00 MI-07
***LEUCONOSTOC MESENTEROIDES* STRAIN WH8 AND *ENTEROCOCCUS HIRAE* STRAIN WF5 ISOLATED FROM WALNUT AS BIOCONTROL AGENTS AGAINST WALNUT PHYTOPATHOGENS**
Wagner V, Vasquetto, A, Silva, JA, Pellegrino, MS, Príncipe A

10:15 MI-08
REGULATION OF MFD-MEDIATED MUTAGENESIS BY MUTS PREVENTS THE EMERGENCE OF ANTIBIOTIC RESISTANCE IN *BACILLUS SUBTILIS*
Ibañez Busseti MI and Monti MR

Lipids. Salón de Actos
Chairpersons: *Dr. Edith Guaytima- Dr. Ariel Quiroga*

8:30 LI-01
THE PLANT UREASE "JACK BEAN UREASE" IMPAIRS LIPID METABOLISM ON THE FAT BODY-OVARY AXIS IN THE CHAGAS DISEASE VECTOR *RHODNIUS PROLIXUS*
Paglione PA, Carvalho MF, Leyria J, Fruttero LL, Atella GC, Canavoso LE

8:45 LI-02
GLIAL EXTRACELLULAR VESICLES PRODUCTION AND LIPID-DEPENDENT NEUROPROTECTION: A NEW CASE STUDY
Benzi Juncos, ON, Alza, NP, Monyror J, Sipione, S, Salvador, GA

9:00 LI-03
ACSL4 REMODELS MICRORNA PROFILE IN BREAST CANCER: FOCUS ON MIR-99A
Quevedo LM, Bulian VC, Mele P, Nudler S, Orlando UD, Castillo AF

9:15 LI-04
NEUROTOXIC EFFECTS OF GLIAL-DERIVED 24-S-HYDROXYCHOLESTEROL: IMPACT ON SYNAPSE STRUCTURE, NEURONAL VIABILITY AND POTENTIAL AMYLOIDOGENIC ROLE
Perona A, Martin MG

9:30 LI-05
GLYCEROLIPID METABOLISM ACTIVATION IS ESSENTIAL FOR EPITHELIAL RESTITUTION AFTER CALCIUM OXALATE INJURY
Parra L, Sendyk DE, Verstraeten SV, Salafia A, Morel Gómez E, Fernández Tome MC, Casali CI

9:45 LI-06
ENDOCRINE DISRUPTOR NONYLPHENOL IMPAIRS LIPID METABOLISM AND MEIOTIC ENTRY IN PREPUBERTAL MOUSE TESTIS EXPLANTS
Tajes Ardanaz OJ, Sánchez Chaves MA, Luquez JM, Arias AH, Oresti GM

10:00 LI-07
GLYCOSPHINGOLIPIDS AS CRITICAL REGULATORS OF EPITHELIAL MORPHOGENESIS
Alvarez MB, Krivocapich C, Bardinella NG, Favale NO, Pescio LG

10:15 LI-08
ADAPTIVE LIPID METABOLISM IN DROSOPHILA: HOW DIETARY LIPIDS REWIRES *ORSAI* REGULATION
Mares ML,Dekanty A,Ceriani MF,Romero JI

ORAL COMMUNICATIONS- Wednesday, October 29th

Plants. Sala de las Américas
Chairpersons: *Dr. Ana Laura Villasuso – Dr. Laura Saavedra*

8:30 PL-01
ALTERNATIVE SPLICING AS A SOURCE OF REGULATORY LONG NON-CODING RNAs ARISING FROM CODING GENES IN PLANTS
Rodriguez FS, Pulichino L, Tognacca RS, Mammi PA, Aballay FE, Servi L, Gaggion N, Legascue MF, Ariel F, Crespi M, Petrillo E.

8:45 PL-02
LINKING MICRORNA BIOGENESIS AND MOBILITY TO SYSTEMIC DEFENSE IN PLANTS
Musso M, Alanie N, Quevedeo L, Trenchi A. Cecchini NM, Lascano HR, Cambiagno DA.

9:00 PL-03
COORDINATION OF SNRK1-MEDIATED ADAPTATIVE RESPONSES TO CHANGING GROWTH CONDITIONS
Brugnara C, Diaz MC, Aguilar Lucero DA, Bultri JG, 1, Fusari CM, Dengjel J, Levi V, Blanco NE.

9:15 PL-04
MINION T, THE BiTVOX PLAYER
Becerra-Agudelo E, Welchen E.

9:30 PL-05
UNCOVERING A NOVEL PATHWAY OF ANTHOCYANIN REPRESSION IN *ARABIDOPSIS THALIANA*
Jure RM, Viola IL, González DH.

9:45 PL-06
ADVANCES IN THE FUNCTIONAL STUDY OF *LsDRIP* GENES IN LETTUCE: EXPLORING NEW STRATEGIES FOR ADAPTATION TO ABIOTIC STRESS
Darqui F[1], Tajima H, Luege D, Sena M, Radonic L, Beracochea V, Blumwald E, López Bilbao M

10:00 PL-07
INTEGRATING OXYGEN ELECTRODE MEASUREMENTS AND IMAGE-BASED GREENNESS INDEX FOR COMPREHENSIVE PHOTOSYNTHESIS AND RESPIRATION ASSESSMENT IN *ARABIDOPSIS THALIANA*
Sena F, Couture C, Berais-Rubio A, Signorelli S

10:15 PL-08
THERMOPRIMING BOOSTS CHLOROPLAST ANTIOXIDANT CAPACITY AND IMPROVES HEAT STRESS SURVIVAL IN *ARABIDOPSIS THALIANA*
Suárez J, Robert G, Lobatto VL, Lascano HR, Lescano López I

Signal Transduction/Biotechnology/Enzymology/ Neurosciences. Salón de Actos

Chairpersons: *Dr. Alicia Degano-Dr. Mariana Bollo*

8:30 BT-01
OPTIMIZATION OF A SOLUBILIZATION AND REFOLDING METHOD FOR RECOMBINANT GLYCEROL KINASE
Faliva A, Espejo PJ, Barra JL, Godino A.

8:45 BT-02
ENGINEERED LACTOCOCCUS LACTIS AS A PLATFORM FOR ENZYME-ENRICHED SILAGE
Gizzi F, Giancristofano T, Taborra ME, Martin M, Guerrero S, Iglesias A, Magni C, Blancato V.

9:00 BT-03
PEG-COATED MAGNETIC NANOPARTICLES AS SAFE NANOTHERANOSTICS: BIODISTRIBUTION AND TARGETING IN A VIRAL ONCOGENESIS MOUSE MODEL
Principe G, Tiburzi S, Lezcano V, Montiel Schneider G, Sives F, Sánchez FH, García BN, Gumilar F, Lassalle V, González-Pardo V.

9:15 EN-01
ADVANCES IN THE STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF AGMATINASE-LIKE PROTEIN (ALP/LIMCH1)
Uribe EA, Reyes M, Fuentes A, Bustamante D, Retama F, Lillo I, Villegas C, Gatica M, Carrasco J, Figueroa M, Neira Y, Martínez J.

9:30 NS-01
DOPAMINE ASSESSMENT IN PARAQUAT-EXPOSED Caenorhabditis elegans: MITIGATION WITH N-ACETYLCYSTEINE
Gonzales-Moreno C, Virgolini MB.

9:45 NS-02
NITRO-OLEIC ACIDS AS A POTENTIAL THERAPEUTIC AGENT IN EXPERIMENTAL CHOROIDAL NEOVASCULARIZATION
Vaglienti MV, Tovo A, Barcelona PF, Bonacci G, Sánchez MC.

10:00 ST-01
ALLOSTERY CHARACTERIZATION ON ANGIOTENSIN CONVERTING ENZYME II
Acebedo Martinez M, Sacerdoti M, Gross L, Gironacci M, Di Lella S, Otero LH, Fernandez M, Klinke S, Biondi RM, Leroux AE.

10:15 NS-03
CALCINEURIN Aβ-MEDIATED MODULATION OF PERK SIGNALING IN REACTIVE ASTROCYTES
Morales C, De Batista J, Asis S, Chen Y, Martin M, Bollo M.

ORAL COMMUNICATIONS- Thursday, October 30th

Cell Biology. Sala de las Américas
Chairpersons: *Dr. Anahi Bignante- Dr. Pablo Aguilar*

8:30 CB-01
ACUTE *TRYPANOZOMA CRUZI* INFECTION REDUCES STARD7 EXPRESSION ASSOCIATED WITH MITOCHONDRIAL DYSFUNCTION, ALTERED LIPID METABOLISM, AND CHANGES IN OXIDATIVE STRESS ENZYME LEVELS IN THE MURINE LIVERS
Flores-Martín JL, Mazzocco YL, Aoki MP, Genti-Raimondi S

8:45 CB-02
IMPACT OF SILENCING A MEIOTIC lncRNA ON SPERMATOGENESIS: DEVELOPMENT OF AN ANTISENSE OLIGONUCLEOTIDE MICROINJECTION-BASED APPROACH
de los Santos-Silva E, Rodríguez-Casuriaga R, Geisinger, A

9:00 CB-03
RECIPROCAL REGULATION BETWEEN ACYL-COA SYNTHETASE 4 AND ANDROGEN RECEPTOR: IMPLICATIONS AS THERAPEUTIC TARGETS IN BREAST CANCER CELLS
Dattilo MA, López PF, Benzo Y, Decono M, Bigi MM, Mansini A, Hoeppner L, Podestá EJ, Paz C, Maloberti PM

9:15 CB-04
THE ROLE AND IMMUNOLOCALIZATION OF ITGB1 AND MERLIN PROTEINS DURING THE PROCESS OF VASCULOGENIC MIMICRY IN AN OVARIAN CANCER CELL LINE.
Santander GN, Silva M, González P, George V, Babbitt N, Canales C, Roa JC, Bizama C, Nualart F, Ravasio A, Bertocchi C, Owen GI

9:30 CB-05
GLYCOSYLTRANSFERASES DIFFERENTIALLY MODULATE THE GOLGI ONCOPROTEIN GOLPH3
Martínez-Koteki N, Rasino S, Lopez PHH, Fidelio GD, Chanaday NL, Vilacaes A

9:45 CB-06
MICRORNA-597 SUPPRESSES TUMOR PROGRESSION IN GASTRIC CANCER BY DIRECTLY TARGETING RUNX1 AND IS MODULATED BY THE LNCRNA KCNQ1OT1
Sandoval-Borquez A, Olivares W, Santoro PM, Carvajal FJ, Torres K, Ávalos-Guajardo Y, Bizama C, Quest A, Corvalán AH

10:00 CB-07
CALCIUM DEPENDENT DYNAMIC ORGANIZATION OF PERK–CALCINEURIN B CO-CLUSTERS REVEALED BY QUANTITATIVE MICROSCOPY
Bairo SM, Quassollo G, Bisbal M, Bollo M

10:15 CB-08

BEYOND GOLGI: NUCLEAR FUCOSYLATION AS AN EMERGING GLYCAN MODIFICATION

Angeloni G, Araoz Argüello AJ, Irazoqui FJ

Microbiology/Biotechnology/Plants: Salón de Actos

Chairpersons: Dr. Georgina Fabro- Dr. Paula Casati

8:30 MI-09

NEW THERAPEUTIC STRATEGIES FOR AMERICAN TEGUMENTARY LEISHMANIASIS BASED ON DRUG REPOSITIONING

Guevara Sola E, Occhionero MA, Gaspar DA, Vázquez ME, Barrientos MC, Zabala BA, Pérez Brandán CM, Minahk CJ, Acuña L, Barraza DE

8:45 MI-10

A TROJAN HORSE STRATEGY TO BROADEN NISIN'S ANTIMICROBIAL ACTION

Lanza L, Masías RE, Chalón MC, Cattaneo M, Delgado MA, Bellomio A

9:00 MI-11

LEISHMANICIDAL POTENTIAL OF *ENTEROCOCCUS MUNDTII* CRL35 AND ITS METABOLITES: FROM *IN VITRO* STUDIES TO MURINE MODELS

Occhionero MA, Vázquez ME, Barraza D, Sandoval R, Saavedra L, Pérez Brandán CM, Corbalán NS, Acuña L

9:15 BT-04

SLPA-BASED VACCINE PLATFORM FOR CHAGAS DISEASE: DUAL-FUNCTION ANTIGEN DELIVERY AND IMMUNOSTIMULATION

Zabala BA, Vázquez ME, Gaspar DA, Barrientos MC, Pérez Brandán C, Corbalán NS, Barraza DE, Acuña L.

9:30 PL-09

SHEDDING LIGHT ON HIDDEN PLAYERS: ALARMONE IS A NOVEL PLANT IMMUNITY REGULATOR

Aballay FE, León I, Galceran F, Rodriguez FS, Tognacca RS, Cecchini NM, Petrillo E.

9:45 PL-10

BEYOND THE PROTEIN: UNCOVERING RNAi CONTRIBUTIONS TO THE HB4® TECHNOLOGY

Vannay GJ, García JE, Schenfeld C, Capella M, Chan RL

10:00 PL-11

CYTOCHROME C LEVELS LINK MITOCHONDRIAL FUNCTION TO CELL CYCLE PROGRESSION AND DIFFERENTIATION IN *ARABIDOPSIS THALIANA*

Roldán F, Barrera V, Wagner M, Mansilla N, Canal MV, Coronel F, Gras DE, Rodriguez RE, Welchen E, Gonzalez, DH

10:15 PL-12

MEET ASER53: A SHAPE-SHIFTING NLR LINKING PRIMING, ALTERNATIVE SPLICING, AND VIRAL IMMUNITY IN *ARABIDOPSIS*

León I, Aballay F, Manacorda C, Benelli C, Contreras M, Asurmendi S, Petrillo E, Cecchini N

ABSTRACTS

CONFERENCES

REGULATION OF THE IMMUNE RESPONSE IN THE CENTRAL NERVOUS SYSTEM BY ASTROCYTES

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Astrocytes are the most abundant glial cells of the central nervous system (CNS). Historically, astrocyte function was thought to be providing support to neurons and other cells of the CNS. However, astrocytes are now shown to perform multiple functions in development, homeostasis and disease. Indeed, we have shown that astrocytes establish bi-directional interactions with CNS-resident and CNS-recruited immune cells. These interactions control both astrocyte and immune cell responses, with important consequences for CNS inflammation and neurodegeneration. In this talk, we will discuss mechanisms of astrocyte-immune cell interactions, novel platforms for their identification, and the relevance of those interactions for CNS physiology and pathology.

BRIDGING THE GAP: OVERCOMING CHALLENGES IN APPLYING TRANSCRIPTION FACTOR RESEARCH FROM MODEL SYSTEMS TO CROP IMPROVEMENT IN FIELD ENVIRONMENTS

Raquel Lía Chan
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Plants have intricate signalling pathways that help them adapt to environmental stresses, and transcription factors (TFs) are key regulators of these responses. Among these TFs, the homeodomain-leucine zipper (HD-Zip) family is unique to the plant kingdom and was associated with developmental events related to abiotic stress. Notably, sunflower and other Asteraceae species have HD-Zip I proteins exhibiting distinctive structural features. HaHB4 and HaHB11 are two of these family divergent members. The former confers tolerance to drought in maize, wheat, and soybeans, whereas the latter enhances yield and flooding tolerance in maize lines and hybrids, as well as in rice and soybeans. HaHB4 soybean and wheat transgenic plants became rare, yet successful, cases commercially approved by worldwide regulatory organisms, and released in 2022. Despite the high sequence similarity between these TFs, their effects on host plants differed but shared an increase in yield under normal growth conditions and fewer penalties than non-transgenic controls under stress, attributed to a higher grain number. To understand the mechanistic basis for these differential responses, we conducted comprehensive molecular analyses including transcriptomics, metabolite evaluation, and histological studies. While these approaches provided valuable insights, they also highlighted the remaining complex open questions. Our investigations led us to hypothesize that the differential traits conferred by these transgenes might be triggered

by small RNAs generated by the plant defence mechanisms. To test this hypothesis, we developed several novel genetic constructs designed to produce elevated levels of small RNAs derived from different domains and regions of the HaHB4 sequence. Transforming Arabidopsis plants with these constructs yielded amazing results: varied but significantly enhanced seed yield. Water deficit tolerance varied among the new genotypes. Crucially, several beneficial phenotypic traits were lost when the constructs were expressed in Arabidopsis mutants unable to generate small RNAs, partially supporting our hypothesis and opening new questions. Although further research is needed to fully elucidate how such small molecules enhance crop desirable characteristics, these findings highlight their immense potential as biotechnological tools for crop improvement. We are currently analysing soybean plants transformed with the same constructs, and our discussion will explore both the mechanistic and biotechnological implications of these discoveries.

DINUINUCLEASES AT THE CROSSROADS OF BACTERIAL SIGNALING AND RNA DEGRADATION

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RNA degradation is crucial for terminating gene expression and nucleotide recycling. The traditional view of this process involves cleavage of RNA by endoribonucleases, followed by exoribonuclease processing to short RNA fragments, which are then degraded into mononucleotides by nanoRNases, such as oligoribonuclease (Orn) and nanoRNase C (NrnC). Recently, we found that Orn and NrnC act as dedicated diribonucleases instead of nanoRNases, suggesting that there is a sequence of discrete steps for RNA degradation from oligoribonucleotides to mononucleotides. Notably, the *orn* and *nrnC* genes are essential in many species, indicating that the accumulation of diribonucleotides has functional consequences in these organisms. Another source of diribonucleotides is the linearization of cyclic dinucleotides, which indicates a functional intersection of RNA degradation and bacterial signaling. We also identified a class of NrnC-related proteins that specifically hydrolyze single-stranded DNA dinucleotides in a sequence-independent manner. The genes encoding these diDNases are found predominantly in genomic islands of Actinomycetes and Clostridia, which, together with their association with phage-defense systems, suggest potential roles in bacterial immunity. We reconstructed and characterized a common ancestor of diDNases and NrnC orthologs. The structures of ancestral and extant dinucleases reveal gradual changes in conformation that gave rise to substrate preference, oligomeric state, and catalytic efficiency. These findings highlight how subtle, concerted structural modifications enable large-scale changes in molecular assembly and functional specialization, harnessing a conserved protein fold. DNA dinucleotide preference in the early ancestor and preservation of DNase activity in all extant enzymes strongly argues for a biological function of DNA dinucleotides.

MOLECULAR AND CELULAR MECHANISMS BEHIND THE MECHANICAL AND BIOLOGICAL PROTECTION OF THE RESPIRATORY SURFACE

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In order to facilitate an operative gas exchange with the environment, the mammalian lung needs to expose and maintain open, at the alveolar spaces, a surface hundred times larger than that exposed at the skin. Such respiratory surface is highly dynamic, opening and closing during the breathing cycles that introduce into the lungs from 15.000 to 50.000 litters of air per day. Within that air, the systemic blood circulation is potentially exposed through the lung vasculature at the alveolar-capillary barrier to the entrance of bacteria, virus, pollutants, inhaled nanoparticles, allergens, and a myriad of potential pathogenic and noxious entities. Thus, to maintain an stabilize the large respiratory surface open and at the same time to protect it from multi-factorial injury, the alveolar epithelium secretes a lipid-protein complex, the pulmonary surfactant system, that rapidly spread and coats the whole surface while it integrates elements in charge of both reducing surface tension at the air-liquid interface and serve as part of the innate immune defence system. Several decades of research have determined the role of the different lipid and protein species in surfactant to define the architecture of the membrane-based surfactant network coating the alveoli, as well as the mechanisms by which the surfactant layer facilitates breathing dynamics. This research has also revealed how the lack or inactivation of an operative surfactant is associated with severe respiratory pathologies, making possible that at least some of them can now be treated by supplementation with exogenous therapeutic surfactant formulations. This lecture will summarize current models on the structure-function determinants of pulmonary surfactant, the fundamental methods at the frontier between biochemistry and interfacial physics that have allowed the characterization of active and diseased surfactant, and the perspectives to develop new diagnostic and therapeutic surfactant-based applications.

TRANSPOSON-MEDIATED CHROMATIN FOLDING DEFINES POL-II TRANSCRIPTIONAL ACTIVITY

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Transposons are mobile elements that are commonly silenced to protect eukaryotic genome integrity. In plants, transposable element (TE)-derived inverted repeats (IRs) are commonly found near genes, where they affect host gene expression. However, the molecular mechanisms of such regulation are unclear in most cases. We found that the expression of these IRs is associated with production of 24-nt small RNAs, methylation of the IRs, and drastic changes in local 3D chromatin organization. Notably, many of these IRs differ between Arabidopsis thaliana accessions, causing variation in short-range chromatin interactions, gene expression and adaptative phenotypic traits. In a proof-of-concept case we found that an inverted-repeat transposon (EFR-associated IR, Ea-IR) located between the loci encoding the pathogen receptor EFR and myosin XI-k (XI-k) affects chromatin organization, promoting the formation of a repressive chromatin loop. Upon pathogen infection, chromatin changes correlate with increased EFR transcription. Arabidopsis accessions lacking Ea-IR have higher

basal EFR levels and resistance to pathogens. We show a scenario in which a transposon, chromatin organization and gene expression interact to fine-tune immune responses, during both the course of infection and the course of evolution. Our data show that insertion of an IR near a gene provides an anchor point for chromatin interactions that profoundly impact the activity of neighbouring loci. This turns IRs into powerful evolutionary agents that can contribute to rapid adaptation. Our recent work in *Fragaria vesca* reveals that this principle extends beyond Arabidopsis: miniature inverted-repeat transposable elements (MITEs) positioned near genes are associated with local changes in DNA methylation, transcription, and in some cases, with phenotypic variation relevant to fruit development and ripening. These findings point to a conserved role for TE-derived IRs as anchor points for chromatin interactions that shape gene regulation. Collectively, our studies highlight how transposon insertions can serve as powerful evolutionary drivers, enabling rapid adaptation through dynamic modulation of chromatin topology and gene expression in both model and crop species.

DIVERSITY OF MORPHOGENETIC PEPTIDOGLYCAN SYNTHASES IN THE DOMAIN BACTERIA

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Bacteria are classified by their genetic makeup that determines the phenotype, including a defined cell shape inherited by the offspring. The morphologies exhibited by bacteria are highly diverse with examples ranging from helicoid, branched, vibrioid twists, filamentous to the most extensively studied coccoid or rod shapes. Investigations in the model bacteria *Escherichia coli* and *Bacillus subtilis* led to the identification of a subset of peptidoglycan (PG) synthases that form part of morphogenetic multiprotein complexes known as elongasome and divisome. In these complexes, the PG synthases connect to bacterial cytoskeletal platforms directed by MreB or FtsZ to coordinate in time and space essential processes like cell elongation and cell division. The biochemical characterization of morphogenetic PG synthases has shown that they are monofunctional enzymes displaying either transpeptidase (TPase) or glycosyl-transferase (GTase) catalytic activities and that interact as pairs to insert new PG material in a defined topological manner. The widely accepted models emerged from studies in non-sporulating rod shaped bacteria infer two pairs of morphogenetic PG synthases, one assembling specifically in the elongasome and the other in the divisome. Our current studies, however, demonstrate many examples of bacteria that break this 1:1 rule in the TPase-GTase ratio. An example are intracellular bacterial pathogens of the genus *Salmonella*, with genomes encoding alternative morphogenetic TPases that can replace the endogenous canonical TPases conserved in all bacteria of the order *Enterobacterales*. Interestingly, *Salmonella* uses these alternative TPases in “specialized” enlogasome and divisome complexes when exposed to environmental cues such as acidic pH and high osmolarity in which they recognise the canonical GTase. Therefore, morphogenetic complexes admit some degree of flexibility and can replace PG synthase components like the TPase depending on external cues. We have recently extended these analyses to the entire domain Bacteria and discovered new combinations of conventional and alternative morphogenetic TPase and GTase in diverse bacterial taxa. Strikingly, our study also unveiled unconventional proteins predicted to play a role in morphogenesis that bear both the TPase and GTase catalytic domains.

INTRACELLULAR PATHOGENS AND AUTOPHAGY: PARTNERS IN A TANGO DANCE

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Autophagy is a degradative cellular process in response to stress conditions or infection with certain pathogens. Numerous pathogens use the invasion of host cells as strategy to shelter from the host immune system but cells have developed powerful means to destroy invading pathogens. Thus, intracellular pathogens use sophisticated mechanisms to overcome host cell defenses and replicate successfully. One mechanism that bacteria use to evade the host's innate immune response is residing in a phagosomal compartment while preventing fusion with lysosomes. Another mechanism is to escape into the cytoplasm to avoid lysosomal killing, thus guaranteeing the progression of the infectious process. A third strategy is to divert trafficking from the normal phagosomal pathway towards the autophagic pathway. Autophagy involves sequestering cytosolic components, such as organelles or microorganisms, within a vacuole known as an autophagosome. This autophagosome then fuses with lysosomes to degrade the enclosed material, allowing for the recycling of molecules for reuse. Several lines of evidence show that certain bacteria, viruses, and parasites avoid or, in contrast, actively subvert autophagy to promote their own replication. We have studied various intracellular pathogens that survive and replicate within host cells, exhibiting different intracellular lifestyles. These include *Mycobacterium tuberculosis*, *M. marinum*, *Coxiella burnetii*, and *Staphylococcus aureus*, among others. Several of these microbes manipulate the autophagic pathway at the molecular level as a strategy to establish persistent infection. However, transit through the autophagy pathway is not beneficial for most pathogens, and autophagic events are critical cell defense mechanisms against invading microorganisms. *S. aureus* is a pathogen that causes serious infectious diseases, eventually leading to septic and toxic shock. One of the key features of *S. aureus* infection is the production of a series of virulence factors, including enzymes and toxins. We have previously demonstrated that alpha-hemolysin (Hla) is responsible for the autophagic response induced by this bacterium. This toxin is used by the pathogen for escaping from its containing phagosome labeled with the autophagic protein LC3. Results from our laboratory indicate that *S. aureus* at early times post-infection generates tubular dynamic structures marked with LC3 and certain GTPases RABs. The formation of these filaments depends on the integrity of cytoskeleton elements and motor proteins. When the formation of these tubular structures was inhibited, there was a significant reduction in the replication of *S. aureus*, indicating that these structures are critical for pathogen persistence. Recent results from our laboratory provide evidence of how this bacterial pathogen shelters from neutralizing antibodies and antimicrobial agents and the importance of autophagy as a modulator of intracellular pathogens' fate.

YOUNG INVESTIGATORS I

NEUROENDOCRINE CONTROL OF REPRODUCTION IN *RHODNIUS PROLIXUS*, MODEL ORGANISM AND CHAGAS DISEASE VECTOR: INSIGHTS INTO JUVENILE HORMONE MODULATION

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In vector insects, reproduction is central from an epidemiological perspective, as it directly drives population growth and dispersal. Reproductive success in these species is tightly regulated by the neuroendocrine system, which integrates hormonal signals in response to environmental and physiological cues. Key regulators of insect reproduction include lipid hormones such as juvenile hormone (JH), neuropeptides like insulin-like peptides (ILPs), and biogenic amines such as octopamine (OA). In *Rhodnius prolixus*, a major Chagas disease vector and classical model in insect physiology, blood feeding triggers a neurohormonal cascade that activates JH production. Using molecular assays, *in vitro* and *ex vivo* approaches, hormone quantification, and protein expression analyses, we show that both ILPs and OA stimulate JH biosynthesis in the *corpora allata* by enhancing biosynthetic enzyme expression and increasing circulating JH levels. These findings identify ILPs and OA as upstream modulators of JH production. Moreover, silencing the nuclear JH receptor, Met, severely compromises reproductive success. Building on this evidence, our next goal is to investigate how JH balances reproduction with immune competence, with particular focus on the dual role of yolk protein precursors in mediating this interaction. By unravelling the neuroendocrine regulation of these interconnected systems, our work seeks to reveal key physiological targets for innovative, species-specific strategies for vector control.

SPLICING THE WAY TO GERMINATION: *RS31* AND *DAG1* SHAPE LIGHT RESPONSES IN *ARABIDOPSIS THALIANA* SEEDS.

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Seeds decide when to germinate, determining the success or failure of seedling establishment. Light is one of the main environmental cues relieving dormancy and promoting germination, and, together with the temperature, influences the release of primary dormancy. These environmental cues trigger molecular and physiological responses (including ABA and GA signalling) and shape the seed transcriptome by affecting each possible level of gene expression (i.e. mRNA splicing, translation, and stability). In Arabidopsis seeds, phytochromes tightly control gene expression, yet the contribution of alternative splicing to the photocontrol

of germination remains poorly understood. Notably, the expression of many genes that regulate dormancy, germination and flowering can be modulated by alternative splicing in response to the environment, and this regulation depends on the expression level and post-translational modification of serine/arginine rich (SR) proteins and other splicing factors. Our previous transcriptome-wide analyses in stratified seeds irradiated with a pulse of red (Rp) or far-red light showed that the Rp alters ~20% of the transcriptome and the alternative splicing pattern of 226 genes associated with mRNA processing, RNA splicing, and mRNA metabolic processes, including the splicing factor *RS31*, are drastically changed. Furthermore, phyB modulates only some of these alternative splicing events. Here, we investigate the role of RS31 and its relation with *DAG1* (a negative regulator of germination) during light-induced seed germination. We show that the *RS31* coding isoform (namely *mRNA1*), ergo the RS31 protein, has a key role in the regulation of seed size, primary and secondary dormancy and the promotion of seed germination by mainly affecting the ABA signalling pathway. Also, *DAG1* expression is higher in seeds overexpressing *mRNA1*(mRNA1ox) than in wild-type. *DAG1* generates four isoforms by alternative splicing, depending on whether or not it retains the intron and/or exitron. Our results suggest that the exitron-retaining coding isoform might be acting as a positive regulator of light-induced seed germination. Interestingly, mRNA1ox seeds continuously express the exitron-containing *DAG1* isoforms. Our findings highlight alternative splicing as a key regulatory layer influencing seed responses to environmental signals.

THE SEGREGASE CDC48 INTEGRATES BLUE LIGHT AND HORMONAL CUES TO REGULATE PLANT DEVELOPMENT

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Photomorphogenesis enables plants to adjust their growth in response to ambient light, thereby optimizing photosynthesis and enhancing survival. This light-driven program depends on protein homeostasis to ensure proper folding, activation, and timely degradation of signaling components. CDC48 (Cell Division Cycle 48) is a conserved and abundant type II AAA+ ATPase that, in conjunction with its cofactors NPL4 and UFD1, functions in protein quality control. While well characterized in yeast and mammals, its role in plant development remains largely unexplored. Here, we show that CDC48A is required for blue light-mediated photomorphogenesis in Arabidopsis. Blue light promoted nuclear accumulation of CDC48A in hypocotyl cells, and *cdc48A* mutants failed to display the typical inhibition of hypocotyl elongation. Similar phenotypes arose in wild-type seedlings treated with a CDC48 inhibitor and in mutants of the adaptor proteins NPL4 or UFD1. Additionally, treatments with exogenous gibberellins (GA) or GA biosynthesis inhibitors revealed that CDC48A negatively regulates GA signaling, likely at the perception or transduction level. Consistently, the inhibition of CDC48A reduced DELLA protein RGA levels. Moreover, UFD1 directly interacted with the GA receptor GID1A, and this association appears to depend on a conserved motif within UFD1. Together, these results reveal an uncharacterized role of CDC48A, together with NPL4 and UFD1, in integrating light and hormonal cues through protein homeostasis to regulate photomorphogenic development.

SUPER-RESOLUTION INSIGHTS INTO CHROMATIN ARCHITECTURE IN NEURONAL DEVELOPMENT

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In eukaryotes, DNA is packaged in a three-dimensional (3D) arrangement within the nucleus, forming a hierarchical architecture that serves as a key regulatory layer of gene expression and cell fate determination. Epigenetic modifications play a central role in shaping this spatial genome organization, with chromatin remodelers and histone marks defining structures at multiple genomic scales. We are particularly interested in how such modifications influence the chromatin 3D organization and impact transcriptional regulation during neuronal differentiation. Our recent work has focused on the Polycomb Repressive Complex 2 (PRC2) and its deposition of H3K27me3, which we find organizes into nanodomains ranging from 50 to 400 nanometers, as revealed by expansion microscopy and STED nanoscopy. Neurons exhibit a more intense and larger size of these nanodomains as they develop, raising the question of what is the role of their spatial organization. Importantly, our studies also indicate a potential interplay between chromatin architecture and the cytoskeleton. By pharmacology perturbing microtubule dynamics, we are exploring how nuclear organization of histone modifications is coupled to cytoskeletal regulation. Complementary experiments with other histone marks, including H3K9me2 and H3K4me3, extend this framework and suggest that chromatin nanodomain formation represents a general mechanism of transcriptional control. Together, these approaches aim to reveal how epigenetic landscapes and chromatin architecture converge to orchestrate neuronal identity.

METHODOLOGICAL ADVANCES

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THE GREEN REVOLUTION IN SCIENCE: SUSTAINABLE AND INNOVATION IN MOLECULAR BIOLOGY LABORATORIES

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Laboratories are essential drivers of scientific progress, but they are also intensive consumers of critical resources such as water and energy, and major generators of waste. Within this context, the life sciences sector has a particularly significant footprint: molecular and cellular biology laboratories alone contribute substantially to global plastic waste, largely due to the reliance on single-use consumables, cellular workflows, and reagent intensive methodologies that inherently demand high resource input. The high frequency of repetitive experimental procedures further amplifies energy consumption and the generation of residuals, creating a systemic challenge for sustainability. This reality, combined with the broader global environmental crisis, has consolidated a scientific consensus, sustainability is no longer optional, but a core principle of modern scientific practice. The transition relies on three key strategies: minimizing waste, adopting safer and less hazardous reagents, and improving energy efficiency. Achieving this transformation requires coordinated action across the scientific community, industry, and regulatory bodies. The market is already responding with eco-friendly and resource-efficient products. In this presentation, we will examine practical strategies to implement these principles and highlight the sustainable solutions currently offered by OneLab to help transform your laboratory into an environmentally responsible hub of innovation. Addressing the environmental impact of cellular and molecular biology is not merely a matter of efficiency, it is a recognition of the scale of the problem and the urgent need for systemic change in how science is practiced.

MICROBIOLOGY

DECODING C-DI-GMP CONTROL OF MN(II) OXIDATION IN *Pseudomonas resinovorans* FOR GROUNDWATER BIOREMEDIATION

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Groundwater is an important drinking-water source, but in many areas of Argentina it contains unacceptable levels of dissolved manganese Mn(II), which impairs water quality, interferes with disinfection, and causes aesthetic, organoleptic, and operational problems. Biological sand filtration is an efficient, eco-friendly method to purify this water, though Mn(II) removal is slow without Manganese-Oxidizing Bacteria (MOB). In previous studies, we isolated and characterized various MOB, selecting those with high adhesion efficiency, biofilm formation, and Mn(II) oxidation capabilities. In *Pseudomonas resinovorans* MOB-513, high levels of c-di-GMP were found to enhance both biofilm formation and, interestingly, Mn(II) oxidation capacity.

To investigate the role of c-di-GMP in Mn(II) oxidation, a transposon mutant library was constructed in MOB-513, selecting mutants that lost or enhanced their ability to oxidize Mn(II). Characterization of these mutants through phenotypic analysis of motility and biofilm formation, transmission electron microscopy (TEM), quantification of intracellular c-di-GMP levels, and proteomic analysis led us to identify key factors that regulate c-di-GMP homeostasis and favor Mn(II) oxidation, such as: (i) Type IV pili and their AlgR-dependent control, which are essential to initiate close cell–cell interactions, build biofilm architecture, and sustain extracellular Mn(II) oxidation; (ii) a two-component system centered on a histidine kinase (HK2948) and a PleD-like response regulator (RR2947) that maintains basal c-di-GMP homeostasis to coordinate Mn(II) oxidation; and (iii) elevation of c-di-GMP by a native diguanylate cyclase (DGC4077) that shifts cells toward a strongly sessile, matrix-centric state that boosts Mn(II) oxidation.
These results demonstrate that Mn(II) oxidation in MOB-513 is a tightly regulated process involving a complex network that links second-messenger signaling and surface behaviors to metal oxidation. This knowledge will contribute to improving future metal bioremediation processes, through the design of robust bacterial inoculants to accelerate the start-up of biological sand filters.

POLYAMINES AS REGULATORS OF OXIDATIVE STRESS RESPONSE IN PHYTOPATHOGENIC BACTERIA

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In the agricultural sector, bacterial infections of plants lead to significant crop losses, posing a major challenge for farmers and the food industry. The outcome of these diseases depends on the complex interactions between the host's defense systems and the survival strategies of pathogens. A major defense mechanism deployed by the host's innate immune system is the generation of reactive oxygen species such as hydrogen peroxide (H_2O_2) to induce oxidative stress and eliminate invading microbes. In response, pathogens have developed different mechanisms to sense and mitigate this stress. Polyamines such as putrescine and spermidine, small polycationic molecules ubiquitous in all living organisms, are crucial for basic cellular processes but also serve as key modulators in the host-pathogen crosstalk. While the role of polyamines in mediating oxidative stress tolerance is established in human pathogens, this link remains largely unexplored in plant-bacterial systems. Therefore, the primary aim of our research is to elucidate the interplay between bacterial polyamine metabolism and oxidative stress responses in the key phytopathogen *Pseudomonas syringae*. We found that exposure to H_2O_2 alters the levels of polyamines both within and outside of cells. Specifically, the bacteria raise putrescine's external levels while decreasing its internal concentrations and keeping spermidine concentrations constant. The addition of exogenous putrescine significantly improved the bacterium's tolerance to H_2O_2, highlighting the protective role of this externalized polyamine. Intriguingly, this protective effect is not due to direct H_2O_2 scavenging. Instead, our research points to a more indirect mechanism. The enhanced oxidative stress tolerance observed was directly correlated with a higher expression of H_2O_2-degrading enzymes, specifically catalases. Furthermore, the presence of external putrescine also improved the stability of the bacterial outer cell membrane, a crucial physical barrier for maintaining cellular integrity. These results suggest that polyamines protect bacterial cells by both promoting the degradation of harmful oxidative molecules and reinforcing their physical structure.

Comparative studies in *Ralstonia solanacearum* and *Pectobacterium* versatile reveal species-specific variations, suggesting this relationship may be more complex than initially thought. Understanding this intricate relationship offers a promising avenue for the development of innovative disease control strategies, targeting these novel defense mechanisms to fight a broad spectrum of infectious diseases.

MEMBRANE AND CELL WALL BIOGENESIS IN GRAM POSITIVE BACTERIA: TWO INDEPENDENT OR COORDINATED PROCESSES?

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The bacterial cell envelope, a complex multilayered structure, plays a crucial role in cell growth and survival by ensuring cellular integrity, serving as a protective barrier to control permeability, and mediating interactions with the environment. In Gram-positive bacteria, the cell envelope comprises the plasma membrane (PM) and a thick peptidoglycan (PG) layer threaded with long anionic polymers known as teichoic and lipoteichoic acids. All of these components must be synthesized, transported, and assembled in a coordinated manner for proper envelope biogenesis and maintenance during cell growth and division. Despite its relevance, and although the individual pathways for PM and PG synthesis are well characterized, the molecular basis of this coordination remains largely a mystery. Gram-positive bacteria use the type II fatty acid synthase (FASII) system to synthesize long-chain acyl-ACPs, which are substrates of the PlsX/PlsY/PlsC pathway. These three acyltransferases sequentially convert acyl-ACPs into phosphatidic acid (PA), the common precursor of all phospholipids (PLs). In this work, we have studied the effect of arresting lipid biosynthesis at different stages on PG homeostasis. We took advantage of the antibiotic cerulenin, which inhibits FASII and thereby arrest PL synthesis, and a set of *plsX*, *plsY*, and *plsC* conditional mutant strains. These mutants are unable to synthesize PA and hence PLs, limiting plasma membrane expansion, but they offer particular cellular backgrounds concerning FASII activity and precursor accumulation: for example, in PlsC-depleted cells, PL biosynthesis is arrested, FASII is active, and free fatty acids accumulate, while depletion of PlsX inhibits both PL biosynthesis and FASII, leading to the accumulation of long-chain acyl-ACPs. Interestingly, we have determined that lipid starvation induces the expression of the PG autolysin gene *yocH* in some of these cellular contexts while in others it does not. The analysis of the different physiological scenarios has led us to identify a candidate signaling molecule that would link PM and PG biosynthesis and to propose a model for their coordination. Altogether, the outcomes derived from these experiments pave the way to elucidate the molecular basis of cell envelope homeostasis in Gram-positive bacteria. Since envelope assembly is crucial for Gram-positive survival, our results may aid in the development of new treatments effective against the growing problem of drug-resistant infections.

HARNESSING THE MICROBIOME OF VERMI-COMPOST TO CONTROL FUNGAL DISEASE

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Tomato (*Solanum lycopersicum* L.), which is consumed globally as a fresh vegetable, has high nutritional value and antioxidant properties. However, its yield and quality can be significantly affected by soilborne diseases, such as bacterial wilt and Fusarium wilt. These diseases are usually managed through the use of agrochemicals that can produce chemical residues, pesticide resistance, and environmental pollution. A promising alternative consists in exploiting the ability of certain microbial communities to inhibit pathogens and promote plant growth and immunity. The fact that most suppressive soils lose their activity when sterilized is strong evidence of the important role played by soil microorganisms in pathogen suppression. The growth and activity of such microbes can be enhanced by the addition of compost, an organic soil amendment. This study evaluated the impact of vermicompost on the growth of tomato, as well as its suppressive effects on phytopathogen *Fusarium oxysporum* f. sp. lycopersici (Fol). It also gathered information about the microbial community within the compost. Vigor parameters (stem length, fresh weight, dry weight, water content, and fluorescence intensity) were measured in seedlings after they had been growing for 21 days in mixtures of sterile and non-sterile compost and peat. They were generally higher in plants grown in the presence of compost than without it. Moreover, seedlings grown in compost showed lower disease severity after being infected with Fol than the controls. Bacterial and fungal isolates from the compost were then tested *in vitro* against the pathogen, through a plate inhibition assay. A *Trichoderma* isolate showed mycoparasitic activity against Fol, an interaction which was subsequently studied through scanning electron microscopy. On the other hand, three bacterial isolates (JL05, JL07 and JL11) significantly reduced the pathogen's radial hyphal growth. Morphological and molecular analyses identified JL05 and JL07 as *Bacillus amyloliquefaciens*, and JL11 as *Bacillus velezensis*. Whole-genome sequencing and average nucleotide identity (ANI) confirmed their taxonomic placement and could provide further insight into their biocontrol mechanisms. The antagonist effects of the cyclic lipopeptides produced by these three strains were also studied in the context of net blotch disease, which is caused in barley by the ascomycete *Pyrenophora teres*. JL05 was able to inhibit *P. teres in vitro* and reduced the severity the disease. These findings highlight the potential of compost-derived microbes for the sustainable and integrated management of fungal disease in valuable crops.

LIPIDS

EXPLORING NATURAL PRODUCTS TO OVERCOME FERROPTOSIS, A FORM OF CELL DEATH DRIVEN BY LIPID PEROXIDATION

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Ferroptosis is a cell death modality driven by iron-dependent lipid peroxidation. Although lipid peroxidation can be initiated through several pathways (spontaneously via the Fenton reaction from the labile iron pool or enzymatically through lipoxygenases), the execution and propagation of ferroptosis rely on polyunsaturated fatty acids (PUFAs) esterified to membrane phospholipids or ether-linked to plasmalogens, along with compromised antioxidant defences. Over the past five years, research on ferroptosis has experienced an exponential growth, mainly due to its pathological implications, particularly in neurodegenerative diseases and its potential relevance in anti-cancer interventions. Given that neurons utilize iron to meet their metabolic requirements and harbor elevated levels of PUFAs, these brain cells are susceptible to ferroptosis. Thus, inhibition of this process could be an emerging and promising strategy in the treatment of neurodegenerative disorders. Accordingly, growing evidence points towards the regulation of lipid metabolism and redox signaling as new therapeutic avenues, the latter focusing on enhancing the cell's intrinsic antioxidant capacity through synthetic radical-trapping agents, and glutathione-dependent support, such as GPX4 mimetics. In this work, we explored natural products and related derivatives as ferroptosis modulators in *in vitro* models associated with neurodegenerative processes related to iron accumulation. In ferroptotic stress scenarios, the natural sesquiterpene lactone deacylcynaropicrin effectively attenuates iron-induced lipid peroxidation and reactive oxygen species formation in human neuroblastoma and glial cells. Mechanistically, the compound promotes the nuclear translocation of the antioxidant transcription factor NRF2 and the upregulation of GCLc. NRF2 and its downstream gene GCLc are considered as the indirect regulatory sphere of ferroptosis. Furthermore, under ferroptotic stress, deacylcynaropicrin exhibited anti-inflammatory activity by suppressing NO production, preventing the nuclear translocation of the pro-inflammatory transcription factor NFκB, and downregulating COX-2 and IL-1β expression in macrophages and glial cells, while also attenuating astrocyte reactivity. Additionally, we constructed a library of triterpenoids and coumarins derivatives predicted *in silico* to cross the blood–brain barrier and tested them in dopaminergic neurons challenged with ferroptosis inducers, erastin and RSL3. Within this paradigm, we identified a synthetic coumarin–chalcone hybrid with significant anti-ferroptotic activity. In conclusion, our findings highlight natural products and their derivatives as promising scaffolds for ferroptosis inhibition. Deacylcynaropicrin and a coumarin-chalcone hybrid stand out as lead candidates for neuroprotective strategies in ferroptosis-associated neurodegeneration, warranting further mechanistic exploration and *in vivo* validation.

WHEN MEMBRANE-BOUND MEET MEMBRANE-LESS: WETTING, REMODELING, AND DAMAGE STABILIZATION

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In addition to membrane-bound organelles, cells compartmentalize their interiors into membrane-less organelles, also known as biomolecular condensates; some examples include the nucleolus, Cajal bodies, P-bodies, and stress granules. These condensates are liquid-like droplets that arise from the condensation of proteins and genetic material and play a key role in several biological processes across different organisms. Furthermore, dysfunction in condensate assembly can lead to neurodegenerative diseases and cancer. Thus, research on biomolecular condensates has become a highly active, interdisciplinary field.

Recently, the interaction between condensates and membranous organelles has gained attention because they are crucial for diverse cellular functions, including autophagy, T-cell signal transduction, virus capsid protein assembly, tight-junction development, and endocytic vesicle assembly, among others. These findings strongly suggest that membrane-less and membrane-bound condensates correspond to different functional states.
While the field of membrane-condensate interactions is gaining momentum, important cues are still missing in our understanding of the underlying mechanisms associated with the resulting remodeling processes and structural changes. Given the small size of condensates, precise quantifications of these interactions are often precluded in vivo. Thus, critical insight about the material properties and behavior of condensates have been obtained in vitro, taking advantage of their relatively ease of their reconstitution.
In this talk, I will demonstrate how in vitro models can be leveraged to explore how lipids and the membrane interface affect the affinity for condensates and their mutual remodeling. By combining advanced microscopy techniques with spectroscopy, we revealed the interaction and crosstalk mechanisms between these structures: condensate can locally modulate lipid packing and hydration at the contact region. In turn, the degree of membrane lipid packing, modulated by the chain length, saturation, and cholesterol content, act as a regulator of condensate affinity.
Finally, by taking a synergistic approach that combines in vivo, in vitro, and in silico experiments, we uncovered a new, crucial role for stress granules: acting as stabilizers of lysosomal damage. Our research shows that upon chemical stress or infection with *Mycobacterium tuberculosis*, these granules rapidly condense at endolysosomal damage sites, stabilizing and repairing the endomembrane.
Understanding how to modulate condensate-membrane interactions might have broad implications, from designing new ways to control cellular processes and novel therapeutic strategies, to creating smart materials with tunable behavior.

INFLUENCE OF XENOBIOTIC EXPOSURE ON THE DAILY CHANGES OF GLYCEROPHOSPHOLIPID TURNOVER: INSIGHTS FROM TOBACCO HAIRY ROOTS AS A MODEL SYSTEM

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This study explored the distinct but interconnected impact of arsenite (AsIII) and phenol on glycerophospholipid (GPL) turnover and the circadian clock (CC) regulation in *Nicotiana tabacum* hairy roots (HR). Both contaminants profoundly influenced plant membrane dynamics and the rhythmic regulation of metabolic responses, showing a complex interplay between environmental stress, lipid signaling and the internal biological clock. The results found in this study, showed that AsIII treatment significantly modified the turnover activity (TA) profiles of major GPLs. A key finding is the opposite oscillation observed for phosphatidylcholine (PC) and lysophosphatidylcholine (LysoPC), along with an increase in TA for

lysophosphatidylethanolamine (LysoPE) and cardiolipin (CL). Gene expression analysis revealed that key genes involved in GPL turnover, such as *NtLPAT2* and *NtCEK4* showed increased expression and circadian oscillation, with respect to untreated conditions, where no significant changes were observed. These results suggest that AsIII can act as an input signal, with the potential to reset the circadian clock through lipid signaling. In contrast, phenol treatment decreased total PC levels while increased levels of phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and CL, especially during the dark phase were found. Phenol's impact on expression of genes related to GPL metabolism was notable, causing the downregulation of *NtCCT2* and altered expression patterns for *NtPECT1* and *NtAAPT1*. The study links increased phosphatidic acid (PA) level induced by phenol, with the downregulation of core clock genes, resulting in a loss of rhythmic control over metabolic processes. This suggests that phenol also acts as a circadian clock input signal, but through different lipid messengers such as PA. Both pollutants interfere with the circadian oscillation patterns of GPLs and their related gene expression. However, their methods for doing so differ. Arsenic primarily affects the TA and oscillation phases of PC and LysoPC, while phenol's influence is more pronounced on the levels of other GPLs and is directly linked to the downregulation of core clock genes, which could involve PA as a signal molecule. These findings suggest that lipid signaling is a crucial mechanism through which a variety of environmental contaminants can influence and potentially reprogram the plant's internal clock, with broad consequences for metabolic stress responses.

UNCOVERING THE BIOMEDICAL POTENTIAL AND MOLECULAR TARGETS OF LIPIDS DERIVED FROM THE MACROALGAE *GRACILARIA CHILENSIS*

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Gracilex®, a botanical extract derived from the edible red seaweed *Gracilaria chilensis*, exhibits significant promise in addressing both metabolic disorders and cancer therapy due to its unique and diverse biochemical composition. Enriched with polyunsaturated fatty acids, eicosanoids, lipophilic antioxidants (e.g., tocopherols and β-carotene), and other bioactive compounds, Gracilex® selectively activates the PPARγ nuclear receptor, a critical target for insulin sensitization, without inducing adipocyte differentiation. This selective activation mirrors the effects of selective PPARγ modulators (SPPARMs), providing a safer alternative to thiazolidinediones (TZDs), which are commonly used drugs for type 2 diabetes mellitus and are associated with adverse side effects. In a diet-induced obesity model, Gracilex® demonstrated its potential to improve insulin sensitivity, normalize glucose and insulin parameters, and enhance antioxidant activity both in vitro and in vivo, highlighting its nutraceutical value for mitigating metabolic disorders.

Beyond its metabolic benefits, Gracilex® has shown promising antitumoral properties against prostate cancer (PCa), one of the leading causes of cancer-related deaths in men. Gracilex® significantly inhibited tumor growth in a xenograft model, further emphasizing its therapeutic potential in oncology. To better understand the mechanisms underlying these effects, the

extract was fractionated using solid-phase chromatography, allowing the characterization of a free fatty acid fraction enriched in PPARγ activity and the oxylipin 8-HETE. This fraction not only enhanced neuroprotective properties in vitro but also explained the antioxidant effects observed in vivo, as shown in *Caenorhabditis elegans* assays.
To facilitate broader applications, efforts have been made to scale up the production of Gracilex® for commercial use and human safety evaluation. Collectively, these findings position Gracilex® as a versatile botanical extract with dual benefits: improving metabolic health and offering antitumoral effects. Further research into its nutraceutical and therapeutic applications in humans is warranted, paving the way for innovative solutions to significant global health challenges.

PLANTS

THE PLANT SPECIFIC HISTONE DEMETHYLASE *MtPKDM9B* AFFECTS GENE EXPRESSION REPROGRAMMING DURING ROOT DEVELOPMENT AND THE NITROGEN FIXING SYMBIOSIS IN *MEDICAGO TRUNCATULA*

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Legume plants play key roles in both natural and agronomic ecosystems, contributing to biological nitrogen fixation through their symbiotic interaction with soil bacteria collectively known as rhizobia. This interaction results in the formation of a new root organ, called the nodule, where rhizobia are housed and fix atmospheric nitrogen. The establishment of this symbiotic interaction depends on signal exchanges between both partners and the activation of a signal transduction pathway that promotes the reprogramming of gene expression in root cells engaged in symbiosis. These changes in gene expression are modulated at multiple levels including chromatin remodeling, transcriptional, co-transcriptional (e.g., splicing), post-transcriptional, and translational mechanisms. Previously, we characterized changes in the steady-state levels of mRNAs and their association with the translational machinery at early stages of the interaction between *Medicago truncatula* and its symbiont *Sinorhizobium meliloti*. An mRNA encoding a putative lysine (K) demethylase (MtPKDM9B), which catalyzes the oxidative demethylation of tri-methylated lysine 27 in histone 3 (H3K27me3), was found to be downregulated at the translational level in response to rhizobia. *MtPKDM9B* undergoes alternative splicing due to exon skipping. Retention of exon 3 produces a long variant (*MtPKDM9B*) that encodes the full-length protein, whereas skipping of exon 3 in the short variant (*AS MtPKDM9B*) introduces a premature stop codon. Functional characterization using RNA interference and artificial microRNAs revealed that the long variant, *MtPKDM9B*, is a positive modulator of nodule organogenesis, rhizobia infection and the survival of rhizobia within the nodule. In addition, MtPKDM9B negatively modulates primary root growth, presumably by affecting cell division. Chromatin immunoprecipitation using anti-H3K27me3 followed by DNA sequencing (ChIP-seq) revealed that *MtPKDM9B* is required for demethylation of H3K27me3 at loci containing genes involved in cell cycle progression, sugar transport, and auxin transport/signaling/response under non-symbiotic conditions. Moreover, under symbiotic conditions, MtPKDM9B participates in the demethylation of H3K27me3 and transcriptional activation of early symbiotic genes required for bacterial infection, such as those involved in cell wall remodeling and membrane trafficking, as well as genes required for

nodule organogenesis, including those encoding transcription factors of the GRAS and NF-Y families and proteins involved in auxin biosynthesis and signaling. These results highlight the importance of fine-tuned modulation of H3K27me3 methylation/demethylation dynamics for root development and the successful establishment of nitrogen-fixing symbiosis.

THE ROLE OF PROLINE BIOSYNTHESIS IN PLANTS: A BIOCHEMICAL PERSPECTIVE

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Proline biosynthesis is induced under abiotic stress conditions, leading to proline accumulation. Several roles have been proposed to both proline and its biosynthesis under stress, such as antioxidant role, osmoprotection, redox buffering, carbon and nitrogen storage, and signalling. However, direct evidence related to these putative roles in plants is scarce. Our group has investigated some of these roles by multidisciplinary approaches. We have observed that proline accumulation is unlikely to be a conserved response of plant due to a direct antioxidant role. We also investigated the proteome and photosynthetic activity of *Arabidopsis thaliana* proline-accumulating mutants (*p5cs1-1* and *p5cs1-4*) under control, saline, and drought stress conditions. We analysed steady-state parameters of photosystem I (PSI) and photosystem II (PSII). Light curve experiments revealed that these mutants often exhibited a reduced electron transport rate even under control conditions compared to the wild type (WT). At the proteomic level, we found increased abundance of the TOR complex in proline-accumulating mutants under control conditions, while several proteins involved in photosynthesis were less abundant under stress conditions. To further explore the potential link between proline biosynthesis and photosynthetic activity, we characterized the photosynthetic performance of crr2-2, nadp-mdh mutants (each known to be affected in either cyclic electron flow or NADP+ regeneration), *p5cs1-1.nadp-mdh* and *p5cs1-1.crr2-2* double mutant lines under saline and high-light stress. Our preliminary results suggest that proline biosynthesis does not seem to contribute significantly to chloroplast NADPH consumption, hence limiting its possibility to alleviate the light reactions of photosynthesis under stress conditions. We observed, however, that proline-accumulating mutants display a constitutive stress response, in which they seem to limit growth promotion and enhance stress responses even under control conditions. Although, this molecular signature, does not result in an apparent reduced growth of plants. Our data, strengthen the idea that proline metabolism is more likely to play a role as a redox buffer between the cytosol and the mitochondria, while proline itself can serve as a carbon and nitrogen storage and as a cosmotropic agent, protecting proteins under harsh conditions. Moreover, evidence form our groups and others point to a possible role of proline metabolism that we are still far to understand.

INTEGRATING MITOCHONDRIAL ENERGETICS AND VACUOLAR SIGNALING TO REGULATE GROWTH–DEFENSE TRADE-OFFS IN PLANTS

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Sessile organisms, such as plants, face a constant challenge: allocating limited resources between growth and survival. Growth relies on energy-intensive anabolic processes, while survival requires activating defence pathways and catabolic recycling under stress. These competing demands create the well-known growth–defence trade-off, where investment in one process often limits the other. We establish a mitochondrial energy-sensing pathway where the status of the Cytochrome c (CYTc)-dependent electron transport chain serves as a direct potentiometer of cellular bioenergetic capacity. Using genetic and biochemical approaches in *Arabidopsis thaliana*, we demonstrate that mild CYTc deficiency reduces mitochondrial membrane potential and cellular ATP levels. This energy deficit signal is transduced by the energy-stress sensor SnRK1, which becomes activated independently of sugar levels. Activated SnRK1 subsequently inhibits the TOR kinase, leading to growth arrest, constitutive autophagy, and a pre-acclimated state that enhances tolerance to osmotic stress.
Furthermore, we introduce a novel, convergent signalling module at the vacuolar membrane that integrates hormonal and nutrient cues to command growth. We propose that the protein AtMinionT, which functions as a scaffold at the tonoplast, connecting the TOR complex to the V(H+)-ATPase, a key component of the nutrient-sensing machinery. This interaction is modulated by the Brassinosteroids (BR) hormonal pathway. In the presence of growth-promoting BRs, the inhibitory kinase BIN2 is inactivated, allowing AtMinionT to effectively assemble and stabilise the TOR complex for activation in a nutrient-dependent manner. AtMinionT thus acts as a coincidence detector, ensuring TOR is activated only when both pro-growth hormonal commands and sufficient nutrient availability are present.
We proposed a dual-input model in which the TOR kinase acts as the central processor. A systemic "capacity" signal transmitted by the CYTc from mitochondria, and mediated by SnRK1, provides a non-negotiable brake on growth during energy-deficient conditions. Concurrently, a localised "command" signal at the vacuole, mediated by the AtMinionT-BR-V-ATPase module, provides a conditional accelerator. This organellar control system ensures that plants commit to the energetically expensive process of growth only when they possess both the metabolic capacity and the developmental mandate to do so, providing a robust mechanism to navigate the fundamental growth-defence interplay.

UNDERSTANDING THE WHYS, HOWS, AND CONSEQUENCES OF ALTERNATIVE SPLICING REGULATION IN PLANTS

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Alternative splicing is a fundamental feature of gene expression in plants, as it is in animals. Traditionally, it is viewed as a mechanism to enhance proteome diversity, allowing a single gene to generate multiple transcript variants—and, consequently, different proteins. However, this classical perspective presents a paradox in plants, where the predominant form of alternative splicing is intron retention. Intronic sequences, at least when considering canonical

ones, contain codons that can be recognized as premature stop signals by ribosomes, typically leading to transcript degradation via the nonsense-mediated mRNA decay (NMD) pathway.
Curiously, rather than being eliminated, intron-retaining transcripts persist under specific physiological conditions, suggesting a regulated process rather than an error in splicing. To better understand this phenomenon, we investigate the splicing regulation of AT3G618690 (At-RS31) in Arabidopsis thaliana. Through this study, we aim to unravel how different transcript variants are directed toward distinct fates and functions—undergoing nuclear retention, efficient translation, or degradation via NMD.
Unravelling these regulatory mechanisms will deepen our understanding of alternative splicing in plants and reveal its broader implications for development and environmental adaptation.

SIGNAL TRANSDUCTION

SIGNAL TRANSDUCTION IN THE CONTEXT OF AIDS RELATED MALIGNANCIES: KAPOSI SARCOMA AND BEYOND

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Kaposi's sarcoma (KS) is the primary cancer associated with Acquired Immune Deficiency Syndrome (AIDS) and is caused by infection with the Kaposi's sarcoma-associated herpesvirus (KSHV). The virus encodes a variety of genes related to signaling pathways in the host cells. Expression of the vGPCR gene (a constitutively active G protein-coupled receptor) is a requirement for cell transformation that is triggered by an imbalance in the activity of signaling pathways such as Growth Factors and its receptors (PDFG, VEGF), G proteins (Ras, Rac), protein kinases, and transcription factors. We have previously shown a pivotal role for Rac in the activation of MAPK family memberss. We are currently studying G proteins and MAPKs as integral members of pathways for signaling from vGPCR to the cellular genes for COX-2 and HO-1, enzymes whose inhibition attenuates the parameters of cell transformation by vGPCR. We observed that vGPCRs and their signaling intermediaries control the expression of these enzymes at the promoter level, but also at the level of regulating mRNA stability. At the promoter level, we focused on the transcription factor Nrf2. Our data indicates its importance in controlling vGPCR effectors and cellular transformation. We are currently studying pharmacological inhibitors for cell transformation in the context of Kaposi Sarcoma. In particular, a rational design for a molecular clip that allows Nrf2 inhibition as a therapeutic strategy for modulating cell signaling in the context of AIDS-related disease research. We will discuss these results as well as strategies to potentiate and expand the reach of our discoveries in the local context and beyond the equator.

SURVIVING A HOSTILE WORLD: NEURAL COORDINATION OF STRESSOR-SPECIFIC STRATEGIES IN *C. ELEGANS*

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To navigate a hostile and changing environment, animals must dynamically switch between competing survival strategies. Our research in *C. elegans* elucidates how two antagonistic neuromodulators—tyramine (TA, an invertebrate analog of noradrenaline (NA)) and serotonin (5-HT)—orchestrate this critical decision-making process.
Facing an acute threat such as a predator, the animal releases TA to initiate a fight-or-flight response. TA signals through an intestinal adrenergic-like GPCR, triggering a cascade that stimulates secretion of the insulin-like peptide INS-3. INS-3 then activates the insulin receptor DAF-2, inhibiting the transcription factor DAF-16/FOXO and its cytoprotective gene program. This mechanism prioritizes a rapid, energy-demanding escape response at the direct expense of long-term cellular maintenance.
In stark contrast, environmental stressors such as hunger, heat, or oxidation often appear more gradually but are long-lasting. Critically, these chronic stressors suppress the activity of tyraminergic neurons, reducing TA release. This suppression is adaptive, lifting the inhibition on cellular defense pathways to allow DAF-16 activation. Collectively, this demonstrates that tyraminergic signaling acts as a neural switch, coordinating the appropriate stress resistance strategy based on the nature of the threat.
Crucially, this drop in TA also primes the animal for future resource exploitation. Real-time calcium imaging reveals that TA depletion during fasting removes a tonic inhibition on serotonergic neurons, which permits their hyperactivation and a consequential surge of 5-HT release upon food encounter. This amplified neuromodulatory response dramatically enhances foraging efficiency and slowing, enabling rapid restoration of nutritional status. Genetic and functional analyses solidify this model of cross-inhibition. Mutants lacking TA exhibit exaggerated behavioral and serotonergic responses to food, with neuronal imaging confirming significantly heightened calcium transients, unequivocally validating the disinhibition mechanism. Thus, the suppression of a single stress hormone, TA, delivers a dual advantage: it ensures survival during chronic stress and proactively prepares the animal to capitalize on imminent opportunities.
The deep conservation of these pathways (TA/NA, 5-HT, insulin signaling) underscores a fundamental principle of survival: neural circuits compute adaptive strategies by dynamically balancing opposing neuromodulatory systems to optimize fitness in unpredictable and challenging environments.

WHY DO WE SLEEP? INSIGHTS FROM *DROSOPHILA*

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Sleep is a fundamental and evolutionarily conserved process observed across all animals, yet its core biological function remains unresolved. Classical hypotheses suggest that sleep facilitates memory consolidation, synaptic pruning, and the clearance of metabolites

accumulated during wakefulness. However, recent findings in zebrafish have introduced a compelling new hypothesis: repairing DNA damage accumulated during wakefulness may represent a primary, evolutionarily conserved function of sleep. This raises critical questions: Are DNA repair proteins central regulators of sleep drive? How does a cellular mechanism such as DNA repair translate into a systems-level behavioral output? Are canonical sleep–wake centers or specific neuronal populations engaged in this process? To address these questions, we are investigating the role of DNA damage and repair in regulating sleep in Drosophila melanogaster, a genetically tractable model with well-characterized sleep behavior. First, we employ established methods to induce DNA damage and analyze consequent changes in sleep. We focus on PARP1, a key sensor of DNA damage proposed to mediate sleep induction in zebrafish, to determine whether its role is conserved in insects. Additionally, we are exploring the involvement of other DNA repair-related proteins, including Rad51, Ku70, and Ku80, in modulating sleep behavior. Complementary to these molecular approaches, we are conducting a thermogenetic neuronal screen to identify specific neural populations responsible for translating DNA repair signals into increased sleep drive. These studies combine sleep behavior assays with molecular biology techniques and imaging approaches to monitor DNA damage and repair in the fly brain. Understanding how DNA repair proteins act as potential molecular components of the sleep homeostat, and whether specific neural circuits mediate this link, will provide crucial insights into the fundamental biological role of sleep and its conservation across species.

BEYOND KINASE ACTIVITY: UNLOCKING THE THERAPEUTIC POTENTIAL OF GRK2 RH DOMAIN FOR HEART DISEASE

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G protein-coupled receptor (GPCR) kinases (GRKs) mediate receptor desensitization in response to prolonged or repeated stimulation of signaling systems. These enzymes play a major role in receptor regulation and, consequently, in cell biology and physiology. Among them, GRK2 is a multidomain protein that modulates numerous receptors, including α- and β-adrenergic receptors, as well as angiotensin, endothelin, and histamine receptors, among others. GRK2 mediates desensitization through both its kinase domain, which phosphorylates the receptor to promote G protein uncoupling, and its RGS homology (RH) domain, which can interrupt G protein signaling. The dysregulation of GRK2 is associated with cardiovascular diseases and has been validated as a therapeutic target for cardiac hypertrophy, hypertension, and heart failure. While inhibitors of GRK2 kinase activity showed encouraging results, limitations such as a lack of potency, unspecific effects, or poor pharmacokinetics have prevented them from advancing to clinical trials. In this work, we describe the design and development of novel inhibitors that target the RH domain of GRK2. These compounds are potent at enhancing GPCR signaling and reducing GRK2-mediated desensitization. Furthermore, they are effective as antihypertensive and antihypertrophic agents in spontaneously hypertensive rats, with a good safety profile. These new GRK2 inhibitors represent promising candidates for treating cardiovascular diseases where GRK2 plays a key role.

CELL BIOLOGY

REGULATION OF CELL CYCLES AND CELL BIOLOGY PROCESSES DRIVING PLANT ORGAN GROWTH

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Plant organ growth relies on the coordinated action of developmental zones that are spatially separated and functionally coordinated. In meristems, stem cells generate new cell types, which are amplified in number through cell proliferation. Post-mitotic cells then initiate massive differentiation programs, prominently characterized by anisotropic diffuse cell expansion.
Within plant meristems, the mitotic cell cycle generates new cells that subsequently undergo expansion and differentiation, often coupled to endoreplication (ER), a variant cell cycle that increases somatic polyploidy. While mitotic progression is coordinated by well-known regulators such as E2F and MYB3R transcription factors, genome-wide studies revealed that many phase-specific genes are controlled by additional, yet unidentified regulators.
We recently identified SCARECROW-LIKE 28 (SCL28), a GRAS transcription factor that peaks in G2/M and is itself under MYB3R control. Functional analyses demonstrate that SCL28 modulates G2/M progression, influences division-plane orientation, and unexpectedly regulates SIAMESE-RELATED genes encoding CDK inhibitors that drive mitotic cell cycle exit and endoreplication. Consistent with this, mutants in SCL28 display reduced endoreplication and compromised cell expansion. Moreover, transcriptomic evidence points a role of SCL28 in cytoskeleton and cell-wall dynamics during post-mitotic cell expansion.
Together, these findings position SCL28 as a central regulator of plant organ growth, modulating cell proliferation, mitotic cell cycle exit, endoreplication, and cell expansion and differentiation programs.

MODIFIED MIRNAS RESTORE CHONDROCYTE FUNCTION UNDER INFLAMMATORY STRESS: TOWARD A RELIABLE DISEASE-MODIFYING STRATEGY FOR OSTEOARTHRITIS

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Osteoarthritis (OA) is a degenerative joint disease characterized by progressive cartilage loss and impaired chondrocyte function that represents an unmet clinical need, as no disease-modifying therapies are currently available. We aimed to develop engineered microRNAs (miRNAs) with enhanced regulatory potential and to establish a human ex vivo OA-like model for robust preclinical validation.
Stage-specific dysregulated miRNAs were identified through bioinformatic analysis and quantified in synovial fluid from 80 OA patients. Candidate miRNAs were correlated with WOMAC clinical scores and rationally modified to improve passenger-strand activity and reduce off-target effects. To establish an ex vivo screening platform, primary human articular chondrocytes (HACs) were isolated from knee arthroplasties, characterized by bioinformatics, and cultured under inflammatory, OA-mimicking conditions. Phenotypic and functional

assessments were performed by flow cytometry, qPCR, and Western blot. Inflammatory-challenged HACs exhibited hypertrophic-like features.
Treatment of inflamed HACs with miR-140a and miR-92a-3p (50 nM, 72–96 h) significantly downregulated ADAMTS4 ($p<0.05$), upregulated aggrecan ($p<0.01$), increased COL2/SOX9 ($p<0.05$), and reduced MMP13/RUNX2 ($p<0.01$) expression. Moreover, rationally engineered miRDs (50 nM, 72–96 h) further enhanced chondrogenic marker expression and restored functional phenotype ratios more effectively than native miRNAs ($p<0.05$). Finally, dual activity of miRDs was confirmed by qPCR of specific passenger-strand siRNA targets ($p<0.01$).
In summary, our findings support engineered miRNAs as promising OA therapeutic candidates, capable of restoring chondrocyte homeostasis under inflammatory conditions. The combination of ex vivo human models and rational miRNA design provides a robust translational platform for musculoskeletal regenerative strategies.

INTRACELLULAR TRAFFICKING DEFECTS UNDERLY DYSFUNCTIONS IN REELIN SIGNALING: IMPLICATIONS FOR A RARE GENETIC DISEASE AFFECTING THE CENTRAL NERVOUS SYSTEM

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Reelin is a secreted glycoprotein that regulates the brain´s development, function, and plasticity. Reelin and its receptors, ApoER2 and VLDLR, have neuroprotective roles in Alzheimer´s and Parkinson´s. Decreased Reelin is found in neuropsychiatric disorders, including depression, schizophrenia, Fragile X syndrome, and autism spectrum disorder and causes brain defects like microcephaly and thin corpus callosum.
ApoER2 is internalized in a clathrin-mediated pathway and recycled to the plasma membrane from early endosomes. Recently, we showed the role of endosomal recycling and the biosynthetic and polarized trafficking of ApoER2 in Reelin signaling; when these routes are affected by specific disease conditions, Reelin responses are impaired. In its biosynthetic traffic, ApoER2 interacts with the Golgi-associated adaptor complex AP4, mutated in Hereditary Spastic Paraplegia (HSP-AP4), a rare condition characterized by neonatal hypotonia, progressive spasticity, developmental delay, and seizures. In AP4-deficient mouse and human cortical neurons, ApoER2 axonal distribution and total levels significantly decrease, affecting relevant Reelin responses.
We propose that in conditions like HSP-AP4, or related to them, relevant functions of Reelin signaling associated with neurodevelopment and learning and memory processes are affected, explaining part of the patient's neurologic phenotypic features found in these diseases.

"SMAUG, HYDRA, OR DRAGON? CONDENSATION OF A POST-TRANSCRIPTIONAL REGULATOR INTO MEMBRANELESS ORGANELLES"

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Membraneless organelles and biomolecular condensates (BMCs) have emerged as key players in RNA regulation. Smaug is a conserved post-transcriptional regulator that binds numerous mRNAs, thereby modulating mitochondrial function and responses to nutrient deprivation. Smaug orthologs assemble into cytosolic BMCs in mammals, insects, and yeast. Notably, non-

canonical activation of the Smoothened (SMO)–AMPK pathway triggers the rapid disassembly of Smaug BMCs, leading to the release and translational activation of bound mRNAs. Our results uncover dynamic Smaug condensation as a novel arm of metabolic regulation downstream of the SMO–AMPK axis.

YOUNG INVESTIGATORS II

REGULATORY MECHANISMS AT THE MATERNAL–FETAL INTERFACE: INSIGHTS FROM PREGNANCY PATHOLOGIES AND CONNECTION TO CANCER

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The placenta is a transient but essential organ that ensures nutrient and oxygen transport to the fetus, immune tolerance at the maternal-fetal interface, and protection against external insults. It is composed of multiple cell types, among which cytotrophoblasts represent the specialized proliferative population. Cytotrophoblasts can differentiate into two main lineages: villous cytotrophoblasts, which sustain nutrient and gas exchange, and extravillous cytotrophoblasts (EVTs), which acquire a migratory and invasive phenotype that allows placental anchoring to the maternal uterus. EVT differentiation involves a physiological epithelial-to-mesenchymal transition regulated by the low oxygen environment of early pregnancy. Once anchoring is achieved, rising oxygen levels resulting from spiral artery remodelling restrain migration and support placental homeostasis. These processes are tightly regulated, and dysregulation underlies pregnancy pathologies. Herein, we investigated the interplay between two transcription factors central to placental biology under low oxygen tension: HIF-1α, the master regulator of the events that occur under hypoxia, and KLF6, a tumor suppressor highly expressed in the placenta. Using HTR-8/SVneo trophoblast cultures, we demonstrate that their interaction forms a negative feedback loop on HIF-1α involving NFκB signalling, reactive oxygen species, and protein degradation, as shown by western blot, qRT-PCR, flow cytometry, and the use of dominant-negative constructs. This regulatory circuit modulates EVT migration and angiogenesis under hypoxia, as evidenced by wound-healing assays and molecular markers. *In vitro* low oxygen cultures were modelled using a hypoxia chamber and 3 dimensional cultures.
Pathological placentas from preeclamptic and abnormally invasive pregnancies display reduced or excessive trophoblast migration, respectively, together with altered expression of these factors, consistent with our findings. Because the placenta and tumors share features such as migration and angiogenesis, although tightly regulated in one and dysregulated in the other, studying both systems in parallel may yield novel therapeutic insights. In line with this, our results reveal a context-dependent HIF1α–KLF6 interplay in tumor models that warrants further investigation.

REDOX HOMEOSTASIS AND TOR KINASE AS CENTRAL REGULATORS OF PRIMARY NITRATE RESPONSE IN PLANTS

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Meeting the food demands of a growing global population poses a major challenge to agriculture, particularly under climate change and soil degradation. Although nitrogen (N) fertilizers are widely applied, N deficiency still restricts plant growth and yield, while excessive use contributes to environmental damage. Improving nitrogen use efficiency (NUE) requires optimizing nitrate uptake and its integration into signaling networks. Over recent years, we have investigated the effects of expressing nitric oxide synthase (NOS) in plants and observed enhanced NUE through significant adjustments in N metabolism under N-deficient conditions. Beyond being a nutrient, nitrate also acts as a signal that triggers the Primary Nitrate Response (PNR). Redox signaling plays a central role in this process, as nitric oxide (NO) serves as a key second messenger in nitrate perception. Nitrate resupply also remodels cellular redox homeostasis by reducing reactive oxygen species (ROS) levels and activating antioxidant enzymes such as catalase and ascorbate peroxidase. Furthermore, we evaluated hydrogen sulfide (H_2S), another gasotransmitter and redox-active molecule, and found that nitrate treatment modified protein persulfidation profiles. Our results demonstrate that the nutrient-sensing kinase Target of Rapamycin (TOR) is activated during PNR. Using *Arabidopsis thaliana* seedlings, we showed that TOR activity depends on extracellular calcium and NO. Together, these findings reveal that NO, H_2S, and calcium converge on redox and nutrient signaling to modulate TOR activity, uncovering novel mechanisms of nitrate signal integration.

SYSTEMIC RESISTANCE TO PATHOGENS IN ARABIDOPSIS REQUIRES HASTY-DEPENDENT MIRNA CELL-TO-CELL MOVEMENT

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Plant defenses against pathogens are tightly regulated through complex gene expression control mechanisms. The precise activation and repression of defense-related genes are crucial to balancing the trade-off between growth and immunity. MicroRNAs (miRNAs) play a well-established role in the local regulation of plant-microbe interactions. While some miRNAs are also essential for systemic defense responses, their mechanisms of action, biogenesis, and long-distance mobility remain largely unexplored. Here, we show that HASTY (HST), a key factor in the miRNA biogenesis and intercellular movement, is required for systemic defense activation. The impaired mobility of miRNAs in these mutants correlates with a lack of systemic responses. In infected tissues, HST may enhance the co-transcriptional processing of specific pri-miRNAs, which promotes the cell-to-cell movement of their mature miRNAs and contributes to the activation of systemic defenses. Furthermore, two miRNAs that exhibit increased mobility during systemic defense induction are required for a proper systemic response. Interestingly, complementing *hst* mutants with a version of HST expressed exclusively in companion cells is sufficient to restore systemic defense induction, highlighting the role of miRNA cell-to-cell movement. These findings shed light on the role of HST in plant immunity, linking miRNA biogenesis and mobility to the fine-tuned regulation of systemic defenses.

FROM BUD TO BRANCH: GENE EXPRESSION REGULATION AT MULTIPLE LEVELS DICTATES PLANT ARCHITECTURE

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A key process that determines plant architecture is the branching pattern. Typically, plants develop a main inflorescence and lateral inflorescences that repeat a similar pattern; together, these structures determine the final plant architecture. Lateral branches emerge from meristematic buds located in the axils of leaves. These buds remain dormant until internal and external cues alter their fate. Since lateral branches develop into flowers and, consequently, fruits, branching pattern is also a critical trait defining plant productivity. Therefore, unravelling the molecular mechanisms that regulate gene expression in axillary buds is a major focus of research in both model and crop species. My research integrates developmental biology and gene regulation studies in both model and crop plants to unravel the molecular mechanisms controlling this process. I will present my contributions to understanding the evolutionary significance of conserved and divergent regulatory pathways. My work in the field covers a plethora of components that affect chromatin topology and, consequently, gene expression, including transcription factors, Polycomb proteins, and lncRNAs. Finally, I will introduce novel findings on a poorly explored facet of bud dormancy: epigenetic regulation. Our ongoing work in *Arabidopsis thaliana* will reveal new roles for the epigenetic silencing machinery in controlling plant development.

ORAL COMMUNICATIONS

MICROBIOLOGY

MI-1

β-LACTAMASE SUB-CELLULAR LOCALIZATION

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Antibiotic resistance is recognized by the World Health Organization as one of the most pressing global health challenges. Among the diverse mechanisms driving resistance, the expression of β-lactamases plays a central role by hydrolyzing and inactivating β-lactam antibiotics. While these enzymes have been thoroughly investigated in terms of structure, function, and evolution, their subcellular localization remains comparatively underexplored.
In this study, we analyzed all sequences deposited in the Beta-Lactamase DataBase using a Python-based pipeline combined with SignalP 6.0. signal peptides analysis allowed us to infer potential translocation pathways and subcellular destinations. As expected, the most frequent system identified was Sec/SpI, which directs soluble proteins to the periplasm of Gram-negative bacteria, in line with the prevailing view of β-lactamases as periplasmic soluble enzymes. However, a notable fraction of sequences was predicted to undergo lipidation (Sec/SpII), suggesting membrane anchoring. This feature could have important biological implications, including altered enzyme stability, incorporation into outer membrane vesicles, and modulation of host detection mechanisms.
To experimentally validate these predictions, we assessed the localization of selected β-lactamases of both clinical and environmental origin in different bacterial hosts. Resistance phenotypes were evaluated, and in the case of clinically relevant enzymes, we further demonstrated their incorporation into outer membrane vesicles and their capacity to confer protection to otherwise susceptible populations.
Together, our findings provide new insights into the biology of β-lactamases, highlighting subcellular localization as a critical but often overlooked determinant of antibiotic resistance dissemination.

MI-2

SPATIAL PATTERNS OF SURVIVAL AND REGROWTH OF CELL SUBPOPULATIONS WITHIN *ESCHERICHIA COLI* BIOFILMS FOLLOWING ANTIBIOTIC TREATMENT

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Bacterial pathogens such as *Escherichia coli* exploit their capacity to build extracellular matrix (ECM)–embedded communities, called biofilms, to establish persistent infections. Within biofilms, subpopulations survive antibiotic treatments, facilitating infection resurgence. Despite extensive research, it remains unclear which biofilm regions provide better survival conditions and how surviving cells regrow to rebuild the community. Leveraging our understanding of

heterogeneity in *E. coli* physiology and ECM production in macrocolony biofilms, and applying an approach to distinguish dead and live cells, we began to reveal spatial patterns of death and survival within kanamycin-treated biofilms. By analyzing transverse sections along the macrocolony radius, we found that in the border region, the youngest area with rapidly growing cells, kanamycin killed all bacteria. In contrast, in mature regions towards the center, we observed clearly defined and interspersed zones of bacterial death and survival, categorized as "susceptibility zones" and "tolerance zones". The tolerance zones corresponded to the upper part of the upper stratum (tolerance zone I) inhabited by starving, ECM-encased cells, and the inner part of the lower stratum (tolerance zone II). The lower stratum, situated between the upper stratum and the agar, is characterized by suboptimal growth, limited oxygen supply, and absence of ECM production. Based on these findings, we hypothesized that biofilm reconstitution post-treatment would rely on regrowth of surviving cells from the tolerance zones. To test this, we established a regrowth assay in which kanamycin-treated *E. coli* macrocolonies were incubated without antibiotic. We combined this with *E. coli* AR3110 carrying a plasmid-encoded P_{ind}::*gfp* fusion, whose expression in macrocolonies was induced by IPTG during regrowth to report newly synthesized proteins and active growth. Similarly, we used *E. coli* AR3110 with a plasmid-encoded P_{BAD}::*dsRed* fusion, where DsRed was induced with arabinose during regrowth also to report active growth. Interestingly, after 48 h of macrocolony regrowth post-treatment, cells in tolerance zone II exhibited exclusive red or green fluorescence from the respective reporters, indicating that only they resumed growth. The lack of growth of starved, ECM-encased cells in tolerance zone I is likely due to deep dormancy, requiring more time or other conditions to activate. Since border cells were killed by treatment, horizontal biofilm expansion during regrowth was limited. Instead, the regrown subpopulation in tolerance zone II drove expansion primarily in the vertical dimension. Overall, our study addresses regrowth of *E. coli* biofilms after antibiotic treatment, showing that, despite survival, distinct tolerant subpopulations exhibit differential capacities to resume growth. This has significant implications for biofilm repopulation and provides novel insights into how surviving cells persist within biofilms.

MI-3

INTRACLONAL DIVERSIFICATION THROUGH RECOMBINATION DRIVES THE EMERGENCE OF NOVEL EPIDEMIC CARBAPENEMASE-PRODUCING *KLEBSIELLA PNEUMONIAE* ST258 SUBLINEAGES

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Carbapenemase-producing *Enterobacterales* are multidrug-resistant critical priority pathogens due to their global spread and limited treatment options. Among these, carbapenemase-producing *Klebsiella pneumoniae* (Kpn-CP) is the predominant species. Global dissemination of Kpn-CP is largely due to sequence type 258 (ST258) lineage, first introduced in Argentina in 2008. This lineage originated from large-scale recombination events and the acquisition of novel genes. To investigate the molecular mechanisms underlying the highly successful epidemiology of Kpn-CP, we carried out a retrospective study across 14 healthcare institutions (Córdoba, Argentina), involving whole-genome sequencing (Illumina MiSeq), molecular typing (MLST), carbapenemase typing (AMRFinder) and pangenome

analysis (PanX) of 67 Kpn-CP isolates collected between 2016 and 2024. Pangenome analysis revealed 3,861 core genes, which were used for phylogenetic reconstruction of ST258 based on non-recombinant core SNPs via GATK and Gubbins. Recombinant regions were predicted using Gubbins. Phylogeny was inferred using IQ-TREE2 under the HKY+F+I model with 1,000 bootstraps (82% ± 15% support). MLST identified ST258 as the most prevalent lineage (40%) and bla_{KPC} was the dominant carbapenemase (81%), followed by bla_{NDM} (16%), bla_{KPC}+bla_{NDM} (2%) and bla_{OXA} (1%). Four distinct ST258 subclades were identified based on core-genome SNPs and recombination profiles: international clade I (11%), international clade II (30%), and two novel epidemic clades named C (26%) and D (33%), which were distributed across 4 and 6 institutions, respectively. Recombination region sizes significantly differed between clades I (59.5 ± 35 Kbp), II (98.9 ± 3.5 Kbp), C (207.5 ± 16.6 Kbp), and D (329.3 ± 5.3 Kbp) (ANOVA, $p < 0.0001$), with C and D showing significantly larger sizes (Tukey, $p < 0.0001$). Phylogenetic analysis by hierarchical clustering of non-recombinant core SNPs (root-to-tip distances), indicated that clades C and D likely evolved from international clade II. Interestingly, in clades C and D, major recombination events affected virulence-associated loci: the K (capsule) and O (O-specific polysaccharide) antigen cluster (~64 Kbp), which carried K type KL20 and O antigen O3/O3a, and the yersiniabactin biosynthesis operon (~50 Kbp), corresponding to *ybt15*. Importantly, these alleles have been previously reported in strains with increased virulence. In addition, clade D exhibited mutations in the ATP-dependent DNA helicase RecG (Thr368Ala, Ser989Ala), acquired by recombination of a 122 Kbp region including the *mrk* fimbriae. In conclusion, these findings identified new Kp-CP ST258 epidemic clades and reveal that this pathogen exploits intraclonal diversification involving recombination in virulence and homologous recombination pathways, as a strategy for successful dissemination.

MI-4

ULTRASTRUCTURAL ANALYSIS OF *STAPHYLOCOCCUS AUREUS* EXPOSED TO *LACTOCOCCUS SPP*., PENICILLIN G, AND THEIR COMBINATION.

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Staphylococcus aureus is a relevant pathogen within the One Health framework, being present in humans, animals, and the environment. In dairy cattle, it is one of the main causative agents of mastitis, a disease that results in economic losses due to decreased milk yield and quality. Penicillin G (PEN) is routinely employed; however, bacterial resistance compromises its efficacy, making it necessary to optimize its use in order to preserve its therapeutic value and avoid the need for last-line antimicrobials. Lactic acid bacteria with probiotic properties have been proposed as alternatives or adjuvants; nevertheless, morphological evidence of their combined effects with antibiotics against *S. aureus* remains limited. In this context, the objective of this study was to describe, through transmission electron microscopy (TEM), the ultrastructural alterations in PEN–resistant *S. aureus* (SAR) following treatment with Lactococcus spp. (LC), PEN, and their combination. SAR and LC strains were obtained from the working group's strain collection. Five experimental groups were established: G1 (SAR), G2 (SAR+PEN), G3 (SAR+LC), G4 (SAR+LC+PEN), and G5 (LC). Bacterial suspensions were prepared from fresh cultures, incubated for 24 h at 37 °C, and processed according to

standardized TEM protocols (fixation in glutaraldehyde/OsO_4, embedding in Spurr resin, and staining with uranyl acetate and lead citrate). As culture medium, a mixture of 90% cation-adjusted Mueller–Hinton broth and 10% Man, Rogosa and Sharpe (MRS) broth was used, which supported the growth of both bacteria and favored co-culture conditions. Micrographs were obtained at 80 kV, and changes in the cell wall, cytoplasm, and structural integrity were assessed. Observations from the control groups (G1 and G5) were employed as a reference to evaluate the changes observed in the co-culture treatment groups (G2, G3, and G4). In G1, SAR strains maintained their characteristic coccoid morphology with a thick cell wall and homogeneous cytoplasm, whereas in G5, LC strains showed arrangements in diplococci or short chains, with dense cytoplasm and regular cell walls. Treatment with PEN (G2) revealed focal weakening of the SAR cell wall without evident rupture. In contrast, treatments with LC alone (G3) and in combination with PEN (G4) induced pronounced alterations, including irregular thickening of the cell wall, focal detachment of the membrane, loss of cytoplasmic homogeneity, pore formation, and release of intracellular material. Damage was more severe in G4, with rupture of the cell envelope and cytoplasmic condensation, suggesting a complementary effect between LC-derived metabolites and PEN. These findings highlight TEM as a valuable tool to visualize ultrastructural alterations in *S. aureus*, providing morphological evidence of a potential synergistic effect between bacteria with probiotic potential and antimicrobials, thereby contributing to the mitigation of bacterial resistance.

MI-5

MICROCINS AS THERAPEUTIC AGENTS AGAINST EPIDEMIOLOGICALLY RELEVANT *SALMONELLA* STRAINS IN SALTA PROVINCE

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Salmonella enterica is a Gram-negative zoonotic pathogen and represents a major public health concern. Human infections result in salmonellosis, which may manifest as typhoidal or non-typhoidal forms, depending on the serotype involved. In Argentina, since November 2017, the province of Salta has faced recurrent outbreaks of paratyphoid fever, with increasing cases, particularly in summer. *Salmonella* Paratyphi B (SPB) has been identified as the etiological agent of these outbreaks. Positive samples were obtained from blood cultures of patients with sepsis; some developed complications such as intestinal perforation, urinary tract infections, gallstones, and liver abscesses. The most affected population was children and adolescents. Antimicrobial resistance (AMR) in *Salmonella* complicates therapeutic management, increases healthcare costs, and poses a serious threat to patient survival. In this context, antimicrobial peptides have emerged as promising therapeutic alternatives owing to their efficacy, safety, and structural and functional diversity, which limit bacterial resistance. Microcin J25(G12Y) (MccJ25(G12Y)) has been shown to be active against *Salmonella*, *Shigella*, and enteropathogenic *Escherichia coli* (including *E. coli* O157:H7), making it a strong candidate for

the treatment of salmonellosis. In this study, the probiotic strain, *E. coli* Nissle 1917 (EcN), was transformed with a plasmid to produce the antimicrobial peptide MccJ25(G12Y). The resulting strain, hereafter referred to as EcN(G12Y), was evaluated for its *in vitro* probiotic properties. The peptide was purified from the supernatant of a stationary-phase culture, and its identity was verified by mass spectrometry. The antimicrobial activity of both EcN(G12Y) and the purified peptide was evaluated against clinical isolates of SPB and non-SPB strains provided by the Hospital Público Materno Infantil of Salta, Argentina. The experimental results confirmed that EcN(G12Y) retained its probiotic properties, showing moderate tolerance to acidity (>65%) and high stability in the presence of bile salts (>95%). It also exhibited moderate biofilm-forming capacity, autoaggregation and coaggregation abilities, and low cell surface hydrophobicity, which were comparable to those of the parental strain. The identity and purity of the purified MccJ25(G12Y) were confirmed using MALDI-TOF mass spectrometry. For the first time, a monoisotopic mass of 2212 Da was determined, which agreed with the theoretical mass expected from the glycine-to-tyrosine substitution at position 12 of the parental MccJ25. Antimicrobial activity assays revealed that both SPB and non-SPB strains were highly susceptible to MccJ25(G12Y), showing inhibition zones comparable to those observed with commercial antibiotics. These findings support the potential of MccJ25(G12Y) as a therapeutic alternative for the control of epidemiologically relevant *Salmonella* strains, highlighting its promise for future clinical applications.

MI-6

INSIGHTS OF THE RESPONSE MECHANISM TO ALBUMIN AND CALCIUM ON BIOFLM FORMATION IN *BORDETELLA BRONCHISEPTICA*

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Bordetella bronchiseptica is a Gram-negative coccobacillus that infects the respiratory tract of a wide range of mammalian hosts. It has been reported that this bacterium can form biofilm-like structures both *in vitro* and *in vivo*, which contribute to its ability to colonize host tissues. A key regulator of biofilm formation, motility, and virulence in *B. bronchiseptica* is the second messenger c-di-GMP. Intracellular concentrations of this messenger influence bacterial lifestyle: elevated levels promote a sessile, biofilm-forming state, while reduced levels are associated with a planktonic, motile one. These levels are modulated by diguanylate cyclases (DGCs) and phosphodiesterases (PDEs), which synthesize and degrade c-di-GMP, respectively. Additionally, several proteins have been reported as contributing to biofilm formation in *B. bronchiseptica*. We previously described that two of the main components of the host's respiratory secretions, albumin and calcium (here referred to as BSAC), impair biofilm formation by lowering c-d-GMP intracellular levels.
In this work, we used a fine-tuned macrocolony assay to study how BSAC influence both morphology and dye uptake in the WT strain. In standard medium, macrocolonies displayed a central cotton-like region with poor dye uptake and wide, colored edges. In contrast, supplementation with BSAC resulted in smooth, continuous colonies lacking differentiated regions, and the overall coloration was uniform, but lighter. These observations suggest that BSAC affect the structure and composition of the biofilm matrix
Among the proteins required for biofilm formation, the adhesin BrtA plays a central role. BrtA, part of the Lap system, is regulated by c-di-GMP. At high c-di-GMP levels, BrtA is retained on

the bacterial surface, promoting biofilm formation, whereas at low levels, it is released, leading to reduced biofilm. This process is likely mediated by one or more specific PDEs. We performed biofilm assays with four single PDE mutants. Only the *pdeA* mutant showed a partial phenotype, displaying a 50% reduction in biofilm formation when compared to the WT strain in the presence of BSAC. This suggests a role for PdeA in the regulatory response to BSAC. Additionally, seven single DGC mutants were tested to identify potential contributors of c-d-GMP to the Lap system, but none showed significant differences compared to the WT strain.
In summary, our results expand the understanding of how BSAC modulates biofilm formation in *B. bronchiseptica* and suggest that PdeA contributes to the mechanism underlying this response.

MI-7

LEUCONOSTOC MESENTEROIDES STRAIN WH8 AND _ENTEROCOCCUS HIRAE_ STRAIN WF5 ISOLATED FROM WALNUT AS BIOCONTROL AGENTS AGAINST WALNUT PHYTOPATHOGENS

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The walnut (*Juglans regia* L.) is one of the main regional crops produced in different regions of Argentina. Crop losses due to different diseases, including canker and brown apical necrosis, can reach alarming levels despite repeated treatments such as copper fumigations.
To develop an effective biocontrol method against these bacterial pathogens, lactic acid bacteria (LAB) were isolated from walnut cultivars and tested as biocontrol agents. The isolation of bacteria was conducted through sampling of leaves, fruits and flowers of walnut trees, and inoculated on MRS agar. Then, biochemical identification was carried out to select only LAB. Thirty isolates were screened for their *in vitro* antagonistic activity. The isolates WF5 and WH8, identified by partial sequencing of 16S rRNA and MALDI-TOF as *Enterococcus hirae* and *Leuconostoc mesenteroides,* respectively, were found to considerably inhibit the growth of *Pectobacterium actinidiae* S2FC (causal agent of canker in walnut) and *Pantoea agglomerans* M10642 (brown apical necrosis).
Through the cross-streak method, we analysed the antibacterial effects of bacterial suspensions of both LAB strains against these phytopathogens and found that they exhibited inhibition zone sizes larger than 16 mm for WF5 and 30 mm for WH8. We also evaluated the inhibition capacity of their cell-free supernatants (CFS), which were obtained from a 48-h culture in MRS broth, followed by centrifugation at 10.000 rpm and sterilization with 0.2 µm filters. After executing the wells method, inhibition halo sizes were greater than 20 mm for both strains against the pathogens tested. Moreover, the CFS exhibited strong thermostability (up to 100°C), although a remarkable decrease in antibacterial activity was observed at neutral pH (pH 6,5). In a subsequent step, the CFS of WF5 was tested *in vitro* against both phytopathogens on immature walnut fruits through a pathogenicity test. For this, previously disinfected fruits were inoculated either with S2FC or with M10642, and the supernatant was then applied at the point of infection. The fruits were kept in a humid chamber for 15 days to evaluate the progression of symptoms. Fruits infected with the pathogens but left untreated served as the controls. In these fruits, characteristic symptoms started to develop 5 days after inoculation (DAI), and black lesions began appearing 7 DAI. By day 13, some fruits were entirely covered in black lesions, and others were rotting. In contrast, infection by S2FC and by

M10642 was respectively 62% and 70% lower in fruits treated with the CFS of WF5 than in the controls. These findings demonstrate the potential of two LAB strains, *E. hirae* WF5 and *L. mesenteroides* WH8, as biocontrol agents against common walnut diseases. Both strains and their supernatants could be incorporated into sustainable disease management strategies seeking to reduce the risks of chemical bactericides in large-scale walnut orchards.

MI-8

REGULATION OF MFD-MEDIATED MUTAGENESIS BY MUTS PREVENTS THE EMERGENCE OF ANTIBIOTIC RESISTANCE IN *BACILLUS SUBTILIS*

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DNA replication and transcription machineries use the same DNA template and occur concurrently in bacteria without temporal and spatial separations. These transcription-replication conflicts (TRCs) have detrimental consequences on replication and cell viability, as well as they promote mutagenesis in highly transcribed genomic regions. The TRCs-induced mutagenesis is produced by the DNA synthesis catalyzed by low-fidelity DNA polymerases (LF-Pols) in the nucleotide excision repair (NER). Briefly, the Mfd protein recognizes RNA polymerases stalled at DNA lesions and subsequently displaces them from DNA. The exposed lesion is excised by the NER proteins, leaving a single nucleotide gap, which is filled in by LF-Pols. Our previous results have demonstrated that the Mismatch Repair protein, MutS, modulates the access of LF-Pols to replication sites during translesion synthesis by regulating their interaction with the processivity beta clamp factor. In the present study, we analyzed whether this novel MutS-dependent mechanism modulates the TRCs-mutagenesis induced by LF-Pols in *Bacillus subtilis*. With this aim, mutation rates in endogenous genes with low (*thyA*), medium (*rpsL*) and high (*rpoB*) transcription levels were estimated in a *mutSβ* strain, which expressed a MutS mutant that does not bind to β clamp and therefore does not control LF-Pols, compared to the wild type (WT) strain. We found a significant increase in the mutation rates to resistance to rifampicin (target gene: *rpoB*, *rifR*) in the *mutSβ* strain relative to the WT strain. In contrast, both strains showed similar mutation rates to streptomicin (target gene: *rpsL*, *smR*) and trimetoprim resistance (target gene: *thyA*, *tmpR*). Then, we tested if Mfd, the NER factors UvrA and UvrB, and the LF-Pols, Pol I, PolY1 and PolY2, are involved in the mutagenesis observed in mutSβ. Inactivation of Mfd, UvrA/UvrB and Pol I specifically decreased mutation rates to *rifR* and *smR* but not to *tmpR* in the *mutSβ* genetic background. Similar results were obtained with the exogenous *thyP3* reporter gene, which was placed under an IPTG-inducible promoter. Finally, using a laboratory evolution assay, we found that the *mutSβ* and *mutS*-deficient strains showed an accelerated acquisition of resistance to different antibiotics, reaching up to 16- and 130-fold higher MICs, an effect dependent on Mfd, UvrA and Pol I factors. In conclusion, these results suggest that MutS regulates the action of the low-fidelity Pol I in the Mfd-dependent mutagenesis resulting from TRCs, a mechanism critical for preventing the emergence of antibiotic resistance. Currently, we are analyzing the molecular signatures of this process in *B. subtilis* genomes.

MI-9

NEW THERAPEUTIC STRATEGIES FOR AMERICAN TEGUMENTARY LEISHMANIASIS BASED ON DRUG REPOSITIONING

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American tegumentary leishmaniasis (ATL) is a neglected tropical disease caused by protozoa of the genus *Leishmania*. It is endemic in several regions of Argentina, with high prevalence in the province of Salta, where it is a major public health concern. Current therapeutic options, like pentavalent antimonials and amphotericin B (AmpB), have important limitations: they are toxic, painful, and poorly tolerated, leading many patients to abandon treatment. This scenario highlights the urgent need to identify safer and more effective therapeutic alternatives.

In this context, drug repositioning has emerged as a promising strategy, as it allows the use of compounds already approved for other diseases, thereby reducing costs and development time. Our group previously evaluated acetylcholinesterase inhibitors, which are currently marketed for neurodegenerative disorders, and demonstrated their *in vitro* activity against *Leishmania* (*L.*) *amazonensis*. These molecules displayed a marked synergistic effect when combined with AmpB, with the compound FR2 showing the strongest potential. Based on these findings, we investigated the therapeutic value of FR2, alone or combined with AmpB, through *in vitro* and *in vivo* approaches. *In vitro* assays were performed against *L.* (*L.*) *amazonensis* promastigotes and *L.* (*Viannia*) *braziliensis* axenic amastigotes. Parasites were exposed to FR2, AmpB, or their combination, and morphological changes were analyzed by light and scanning electron microscopy. For *in vivo* evaluation, BALB/c mice were infected with *L.* (*L.*) *amazonensis* promastigotes and divided into five groups: placebo, AmpB, FR2, FR2-AmpB, and healthy controls. Treatments were administered for six weeks by intraperitoneal (AmpB) or oral (FR2) routes, and lesion progression, body weight, and hematological parameters were monitored. *In vitro* analyses revealed that while AmpB and FR2 alone induced moderate alterations, such as reduced parasite size, flagellar shortening, and surface irregularities, the combination therapy caused more profound damage, including twisted cell bodies, flagellar abnormalities, and membrane pores, suggesting a synergistic mechanism of action. Comparable effects were observed in axenic amastigotes of *L.* (*V.*) *braziliensis*, reinforcing the broader applicability of this strategy across species. In the murine model, the FR2-AmpB combination yielded the most significant therapeutic benefits, with marked reductions in lesion size and parasite burden compared with the placebo. Moreover, hematological parameters, such as hematocrit and total IgG, remained stable, indicating improved tolerability.

Our findings support the FR2-AmpB combination as a novel and potentially less toxic therapeutic alternative for ATL. This study highlights the value of drug repositioning to expand the therapeutic arsenal against neglected tropical diseases and offers a promising avenue for future clinical applications in vulnerable populations disproportionately affected by ATL.

MI-10

A TROJAN HORSE STRATEGY TO BROADEN NISIN'S ANTIMICROBIAL ACTION

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Nisin is a well-established antimicrobial peptide widely employed as a biopreservative to extend shelf life and control foodborne pathogens, particularly Gram-positive bacteria such as *Listeria monocytogenes*. However, its effectiveness against Gram-negative bacteria is limited, primarily due to its inability to penetrate their outer membrane, which prevents nisin from accessing its target, lipid II, located in the inner membrane. To overcome this barrier, outer membrane-permeabilizing agents—or innovative delivery strategies—are required. In this study, we aimed to expand nisin's spectrum of activity against Gram-negative bacteria by conjugating it to salmochelin S4, a siderophore produced by *Salmonella enterica*. Siderophores are iron-chelating molecules that enter bacterial cells through specific outer membrane receptors. This mechanism forms the basis of the "Trojan horse" strategy, an emerging approach in antimicrobial design that exploits siderophore-mediated transport to smuggle antibiotics across the outer membrane of Gram-negative bacteria. This concept is particularly relevant considering the increasing threat posed by antimicrobial resistance. Commercially available nisin was purified and characterized alongside salmochelin S4, which was extracted from *S. enterica* strain H5547. Both compounds were purified using chromatographic techniques and characterized via mass spectrometry. To synthesize nisin derivatives with enhanced Gram-negative activity, we employed a copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC) "click" reaction. Nisin was first functionalized at its C-terminal end with an alkyne group, while salmochelin S4 was modified with an azide moiety. The successful functionalization of each component was confirmed by HPLC-MS analysis. The resulting Nisin–Salmochelin (N–S) conjugates were purified and analyzed by reversed-phase chromatography, revealing distinct retention times corresponding to the different variants. The N–S conjugates displayed antimicrobial activity not only against Gram-positive *L. monocytogenes* FBUNT, but also against Gram-negative strains, including *S. enterica* serovar Typhimurium 14028 and the uropathogenic *Escherichia. coli* isolate EC3. This dual activity demonstrates the potential of the conjugate as both a food biopreservative and a candidate therapeutic agent. While further studies are needed to assess its therapeutic applicability, our results provide a promising foundation for the development of broad-spectrum bacteriocins through siderophore conjugation strategies.

MI-11

LEISHMANICIDAL POTENTIAL OF *ENTEROCOCCUS MUNDTII* CRL35 AND ITS METABOLITES: FROM *IN VITRO* STUDIES TO MURINE MODELS

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Cutaneous leishmaniasis is a neglected tropical disease that still lacks safe and effective therapies, driving the search for innovative alternatives. Several strains of *Enterococcus* produce certain metabolites with biotechnological and therapeutic potential, such as antimicrobial peptides. Although their activity against pathogenic bacteria is well documented, their effects on parasites remain poorly studied. In this study, we evaluated the antileishmanial activity of bacterial supernatants obtained from *Enterococcus mundtii* CRL35 against *Leishmania* (*L.*) *amazonensis* ***in vitro*** and in a murine model. The CRL35 strain was cultured in BHI medium (37 °C, 48 h), and the supernatant was centrifuged and filtered. The ***in vitro*** assays included: (i) activity against promastigotes (2×10^6 parasites/well, 48 h, 23 °C), (ii) cytotoxicity on murine macrophages RAW 264.7 (4×10^4 cells/well, 48 h, 37 °C, 5% CO_2), and (iii) activity against intracellular amastigotes (2×10^4 macrophages infected at a 20:1 ratio). Cell and parasite viability was measured by the MTT assay, and IC_{50} and CC_{50} values were obtained using dose-response curves. For the ***in vivo*** model, BALB/c mice were infected with 2×10^7 promastigotes at the base of the tail. Animals were divided into six groups: topical treatment with CRL35 supernatant, ad libitum administration of live *E. mundtii* CRL35, combined treatment (topical supernatant + ad libitum cells), and amphotericin B (positive treatment control), untreated infected (negative treatment control), and healthy mice. The lesion size, body weight, and blood samples were monitored over a period of 60 days. At the endpoint, the parasite load in lesions was quantified by critical dilutions, and the liver and spleen tissue samples were analyzed using qPCR. Serological assays were performed to assess the humoral response. The results demonstrated that CRL35 supernatants exhibited strong antileishmanial activity ***in vitro***, with an efficacy 4-fold against intracellular amastigotes than promastigotes and a low cytotoxicity, indicating a favorable selectivity index. Topical and combined treatments significantly reduced lesion size and parasite burden in the murine model. A treatment-associated beneficial immunomodulatory response is necessary for a complementary analysis. In summary, metabolites derived from *E. mundtii* CRL35 demonstrated potent antileishmanial activity both ***in vitro*** and ***in vivo***, with good safety and tolerability. These findings highlight the potential of *E. mundtii* CRL35 and its metabolites as promising candidates for the development of innovative therapeutic strategies against cutaneous leishmaniasis.

LIPIDS

LI-1

THE PLANT UREASE “JACK BEAN UREASE” IMPAIRS LIPID METABOLISM ON THE FAT BODY-OVARY AXIS IN THE CHAGAS DISEASE VECTOR *RHODNIUS PROLIXUS*

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Ureases are nickel-dependent enzymes that primarily generate bioavailable nitrogen for plants. Jack Bean Urease (JBU), the main isoform from *Canavalia ensiformis*, also exhibits non-catalytic properties, such as entomotoxicity. Triatomines are hematophagous insects of major public health concern as vectors of *Trypanosoma cruzi*, the etiological agent of Chagas disease. *Rhodnius prolixus* is both a primary vector species and a common model organism. In insects, vitellogenesis is a finely regulated process in which the fat body—an organ analogous to the vertebrate liver and adipose tissue—synthesizes and secretes substantial amounts of yolk protein precursors into the circulation for uptake by developing oocytes. This process also involves large-scale lipid mobilization from the fat body to the oocytes via lipoproteins. Although JBU's lethality to insects like *R. prolixus* is documented, the mechanism of its toxicity remains poorly understood, limiting its biotechnological application. Moreover, its potential effects on insect oogenesis and reproduction have not yet been addressed. To identify the targets of JBU toxicity, we investigated its effects on the fat body-ovary axis, focusing on lipid metabolism. Female *R. prolixus* were injected with a sublethal dose of JBU, and oviposition was evaluated together with biochemical, cellular, and molecular parameters. JBU treatment significantly reduced oviposition, delayed ovarian development, and induced follicular atresia. Ultrastructural alterations were also observed, including vacuolization and swelling of nuclear membranes and mitochondria, compatible with necrosis in both the fat body and the ovary. Apoptotic and multivesicular bodies were detected in the ovary, indicative of apoptosis and autophagy. qPCR assays revealed a significant upregulation of fat body genes involved in lipid metabolism—*Brummer lipase* (lipolysis), *acetyl-CoA carboxylase* (synthesis), and *perilipin* (storage)—while tissue staining with Bodipy indicated that adiposomes were significantly larger in JBU-treated females than in controls. Analysis of lipid composition in the fat body and ovaries of treated females further showed changes in different lipid classes, particularly triacylglycerol, monoacylglycerol, and fatty acid, indicating that JBU interferes with lipid mobilization and accumulation in these organs. Together, these findings demonstrate that JBU impairs oogenesis by disrupting lipid metabolism in the fat body-ovary axis. This unveils a novel entomotoxic mechanism in which JBU disrupts reproductive energy metabolism, advancing our understanding of urease effects on insects and providing a basis for developing targeted control strategies against disease vectors.

LI-2

GLIAL EXTRACELLULAR VESICLES PRODUCTION AND LIPID-DEPENDENT NEUROPROTECTION: A NEW CASE STUDY

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The dithiocarbamate pesticide maneb has been associated with parkinsonism after chronic exposure. We have previously demonstrated that astrocytes' secretome triggers protective signals associated with resolution pathways in neuronal cells exposed to maneb. In the present study, we aimed to characterize the secretome of primary glial cells, specifically their extracellular vesicle (EV) population and its role in neuroprotection. EVs are membranous nanostructures secreted under both normal and pathological conditions, thus playing a crucial role in neuron-glia communication. Firstly, we isolated glial cells from mice brains and obtained mixed glial cultures (MGC) and purified microglia and astrocytes, which were characterized by assessing activation markers using RT-qPCR. Microglia showed increased mRNA expression of *Tnf-α*, with no changes in *Il-1β* or *Il-6*, and increased *Il-10* and *Tgf-β,* along with higher *Arg-1* levels, consistent with a M2 phenotype. In contrast, astrocytes displayed increased *Tgf-β* but no changes in the A1 marker *C3* or other cytokines. Conversely, MGC exhibited a decreased expression of *Arg-1* and *C3*, alongside higher expression of *Il1-β* and the A2 marker Emp*1*, demonstrating differential responses in mixed and isolated cultures. To study EVs derived from the different glial cultures challenged with maneb, size-exclusion chromatography was used for their isolation from the secretome. Through micro-flow cytometry, we found that maneb-exposed astrocytes and microglia, but not MGC, secreted nearly fivefold more EVs than controls, with no change in total EV protein content. Characterization by nanoparticle tracking analysis revealed a broader size distribution with bigger EVs compared to controls for the isolated cultures, coinciding with increased levels of total phospholipids (PL). In addition, GC-MS analysis of fatty acids from PL displayed a differential contribution of the polyunsaturated fatty acids 22:6 and 20:4 in EVs derived from maneb-exposed astrocytes. Moreover, live imaging revealed a significant reduction of EV uptake in neurons under pesticide exposure. Interestingly, pesticide-induced mitochondrial alterations in neurons were attenuated by EVs derived from maneb-exposed astrocytes with no changes in cell viability. This contrasted with the neuroprotective role of the astrocytes' secretome, which was associated with resolution mechanisms depending on FPR2/ALX activation mediated by lipoxin A4, a pro-resolving lipid mediator derived from 20:4. Our results suggest that astrocytes adapt their EV formation and composition in response to pesticide-induced toxicity, a mechanism that may play a pivotal role in mediating astrocytic neuroprotection and that can synergize with pro-resolving factors of a lipid nature.

LI-3

ACSL4 REMODELS MICRORNA PROFILE IN BREAST CANCER: FOCUS ON MIR-99A

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Acyl-CoA synthetase 4 (ACSL4) is a key enzyme in lipid metabolism that catalyzes the esterification of long-chain fatty acids, with a preference for arachidonic acid. Beyond its metabolic role, ACSL4 has been implicated in the regulation of cellular pathways associated with tumor progression. Our aim is to unravel the molecular mechanisms driven by this enzyme that contribute to an aggressive tumor phenotype, focusing on microRNAs (miRNAs) as potential mediators. miRNAs are small non-coding RNAs that silence genes by specifically targeting mRNAs. Through RNA sequencing, we showed that ACSL4 alters the expression of a large set of genes, including several precursor transcripts of miRNAs. Here, we investigated how both mature miRNAs and their precursors respond to changes in ACSL4 expression. We performed miRNA sequencing (miRNA-seq) in MCF-7 breast cancer cells stably overexpressing ACSL4, and identified differentially expressed miRNAs compared with control cells (Log2 fold change > |0.5|). Using databases such as KEGG Pathway, WikiPathways, and miRPathDB, we conducted predictive analyses to explore the potential roles of these miRNAs in biological pathways, physiological processes, and pathological conditions. Several candidates were validated by RT-qPCR. Among them, we focused on miR-99a-3p, given its reported involvement in cancer-related pathways. Using cellular models of ACSL4 overexpression and knockdown, we found that miR-99a-3p is consistently downregulated in breast cancer models with high ACSL4 expression, and upregulated in models with low ACSL4 expression. Additionally, its precursor transcript and the associated miR-99a-5p followed the same regulatory trend, showing significant downregulation upon ACSL4 overexpression. To explore the regulatory mechanism, we identified the host gene MIR99AHG and its putative promoter region. Reporter gene assays confirmed decreased promoter activity under ACSL4 overexpression. Using PROMOTER 2.0 and FIMO (JASPAR databases), we predicted candidate transcription factor binding sites. Integration of RNA-seq data and qPCR validation revealed ACSL4-dependent changes in the expression of several transcription factors, some of which have been associated with lipid-related pathways according to functional enrichment analyses (Gene Ontology, KEGG Pathways). In summary, we describe and validate a miRNA expression profile reshaped by ACSL4 in breast cancer cells. Our findings indicate that ACSL4 contributes to a pro-tumorigenic phenotype in breast cancer, at least in part through the transcriptional repression of miR-99a-3p, likely involving lipid metabolism-associated transcription factors.

LI-4

NEUROTOXIC EFFECTS OF GLIAL-DERIVED 24-S-HYDROXYCHOLESTEROL: IMPACT ON SYNAPSE STRUCTURE, NEURONAL VIABILITY AND POTENTIAL AMYLOIDOGENIC ROLE

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The brain is the richest organ in cholesterol, containing nearly 25% of the total cholesterol present in humans. This high proportion reflects the crucial importance of this molecule in the central nervous system, where it is involved in processes such as synaptogenesis, synaptic plasticity, and axonal and dendritic development. In adulthood, cholesterol synthesis in the brain occurs almost exclusively in astrocytes, while its excess is eliminated through conversion to 24-S-hydroxycholesterol (24-OHC) by the cholesterol 24-hydroxylase enzyme (CYP46A1). Although this enzyme is expressed in neurons under physiological conditions, its expression

has been reported in reactive astrocytes in the context of brain injury, such as Alzheimer's disease or traumatic insults. Our results suggest that reactive astrocytes can produce high amounts of 24-OHC under inflammatory conditions; however, the functional role of this molecule in pathological states has not yet been clearly elucidated. Therefore, we evaluated the effect of 24-OHC in primary cultures of rat cortical neurons, analyzing neuronal viability, synaptic contact density, and its ability to induce the Amyloid Precursor Protein (APP), the main player in Alzheimer´s disease development. We observed a significant alteration in synaptic structure at minimal doses of 24-OHC, whereas higher concentrations impaired neuronal viability. Furthermore, exposure of primary neurons to 1 µM 24-OHC induced APP synthesis, suggesting a possible predisposing role in AD at higher concentrations of this sterol. Our findings suggest that, under inflammatory conditions, 24-OHC derived from reactive astrocytes may act as a neurotoxic agent that could contribute to the pathophysiology of Alzheimer's disease.

LI-5

GLYCEROLIPID METABOLISM ACTIVATION IS ESSENTIAL FOR EPITHELIAL RESTITUTION AFTER CALCIUM OXALATE INJURY

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Calcium oxalate (CaOx) is the main component of kidney stones. CaOx crystals interact with the surface of renal epithelial cells and initiate injury. In differentiated renal epithelial cells (DREC), we showed that oxalate (Oxa) injures monolayers, which undergo type II EMT (first 24 h, damage period). Thereafter, cells gradually recover their morphology, restituting the monolayer between 48 and 72 h (restitution period). Since Oxa induces lipid peroxidation (LPO) which disrupts membrane homeostasis, we hypothesize that epithelial restitution occurs after the activation of lipid metabolism and the restoration of cellular membrane integrity. In this study, we determined the role of glycerolipid (GL) metabolism in DREC monolayers survival and restitution after Oxa injury. DREC monolayers were incubated with 1.5 mM Oxa for 0, 4, 8, 24, 48, and 72 h. At every time, we evaluated cell morphology (Fluorescence microscopy), number and viability (Trypan blue assay), lipid peroxidation (TBARS), membrane biophysical properties (Pyrene probe) and composition (TLC). We determined the dynamics of GL metabolism, with emphasis on glycerophospholipid (GP) and triacylglyceride (TG), by measuring the *de novo* synthesis (^{14}C-Gly labeling) in the absence or presence of propranolol, an inhibitor of lipins, key enzymes in this biosynthetic pathway. During damage period, we found an altered DREC monolayer, a decrease in cell number, and a significant increase in TBARs. Oxa induced changes in membrane biophysical properties and in its composition, increasing phosphatidylinositol (PI) and phosphatidylserine (PS) and decreasing phosphatidylcholine (PC) contents. These changes were along with the activation of GP and TG synthesis and with an increase in the number of LD. Propranolol impaired GP and TG

synthesis, completely preventing DREC monolayer restitution as seen by florescence microscopy. Our results showed that Oxa-induced LPO disrupts DREC membrane properties, changing its biophysics and composition which affect cell physiology. To restore cell homeostasis, GL synthesis and LD biogenesis are activated, allowing the gradual recovery of DREC phenotype. This work highlights the importance of membrane structure maintenance in cell survival and epithelial restitution after calcium oxalate injury.

LI-6

ENDOCRINE DISRUPTOR NONYLPHENOL IMPAIRS LIPID METABOLISM AND MEIOTIC ENTRY IN PREPUBERTAL MOUSE TESTIS EXPLANTS

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Nonylphenol (NP) is an emerging endocrine-disrupting pollutant that can be present as a residual additive in plastics and can also adsorb onto, and later desorb from, microplastics, thereby enhancing its persistence and bioavailability in aquatic environments. According to the PlastChem Project, which has identified over 16,000 chemicals used in plastics, NP is listed among 3,651 hazardous substances on the Red List that remain internationally unregulated and should be subject to restriction. This toxicant has recently been associated with multiple adverse effects on male fertility in both laboratory animal models and wild species. Previously, using a gas–liquid interphase testicular tissue culture model, we demonstrated that NP disrupts steroidogenesis and lipid homeostasis while simultaneously impairing *in vitro* spermatogenesis. In this study, we focused on alterations in testicular lipid and fatty acid composition and content, as well as on changes in the expression of genes encoding proteins involved in lipid metabolism. Our results show that NP impairs the normal progression of spermatogenesis *in vitro* by reducing the number of seminiferous tubules (ST) that reach the meiotic stage. Interestingly, after 22 days in culture, NP-exposed testicular tissue exhibited a slight increase in choline glycerophospholipids accompanied by a decrease in sphingomyelin and ethanolamine glycerophospholipids. In addition, there was a significant accumulation of neutral lipids, including triacylglycerols (TAG) and alkyl-diacylglycerols (ADG). This finding was consistent with results obtained using the fluorescent marker Nile Red, which revealed that NP increases the abundance of neutral lipid droplets in the ST and interstitium. While the fatty acid composition of glycerophospholipids remained unchanged, TAG, ADG, and cholesterol esters (CE) contained higher levels of saturated fatty acids (SFA) and lower levels of polyunsaturated fatty acids (PUFA). These alterations suggest that, in the presence of NP, testicular neutral lipids are unable to incorporate PUFA, which are essential for normal spermatogenic progression. Concomitantly, the mRNA levels of genes involved in fatty acid elongation and hydroxylation, such as very-long-chain elongase 2 (*Elovl2*) and fatty acid 2-hydroxylase (*Fa2h*) were down-regulated. In contrast, mRNA levels of acyl-CoA:diacylglycerol acyltransferase 1 (*Dgat1*), involved in lipid droplet biogenesis, were upregulated at 22 days of culture. Moreover, NP-exposed testes accumulated cholesterol, consistent with the observed disruption of steroid hormone production. Together, these results highlight the detrimental impact of NP on prepubertal spermatogenesis and germ cell maintenance, unveiling part of the mechanisms through which this compound exerts its toxic effects, namely alterations in lipid metabolism and steroid hormone synthesis. *Supported by FONCyT [PICT2020-02056 to GMO] and SGCyT UNS [PGI 24/B341 to GMO and JML].*

LI-7

GLYCOSPHINGOLIPIDS AS CRITICAL REGULATORS OF EPITHELIAL MORPHOGENESIS

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Epithelial morphogenesis involves key cellular events such as proliferation, polarization, and lumen formation, which are essential for the architecture and function of epithelial tissues. Three-dimensional (3D) cultures recapitulate the natural organization of tissues in vivo. When embedded in an extracellular matrix, MDCK cells form polarized spherical cysts with their apical membranes oriented toward a single central lumen. While extensive research on protein complexes has provided major insights into epithelial morphogenesis, the role of lipids in this process remains poorly understood. De novo lumen formation in MDCK cysts begins during the first mitosis and requires polarized exocytosis of vesicles carrying apical markers along the mitotic spindle to the site of abscission. During subsequent divisions, maintenance of a single central lumen depends on planar orientation of the mitotic spindle in metaphase. We have previously shown that glycosphingolipid depletion leads to the formation of multilumen cysts and abnormal primary ciliogenesis. In the present study, we further investigated the effects of glycosphingolipid inhibition by analyzing lumen positioning and spindle orientation during metaphase. MDCK cells were embedded in a collagen matrix to generate 3D cysts, treated on day 3 with D-PDMP, a glucosylceramide synthase inhibitor, and immunostained on day 9 for actin and acetylated tubulin. Confocal fluorescence microscopy revealed that glycosphingolipid depletion disrupts lumen formation, increasing the proportion of cysts with atypical actin distribution and misoriented mitotic spindles. Quantitative image analysis demonstrated that the distribution of mitotic spindle angles was significantly broader in treated cysts compared with controls, suggesting that impaired spindle orientation may underlie the observed multilumen phenotype. Together, these findings highlight glycosphingolipids as critical regulators of epithelial morphogenesis, influencing cell polarization, ciliogenesis, and lumen formation. The use of 3D cyst cultures provides a powerful model to dissect the contribution of lipids to epithelial morphogenesis and offers valuable insights into ciliopathies such as polycystic kidney disease, reinforcing their relevance for both basic and translational research.

LI-8

ADAPTIVE LIPID METABOLISM IN DROSOPHILA: HOW DIETARY LIPIDS REWIRES *ORSAI* REGULATION

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Organisms must effectively adapt to fluctuating environmental conditions and nutritional states to ensure proper development, primarily by modulating metabolism and behavior (Koyama et al, 2020). The ability to sense both internal nutritional status and external environmental cues is therefore critical. In *Drosophila melanogaster*, the fat body functions as

the central nutrient-sensing organ, analogous to the mammalian liver and adipose tissue (Colombani et al, 2003) and plays a key role in mediating the impact of dietary alterations on larval growth and development (Koyama et al, 2020). Maintaining lipid metabolic homeostasis is particularly vital for environmental adaptation and organ growth progression through development, as lipids are essential structural components of membranes, serve as energy reservoirs, and function as signaling molecules influencing developmental pathways and gene expression via chromatin organization (Yao et al, 2019). Consequently, understanding how lipid metabolism responds to environmental inputs is crucial for deciphering the gene regulatory networks governing development. We identified Orsai (Osi) as a key metabolic factor involved in fatty acid beta-oxidation. Given the significant developmental phenotypes associated with Osi loss of function, particularly in the fat body, and its known responsiveness to various stimuli (Brown et al., 2014), it is fundamental to elucidate its regulatory mechanisms in this organ. Thus we investigated *osi* expression and Osi protein levels/localization in the fat body under different nutritional conditions. Our findings reveal that a high-fat diet transiently induces an acute adaptive response, initially increasing *osi* mRNA and protein levels, but with a quick turnover. Furthermore, prolonged exposure results in attenuated protein levels. This suggests that dietary lipid intake triggers the adaptive regulation of genes and proteins involved in fatty acid beta-oxidation. Further characterization of these regulatory dynamics is essential to understanding metabolic adaptation and its implications for nutrition and health.

PLANTS

PL-1

ALTERNATIVE SPLICING AS A SOURCE OF REGULATORY LONG NON-CODING RNAs ARISING FROM CODING GENES IN PLANTS

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Gene expression in eukaryotes is a complex process involving various regulatory mechanisms. RNA processing, which involves a series of events generating mature transcripts, represents a crucial regulatory layer. Alternative splicing (AS) is a mechanism capable of producing multiple mature transcripts from a single gene through variable and regulated selection of splice sites, and it contributes to the diversity of the transcriptome and proteome. Being plants sessile organisms, AS provides an important adaptive mechanism and it is tightly regulated by environmental conditions, including light, which is a key determinant of growth, development, and adaptation. In general, different isoforms can be translated into different proteins, but it can also give rise to non-coding variants, which tend to be degraded, allowing fine regulation of the levels of coding isoforms and the amount of protein produced. The selection of alternative splicing sites is mediated by trans-acting splicing factors, such as Serine/Arginine-rich (SR) proteins, highly conserved RNA-binding proteins that are unusually numerous and diverse in plants. In this work, we aim to analyze why most plant *SR* genes produce at least

one non-coding isoform through intron retention, which are accumulated in the nucleus. *At-RS31*, a member of the plant-specific RS sub-family, is one of the most affected genes in terms of AS regulation by light. The AS derived isoforms are: *mRNA1*, that accumulates in light; and two non-coding isoforms: *mRNA2*, that is subjected to degradation via nonsense-mediated mRNA decay (NMD) due to a premature termination codon (PTC), and *mRNA3*, which despite of having the same PTC, accumulates to elevated levels in darkness and is retained in the nucleus. We demonstrated that *Arabidopsis* seedlings overexpressing *mRNA1* (mRNA1ox) show a deleterious phenotype in several developmental stages, while overexpression of the genomic construct results in seedlings with a normal phenotype. Although *mRNA3* overexpression alone does not produce visible phenotypes, its expression in an mRNA1ox background is able to suppress the developmental defects caused by *mRNA1* overaccumulation. RNA immunoprecipitation assays demonstrate that *mRNA3* binds to the RS31 protein, suggesting that it functions as a long non-coding RNA that modulates the activity of its own protein. This interaction is enhanced in darkness, indicating that light conditions may influence this regulatory mechanism. Altogether, our results support a novel model in which non-coding isoforms generated by AS are not a passive by-product, but instead, act as regulatory RNAs capable of binding and titrating splicing regulators, including their own gene products, thereby modulating AS in response to environment. Our results uncover a new layer of gene expression regulation shedding light on how plants fine-tune their responses to the environment, giving AS a novel role and reaffirming the complexity of biology.

PL-2

LINKING MICRORNA BIOGENESIS AND MOBILITY TO SYSTEMIC DEFENSE IN PLANTS

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Plant immunity against pathogens is orchestrated through complex gene expression programs that ensure the fine-tuning of defense activation. MicroRNAs (miRNAs) are central regulators of local plant–microbe interactions; however, their contribution to systemic defense and the mechanisms underlying their mobility as signal molecules remain poorly understood. Here, we show that the induction of systemic defenses correlates with increased miRNA loading into the phloem, which is ultimately associated with the activation of systemic immunity. Consistently, mutation of *HASTY (HST)*—a factor involved in miRNA biogenesis and cell-to-cell movement—abolishes systemic defense activation. We further found that, in infected tissues, pri-miRNAs producing non-cell-autonomous miRNAs undergo enhanced co-transcriptional processing, thereby promoting their mobility. In addition, we demonstrate that two of these mobile miRNAs are essential for the activation of the systemic program. Remarkably, expression of HST exclusively in phloem companion cells of *hst* mutants was sufficient to restore systemic defense activation, underscoring the role of cell-to-cell miRNA movement. Moreover, in a *hws/hst* double mutant—where mutation of *HAWAIIAN SKIRT (HWS)* restores miRNA mobility but not abundance in *hst*—systemic resistance was also complemented. Altogether, these findings identify HST as a key regulator linking miRNA biogenesis and mobility to systemic plant immunity.

PL-3

COORDINATION OF SNRK1-MEDIATED ADAPTATIVE RESPONSES TO CHANGING GROWTH CONDITIONS

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A proper energy balance is crucial for organisms to survive and adapt to their environment. In plants, the Sucrose non-Fermenting 1 (SNF1)-Related Kinase 1 (SnRK1) complex is a central player in securing cellular energy homeostasis against changes in growth conditions. The catalytic α–subunit of this kinase complex, known as SnRK1.1, plays a key role in sensing cellular energy status and coordinating metabolic reprogramming to restore energy balance. The kinase activity of SnRK1.1 has been proposed to not be regulated by phosphorylation of the T-loop of SnRK1.1, the activation site of all the kinases of the SnRK1/AMPK/SNF1 family. In contrast with its opisthokont orthologues (AMPK in mammals and SNF1 in yeast), the stable phosphorylation status of the SnRK1.1 T-loop suggests a distinct regulatory mechanism in photosynthetic organisms. Our lab has demonstrated that SnRK1.1 exhibits a dual and dynamic distribution between the endoplasmic reticulum (ER) and the nucleus. We hypothesize that this intracellular distribution is related to the sensing of stimulus signals and the coordination of the response to face this energy imbalance. In this work, we evaluated the response to energy imbalances induced by different stress conditions by tracking SnRK1.1 intracellular distribution. Using SnRK1.1-eGFP expressing plants (SnRK1.1-OE), the SnRK1-mediated response was analyzed by triggering different energy imbalances including extended darkness, blockage of the photosynthetic performance (DCMU, DBMIB) and treatment with abscisic acid (ABA). We analyzed and quantified the intracellular distribution of SnRK1.1 using Z-stack sets of images of SnRK1.1-OE pavement cells using Laser Scanner Confocal Microscopy (LSCM) and analyzed by a recently developed pipeline generated in IMAGEJ/Fiji. Based on this analysis, we calculated the N/ER index, which is a proxy of the SnRK1 response and cell energy status. In addition, we examined SnRK1.1 expression and T-loop phosphorylation levels in treated SnRK1.1-OE lines by western blot analysis. Our results revealed profound changes in SnRK1.1 distribution, without significant changes in total SnRK1.1 protein levels or T-loop phosphorylation. These findings support our hypothesis that changes in SnRK1.1 intracellular distribution are key in the mechanisms that sustain plant cell homeostasis.

PL-4

MINION T, THE BiTVOX PLAYER

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Plant growth and development are processes intricately regulated by a complex signaling network of kinases and hormonal pathways, which, in conjunction with ROS, orchestrate responses to both developmental cues and adverse environmental conditions. The *Arabidopsis* protein MinionT was identified in our laboratory during a screen for novel regulators of these pathways. Previously, our laboratory demonstrated that *minionT* loss-of-function mutants exhibit a reduced growth phenotype and diminished activity of the Target of Rapamycin (TOR) pathway, a central integrator of hormonal and energetic signals. Supplementation of the growth medium with Brassinosteroids (BR) exerted a differential effect on hypocotyl and root growth in *minionT* mutants, while restoring TOR activity to wild-type (WT) levels, suggesting a functional linkage between MinionT, the BR pathway, and TOR. To further elucidate this connection, we conducted genetic crosses with key mutants of the BR signaling pathway. In the BR-deficient *det2-1* background, MinionT overexpression rescued the growth phenotype to near-WT levels, whereas the *minionT* loss-of-function led to a severe exacerbation of the phenotype. Conversely, in the *bin2-1* gain-of-function mutant, MinionT overexpression reverted the characteristic dwarf phenotype. Furthermore, bimolecular fluorescence complementation (BiFC) assays confirmed a direct physical interaction between MinionT and BIN2. We hypothesize that MinionT functions as a positive regulator of the BR pathway, mediated, in part, by its direct interaction with the pathway's negative regulator, BIN2. In parallel, independent studies have shown that mutants in a vacuolar proton pump V(H+)-ATPase subunit (*vha-2*) display altered BR signaling, and that mammalian homologs of MinionT interact with V(H+)-ATPase subunits. Accordingly, we investigated the participation of MinionT in this regulatory axis. Assays revealed a significant reduction in V-ATPase activity in *minionT* mutants and an increase in lines overexpressing MinionT (*oeMinionT*). Notably, *oeMinionT* expression in the *vha-2* mutant background partially rescued the phenotype, implicating MinionT as a stabilizer of V(H+)-ATPase activity. In conclusion, our results indicate that MinionT functions as a growth promoter by enhancing the BR pathway, modulating V-ATPase activity, and consequently impacting TOR activation. We propose a mechanistic model wherein MinionT, via its C-terminal domain, serves as a molecular link between the BR pathway, the vacuolar proton pump, and the TOR signaling cascade, thereby integrating the hormonal and metabolic cues that govern plant growth.

PL-5

UNCOVERING A NOVEL PATHWAY OF ANTHOCYANIN REPRESSION IN *ARABIDOPSIS THALIANA*

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Anthocyanins are protective secondary metabolites that enable plants to withstand adverse environmental conditions, such as abrupt increases in light intensity. Their accumulation is stringently regulated at the transcriptional level, predominantly through the activity of the MBW complex and its associated modulators, underscoring the complexity of the regulatory networks orchestrating pigment biosynthesis. Here, we report a novel repressive pathway in *Arabidopsis thaliana*, mediated by class I TCP (TEOSINTE BRANCHED 1, CYCLOIDEA, and PROLIFERATING CELL FACTOR) transcription factors in association with PHYTOCHROME INTERACTING FACTOR 4 (PIF4). Our data demonstrate that TCP15 and PIF4 negatively regulate anthocyanin biosynthesis by directly promoting the expression of GOLVEN1/CLE-LIKE6 (GLV1/CLEL6), a previously characterized repressor of anthocyanin accumulation. Both

transcription factors associate with a specific region of the *GLV1/CLEL6* promoter that contains adjacent TCP-box and G-box motifs. Moreover, PIF4 binding to the *GLV1/CLEL6* promoter is affected in the *tcp15 tcp14* double mutant, suggesting the existence of a functional interaction between these transcription factors. In addition, TCP15 and PIF4 contribute to gibberellin-mediated repression of anthocyanin biosynthesis, acting at distinct regulatory levels and independently of GLV1/CLEL6. Collectively, our findings provide novel mechanistic insights into the transcriptional control of anthocyanin accumulation in plants.

PL-6

ADVANCES IN THE FUNCTIONAL STUDY OF *LsDRIP* GENES IN LETTUCE: EXPLORING NEW STRATEGIES FOR ADAPTATION TO ABIOTIC STRESS

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The study of the mechanisms regulating plant responses to abiotic stress has become a priority in the context of global climate change and the increasing frequency of extreme events, such as recurrent droughts, abrupt temperature fluctuations, and rising soil salinity. DREB proteins (Dehydration Responsive Element-Binding Proteins) are transcription factors that activate genes associated with abiotic stress responses. The first *DREB* genes were characterized in *Arabidopsis thaliana*, including *AtDREB2A*, which is activated in response to drought, salinity, and high temperatures. Under normal conditions, the accumulation of AtDREB2A is controlled by AtDRIP1 and AtDRIP2, which are DRIP (DREB2A-Interacting Protein) ubiquitin E3 ligases, that act as negative regulators. These proteins recognize, ubiquitinate, and target AtDREB2A to the 26S proteasome, thereby preventing the premature activation of stress-responsive genes. When the plant is exposed to abiotic stress, AtDRIP activity decreases, allowing AtDREB2A to stabilize and accumulate, which in turn promotes the induction of protective genes. Previously, our group studied the effect of the loss of function of the lettuce ortholog *LsDREB2A* using CRISPR/Cas9-generated knockout plants, evaluated under both control and abiotic stress conditions. The results indicated that the knockout of *LsDREB2A* gene impaired lettuce responses to salt, drought, and heat stress. Based on these findings, the functional study of LsDRIP genes in lettuce is relevant to determine their contribution to the abiotic stress response and to evaluate their potential as candidate genes for future breeding strategies. Although *LsDRIP* genes had not been functionally characterized in lettuce until now, two predicted *LsDRIP* transcript sequences had been identified based on whole-genome sequencing of lettuce. To confirm their functionality, we verified their transcription by RT-PCR. Subsequently, to evaluate their role in the abiotic stress response, we generated loss-of-function plants for these genes through a multiplex gene-editing strategy mediated by CRISPR/Cas9. This work describes the RT-PCR experiments, the method used to obtain non-transgenic knockout lines, the procedure for detecting edits, and the types of mutations identified. It also presents preliminary results from abiotic stress assays performed on *in vitro*-grown seedlings.

PL-7

INTEGRATING OXYGEN ELECTRODE MEASUREMENTS AND IMAGE-BASED GREENNESS INDEX FOR COMPREHENSIVE PHOTOSYNTHESIS AND RESPIRATION ASSESSMENT IN *ARABIDOPSIS THALIANA*

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Photosynthesis and respiration are fundamental, tightly interconnected metabolic processes in plants, linked through shared substrates, energy flows, and redox balance. In *Arabidopsis thaliana*, the small size of intact seedlings presents challenges for directly quantifying these processes with conventional gas-exchange or fluorescence-based methods. Here, we present and validate a sensitive approach using Clark-type oxygen electrodes (Hansatech Oxytherm+P) to simultaneously monitor photosynthetic oxygen evolution and respiratory oxygen consumption in intact seedlings. By alternating dark and light phases, we distinguished mitochondrial respiration from photosynthetic activity and demonstrated the influence of tissue biomass, light intensity, developmental stage, and abiotic stress conditions on these parameters. Specific inhibitors, including potassium cyanide and paraquat or DCMU, confirmed that the observed oxygen dynamics reflected mitochondrial cytochrome oxidase activity and photosystem electron transport, respectively. Bicarbonate supplementation significantly increased oxygen evolution, highlighting the method's responsiveness to carbon fixation. To complement oxygen-based measurements with a visual, non-invasive proxy, we incorporated the Green Index (GI), an open-source image-based phenotyping tool that quantifies leaf greenness from standard RGB images without advanced computational requirements. GI accurately tracked progressive greening during de-etiolation, discriminated developmental stages and stress responses, and correlated strongly with chlorophyll content. Notably, GI values showed significant correlation with photosynthetic activity measured via Clark-type electrodes, linking visual pigment dynamics with physiological performance. This integrated platform offers a robust and scalable framework for assessing photosynthesis, respiration, and pigment-related traits in *Arabidopsis* knockout mutants, CRISPR-edited lines, overexpression lines, ecotypes, and other small plant tissues. By combining real-time oxygen exchange analysis with accessible image-based phenotyping, it enables a comprehensive characterization of plant performance under diverse developmental and environmental conditions and provides a versatile foundation for studies at the intersection of physiology, genetics, and stress biology.

PL-8

THERMOPRIMING BOOSTS CHLOROPLAST ANTIOXIDANT CAPACITY AND IMPROVES HEAT STRESS SURVIVAL IN *ARABIDOPSIS THALIANA*

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High temperatures and heat waves are major threats for agriculture worldwide. Plants have evolved rapid responses to elevated temperatures, including the activation of acquired thermotolerance or thermopriming, where a mild heat treatment enhances tolerance to

subsequent severe heat stress. Chloroplasts act as key heat sensors, where photosynthetic electron transport generates reactive oxygen species (ROS), including singlet oxygen ($^1O_2$) in photosystem II (PSII) and superoxide (O_2^-) in photosystem I (PSI), the latter converted to hydrogen peroxide (H_2O_2). Chloroplast-derived ROS can function as signaling molecules, triggering retrograde communication to the nucleus. Here, we investigated how thermopriming alters chloroplast redox states and ROS production, and whether these changes promote plant acclimation and heat tolerance. To this end, *Arabidopsis thaliana* seedlings were exposed to either 22°C or 37°C for 1 h (mild heat stress) and, two days later, challenged at 45°C (severe heat stress). Growth phenotype and survival were assessed in parallel with redox and ROS dynamics using genetically encoded biosensors (Grx1-roGFP2 and roGFP2-Orp1) and chemical probes for $^1O_2$, H_2O_2, and O_2^-. Photosynthesis inhibitors were applied to selectively induce ROS overproduction in chloroplasts. Mild heat treatment at 37°C enhanced chloroplast redox buffering capacity. In contrast, photosynthetic inhibitors triggered oxidation in chloroplasts. Remarkably, plants pretreated at 37°C or exposed to ROS-inducing compounds displayed lower oxidation when subsequently exposed to 45°C. However, thermotolerance required specific ROS: promoting $^1O_2$ production significantly increased plant survival under severe heat stress. These results demonstrate that thermopriming strengthens chloroplast antioxidant capacity, fine-tunes ROS signaling, and enhances heat tolerance in Arabidopsis. Future work will dissect the contribution of distinct ROS species to chloroplast-to-nucleus retrograde signaling and its integration with nuclear transcriptional programs, offering potential strategies to improve thermotolerance in crops facing climate change.

PL-9

SHEDDING LIGHT ON HIDDEN PLAYERS: ALARMONE IS A NOVEL PLANT IMMUNITY REGULATOR

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In order to adapt to the environment, plants must fine-tune their gene expression, ergo, their physiology, especially if any stress affects them. Throughout plant evolution, novel phylum-specific adaptive mechanisms have been developed to face challenging conditions. However, some ancestral mechanisms shared among bacteria and plants are still active. Guanosine tetraphosphate and guanosine pentaphosphate ((p)ppGpp, collectively known as alarmone) are hyperphosphorylated nucleotides produced by bacteria in response to stringent conditions. In plants, its metabolism is controlled by RELA/SPOT HOMOLOGUE (RSH) enzymes. *Arabidopsis thaliana* encodes four plastid-localized enzymes in the nuclear transcriptome: *RSH1*, *RSH2*, *RSH3* and *cRSH*. It has been reported this molecule is accumulated under different stimuli, i.e: flg22 stimulation and jasmonic acid (JA) treatment. However, its downstream responses remain poorly understood. The aim of this work is to uncover the role of alarmone as a novel plant immunity regulator using the model plant *Arabidopsis thaliana*. To mainly address this question, we performed an RNA sequencing experiment of alarmone-over accumulating plants (RSH3OX) and alarmone-null plants (*rsh1rsh2rsh3crsh*, namely *rshq*), in non-infection conditions. We found that alarmone regulates the expression and alternative splicing of many senescence and immunity-related genes such as *BSMT1*, *JOX4*,

NATA1 and *PSD3*. Since these transcripts encode for salicylic acid (SA) and JA signalling pathway-involved enzymes, we decided to perform infections with *Pseudomonas syringae* DC3000 (*Pst,* hemibiotrophic) and *Botrytis cinerea* (necrotrophic). We found RSH3OX plants are hypersensitive to *Pst* whereas *rshq* plants are resistant to the infection. In contrast, RSH3OX plants are as sensitive as wild type, while *rshq* plants are hypersensitive to *B. cinerea*. Furthermore, by infecting such plants with a T3SS-deficient *Pst* strain (ΔhrcC), to assess immune functionality suppresion, RSH3OX plants displayed impaired callose deposition; strikingly, *rshq* plants exhibit lower callose deposition as well. To deeply understand the role of alarmone *in planta,* specifically the possible molecular targets of it, we performed an *in silico* homology-based analysis using bacterial alarmone interactome. By molecular docking simulation, we found alarmone is predicted to bind to GSTU19, a glutathione transferase involved in JA biosynthesis, at its catalytic site possibly acting as a competitive inhibitor. In conclusion, our results suggest that alarmone might act as (1) a novel plastid-based signal able to regulate nuclear transcriptome and (2) a negative regulator of plant immunity and adaptation to biotic stress, through the antagonistic regulation of both JA and SA pathways.

PL-10

BEYOND THE PROTEIN: UNCOVERING RNAi CONTRIBUTIONS TO THE HB4® TECHNOLOGY

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The HB4® technology consists of a genetic construct carrying the sunflower *HaHB4* (*Helianthus annuus Homeobox 4*) gene, which encodes a transcription factor involved in developmental processes linked to plant adaptation to environmental conditions. The expression of *HaHB4*, which has been extensively studied in our group, enhances crop tolerance to drought and heat stress, as demonstrated in a wide network of field trials across diverse environments. Despite the accumulated knowledge about the action of *HaHB4*, the molecular mechanisms by which this transcription factor improves seed yield and stress tolerance remain unclear. While direct detection of the protein has not been achieved using standard approaches, small RNA sequencing data from WT and HB4® soybean plants revealed the presence of *HaHB4*-derived small interfering RNAs (siRNAs). Since plants protect themselves against transgenes mainly through this pathway, largely mediated by RDR6, we hypothesized that the effect of HB4® technology could be mediated, at least in part, by RNA interference (RNAi).
To unravel whether the beneficial traits observed in plants expressing *HaHB4* are due to the protein or to RNAi activity, we developed two experimental approaches in *Arabidopsis thaliana*. To test this, we performed genetic crosses with *rdr6* and *rdr2* mutants, which are impaired in siRNA biogenesis, aiming to study the effect of the protein in the absence of RNAi. Unexpectedly, these crosses showed reduced to nearly undetectable *HaHB4* expression. These plants were further evaluated to compare phenotypic traits linked to HB4 and to assess their differences.
To directly assess the contribution of *HaHB4*-derived siRNAs to specific beneficial outcomes, we generated RNAi constructs using *HaHB4* cDNA to test whether RNAi alone could have an effect. Remarkably, these RNAi lines produced 70–110% more seeds compared to controls, largely due to increased axillary and cauline branching, higher pod number, and wider stems. In addition, they developed longer primary roots, more lateral roots, and greater root density.

Importantly, these lines also maintained their enhanced seed yield under moderate water deficit conditions.
Altogether, our results suggest that small RNAs may contribute to the *HaHB4*-associated phenotype. Future work will be required to clarify whether they act independently or synergistically with the protein to enhance stress tolerance and productivity.

PL-11

CYTOCHROME C LEVELS LINK MITOCHONDRIAL FUNCTION TO CELL CYCLE PROGRESSION AND DIFFERENTIATION IN *ARABIDOPSIS THALIANA*

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Mitochondria integrate cellular energy status with developmental programs through interaction with different signalling pathways. In this work, we studied how changes in cytochrome c (CYTc), a soluble heme-protein that serves as an electron carrier in the mitochondrial electron transport chain, affect root growth characteristics at the cellular level. Plants with reduced CYTc levels displayed decreased root growth rate, reduced meristematic cell number, and shorter mature cells, linked to a premature entry into differentiation and reduced nuclear endoreplication. Cell cycle analysis using the PlaCCI reporter revealed an accumulation of meristematic cells in G1 and a concomitant reduction in S-phase cells, indicating a delay in the G1–S transition. This was accompanied by a decrease in the auxin response domain, changes in cytokinin sensitivity, and induction of the auxin repressor SHY2 and the cyclin-dependent kinase inhibitor SMR7. Genetic analysis showed that these alterations were associated with the action of the protein kinase YAK1, probably due to changes in the activity of the TOR and SnRK1 energy-sensing pathways. Collectively, our findings establish CYTc as a central integrator of mitochondrial metabolism, energy signaling, and hormonal pathways, ultimately determining the balance between proliferation and differentiation in the root meristem.

PL-12

MEET ASER53: A SHAPE-SHIFTING NLR LINKING PRIMING, ALTERNATIVE SPLICING, AND VIRAL IMMUNITY IN *ARABIDOPSIS*

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Nucleotide-binding Leucine-rich repeat (NLR) proteins are crucial receptors in the plant immune system. They detect pathogen-secreted effectors and trigger local and systemic

defenses, the latter often linked to a key type of immunological memory known as priming. Co-/post-transcriptional processes, such as alternative splicing (AS), may significantly expand plants NLR repertoire. Exitrons are a unique class of cryptic coding introns with dual characteristics between exons and introns, which might have great potential to increase NLR functional diversity. Here, we explored whether previously uncharacterized NLR isoforms in Arabidopsis thaliana could arise from exitron AS in response to different defense induction programs. We identified exitrons in 16 NLR-coding genes. Transcript analyses revealed that the abundance of these isoforms change in response to pathogen infection or the primed state inducers azelaic acid, pipecolic acid, and β-aminobutyric acid. In some NLRs, the new isoform may regulate receptor activation or disrupt immune complex interactions / assembly. That seems to be the case of a fascinating CNL-class NLR, whose exitron AS ratio, subcellular localization and gene expression respond to treatment with the priming inducers and Turnip Mosaic Virus (TuMV) infection. We called it ASER53. In other NLRs, exitron splicing affects domains involved in effector perception, signaling, or leads to truncated, unstable, or hyperactive receptors. Notably, in one NLR from a key subclass known as helper-NLRs, the exitron AS might potentially regulate the activity of other NLRs. These findings suggest that exitron AS contributes to modulating NLR function, particularly during immune memory establishment. Further functional studies on exitron AS dynamics may provide key insights into plant immunobiology.

BIOTECHNOLOGY

BT-1

OPTIMIZATION OF A SOLUBILIZATION AND REFOLDING METHOD FOR RECOMBINANT GLYCEROL KINASE

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The formation of inclusion bodies (IBs) represents a common challenge in the production of recombinant proteins. These insoluble aggregates concentrate proteins at a high degree of purity, although in misfolded or partially folded states. Thus, IBs represent a source for the purification of the active enzyme through solubilization and refolding processes. In this work, the recombinant glycerol kinase (GK), an enzyme widely used in diagnostics for the quantification of fatty acids, was produced in *Escherichia coli* as a model for the obtention of IBs and their subsequent recovery in the native form. Three expression conditions were evaluated: incubation at 20 °C overnight (ON), and at 37 °C for 1 h and 4 h. Comparable amounts of total protein were observed under the 20 °C ON and 37 °C 4 h conditions per unit of mass. However, the proportion of IBs compared with the soluble fraction was higher in the 20 °C ON condition. These two conditions were selected for further IBs solubilization. Based on the assumption that IBs may contain partially folded protein, a mild solubilization method (*n*-propanol 6M + urea 2M) was evaluated in contrast to a strong solubilization condition (urea 6M). The mild solubilization condition was not effective for GK solubilization, whereas strong urea 6M allowed the recovery of the protein in soluble form in both experimental groups (37 °C 4 h and 20 °C ON). In addition, IB obtained at 37 °C for 4 h showed a more efficient dissolution, suggesting that in this condition less compact aggregates are formed, which are

more susceptible to denaturing agents compared with IBs obtained at 20 °C ON. Subsequently, the enzyme denatured in 6M urea was purified by affinity chromatography (His-tag). A partial recovery of the purified enzyme was observed, since most of it remained in the unbound fraction (flow-through). Taken together, these results provide valuable information to guide strategies for the production and solubilization of the recombinant GK enzyme from inclusion bodies. Nevertheless, optimization of the purification of the denatured enzyme and further protein refolding is still required to maximize its recovery in an active form.

BT-2

ENGINEERED *LACTOCOCCUS LACTIS* AS A PLATFORM FOR ENZYME-ENRICHED SILAGE

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Sorghum silage is one of the most effective techniques for improving and preserving forages for feeding beef cattle. The capacity of ruminants to transform plant biomass into meat and milk is highly dependent on the digestibility of plant cell walls. However, the efficiency of this process is often constrained by the complex structure of plant cell walls, mainly composed of cellulose, hemicellulose, and lignin, which limit the accessibility of fermentable carbohydrates. Carbohydrate-active enzymes (CAZymes), such as cellulases and xylanases, can facilitate the breakdown of these polysaccharides, thereby enhancing digestibility and feed efficiency, making carbohydrates more accessible to ruminal microorganisms and potentially increasing animal productivity. The key factor in this process is the transformation of biomass through lactic acid fermentation and enzymatic activity; thus, lactic acid bacteria (LAB) play a crucial role in this process. Inoculation with LAB is a well-known technique recognized for enhancing the quality of cattle feed, with examples including improved digestibility, faster stabilization, prevention of pathogen growth, and, most importantly, increased milk and meat production. The goal of this work is to examine the potential of recombinant *Lactococcus lactis* strains engineered to express CAZymes in sorghum silage in a longitudinal experiment. We aimed to assess the survival of these strains and their enzyme production in mini-silages. Mini-silage systems were inoculated and monitored for strain persistence (by qPCR), pH dynamics, lactic acid production, and presence of recombinant enzymes. The mini-silages were prepared with: water (negative control), *L. lactis* with empty plasmid, *L. lactis* with endoglucanase-encoding plasmid, and a combination of endoglucanase- and xylanase-encoding plasmid *L. lactis* strains. Total bacterial population, lactobacillus strains, *Lactococcus* strain, and NZ9000-specific CAZyme recombinant strains were measured in separate qPCR reactions. Measurements were taken at several time points: 0, 7, 21, and 56 days. The qPCR experiments showed a stable total bacterial population, measured as CFU per gram of sorghum, across all treatments throughout the time course. In addition, the presence of *Lactococcus* strains was consistently higher compared with the water control at every time point. Regarding the *L. lactis* strains carrying CAZyme-encoding plasmids, a persistent presence was detected up to 56 days of incubation, reaching values of 2.1×10^{6} CFU per gram of sorghum. Enzyme production was further confirmed by Western blot analysis up to 21 days, demonstrating the ability of the recombinant strains not only to survive under silage conditions but also to secrete a measurable amount of CAZymes. Overall, this strategy may contribute to a cost-effective and

scalable solution for improving forage digestibility, with the potential to enhance animal productivity while reducing reliance on external enzyme supplements.

BT-3

PEG-COATED MAGNETIC NANOPARTICLES AS SAFE NANOTHERANOSTICS: BIODISTRIBUTION AND TARGETING IN A VIRAL ONCOGENESIS MOUSE MODEL

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Magnetic nanotheranostics, which combine both diagnostic and therapeutic capabilities, represent a promising alternative to conventional approaches for several pathologies. In our previous work, we evaluated polyethylene glycol-coated iron oxide nanoparticles (MAG@PEG) in endothelial cells stably expressing the Kaposi's sarcoma-associated herpesvirus G protein-coupled receptor (vGPCR), highlighting their potential as a novel drug delivery system with diagnostic applications. In this study, we investigated the toxicity and biodistribution of MAG@PEG in tumor-bearing mice, using a moderate-power magnet to direct MAG@PEG toward the tumor. To establish the viral oncogenesis animal model, 2 million vGPCR cells were injected subcutaneously into the right flank of female nude mice. Animals were randomly assigned to three groups: G1, vehicle (saline); G2, MAG@PEG (10 mg/kg); and G3, MAG@PEG (10 mg/kg) plus magnet exposure for 1 h. Treatments were administered intraperitoneally once per week for one month. Body weight and tumor growth were monitored throughout the study. Results showed that body weight remained unaffected by treatment, whereas tumor growth was impaired only in G2, the group treated with the nanoformulation without magnet exposure. At the end of the study, tumors and major organs (brain, heart, spleen, liver, stomach, intestine, kidneys, and lungs) were dissected, and blood samples were collected by cardiac puncture. Magnetization studies of the extracted organs were conducted to quantify the accumulation of MAG@PEG in each tumor/organ. MAG@PEG were found in higher amounts in stomach, spleen, lungs, and tumors, with greater accumulation in G2. Additionally, H&E and Prussian blue staining confirmed the presence of MAG@PEG in the organs of interest. Furthermore, serum biochemical analyses revealed no evidence of kidney or liver damage, as urea, creatinine, alanine aminotransferase, aspartate aminotransferase, and ALP levels remained unchanged. In conclusion, these findings suggest that MAG@PEG is a safe nanosystem at the tested dose and display suitable magnetization properties for biodistribution studies. Future research should focus on optimizing administration routes and magnetic targeting strategies to reduce off-target accumulation and enhance tumor specificity.

BT-4

SLPA-BASED VACCINE PLATFORM FOR CHAGAS DISEASE: DUAL-FUNCTION ANTIGEN DELIVERY AND IMMUNOSTIMULATION

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The development of affordable and effective vaccines remains a critical global health priority, particularly for diseases without prophylactic options. Chagas disease, caused by the intracellular parasite *Trypanosoma cruzi*, exemplifies this gap, as no vaccine has reached approval despite decades of effort. Conventional platforms often face limitations in terms of scalability, safety, or immunogenicity, especially when targeting complex parasitic infections. Although *Lactobacillus acidophilus* has been explored as a probiotic vector, its use in customizable vaccine platforms remains underdeveloped. In this study, we explored the potential of SlpA, the major surface-layer (S-layer) protein of *L. acidophilus*, as a structurally stable and immunostimulatory scaffold for antigen delivery. We engineered a biosafe, recombinant-free system based on the fusion of SlpA with Tc52, a well-characterized *T. cruzi* antigen associated with partial protection, and subsequently evaluated both purified N-Tc52/SlpA protein and SlpA-coated probiotic bacteria for protective efficacy. The N-Tc52/SlpA fusion consistently enhanced both the magnitude and quality of antibody responses, inducing balanced IgG2a/IgG1 ratios. Upon challenge, animals receiving N-Tc52/SlpA, either in purified form or displayed on *L. acidophilus*, exhibited reductions in parasite burden in heart and skeletal muscle tissues. Cellular analyses further revealed that both formats promoted activation of splenic $CD4^+$ and $CD8^+$ T cells and expansion of effector and central memory $CD4^+$ subsets, highlighting SlpA's ability to sustain T cell responses. These findings highlight SlpA's dual functionality as antigen carrier and immune enhancer, and establish a proof of concept for SlpA-based delivery systems adaptable to other intracellular pathogens, particularly where affordability, safety, and stability are crucial.

EN-1

ADVANCES IN THE STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF *AGMATINASE-LIKE PROTEIN* (ALP/LIMCH1)

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Agmatine is a biogenic amine with neurotransmitter functions and well-established anticonvulsant, antineurotoxic, and antidepressant properties. In mammals, it is metabolized into putrescine and urea by agmatinases or an agmatinase-like protein (ALP), which corresponds to the C-terminal region of the LIMCH1 protein. ALP/LIMCH1 requires Mn^{2+} ions for its activity; however, it lacks the conserved Mn^{2+}-binding residues typical of classical ureohydrolases, and its three-dimensional structure remains unresolved, even with advanced

prediction tools such as AlphaFold.
In this study, we characterized the secondary structure of a truncated ALP variant (ΔLIM-ALP, 457 amino acids) and the full-length LIMCH1 protein (1056 amino acids). Additionally, five single-point mutants were designed to investigate potential Mn^{2+}-binding residues in ΔLIM-ALP. Both proteins were successfully expressed, purified, and analyzed via circular dichroism (CD) spectroscopy. CD data revealed a predominance of disordered regions (~60%) and β-structures (~30%), with minimal α-helical content in both constructs. This structural profile contrasts sharply with the α/β/α sandwich fold typically observed in bacterial ureohydrolases, such as *E. coli* agmatinase.
To probe the functional role of candidate Mn^{2+}-binding residues, five single-point mutations (N213A, Q215A, D217A, E288A, K290A) were introduced. Although these mutations did not significantly alter Mn^{2+} binding or total metal content, four variants exhibited a reduced K_m for agmatine, accompanied by a decrease in V_{max} normalized to protein concentration. These findings suggest that, while the targeted residues may not directly coordinate Mn^{2+}, they likely play a role in substrate positioning or contribute to the structural integrity of the active site.
Collectively, our results enhance the current understanding of agmatine metabolism in mammals and reveal an unconventional enzymatic framework for LIMCH1, underscoring its functional divergence from classical ureohydrolase models.
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NS-1

DOPAMINE ASSESSMENT IN PARAQUAT-EXPOSED *Caenorhabditis elegans*: MITIGATION WITH N-ACETYLCYSTEINE

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Paraquat (PQ) is an herbicide that disrupts the glutathione redox cycle, induces mitochondrial dysfunction, and leads to cell death, particularly affecting the dopaminergic neurons, associated with Parkinson's disease. The therapeutic approach using the antioxidant N-acetylcysteine (NAC) emerges as a viable alternative to mitigate and/or prevent the toxic effects induced by PQ. The present study sought to evaluate a dopamine (DA)-dependent behavior in response to non-lethal PQ concentrations. Wild-type N2, TK22 (PQ-sensitive), and TK66 (PQ-resistant) worms were synchronized and co-exposed at the L4 stage to 5 mM PQ alone or in combination with 0.5 mM NAC in liquid medium for 24 h to assess lethality. Non-PQ-exposed worms (control group) from the N2 train showed no mortality, whereas the PQ-exposed group exhibited a 5% mortality. While the TK66 worms exhibited a 1% lethality, 21% of the animals from the TK22 strain died. Interestingly, co-exposure to PQ and NAC significantly reduced mortality in all strains. On the other side, from a functional perspective, the DA present in *C. elegans* regulates the locomotor activity in a food/no food situation, known as the "basal slowing response" (BSR). Since decreased locomotion in the presence of food is characteristic of well-fed wild-type worms and a healthy dopaminergic system, alterations in this behavior are indicative of DA dysfunction. To evaluate this behavior, wild-type N2, TK22, and TK66 strains were assessed, along with the MT15620 strain, which serves as a positive control due to its non-functional cat-2 gene (tyrosine hydroxylase, TH in humans), a protein essential for DA biosynthesis. Thus, synchronized L4 worms from the four strains were co-exposed to 5 mM PQ alone or in combination with 0.5 mM NAC in liquid medium for 24 h. After

exposure, they were allowed to recover for another 24 h on food agar plates to perform the BSR assay, in which the difference (delta) in the distance traveled (mm) and average speed (mm/s) in the food/non-food conditions was recorded. As expected, the MT15620 strain (positive control) presented small and, in some cases, negative deltas, whereas worms from the TK22 and N2 strains exposed to PQ exhibited significantly smaller deltas compared to the control worms. Interestingly, NAC was able to fully mitigate this behavior selectively in the TK-22 worms and partially in the N2 animals. On the contrary, the TK66 strain showed no differents deltas from the controls in all the groups evaluated. Collectively, these results demonstrate that according to PQ susceptibility, the herbicide induces adverse effects on DA functionality and NAC's ability to mitigate the observed damage. This provides evidence for the environmental bases of neurodegenerative diseases theory. Furthermore, future studies aim to evaluate the morphology of dopaminergic neurons by using transgenic *C. elegans* strains after PQ exposure to induce a Parkinsonian phenotype in a pro-oxidant environment.

NS-2

NITRO-OLEIC ACIDS AS A POTENTIAL THERAPEUTIC AGENT IN EXPERIMENTAL CHOROIDAL NEOVASCULARIZATION

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Age-related Macular Degeneration (AMD) is a leading cause of blindness in older adults worldwide. Inflammation, ROS, genetics and environmental factors were involved. Nitro-fatty acids are important electrophilic signaling mediators with anti-inflammatory, antioxidant and cytoprotective properties. Our aim was to evaluate the effect of nitro-oleic acid (NO_2-OA) in an animal Model of Choroidal Neovascularization (CNV) and microglial cells. CNV mice were intraperitoneal injected with 15mg/Kg of NO_2-OA or vehicle every other day since the laser day (n=6). Four- and seven-days post laser mice were sacrificed. Samples included RPE-Choroid flat-mounts and extracts were processed for Flow cytometry and Western blot (WB). Antioxidant expression was measured in BV2 cells treated 8h with NO_2-OA (n=4). In addition, TNF-α expression was measured in BV2 cells treated with LPS (10 ng/ml) plus NO_2-OA (5 μM) (n=4). Migration ability of BV2 cells were measured by wound healing assay (n=3) after treatment with LPS (100 ng/ml) plus NO_2-OA (5 μM). Finally, Thp1 cells were treated with NO_2-OA previous challenged with LPS plus INF-ϒ or IL-4 and the polarization toward an M1 or M2 profile was measured by flow cytometry. The area and the perimeter of choroidal neovessels were significantly reduced in RPE-Choroid flat-mounts of mice treated with NO_2-OA ($p= 0.0064$ and $p= 0.0062$). At 4 days post laser, an increase in mononuclear phagocytes cells number was found, both in the retina ($p= 0,0115$) and in the RPE-choroid ($p= 0,0024$), which not was prevented with NO_2-OA. Under 5μM of NO2-OA (8h) treatment, BV2 cells strongly increased Nrf2 downstream gene expression such as HO-1 ($p<0.001$). LPS (10 ng/ml) significantly increased TNF-α expression (8h) ($p<0.001$) compared with control and 5μM of NO_2-OA prevented the increase in TNF-α ($p>0.05$). LPS (100 ng/ml) significantly increased migration (24h) ($p<0.05$) of BV2 cells compared with control and 5μM of NO_2-OA prevented the increase induced by LPS ($p>0.05$). Finally, preliminary results showed LPS and IFN-gamma polarized Thp 1 cells toward an M1 and nitro polarized it toward an M2 profile. Collectively, these results highlight the role of NO_2-OA as potential treatment in CNV in order to attenuate vascular and non-vascular alterations.

NS-3

CALCINEURIN Aβ-MEDIATED MODULATION OF PERK SIGNALING IN REACTIVE ASTROCYTES

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Astrocytes, which are essential for CNS homeostasis, undergo astrogliosis, a set of diverse reactive states, in response to pathological cues. This process represents a spectrum of context-dependent adaptations, encompassing a diverse continuum of graded responses that can give rise to either adaptive or maladaptive states, which are closely associated with the progression of neuroinflammation. Despite varied clinical presentations, neurodegenerative disorders like Alzheimer's, Huntington's (HD), and multiple sclerosis (MS) share increased neuroinflammation and misfolded protein accumulation. Elucidating the signaling mechanisms that mediate the astrogliosis heterogeneity is therefore paramount. Endoplasmic reticulum (ER) stress activates the unfolded protein response (UPR). Its kinase PERK branch phosphorylates eIF2α (P-eIF2α), leading to reduced protein synthesis and enhanced translation of the transcription factor ATF4. Although initially cytoprotective, prolonged ER stress can trigger cell death. However, little is known about UPR regulation during the early stage and the transition to chronic activation. Our previous research showed the beta isoform of the calcineurin subunit A (CNAβ) plays a crucial non-canonical cytoprotective role, promoting cell survival during the acute UPR phase in primary mouse and human astrocyte cultures, and in two brain injury models: photothrombotic stroke and traumatic brain injury (TBI). We demonstrated that CNAβ rapidly increases in astrocytes after injury and directly interacts with PERK's cytosolic domain, promoting autophosphorylation and oligomerization, which further reduces protein synthesis. We propose the CNAβ-PERK pathway is critical for activating astrogliosis. We first tested this using human astrocytes treated with interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), two cytokines linked to MS, establishing a temporal profile of pathway components and reactivity markers. We also validated this using mouse CNAβ knockout (KO) astrocytes followed by a rescue experiment. KO astrocytes exhibited a blunted increase in P-eIF2α and early reactivity marker S100a10 (S100 calcium-binding protein a10) levels compared to wild type (WT). Notably, P-eIF2α and S100a10 expression was restored after CNAβ transfection. Cytokine treatment also induced ATF4 nuclear translocation in the early hours, even with calcineurin phosphatase inhibitors present, supporting CNAβ's non-canonical role. Moreover, in vivo data revealed co-expression of CNAβ and S100a10, at the early stage in an MS mouse model. Overall, our findings suggest that the CNAβ-PERK signaling pathway is a key regulator of astrogliosis during the early UPR.

ST-01

ALLOSTERY CHARACTERIZATION ON ANGIOTENSIN CONVERTING ENZYME II

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Allostery is a phenomenon in which a conformational change at one site of a protein is triggered by a structural modification at a distant site. Over the past 20 years, our group has focused on characterizing allosteric regulation in protein kinases, identifying several small molecules that modulate enzymatic activity as well as interactions with kinase substrates by the reverse allosteric mechanism. Recently, we aimed to determine whether the “reverse allosteric” mechanism of conformational modulation could be extended to other protein systems. Angiotensin-converting enzyme 2 (ACE2) plays a key role as a counter-regulator of the renin-angiotensin-aldosterone system and serves as the primary receptor for SARS-CoV-2, positioning it as a potential therapeutic target for both cardiovascular diseases and viral infections.

The objective of this study is to characterize the allosteric regulation of ACE2 as well as the possible regulation of its interaction with the spike protein from coronaviruses. We applied a chemical biology approach to evaluate the effects of small molecules using enzymatic activity assays with fluorogenic substrates and AngII, as well as ACE2-Spike interaction assays (AlphaScreen). Preliminary studies identified allosteric regulation of ACE2. Using thermal shift assays and *in silico* docking, we explored potential binding mechanisms of these compounds. The quality and homogeneity of the purified protein were confirmed by negative-stain electron microscopy, and X-ray crystallography experiments are currently underway to elucidate the specific binding site and structural basis of modulation.

In conclusion, these very initial results support the model of ACE2 as an allosteric protein. The validation of our preliminary findings represents a step toward the development of novel therapeutic strategies for disorders where ACE2 is involved.

CELL BIOLOGY

CB-1

ACUTE *TRYPANOZOMA CRUZI* INFECTION REDUCES STARD7 EXPRESSION ASSOCIATED WITH MITOCHONDRIAL DYSFUNCTION, ALTERED LIPID METABOLISM, AND CHANGES IN OXIDATIVE STRESS ENZYME LEVELS IN THE MURINE LIVERS

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The infection with *Trypanosoma (T.) cruzi* causes Chagas disease and induces alterations in the lipid levels, subverting the host lipid metabolism and several cellular processes. StarD7 is a ubiquitous phosphatidylcholine (PC) transfer protein that belongs to the START superfamily, which is involved in metabolism, transport, and intracellular signaling of lipids. StarD7 is required to maintain mitochondrial morphology, functionality, and dynamics. Here, we aimed to monitor the StarD7 expression in the livers of *T. cruzi*-infected mice fed with a control (CT) or a diabetogenic (DBT) diet. Furthermore, this study explored the alterations of liver proteins implicated in mitochondrial function, lipid metabolism, autophagy markers, and oxidative and endoplasmic reticulum stress response. qRT-PCR and Western blot experiments demonstrated that *T. cruzi* acute infection leads to decreased StarD7 mRNA and protein levels in the livers of infected animals, independent of the diet. In addition, changes in glycolysis along with alterations in proteins involved in mitochondrial dynamics and biogenesis occur in the livers of infected mice as well as in the uninfected CT fed with the diabetogenic diet. We found that TFAM mRNA expression, an essential mitochondrial DNA binding protein, and mRNA and protein levels of PGC1α, a main regulator of mitochondrial biogenesis, were reduced in the livers of infected mice compared with CT. In addition, a significant decrease of Mfn2 and Drp1proteins, which are involved in mitochondrial outer membrane fusion and mitochondrial fission, respectively, was detected in the livers of infected and uninfected DBT mice compared with those with CT fed. The enzyme levels involved in *de novo* lipogenesis (FASN, ACLY, SCD) and the transcription factor Srep1c, the antioxidant catalase, and IRE1α proteins were significantly reduced in the livers of infected mice compared with uninfected animals. In contrast, the antioxidant HMOX1 and BIP protein levels were upregulated. Finally, induction of the autophagy pathway was detected in the livers of infected mice. Taken together, these findings indicate that *T. cruzi* infection leads to a significant reduction of StarD7 expression and thereby modulating mitochondrial function, lipid metabolism, autophagy, and oxidative and stress response in the mouse liver, independently of the diet.

CB-2

IMPACT OF SILENCING A MEIOTIC lncRNA ON SPERMATOGENESIS: DEVELOPMENT OF AN ANTISENSE OLIGONUCLEOTIDE MICROINJECTION-BASED APPROACH

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Long non-coding RNAs (lncRNAs) are transcripts longer than 200 nucleotides with little or no protein-coding potential. Approximately 40% of lncRNAs are intergenic, while the remaining 60% overlap with, or are located adjacent to protein-coding genes. The biological functions of most lncRNAs, particularly of the antisense ones, remain poorly understood due to technical limitations. Current CRISPR-based approaches for lncRNA depletion are effective for those located far from coding genes, but not suitable for most of them, as they may interfere with their adjacent or overlapping genes. Conversely, RNA interference (RNAi)-mediated knocking-down acts through the microRNA pathway and is therefore more efficient at silencing cytoplasmic transcripts, with limited effectiveness in nuclear contexts. Given these limitations, our aim was to establish an *in vivo* knock-down strategy for lncRNAs through testicular microinjection of chemically modified antisense oligonucleotides (ASOs). This required the

optimization of a delivery system via *rete testis* microinjection. In this study, we focus on the functional characterization of an antisense lncRNA showing meiotic differential expression. RNA-FISH revealed its nuclear localization in prophase I meiotic cells, closely associated with paired homologous chromosomes, and suggesting a potential role in homology search during meiosis. Using this approach, we achieved ~50% reduction of the target lncRNA. Although this silencing efficiency is lower than that typically expected for RNAi (>70%), it was enough to significantly reduce testis size and seminiferous tubules diameter. TUNEL assays showed increased testicular cell death upon decreased levels of the lncRNA. While the precise function of this lncRNA remains to be determined, our findings highlight its importance in meiosis. Furthermore, the development of this methodology represents an important advance, as it enables the *in vivo* functional characterization of lncRNAs in a complex system such as the testis, and may be extended to the study of antisense lncRNAs in other organs and tissues.

CB-3

RECIPROCAL REGULATION BETWEEN ACYL-COA SYNTHETASE 4 AND ANDROGEN RECEPTOR: IMPLICATIONS AS THERAPEUTIC TARGETS IN BREAST CANCER CELLS

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Acyl-CoA synthetase 4 (ACSL4) is an enzyme involved in arachidonic acid metabolism and has been implicated in the progression of several aggressive cancers, including breast cancer. Notably, its overexpression is associated with poor prognosis and increased tumor aggressiveness. In triple-negative breast cancer (TNBC), a subtype lacking estrogen and progesterone receptors and having normal expression of HER-2, the androgen receptor (AR) has emerged as a potential biomarker and therapeutic target, particularly in the subset of AR-positive TNBC. However, for TNBCs that do not express AR, effective treatment options remain limited. The aim of this study was to explore the reciprocal regulation between ACSL4 and AR, and to evaluate whether dual targeting of these proteins could serve as a novel therapeutic strategy in breast cancer. We previously showed that AR represses ACSL4 transcription in breast cancer cell lines. Here, we investigated whether ACSL4 modulates AR expression. Using the MCF-7 TetOff/ACSL4 model, we found that ACSL4 overexpression leads to a significant reduction in AR protein levels compared to control cells. Conversely, stable silencing of ACSL4 in MDA-MB-231 cells restored both AR mRNA and protein expression. Inhibition of ACSL4 with PRGL493, a compound developed in our laboratory, also increased AR levels in MDA-MB-231, with effects evident at concentrations ≥10 μM. Similar results were obtained in BT-20 cells, another TNBC cell model that do not express AR, further supporting the hypothesis that ACSL4 negatively modulates AR expression. Given that ACSL4 inhibition appears to restore AR expression in breast cancer cells, we hypothesized that this effect could sensitize AR-negative TNBC cells to antiandrogen therapy. In this way, we evaluated the combined effect of PRGL493 and bicalutamide (BICA), an AR antagonist, on tumor aggressiveness parameters. In MDA-MB-231 cells, the combination of submaximal doses of PRGL493 and BICA produced a synergistic reduction in cell proliferation, migration, and invasion. These findings were validated *in vivo* using a zebrafish xenograft model, where the

dual treatment more effectively inhibited tumor cell extravasation — a critical step in metastasis — than either agent alone. Altogether, our data show that the regulatory relationship between ACSL4 and AR in breast cancer models is reciprocal. Furthermore, they highlight the therapeutic potential of combining ACSL4 and AR inhibitors, particularly in TNBC subtypes lacking effective treatment options. This approach might offer basis for exploring novel targeted therapies in aggressive breast cancer subtypes with currently limited treatment options.

CB-4

THE ROLE AND IMMUNOLOCALIZATION OF ITGB1 AND MERLIN PROTEINS DURING THE PROCESS OF VASCULOGENIC MIMICRY IN AN OVARIAN CANCER CELL LINE.

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Vasculogenic mimicry (VM) is a dynamic phenomenon by which cancer cells can form vessel-like structures in an endothelial-free fashion to receive nutrients, oxygen and to dispose waste products. In our laboratory we have established an *in vitro* model where cancer cells form VM when growing on Matrigel. This process requires the recruitment of cancer cells and coordinated protein expression and localization. We hypothesize that proteins related to matrix interaction and the mechanical sensing like ITGB1 and Merlin proteins allow the capacitation of cancer cells and the subsequent formation of VM. Using our *in vitro* models of cancer cell lines, we analyze by immunostaining and live cell imaging with Spining disk and Airyscan microscopy the spatio-temporal organization of ITGB1 and Merlin in cells during the process of tubular formation. Results show that the silencing of ITGB1 blocks completely the VM formation and the Merlin knockdown stops the formation after the alignment. Results from a phophoarray assay shows an increase in the phosphorylation site serine 10 of merlin during VM. Interestingly Merlin phosphorylation at serine 10 reveals cellular selective nuclear exclusion during the early stages of Vasculogenic mimicry suggesting cellular specialization for VM formation.

CB-5

GLYCOSYLTRANSFERASES DIFFERENTIALLY MODULATE THE GOLGI ONCOPROTEIN GOLPH3

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Glycolipid glycosyltransferases (GGTs) are Golgi-resident type II membrane enzymes that catalyze the stepwise synthesis of gangliosides, key modulators of cell signaling. Their N-terminal domains (NTD) govern Golgi retention and mediate multienzyme complex formation,

enabling reciprocal regulation among GGTs. GOLPH3, the first identified Golgi-resident oncoprotein, is overexpressed in many cancers and correlates with poor prognosis. Previous studies in our lab showed that GOLPH3 mediates the physical association between the GGTs ST3Gal-2 and β3GalT-4 through their NTD. As GGTs can influence the localization and activity of their partners, we hypothesized that they are also able to modulate GOLPH3 levels. To test this, we first assessed GOLPH3 expression by combining biochemical and imaging methods in CHOK1 cells stable expressing ST8Sia-1 or β4GalNAcT-1 together with β3GalT-4. Stable expression of these GGTs led to a significant increase in GOLPH3 levels compared to wild-type cells. The effect was independent of ganglioside biosynthesis, as treatment with P4, a specific inhibitor of GlcCer synthase, did not alter GOLPH3 expression. Also, the transient expression of β3GalT-4 and ST3Gal-2 NTDs (lacking the catalytic domain) were sufficient to induce GOLPH3 upregulation. As GOLPH3 is frequently overexpressed in breast cancer, we extended our analysis to breast epithelial (MCF10) and tumorigenic (MCF7 and MDA-MB-231) cell lines. ST8Sia-1 NTD upregulated GOLPH3 in MCF7 and MCF10 cells, whereas β3GalT-4 NTD and ST3Gal-2 NTD had no effect in any of these cell lines. Interestingly, β4GalT-5 NTD did no alter GOLPH3 levels across the three cell lines, whereas its homolog β4GalT-6 NTD selectively downregulated GOLPH3 in the tumor-derived MCF7 and MDA-MB-231 cells, with no detectable change observed in MCF10 cells. These results highlight the selectivity of this regulatory mechanism, reflecting a differential capacity of individual GGTs to modulate GOLPH3 expression across cell types. Considering GOLPH3 involvement in tumor progression, we investigated whether its modulation by β4GalT-6 NTD could influence epithelial to mesenchymal transition (EMT). We transduced MCF10 and MDA-MB-231 cells with a β4GalT-6 lentiviral vector and treated them with TGF-β for 4 days. Compared with non-transduced, the transduced cells showed a marked reduction in vimentin expression, a mesenchymal marker highly expressed during EMT, revealing a previously unrecognized mechanism through which GGTs influence tumor cell plasticity. Together, these results demonstrate that GGT expression can differentially modulate GOLPH3 levels independently of their catalytic activity and that β4GalT-6 NTD can counteract TGF-β–induced EMT, revealing a selective crosstalk between GOLPH3 and specific GGTs with potential relevance for cancer biology.

CB-6

MICRORNA-597 SUPPRESSES TUMOR PROGRESSION IN GASTRIC CANCER BY DIRECTLY TARGETING RUNX1 AND IS MODULATED BY THE LNCRNA KCNQ1OT1

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Gastric cancer (GC) remains a major cause of cancer mortality in high-incidence regions. We investigated microRNA-597 (miR-597) to define its clinical relevance, functional impact, and regulatory circuitry in GC. In 75 GC tumors, miR-597 quantified by qRT-PCR was lower in the intestinal subtype ($p = 0.002$) and in stage III–IV disease ($p = 0.048$). In AGS cells, miR-597 gain-of-function curtailed migration (Transwell), wound closure (10–20 h), Matrigel invasion

(~20 h), clonogenic growth (14 days), and short-term viability (MTS and Trypan blue at 12–48 h), whereas inhibition produced opposite effects; in MKN74, miR-597 mimics likewise reduced migration and invasion. In patient-derived organoids (PDOs), miR-597 transfection increased miR-597 levels and reduced colony formation and viability. A focused array of 62 invasion/progression genes identified 19 genes downregulated upon miR-597 overexpression and restored by inhibition. Integrative network analyses placed RUNX1 as the central hub connecting this program; notably, only RUNX1 harbors a conserved miR-597 site (TargetScan/IntaRNA), and dual-luciferase in AGS and KATOIII confirmed direct binding ($p < 0.001$). Downstream, RUNX1-dependent genes encompassed SPP1, CCNE2, FGF8, MYC, TIMP2, CTGF, MMP10, HMGB1, CD44, CTSB, and MAP2K, indicating that miR-597 indirectly restrains a broader invasion axis via RUNX1. The long non-coding RNA KCNQ1OT1 bound miR-597 (reporter assay); its silencing reduced endogenous RUNX1 in NCI-N87 cells, and KCNQ1OT1 positively correlated with RUNX1 in 69 tumors ($r^2 = 0.631$; $p < 0.0001$), consistent with miR-597 sponging that blunts RUNX1 endonucleolytic degradation. Altogether, miR-597 suppresses invasion and tumor progression in GC by directly targeting RUNX1 and constraining a RUNX1-centered network, whereas KCNQ1OT1 counteracts this control. Restoring miR-597 or disrupting the KCNQ1OT1–miR-597–RUNX1 interaction warrants exploration for prognostication and therapy in GC.

CB-7

CALCIUM DEPENDENT DYNAMIC ORGANIZATION OF PERK–CALCINEURIN B CO-CLUSTERS REVEALED BY QUANTITATIVE MICROSCOPY

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The accumulation of misfolded proteins into the lumen induces Endoplasmic Reticulum (ER) stress, which activates a signal transduction cascade known as the Unfolded Protein Response (UPR). In its acute phase, the UPR attempts to restore homeostasis; but during severe or prolonged stress it shifts to a chronic response that can trigger apoptosis. PERK (PKR-like ER-associated kinase), one of the three UPR sensors, is an ER transmembrane protein normally kept inactive by its interaction with the chaperone BiP. Under stress, BiP dissociates, enabling PERK oligomerization and autophosphorylation, which activate its kinase activity and promote phosphorylation of the α-subunit of the eukaryotic initiation factor 2 (eIF2α-P), thereby inhibiting protein synthesis. The PERK signaling pathway is the only UPR branch mediates both cytoprotective and pro-apoptotic responses. In this context, our group described a non-canonical function of calcineurin (CN), a heterodimer composed of a catalytic subunit (CN-A) with two isoforms (α and β), and a Ca^{2+}-binding regulatory subunit (CN-B), which promotes cell survival during the acute phase of the UPR. We observed that CN-Aβ/B directly interacts with the cytosolic domain of PERK, promoting its autophosphorylation and oligomerization, thereby enhancing inhibition of protein synthesis. This interaction is significantly favored by cytosolic Ca^{2+} increases that mimic stress conditions. Here, using super-resolution microscopy and human astrocytes along with two different cell lines as cellular models, we show that under tunicamycin (Tm)-induced stress, an inhibitor of N-glycosylation, PERK and the CN-B subunit form co-clusters with a spherical or fusion-prone morphology, consistent with phase-separated liquid condensates. Interestingly, BAPTA, a Ca^{2+} chelator, significantly disrupted these co-clusters. Consistently, they were absent in cells expressing a truncated CN-B variant

lacking Ca^{2+}-binding sites, which instead formed amorphous aggregates. Super-resolution imaging, which provides a level of detail well beyond the size of the condensates, combined with modulation of critical parameters, quantitative data analysis, and compelling evidence of functional consequences, allows us to validate these liquid-liquid phase separations at the cellular level. Overall, these data suggest that the EF-hand domains of CN-B may act as a switch-on mechanism for PERK activation, as their loss appears to leave the cell with a constitutively active UPR.

CB-8

BEYOND GOLGI: NUCLEAR FUCOSYLATION AS AN EMERGING GLYCAN MODIFICATION

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Aberrant glycosylation is a universal hallmark of tumor biology. These post-translational modifications occur mainly in the Golgi complex and endoplasmic reticulum, but O-GlcNAc and O-GalNAc glycosyltransferase activity has also been described in the cell nucleus. Fucosylation, catalyzed by thirteen human fucosyltransferase enzymes (FUTs), is of particular interest since overexpression of fucosylated epitopes and their respective FUTs has been reported in several types of cancer, linked to cancer progression and prognosis. Given that many nuclear proteins are targeted by these modifications and may have distinct roles in contexts of aberrant glycosylation, we propose to study the significance of nuclear fucosylation level in nuclear physiology and tumor biology.
Here, we have evidence of the presence and enzymatic activity of FUTs in the nuclear compartment. Using confocal microscopy and subcellular fractionation, we demonstrate nuclear localization of FUT9, a FUT associated with tumor-initiating cells. Enzymatic assays on purified nuclei demonstrated that GDP-fucose can be used by this organelle to modify proteins independently of the cytoplasm. Using the HCT116 colorectal cancer cell line, which lacks *de novo* GDP-fucose biosynthesis pathway, we dynamically modulated fucosylation levels by extracellular fucose supplementation. This revealed a tightly regulated process, impacting on key nuclear proteins involved in transcription and chromatin organization. Modulating fucosylation levels significantly impacted the cellular proteome, particularly altering proteins related to energetic metabolism, RNA processing and proliferation. Bioinformatic analysis of the modulated proteins by fucosylation highlighted the transcription factor ATF2 (activating transcription factor 2) as a putative master regulator within this network. Interestingly, ATF2 target genes derived from our proteomic dataset displayed prognostic value for overall and disease-free survival in colorectal cancer patients from the TCGA-COAD cohort. Functionally, fucose supplementation led to reduced proliferation without affecting cell viability.
Our findings suggest that nuclear fucosylation represents a novel aspect of post-translational regulation, with implications for cell physiology and diseases like cancer, where glycosylation processes are deeply altered. This study raises new questions about how nuclear glycosylation influences gene expression and cellular function, highlighting its relevance as a novel regulatory mechanism within the nucleus and a potential target for future therapeutic strategies.

POSTERS

MICROBIOLOGY

MI-12

THE KTR POTASSIUM TRANSPORT SYSTEM IN *ENTEROCOCCUS FAECALIS*

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Enterococcus faecalis is a Gram-positive bacterium belonging to the lactic acid bacteria. Some strains of this microorganism are employed as probiotics, others are part of the natural human gut microbiota, and some are found in artisanal cheeses. However, this species has emerged as an opportunistic multidrug-resistant pathogen, raising concerns about its impact on human health in hospital settings. Its ability to tolerate multiple and diverse environmental stress conditions is partly due to its sophisticated ion transport systems. In this context, the coordination of potassium (K^+), proton, and sodium transport plays a pivotal role in maintaining cellular function and survival under stress conditions. In our previous work, we identified and characterized multiple potassium transporter systems in *E. faecalis*, which enabled this bacterium to adjust its internal K^+ pool with remarkable precision. These transporters include KtrAB, KtrAD, Kup, KimA and Kdp complex (KdpFABC). In this study, we focused particularly on the Ktr systems. For this purpose, we evaluated the potassium uptake kinetics of a wild type strain (JH2-2) and different mutants, in the context of KCl as the limiting substrate. The $KtrAB^+$ mutant (lacking *kup*, *kimA*, and *ktrD*) achieved higher growth yields than the wild-type strain under alkaline pH conditions. This was compared to mutants deficient in *ktrB* (with all other systems present), *ktrD* (with all other systems present), *ktrA* (with all other systems present), or *ktrA* and *ktrD* (with *kimA* and *kup* present). Analysis of the expression of the *ktrA*, *ktrB*, and *ktrD* genes involved in the system by qPCR showed that *ktrA* is expressed at higher levels than *ktrB* and *ktrD*. Furthermore, the expression of none of these genes was modulated by external pH (5.0 vs. 9.0), a characteristic that differentiates this system from KimA and Kup. Finally, a *Galleria mellonella* infection model was used to assess virulence. None of the mutations resulted in significant differences in survival curves from the wild-type strain. In conclusion, our results demonstrate that the Ktr system in *E. faecalis* JH2-2 operates under a distinct regulatory paradigm compared to the Kup and KimA transporters, as evidenced by its constitutive, pH-independent expression. The inability to generate certain multiple mutants ($KtrAD^+$, lacking *kup, kimA*, and *ktrB*) implies an essential, non-redundant role for specific Ktr components in viability. Functional growth assays reveal a complex network of compensation and interaction among potassium uptake systems, where the inactivation of some can surprisingly lead to enhanced fitness under specific conditions. Finally, while central to ion homeostasis and adaptation to stress, the Ktr system does not appear to be a primary determinant of virulence in the *G. mellonella* infection model. This underscores its specialized role in fundamental physiology rather than in pathogenicity.

MI-13

COINFECTION WITH PNEUMOCOCCUS AND SARS-COV-2 IN HUMAN EPITHELIAL CELLS INCREASES THE BACTERIAL INTRACELLULAR SURVIVAL

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The pneumococcus is a major human pathogen responsible for pneumonia, meningitis, and sepsis, causing significant morbidity and mortality, particularly in children and the elderly. It is also well known that the pneumococcus contributes to severe secondary infections following viral infections, as observed during influenza pandemics. Previously, we demonstrated that the pneumococcal two-component system SirRH mediates the synergistic interaction between the pneumococcus and influenza A virus, enhancing bacterial survival in co-infected pulmonary A549 cells. Furthermore, we showed that this increased intracellular survival results from the inhibition of the autophagic pathway in A549 pneumocytes by the viral M2 protein.
Secondary pneumococcal infections have also been reported in COVID-19 patients; however, the interaction between the pneumococcus and SARS-CoV-2 at the cellular level remains poorly understood. In this study, we established the first *in vitro* coinfection model using host cells, including Calu-3, Caco-2, and Vero E6 cells, to evaluate whether SARS-CoV-2 infection alters the intracellular fate of the pneumococcus. Our results showed that infection with the Wuhan strain (Wuhan-Hu-1) of SARS-CoV-2 significantly increased the intracellular survival of the pneumococcus across all tested cell lines. We also observed that this effect was independent of the pneumococcal strain used (D39, R6, and R801). To elucidate the mechanisms underlying the synergistic interaction between the pneumococcus and SARS-CoV-2, we transfected human pulmonary Calu-3 cells with individual SARS-CoV-2 proteins—specifically ORF3a, ORF7a, nsp15, E, and M—prior to subsequent infection with pneumococcal strains. Our findings indicate that select viral proteins, which have been previously implicated in impairing autophagic flux and lysosomal function, were sufficient to reproduce the enhanced intracellular persistence of pneumococci observed in cells infected with SARS-CoV-2.
These findings suggest that SARS-CoV-2 modulates host cell pathways to generate a more permissive intracellular niche that benefits bacterial survival. Further assays are required to confirm these observations, particularly microscopy studies evaluating colocalization of the pneumococcus with phagosomal and lysosomal markers, to fully validate the proposed mechanism. In conclusion, our results reveal a potential mechanism of viral–bacterial synergy in COVID-19 and emphasize the need to consider pneumococcal coinfection in both clinical management and future therapeutic strategies.

MI-14

POLYMORPHIC MEMBRANE PROTEIN C FACILITATES RECOVERY FROM PERSISTENCE AND MAINTAINS *CHLAMYDIA TRACHOMATIS* INTRACELLULAR DISPERSION IN AN *EX VIVO* MURINE UTERINE MODEL

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Chlamydia trachomatis (CT), the leading bacterial cause of sexually transmitted infections globally, undergoes an obligate intracellular cycle alternating between infectious elementary bodies (EBs) and replicative reticulate bodies (RBs) within a host-derived inclusion. Under stress conditions such as interferon- exposure or penicillin treatment, CT halts replication and enters a non-cultivable state known as persistence, characterized by the presence of enlarged, aberrant RBs. Removal of the stressors allows CT to resume replication. Persistence is considered pivotal for CT pathogenesis, yet its molecular mechanisms remain elusive due to challenges in genetically manipulating CT. We previously identified Polymorphic Membrane Protein C (PmpC), a CT-specific autotransporter, as a key factor in persistence and in preventing bacterial aggregation within inclusions in HeLa cells. To further investigate the role of PmpC, we established an *ex vivo* murine uterine horn (MUH) explant model using 8–12-week-old C57BL/6 mice. Uterine horns were sectioned and infected with 2×10^7 EBs of either the parental strain (L2 *wt*) or a *pmpC*-null mutant (L2 *pmpC::GII*). For the untreated condition, tissues were incubated up to 72 hours post-infection (hpi). To induce persistence, penicillin (0.5 IU/ml) was added at 24 hpi for 24 h, followed by recovery in antibiotic-free medium for another 24 h. Infected MUH were homogenized and processed for infectious progeny quantification (IFU assays) in HeLa monolayers. Tissue sections were fixed in 10% formalin, dehydrated and embedded in paraffin. CT inclusions were detected by indirect immunofluorescence using a polyclonal anti-CT043 antibody and visualized by confocal and super-resolution microscopy. Both L2 *wt* and L2 *pmpC::GII* infected MUH and formed aberrant RBs typical of chlamydial persistence upon penicillin treatment. However, upon recovery, the L2 *pmpC::GII* mutant consistently produced fewer infectious progeny than L2 *wt* (5.98×10^2 vs. 1.63×10^3 IFU/µl, $p<0.001$). Super-resolution microscopy further revealed pronounced aggregation of the mutant within inclusions, consistent with our previous HeLa cell observations. These results highlight PmpC as a critical protein for efficient recovery of CT from penicillin-induced persistence and for maintaining bacterial dispersion inside inclusions. Moreover, the MUH explant system provides a physiologically relevant model to investigate CT persistence. By reducing animal use, enhancing reproducibility, and enabling precise control of infection dynamics, this system provides a powerful alternative to traditional *in vivo* models.

MI-15

GENOMIC EPIDEMIOLOGY AND PHYLOGENETIC INSIGHTS INTO THE ARGENTINE VARIANT ST100 OF THE INTERNATIONAL PEDIATRIC CLONE

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Staphylococcus aureus is a pathogen capable of colonizing and causing diverse infections, with a remarkable ability to acquire antimicrobial resistance (AMR), such as methicillin resistance

(MRSA). It has significant potential for dissemination across hospital (HA), community (CA), and livestock-associated (LA) settings through the emergence of high-risk clones (HRCs) with enhanced virulence, transmissibility, and/or AMR. In previous studies (2007–2021), we identified the Argentine variant of the international paediatric clone HA-MRSA ST100 as one of the main lineages colonizing patients at hospital admission and the third most frequent cause of HA-MRSA infections in Argentina.
This lineage exhibited a high propensity to acquire AMR and novel SCCmec structures, including SCCmec IVNv, characterized by a class B mec complex lacking the recombinase *ccrC* and carrying a nuclease-domain protein gene absent in *S. aureus* ZH47 SCCmecIV(2B&5) from Switzerland, the reference strain for SCCmecIV(2B&5), despite their overall high homology. Other ST100 strains recovered in our previous studies carried a non-typeable (NT) SCC*mec*, in which PCR detected only the *mecA* gene among all SCC*mec*-associated elements. The genomic evolution of this Argentine HA-MRSA ST100 variant over time remains poorly understood. The genomic evolution of this Argentine HA-MRSA ST100 variant over time remains poorly understood.
Here, we investigated the resistome, virulome, and phylo-epidemiology of 42 ST100 isolates (23 with typeable SCC*mec* IVNv and 19 NT) recovered in Argentina between 2007–2021, together with five colonizing isolates from healthcare workers in Paraguay, using whole-genome sequencing (Illumina NovaSeq 6000). Phylogenetic analysis revealed two well-supported clades (I and II, 100% bootstrap), each containing distinct subclades. Clade I was largely composed of SCC*mec* NT isolates, carrying *mecA* and *IS431* but lacking *ccr* recombinase genes. Most isolates in both clades harbored aminoglycoside [*aac(6')-aph(2")*] and macrolide/lincosamide [*erm(C)*] resistance genes. Rifampicin resistance was associated with *rpoB* mutations (H481N), while fluoroquinolone resistance was linked to amino acid substitutions: in clade I, *grlA* S80F and *gyrA* S84L; and in clade II, three of five Paraguayan isolates carried *grlA* E84K. Regarding virulence factors, clade I differed from clade II by the presence of a type 7 integrase phage; subclade IA was defined by the absence of *fnbB*; and subclade IIB by the absence of the *egc* cluster within vSaβ.
These findings provide new insights into the phylogenetic diversification of the Argentine HA-MRSA ST100 clone and its acquisition of mobile genetic elements and novel SCC*mec* structures, which may contribute to its long-term persistence in Argentina. This underscores the importance of sustained molecular surveillance to monitor its evolutionary trajectory and public health impact.

MI-16

METAGENOMIC CHARACTERIZATION OF THE MICROBIOTA IN COMPOST BEDDING WITH DIFFERENT SUBSTRATES FOR DAIRY CATTLE

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Compost bedding housing systems have become established in Argentine dairy farming, particularly in Córdoba, due to their advantages in effluent management, reduced water consumption, and improved animal welfare. Characterizing the microbiota in these bedding

systems is crucial for optimizing the composting process and maximizing productive and economic benefits. This study used metagenomic techniques to determine and compare the microbial taxonomic profile in three different compost bedding systems: substrate-free (CF-SS), with peanut hulls (CC-MZ), and with corn stover (CC-MN). DNA was isolated from samples from each system, sequenced on the Illumina NovaSeq 6000 platform, and the reads were analyzed for taxonomic assignment. A common microbial core dominated by the phyla *Actinomycetota, Chloroflexota, Pseudomonadota* and *Bacillota* was identified, whose relative abundances varied significantly depending on the substrate: CF-SS: *Actinomycetota* (37%), *Chloroflexota* (22%), *Pseudomonadota* (19%), *Bacillota* (10%), CC-MZ (peanut): Notable increase of *Actinomycetota* (54%) and *Bacillota* (14%); *Chloroflexota* remained the same (22%) and *Pseudomonadota* decreased slightly (17%), CC-MN (corn): Marked dominance of *Actinomycetota* (58%) and *Chloroflexota* (27%), with a drastic reduction of *Pseudomonadota* (6%) and *Bacillota* (7%). The results demonstrate that the addition of lignocellulosic substrates, such as peanut hulls and corn stover, selectively favors the enrichment of bacterial phyla specialized in the degradation of recalcitrant material (*Actinomycetota* and *Chloroflexota*), while reducing the abundance of bacteria associated with easily degradable compounds (*Pseudomonadota*). By selecting substrates, the microbiota is shaped toward beneficial bacteria that improve composting efficiency and suppress pathogens. This targeted microbial selection is key to improving composting efficiency and litter quality. The benefits will include improved animal health, reduced mastitis, and the utilization of agricultural waste, transforming a common practice into an advanced strategy that contributes to the sustainability of dairy production.

MI-17

DRAFT GENOME SEQUENCE OF AN ENTEROCOCCUS LACTIS STRAIN ISOLATED FROM BOVINE MASTITIS REVEALS VIRULENCE AND ANTIMICROBIAL RESISTANCE GENES

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Enterococcus lactis has recently emerged as a potential etiological agent of bovine mastitis, although its pathogenic mechanisms remain poorly characterized. In this study, we report on the draft genome and phenotypic traits of *E. lactis* strain SU-B150, isolated from a case of subclinical mastitis. Whole-genome sequencing using the Illumina platform revealed a single circular chromosome of 2,486,471 bp with a GC content of 38.60%.
Genomic annotation predicted 2,480 protein-coding sequences (CDSs), of which 67 were annotated as hypothetical proteins. In addition, a complete rRNA operon and 21 tRNA genes were identified.
Taxonomic classification was confirmed by average nucleotide identity analysis (ANI >99%) and digital DNA–DNA hybridization, which placed SU-B150 within the *E. lactis* clade. Phylogenomic analysis was performed using 18 representative *Enterococcus* genomes retrieved from GenBank, including eight strains belonging to the *E. lactis* clade. The identification of orthologous genes revealed 536 single-copy core genes (SCGs) shared among all analyzed genomes. A phylogenetic tree constructed from the alignment of these genes

showed that *E. lactis* SU-B150 clustered within the *E. lactis* clade, forming a well-established monophyletic group. Genomic screening revealed 18 putative virulence genes with ≥90% identity to known virulence determinants in *E. lactis* and *E. faecium*. These include genes associated with adhesion (*acm*, *bepA*, *fms*, *fnm*, *sagA*), metabolic regulation (*ccpA*), and biofilm formation (*empB, empC*).
In addition, multiple antimicrobial resistance genes were identified, including *aac(6')-Ii*, *ermB*, and a *van* operon homologous to the hospital-associated *vanB* cluster.
The genome also revealed the presence of an intact 31.9 kb prophage and a CRISPR-Cas system, suggesting the coexistence of horizontal gene transfer elements with genomic defense mechanisms. The detection of clinically relevant resistance determinants, particularly the *vanB*-type operon, in a mastitis-associated *Enterococcus* strain from a food-producing animal is a significant concern from a One Health perspective. These findings provide crucial genomic evidence supporting the role of *E. lactis* as an emerging mastitis pathogen equipped with a repertoire of virulence and resistance genes. This highlights its potential for cross-species adaptation and underscores the urgent need for enhanced genomic surveillance of non-traditional enterococci at the animal-human-environment interface.

MI-18

DAILY RHYTHMS OF *ACINETOBACTER BAUMANNII* MODULATE VIRULENCE AND INFECTION OUTCOMES IN A MURINE SKIN WOUND MODEL

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Acinetobacter baumannii is a multidrug-resistant (MDR) pathogen ranked among the highest-priority bacterial threats by the World Health Organization (WHO). Previous work from our group has shown that *A. baumannii* can sense and respond to blue light, modulating physiological processes involved in its virulence and pathogenicity. Furthermore, we demonstrated that *A. baumannii* exhibits daily rhythms that are strongly dependent on environmental light-dark (LD) cycles, along with evidence of endogenous circadian rhythms. Under constant light (LL), however, the bacteria exhibit an arrhythmic behavior. We hypothesized that *A. baumannii* may utilize light as a temporal cue to align its physiology with the host's circadian system, potentially optimizing infection. To test whether the bacterial rhythms impact infection outcomes, we employed a murine skin wound infection model designed by our group, in which bacterial cultures used for infection were entrained to either 12 h blue light/12 h dark (12bL:12D) cycles or constant light (bLL), at 23°C, a temperature reflecting the microorganism's environmental life previous to host infection. Infections were initiated at two Zeitgeber times: ZT0 (7 a.m.) and ZT12 (7 p.m.), compatible with the end of the dark and illuminated phases, respectively, in cultures entrained in bLD. Skin tissues were collected at 4 and 7 days post-infection (dpi) for Colony Forming Units (CFUs) determination. Wound healing and degree of inflammation were evaluated using Hematoxylin and Eosin (H&E) staining. Our data show that, regardless of bacterial previous entrainment, infection performed at the end of the dark phase (morning infections) resulted in significantly lower amount of CFUs recovered compared to those recovered at the end of light phase (evening infections) ($p < 0.0001$ at 4 dpi; $p = 0.0630$ at 7 dpi), suggesting enhanced host immune control in the early hours. Morning infections with bLD-entrained bacteria led to higher amounts of

CFUs ($p < 0.0140$) and displayed delayed tissue regeneration with evidence of inflammatory infiltrate, when compared to those infected with LL-grown bacteria, which showed minimal inflammatory signs. In contrast, in evening infections, more CFUs were recovered for bLL-grown bacteria than in the case of bLD-entrained bacteria ($p < 0.0210$), and tissues exhibited moderate inflammation and active wound healing, whereas tissues infected with bLD-grown bacteria showed more advanced healing with reduced inflammation. These findings provide the first substantial evidence that bacterial entrainment conditions (bLD-cycles or bLL) have an influence on infection outcomes. Our results indicate that infection progression in this model depends on both the host's circadian clock and bacterial previous light entrainment. This work contributes to our understanding of host-pathogen interactions and will likely aid in developing novel chronotherapy strategies.

MI-19

CHARACTERIZATION OF A MULTIPLE DIGUANYLATE CYCLASE MUTANT IN *BORDETELLA BRONCHISEPTICA*

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Bordetella bronchiseptica is a Gram-negative coccobacillus that infects a wide variety of mammalian hosts through multiple virulence factors. Among these, its ability to form biofilm structures both *in vivo* and *in vitro* plays a key role in colonization and persistence of the respiratory tract. Biofilm formation is tightly regulated by multiple mechanisms, including the second messenger c-di-GMP. The intracellular concentration of this molecule is controlled by diguanylate cyclases (DGCs), which synthesize it, and phosphodiesterases (PDEs), which degrade it. Low levels of c-di-GMP promote a planktonic, motile lifestyle, whereas high levels drive the transition to a sessile, biofilm-forming state.
To investigate the contribution of individual DGCs to biofilm formation and motility, we constructed and analyzed single and multiple DGC mutants. The strains tested included: BbΔ*bdcC*, BbΔ*bdcB*Δ*bdcC*, BbΔ*bdcB*Δ*bdcC*Δ*bdcJ*, and BbΔ*bdcB*Δ*bdcC*Δ*bdcJ*Δ*bdcF*.
Biofilm formation was first quantified using a crystal violet assay, which revealed a progressive reduction compared to the wild-type, with the most pronounced decrease in the quadruple mutant. To complement this approach, we transformed these mutant strains with a plasmid encoding the Scarlet protein and monitored biofilm formation by microscopy. Consistent with the quantitative assay, microscopic observation revealed that biofilms formed by the wild-type strain exhibited a more compact architecture, whereas DGC-deficient mutants displayed impaired biofilm development, characterized by reduced bacterial adhesion and less structured communities, with unoccupied areas. Moreover, COMSTAT analysis of fluorescence images further revealed a 22% reduction in surface coverage compared to the wild-type strain.
Since c-di-GMP also regulates motility, we examined the same panel of strains on soft agar assays. As predicted, mutants exhibited progressively larger motility halos relative to the wild-type, with the quadruple mutant displaying up to 15% increase.
In summary, our findings demonstrate that the deletion of specific DGCs affects both biofilm formation and motility, underscoring their non-redundant contributions to the regulation of lifestyle transition in *B. bronchiseptica*. These results provide new insights into the complexity of c-di-GMP signaling in this pathogen and point to a modular organization of DGC activities rather than a global, overlapping function.

MI-20

GENOMIC INSIGHTS INTO ANTIMICROBIAL METABOLITES OF *LACTICASEIBACILLUS RHAMNOSUS* CRL 2244 ACTIVE AGAINST MULTIDRUG-RESISTANT *ACINETOBACTER BAUMANNII*

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The global rise of infections caused by multidrug-resistant (MDR) *Acinetobacter baumannii* underscores the urgent need to identify novel antimicrobial agents. We previously identified *Lacticaseibacillus rhamnosus* CRL 2244, isolate from wastewater, exhibiting strong antimicrobial activity against *A. baumannii* and other MDR pathogens. To investigate the genomic basis underlying the production of its active metabolite(s), we performed whole-genome sequencing followed by automated annotation and manual curation, which reduced hypothetical proteins by approximately 47% and improved functional interpretation. Comparative analyses using Average Nucleotide Identity (ANI) and pangenome reconstruction with Roary placed CRL 2244 within the *Lcb. rhamnosus* clade and revealed unique accessory genes potentially linked to antimicrobial metabolite production. Targeted genome mining with antiSMASH and BAGEL4 identified three biosynthetic gene clusters (BGCs), including two RiPP-like regions. One corresponds to a class IIb bacteriocin operon (orf00017–orf00021) encoding a two-component regulatory system, an ABC transporter, an immunity gene, and two double-glycine leader peptides, characteristic of two-peptide bacteriocins. Additional open reading frames (e.g., were predicted by SignalP and PSORTb to be secreted and extracellular, while BLAST searches against DRAMP and CAMP database revealed significant similarity to known enterocins. The integration of functional annotation, pangenome analysis, and specialized mining tools provides a robust framework for identifying candidate antimicrobial metabolites in *Lcb. rhamnosus* CRL 2244. However, these findings remain *in silico* predictions and require experimental validation through chemical identification (LC-MS/MS), metabolite synthesis or isolation, and minimum inhibitory concentration testing to confirm the identity and activity of the active molecule(s).

MI-21

CHARACTERIZATION OF PA1299 (*AITQ*) AS A METALLOCHAPERONE OF THE FE/CO TRANSPORTER *AITP* IN *PSEUDOMONAS AERUGINOSA*

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In the opportunistic pathogen *P. aeruginosa*, members of the Cation Diffusion Facilitator (CDF) family export transition metal ions from the cytosol to the periplasm, allowing the pathogen to adapt to conditions of high and low metal availability. Our laboratory has characterized the CDF family transporter AitP as responsible for exporting intracellular Fe^{2+} and Co^{2+}. The current field working hypothesis suggests that AitP should be assisted by a metallochaperone that coordinates and transfers the cation to the exporter to reduce the intracellular oxidative damage by the free metal. Here, guided by co-occurrence and operon conservation searches in bacterial genomes, we evaluated the role of the locus PA1299 as a putative metallochaperone for AitP. PA1299 codes for a protein of unknown function (DUF3109), it is localized in the cytosol and contains cysteine-rich domains. Alphafold3 3D-structure predicts the presence of two 4Fe-4S iron-sulfur clusters (ISCs) and two surface-exposed acidic domains. Recently, we have expressed and purified the $His_{(6)}$-tagged version of the PA1299 product and named it AitQ. Applying biochemical and electron paramagnetic resonance (EPR) spectroscopy methods to aerobically and anaerobically purified protein, we provide evidence of the presence of ISCs and of transition metal binding capacity in AitQ. We also show the initial phenotypic characterization of an *aitQ* deletion mutant strain generated by CRISPR/Cas9 gene editing system. The results support a role for AitQ as a metallochaperone in Fe^{2+} and Co^{2+} homeostasis in *P. aeruginosa,* and we hypothesize a similar role in other bacterial organisms with AitQ and AitP homologs.

MI-22

FIRST GENOMIC CHARACTERIZATION OF ANCIENT *STREPTOCOCCUS PYOGENES* FROM HUMAN REMAINS (1100–1600 A.D.) PROVIDES EVOLUTIONARY INSIGHTS INTO VIRULENCE AND ANTIBIOTIC RESISTANCE TRAITS

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The field of the so-called “ancient DNA” (aDNA) has allowed the study of different organisms recovered from archaeological sites. This enabled the emergence of “paleomicrobiology”, providing valuable insights into the evolutionary history of various pathogens. The Gram-positive bacterium *Streptococcus pyogenes* is a major human pathogen and the causative agent of many diseases, including pharyngitis, skin and soft tissue infections, osteomyelitis, and toxic shock syndrome. *S. pyogenes* infections are documented in medical records back to the late XIX century; however, the genetic determinants of virulence and antibiotic resistance of ancient *S. pyogenes* remain largely unknown. In this study, aDNA was extracted from dental samples obtained from 64 individuals at archaeological sites in the province of Córdoba, Argentina. An initial metagenomic screening approach suggested positive results for *S. pyogenes* in three individuals, radiocarbon dated to 920±20, 533±42, and 384±41 years before present (~1100-1600 A.D.). Then, library enrichment with specific probes and DNA sequencing to ~20 million reads was used to recover the genomes of *S. pyogenes* from these samples. Reads were mapped against the *S. pyogenes* reference genome (strain NCTC8198).

The Damageprofiler tool was used to identify characteristic aDNA damage patterns. Kraken2 database and KrakenTools were used to select for reads matching *S. pyogenes,* and a K-mer-based screening of virulence and resistance genes was carried out using the KMA algorithm. The presence of aDNA damage patterns in the form of deamination confirmed genuine ancient DNA and discarded modern contamination. Stringent mapping against the reference genome confirmed the presence of *S. pyogenes* in all three samples with a mean per base coverage of 6.4, 9.2, and 11.6X. Resistome analysis revealed no clinically relevant antibiotic resistance genes. The multidrug resistance efflux pump *lmrP*, with uncharacterized functional roles in *S. pyogenes*, was detected in two samples. Virulome analysis indicated the presence of critical *S. pyogenes* virulence genes, including adhesins (*fbp54*, *fbaA*, *cpa*), immune evasion factors (*cppA*, *ideS/mac*, *mrp*), invasins (*hylA*, GRAB, *ska*), DNAses (*mf/spdk*), and exotoxins (*speB*, *speG, sagA*, *slo*). M protein typing for epidemiological surveillance was successfully performed on two samples, revealing 98% coverage and identity with the *emm156.4* allele. Notably, this allele was recently described in a *S. pyogenes* strain isolated from bone infection in the UK. A premature stop codon mutation was identified in one sample for *covS* (c.1246C>T, p.Gln416*), part of the *covRS* two-component regulatory system genes. Importantly, these mutations are associated with hypervirulence in modern *S. pyogenes* strains. In conclusion, we report the first characterization of ancient *S. pyogenes* genomes, providing relevant clues into the evolutionary history of this major human pathogen.

MI-23

HUNTING FOR SIGNALS: A HETEROLOGOUS APPROACH TO DIGUANYLATE CYCLASE REGULATION IN BORDETELLA

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The transition between planktonic and biofilm lifestyles in bacteria is tightly regulated by multiple mechanisms. Over the last decades, the second messenger c-di-GMP has emerged as a central regulator of these processes. Its intracellular concentration is determined by the opposing activities of diguanylate cyclases (DGCs), which synthesize c-di-GMP, and phosphodiesterases (PDEs), which degrade it. While the biochemical mechanisms of c-di-GMP turnover are well understood, the environmental and host-derived signals that regulate these enzymes remain largely unknown. Typically, DGCs and PDEs have their catalytic domain (GGDEF or EAL, respectively) at the C-terminal end of the protein, whereas the N-terminal region corresponds to a sensory or regulatory domain. In membrane-associated DGCs, the sensory domain is usually periplasmic, while the catalytic domain resides in the cytoplasm.
In this work, we adapted an approach previously used to detect DGC ligands in *Pseudomonas fluorescens* to identify potential ligands of DGCs from the respiratory pathogen *Bordetella bronchiseptica*. This bacterium is the etiological agent of atrophic rhinitis in swine and can occasionally infect immunocompromised humans. We have previously shown that c-di-GMP is important for biofilm formation, motility, and virulence in *B. bronchiseptica*. However, the signals that activate its membrane-associated DGCs remain unknown. We employed a *P. fluorescens* mutant strain (*Pf*Δ4), which is unable to form biofilm unless an active DGC is expressed in trans. Biofilm development in this background is further enhanced when a positive allosteric ligand is present in the culture medium. Several predicted membrane-associated

DGCs from *B. bronchiseptica* were cloned and expressed in *Pf*Δ4, and biofilm formation assays were performed in the presence of different candidate ligands.
Using this strategy, we identified significant activation of the DGC BdcJ in the presence of fetal bovine serum (FBS). *Pf*Δ4 expressing *bdcJ* displayed a 100% higher biofilm formation in FBS than in minimal medium. This constitutes the first evidence of a host-derived ligand capable of activating a DGC in *B. bronchiseptica*. Other membrane DGCs, such as BdcA and BdcG, also responded to serum but to a significantly lesser extent. Interestingly, despite structural similarities between BdcA and GcbC (a *P*. fluorescens DGC known to sense citrate), BdcA-driven biofilm formation was not enhanced in the presence of citrate.
In summary, we established a screening method to identify potential ligands of DGCs, applying it to *B. bronchiseptica* but demonstrating its broader applicability to other bacterial species. Our findings provide the first experimental evidence of ligand-mediated regulation of a DGC in *B. bronchiseptica* and represent an important step toward understanding how this pathogen senses and responds to environmental and host-derived signals during infection.

MI-24

SYSTEMATIC STUDY OF PHOSPHODIESTERASES' ROLE IN THE PLANKTONIC-BIOFILM TRANSITION IN *BORDETELLA BRONCHISEPTICA*

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The second messenger c-di-GMP is involved in planktonic and biofilm transition regulation of bacteria. Bacteria within biofilms present high levels of c-di-GMP. In response to usually unknown signals, c-di-GMP levels decrease, catalyzed by phosphodiesterases (PDEs), leading to bacterial motility. This transition is particularly important during pathogenesis, being primarily associated with the symptomatic–asymptomatic life cycle of pathogens. We have previously described that c-di-GMP regulates swimming and biofilm formation in the respiratory pathogen *Bordetella bronchiseptica*. We identified four proteins in the genome with an EAL domain, responsible for PDE activity. One of them, BvgR, lacks a complete active site and has been proposed to function as a c-di-GMP-binding protein. To evaluate how these PDEs are involved in swimming or biofilm formation, we systematically generated single and combined PDE mutants and assessed their effects in these phenotypes.
As described in other bacteria, and confirmed in *B. bronchiseptica*, not all PDEs are involved in both phenotypes. We found no evidence that PdeD regulates either phenotype. In contrast, while BvgR and PdeA are both involved in the regulation of swimming motility, PdeC specifically regulates biofilm formation. Deletion of *bb3116* enhanced biofilm formation, as visualized by fluorescence microscopy. The mutant strain increased 47% the area of surface occupied by bacteria in comparison to the WT strain.
Interestingly, BvgR and PdeA appear to coordinate the regulation of motility. Swimming assays revealed that the deletion of *bvgR* stimulated swimming under conditions where *B. bronchiseptica* wild type does not display motility. However, swimming was abolished when *pdeA* was also deleted in the Δ*bvgR* background, indicating that PdeA is essential for this motility response. This phenotype was not repeated with any other PDE tested.
PdeA contains a C-terminal REC domain, which is predicted to be phosphorylated by a sensor histidine kinase. We constructed two PdeA variants: PdeA-E56D, mimicking constitutive phosphorylation, and PdeA-D56N, unable to accept the phosphate. Overexpression in a wild-

type background revealed distinct effects on motility. The PdeA-E56D strain was unable to enhance swimming, suggesting that phosphorylation inactivates PDE activity. In contrast, PdeA-E56N functioned like the wild-type protein, enhancing motility as expected for an active PDE. These results indicate that phosphorylation of the REC domain acts as a regulatory switch controlling PdeA activity and c-di-GMP–dependent lifestyle transitions.
In summary, our work describes the role of individual PDEs in regulating the transition of *B. bronchiseptica* between biofilm-associated and planktonic states. A better understanding of this regulatory network will provide important insights into the pathogenic processes of this and other respiratory pathogens.

LIPIDS

LI-9

SPHINGOSINE-1-PHOSPHATE MODULATES BRADYKININ-DRIVEN COLLECTIVE DYNAMICS AND JUNCTIONAL STABILITY IN THE DEVELOPING RENAL COLLECTING DUCT

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The maturation of the renal collecting duct (CD), completed postnatally in mammals, requires the coordination of collective migration and the reinforcement of cell–cell junctions. We previously showed that bradykinin (BK), acting through the B2 receptor, promotes the assembly of CD cells into migratory colonies. Sphingosine-1-phosphate (S1P) can act as an intracellular second messenger via sphingosine kinases (SK) or as a ligand for S1P receptors (S1PRs). Here, we examined how S1P modulates BK-induced epithelial organization in primary CD cultures from 10-day-old Wistar rats. We combined selective antagonists (W146, S1PR1; BML-241, S1PR3; JTE-013, S1PR2) with SK inhibitors (tDHS, pan-SK; PF-543, SK1; FTY720-OMe, SK2) and assessed actin architecture, cell–cell junctions, focal adhesions, colony compaction, and migration using immunofluorescence and time-lapse phase-contrast microscopy. BK enhanced colony compaction and strengthened the cortical actin belt, accompanied by aligned lamellipodial protrusions at the leading edge. Dynamic analyses revealed a transient pulse of collective coordination characterized by protrusion alignment that subsequently returned to baseline. Inhibiting SK1 prior to BK shortened the coordination pulse, whereas SK2 inhibition increased cortical dynamics without improving collective behavior. Consistently, under SK inhibition immunofluorescence showed incomplete compaction and fragmentation of the cortical F-actin belt, with micro-gaps in E-cadherin continuity and a more focal/diffuse vinculin distribution at junctions, indicating less stable adhesions. Functionally, colonies transiently increased directional order at the front yet moved more slowly and lost coordination earlier, indicating that front alignment was not converted into sustained advance when SK1/2 activity was compromised. Pharmacological blockade of S1PR1/3 reduced BK-induced compaction and coherence, consistent with pro-collective cues that sustain performance; in contrast, S1PR2 antagonism increased early directional order without improving speed, compatible with a differential role modulating edge contractility rather than

traction. Altogether, our data support a model in which BK and S1P act as a coordinating module that balances motility and adhesion, enabling the developing CD epithelium to elongate and branch while maintaining a cohesive epithelial sheet. In this framework, intracellular S1P (via SK1/2) sustains junctional stability and the duration of BK-evoked coordination, whereas receptor-mediated S1P signaling differentially tunes collective motility, indicating distinct roles in epithelial organization in epithelial organization during postnatal renal development.

LI-10

SINGLE CELL RNASEQ ANALYSIS UNCOVERS LIPID METABOLISM DYNAMICS DURING MOUSE MALE GERM CELLS DIFFERENTIATION

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Spermatogenesis in mammals is one of the most efficient cell production processes in adult organisms and represents an excellent model to study cellular differentiation. Single-cell RNA sequencing (scRNA-seq) allows analysis of gene expression at the single-cell level, facilitating the characterization of transcriptomic profiles and identification of processes associated with differentiation. Although gene expression dynamics and stage-specific markers have been previously reported in mouse germ cells, the regulation of lipid metabolism genes during spermatogenesis remains poorly understood. Our objective was to use scRNA-seq data to analyze lipid metabolism gene expression dynamics during spermatogenesis. We downloaded scRNA-seq data from two 8-week-old C57BL/6J mice testes (GEO-NCBI). Data processing with Seurat v5 in R Studio yielded an expression matrix comprising 2,523 cells and 51,868 genes. Six cell clusters with similar expression patterns were identified: spermatocytes 1 and 2 (SC1, SC2), round spermatids 1 and 2 (RS1, RS2), elongating spermatids (ES), and condensed spermatids (EC). These clusters were validated by known germ cell differentiation stages marker genes. A curated set of 1,749 lipid metabolism–related genes was interrogated, revealing distinct stage-specific expression patterns. Early germ cells (SC and RS) were enriched in transcripts associated with polyunsaturated fatty acid elongation, acyltransferase activity, and lipid transport, whereas later stages (ES and EC) were enriched in transcripts related to neutral lipid biosynthesis, phospholipid metabolism, and remodeling. At the gene level, *Elovl2* and *Ascl6* showed higher expression in SC2 and RS1. The elongated fatty acids produced by these enzymes could serve as substrates for phospholipid biosynthesis in later stages. In this way, an increased expression of *Pcyt1a* and *Lpcat2b* in ES and EC was observed. RS2 accumulated phospholipid transporter transcripts (*Atp8a2*, *Atp10a*, *Pitpnm2*). Stage-specific expression was also evident for glycerol-3-phosphate pathway genes, with *Gpat2* predominating in SC, whereas *Gpat4*, *Agpat2*, and *Agpat3* were enriched in ES and EC. Later stages exhibited upregulation of neutral lipid biosynthesis genes, including *Dgat2*, *Plin2*, and *Lipin1*, consistent with lipid droplet biogenesis, a normal event during spermiogenesis. Genes involved in cardiolipin precursors synthesis (*Cds1*, *Lpgat1*, *Pgs1*) were expressed in early stages (SC1, SC2, RS1), while *Crls1*, encoding cardiolipin synthase, was highly expressed in RS2 and ES. In conclusion, scRNA-seq revealed stage-dependent activation of lipid metabolic pathways during spermatogenesis, highlighting a coordinated program of fatty acid

elongation, phospholipid remodeling, triacylglycerol storage, and cardiolipin biosynthesis. These findings provide new insights into the metabolic regulation underlying male germ cell differentiation. *Supported by SGCyT UNS [PGI 24/B341 to GMO] and [PGI 24/N052 to JAC].*

LI-11

NEURON-ASTROCYTE CROSSTALK: THE NEVER-ENDING STORY OF α-SYNUCLEIN AND LIPID METABOLISM

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In our laboratory, we have previously demonstrated that mild overexpression of α-synuclein in neurons induces a shift in lipid metabolism, promoting lipid droplet and cholesterol accumulation, along with the downregulation of phospholipase D1 (PLD1) and cytoskeletal rearrangements. Additionally, we showed that lipid droplets are essential for neuronal survival under conditions of α-synuclein overexpression. In this study, we aimed to characterize how neuronal α-synuclein overexpression impacts astrocyte biology and metabolism. To this end, we used various glial culture models: mixed primary glia, primary astrocytes, and the C6 astrocytic cell line. These cultures were treated with neuronal conditioned media derived from cells expressing endogenous levels of α-synuclein (e-aSyn CM) or overexpressing α-synuclein (oe-aSyn CM). Both conditions were compared to cultures incubated with regular medium (control). We found that astrocytes exposed to oe-aSyn CM exhibited increased lipid droplets and cholesterol content with the upregulation of diacylglycerol O-acyltransferase expression. Remarkably, these astrocytes also showed an upregulation of PLD1. These metabolic changes were accompanied by actin cytoskeletal rearrangements. Moreover, exposure to oe-aSyn CM also modulated astrocyte proliferative and migratory behavior. Our findings indicate that neuronal α-synuclein overexpression modulates astrocytic lipid metabolism and cytoskeletal architecture and may also affect proliferation and glial scar

LI-12

STRUCTURAL AND FUNCTIONAL CONSECUENCES OF OXIDATIVE STRESS ON PULMONARY SURFACTANT

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Pulmonary surfactant is essential for alveolar stability but is highly susceptible to oxidative injury in inflammatory lung diseases. Hypochlorous acid (HClO), generated by activated neutrophils, is a potent oxidant that may compromise surfactant integrity, yet its molecular impact remains incompletely understood. In this study, the effects of oxidative stress induced

by hypochlorous acid on the biophysical and structural properties of porcine-derived native surfactant were investigated. Its adsorption kinetics at the air–liquid interface, dynamic surface activity under compression–expansion cycles using captive bubble surfactometry (CBS), thermotropic behaviour by differential scanning calorimetry (DSC), and molecular structural changes in both lipids and proteins by FTIR spectroscopy were analysed. Oxidation induced concentration-and time-dependent inhibition of surfactant adsorption and film stability. Langmuir experiments revealed selective disruption of films containing unsaturated phospholipids or native mixtures, while saturated monolayers remained largely unaffected. FTIR spectra showed headgroup perturbations and protein secondary structure rearrangements towards β-sheet-rich aggregates, consistent with oxidative unfolding and aggregation. DSC analyses indicated reduced miscibility and lateral phase separation, with formation of DPPC-enriched gel domains. Functional assays confirmed impaired reduction of surface tension during quasi-static cycling, partially alleviated under dynamic cycling, suggesting displacement of oxidized components from the interface. Overall, HClO selectively targets unsaturated lipids and surfactant proteins, driving lipid phase segregation and protein aggregation that compromise interfacial dynamics. These findings provide mechanistic insight into how neutrophil-derived oxidants inactivate pulmonary surfactant and underscore the need for antioxidant strategies or oxidant-resistant surfactant formulations as potential therapeutic approaches.

LI-13

SPHINGOMYELIN SYNTHASES SUPPORT JUNCTIONAL INTEGRITY AND COLLECTIVE BEHAVIOR IN DEVELOPING COLLECTING DUCT EPITHELIA

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Postnatal morphogenesis of the renal collecting duct (CD) requires epithelial sheets to coordinate collective movement while preserving junctional integrity. Primary CD cultures from 10-day-old rats recapitulate these behaviors and exhibit center–edge heterogeneity relevant to maturation. Thus, we investigated whether sphingomyelin synthases (SMS1/2) support junctional coherence and collective coordination under basal conditions. We performed siRNA knockdown of SMS1 or SMS2 and quantified live dynamics at 24 h and 48 h post-transfection by time-lapse phase-contrast microscopy. Analyses focused on quantitative metrics of collective behavior at the colony scale, including an order parameter of coordination over time (pulse amplitude and duration), velocity and persistence, compaction index, and contour geometry (edge irregularity). Fixed-cell immunofluorescence complemented these readouts by co-labeling E-cadherin and α-catenin to assessed adherens-junction continuity. Our analyses indicated that SMS knockdown weakened the efficiency of the coordination pulse, reduced compaction, and increased edge irregularity, with a more pronounced trend for SMS2 than for SMS1. Consistently, junctional mapping revealed decreased continuity of E-cadherin with less uniform α-catenin; effects at 24 h are subtle and become clearer at 48 h. Because epithelial cohesion and collective motility are coupled during CD maturation, we also tracked morphology-based proxies of epithelial–mesenchymal plasticity—cell shape descriptors,

neighbor exchange frequency at the edge, and local loss of a continuous junctional belt—. In this framework, partial loss of epithelial junction continuity together with increased contour irregularity and reduced persistence was interpreted as early hallmarks of a shift toward mesenchymal-like behavior at colony borders. Together, these measurements indicate that sphingomyelin synthesis contributes to maintaining junctional coherence and the capacity of developing CD epithelia to coordinate movement in basal conditions. This study addresses the gap between membrane-lipid organization and epithelial morphodynamics in a physiologically relevant primary model and sets the stage for mechanistic dissection of lipid–junction coupling and the control of collective epithelial behavior during postnatal CD maturation.

LI-14

POTENTIAL FUNCTIONAL FOOD: *OPUNTIA FICUS-INDICA* MODULATES COX2 IN RENAL EPITHELIAL CELLS AND PREVENTS OXALATE-INDUCED DAMAGE

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Renal lithiasis is the most frequent disease of the urinary tract, with a global prevalence of approximately 12% and a high recurrence rate after the primary episode. Among patients with urolithiasis, more than 60% present calcium oxalate (CaOx) stones. Oxalate nephropathy is characterized by tubulointerstitial deposits of CaOx crystals, interstitial fibrosis, tubulointerstitial inflammation, and progressive renal insufficiency, which may ultimately lead to the development of Chronic Kidney Disease (CKD). In our lab, we have previously demonstrated that the treatment of differentiated renal-epithelial cells with oxalate (Ox) reduced cell number, and surviving cells exhibited a spindle-shaped morphology characteristic of epithelial–mesenchymal transition (EMT) after 24 h. We also demonstrated in renal cells that cyclooxygenase-2 (COX2) is a cytoprotective gene whose expression and activity are essential for renal protection against hyperosmolar stress and mediates epithelial cell monolayer restitution after 72 h of Ox. Functional foods (FF) are natural foods or foods enriched with one or more specific components that confer health benefits and may aid in preventing prevalent diseases such as obesity, diabetes, cancer, and hypertension. In South America, *Opuntia ficus-indica* (Ofi), commonly known as 'nopal', is the most widely distributed cactus. It is particularly abundant in the Argentine Northwest (NOA). Because of its structural composition, wide availability, and low cost, nopal has potential as a food ingredient. In the present study, we evaluate whether different extracts of Ofi can prevent oxalate-induced damage and whether this protective effect is mediated through COX2. To do that, Madin-Darby canine kidney (MDCK) epithelial cells were grown in a hyperosmolar environment (512 mOsm/Kg H2O) for 72 h to obtain a differentiated epithelial monolayer; then cultures were subjected to 1.5 mM Ox in the absence or the presence of 12.5 or 25 μg of gallic acid equivalents (GAE)/g of extract, either from cladode flour or mucilage of Ofi. Extracts were added 30 min before Ox exposure. After 24 h, cells were harvested, and cell number and viability were determined by Trypan Blue exclusion assay. Cell morphology was assessed by fluorescence microscopy using FITC-conjugated phalloidin to stain cortical actin. In addition, the expression of COX2 was assessed by western blot. Pretreatment with Ofi extracts before

Ox addition preserved monolayer integrity, with extensive regions with the typical cobblestone epithelial morphology. This effect was observed with all concentrations used, but mainly with the mucilage extract. Cell number and viability did not change at any concentration evaluated. Also, we observed that Ofi extracts modulate COX2 expression under Ox treatment. These preliminary findings suggest that *Opuntia ficus-indica* extracts exert protective effects against Ox-induced renal epithelial cell damage.

LI-15

SPHINGOSINE KINASE 2 (SK2) TRANSLOCATES TO LIPID DROPLETS DURING EPITHELIAL-MESENCHYMAL TRANSITION OF RENAL EPITHELIAL CELLS

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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. Epithelial cell de-differentiation is known as epithelial-mesenchymal transition (EMT). During EMT, cell adhesion and apical-basal polarity are lost, and the cytoskeleton is reorganized. Previous results from our laboratory showed that fully differentiated MDCK cells at the wound edge undergo EMT during wound healing to acquire a migratory profile through the activation of the S1P receptor 2 (S1PR2)/ERK1/2 pathway. Moreover, we have found that both SK1 and SK2 are capable of modulating cell migration, with SK2 triggering EMT in renal collecting duct cells. This finding is particularly interesting, as most research has focused on SK1 and has left SK2 largely unexplored. In the present study, we investigated SK2 translocation and the potential underlying mechanisms, as well as SK2 implication in EMT. 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). Immunofluorescence studies showed that, during de-differentiation (6 h post wound), SK2 relocates from the Golgi apparatus to vesicular structures identified as lipid droplets (LDs). De-differentiation was associated with a reduction in both the number and size of LDs. Moreover, we demonstrate that this change in LD dynamics depends on the activation and localization of SK2 within these structures. These findings suggest that SK2 may act through the modulation of LD dynamics. These results are particularly interesting, as they establish a connection between a central enzyme in sphingolipid metabolism and triglyceride homeostasis.

LI-16

RETINOIC ACID AND DOCOSAHEXAENOIC ACID COOPERATE TO ENHANCE GERM CELL DIFFERENTIATION AND LIPID REMODELING IN EX VIVO MOUSE TESTICULAR EXPLANTS

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Using a gas-liquid interphase culture system with neonatal mouse testicular explants, we evaluated the combined effects of Retinoic Acid (RA) and Docosahexaenoic Acid (DHA) on spermatogenesis progression, lipid remodeling, and steroidogenic activity during ex vivo maintenance. RA, a well-known regulator of germ cell differentiation, was used as a baseline supplement to support meiotic entry and spermatid formation, while DHA was added to assess its specific contribution within this RA-supported context. RA supplementation alone significantly enhanced spermatogenic progression, as shown by the appearance of round spermatids after 22 days and occasional spermatozoa at 44 days in the explants. RA also induced a remodeling of testicular fatty acid composition, particularly by reducing the presence of atypical n-9 PUFAs (20:3n-9 and 22:4n-9), while promoting enrichment of membrane lipids in C20–C22 n-6 PUFAs. These changes correlated with increased testosterone production and advanced germ cell differentiation, mimicking in vivo postnatal testicular development. When DHA was added in combination with RA, it further enhanced the differentiation process without significantly altering the overall lipid profile. Instead, DHA exerted its effect primarily at the transcriptional level, selectively upregulating 3β-Hydroxysteroid Dehydrogenase (*3β-Hsd1*) among steroidogenesis-related genes, and significantly increasing the expression of key genes involved in meiosis and spermatid formation (*Stra8*, *Sycp3*, *Acr*). In addition, genes encoding polyunsaturated fatty acid elongation and fatty acid transport, such as *Elovl2* and *Fabp9*, were also upregulated in the presence of DHA. This transcriptional activation was associated with a higher incidence of meiotic cells per seminiferous tubules, suggesting a role for DHA in reinforcing germ cell meiotic progression initiated by RA. Together, these findings demonstrate that RA and DHA act through complementary and non-redundant mechanisms to support spermatogenesis in vitro: RA primarily modulates lipid homeostasis and endocrine function, while DHA potentiates germ cell differentiation via gene expression regulation. This cooperative effect positions RA+DHA co-supplementation as a promising strategy to optimize ex vivo testicular culture systems for germ cell development. *Supported by FONCyT [PICT2020-02056 to GMO], CONICET [PIP11220210100420CO to GMO], and SGCyT UNS [PGI 24/B341 and 24/B272 to GMO and JML].*

LI-17

MOLECULAR MECHANISMS ASSOCIATED WITH PGE_2 RECEPTORS ACTIVATION IN THE RESTITUTION OF AN OXALATE-DAMAGED RENAL EPITHELIUM

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Kidney stone disease is the most frequent urinary tract disorder, with a global prevalence of 12%. The main component of kidney stones is calcium oxalate, injuring renal cells and tubular structures, thus contributing to the development of chronic kidney disease. Our Previous results showed that renal differentiated cells treated with Oxalate (Ox) for 24h exhibited spindle-shaped morphology characteristic of epithelial-mesenchymal transition. After 48 h of Ox treatment, cells began to recover their shape, and by 72h, the epithelium was almost restituted. We demonstrated the importance of PGE_2 for epithelial restitution and that COX-2 inhibition impeded this process. EP2 and EP4 receptor expressions were confirmed by q-PCR. The aim of the present work is to evaluate the molecular mechanisms associated with the activation of the PGE_2 receptors in the restitution of renal differentiated epithelial cells after Ox damage. To accomplish this, renal epithelial cells (MDCK) were grown in a hyperosmolar environment (512 mOsm/Kg H2O) for 72 h to differentiate them and then subjected to 1.5 mM Ox for 0h to 72h. Cells were subjected to 10μM PF-04418948 or/and 10μM L-161,982; EP2, and EP4 antagonists, respectively (EP2a/EP4a). Cell number decreased 24h after treatment with EP2a and EP4a + Ox compared to Ox treatment alone, but at 48h it also showed a decrease after treatment with EP2a and/or EP4a compared to Ox. Cell morphology was also evaluated. After 24h, cells treated with antagonists did not show differences compared to cells treated with Ox. However, after 48h Ox cells started to recover their morphology, but this restitution was lower for EP2a-treated cells. Cells treated with EP4a or combined treatment showed more damage compared to Ox. E-cadherin subcellular localization was also altered after treatment with EP2a/EP4a; after 24h, there was an increase in its internalization, and 48h after treatment, its redistribution to the cell membrane was impeded. cAMP levels were measured to evaluate EP2/EP4 activation. Intracellular cAMP increased after 11h of treatment, but this was inhibited after treatment with both antagonists. EP2/EP4 localization was analyzed in stably transfected EP2-AmCyan1 or EP4-mTurq2 cells. Both presented a peripheral localization; however, after 12h of treatment, receptor internalization occurred, accompanied by a loss of peripheral staining. After 72h, they relocalized to the periphery. Confocal microscopy confirmed that this internalization was associated with traffic to the early endosome, as colocalization with EEA was observed. These results provide further insight into the molecular mechanisms triggered by EP2 and EP4 following Ox treatment. Notably, they show that: cAMP levels increase, the relocalization of E-cadherin depends on their activation, and this activation promotes receptor internalization, with receptors subsequently recovering their peripheral localization following restitution.

LI-18

METFORMIN AND 6-AMINONICOTINAMIDE COMBINATION ALTERS LIPID METABOLISM AND REDOX BALANCE IN GLIOBLASTOMA CELLS

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The pentose phosphate pathway (PPP) enables cancer cells to sustain anabolic demands,

including lipid synthesis, and to maintain antioxidant defenses. Metformin (MET), a widely prescribed drug for type 2 diabetes, impairs tumor progression mainly by indirectly inhibiting the mTOR pathway. Our previous results showed that combining MET with 6-aminonicotinamide (6AN), a PPP inhibitor, potentiates their individual cytotoxic effects in tumor cells, including glioblastoma (GB). Here, we investigated the impact of this combination on lipid metabolism in U251 human GB cells. MET/6AN treatment (5 mM and 25 μM, respectively) inhibited acetyl-CoA carboxylase, as shown by increased phosphorylation of ACC (24 h, WB and IF, $p<0.05$), a key enzyme in lipid synthesis. Paradoxically, MET/6AN-treated cells exhibited increased total lipid content (BODIPY and Oil Red staining, flow cytometry (FC), $p<0.01$), accompanied by higher expression of the lipid transporter CD36 (FC, $p<0.01$). Fluorescence microscopy confirmed larger lipid droplets, in agreement with lipid profile analyses that showed elevated triglycerides. Furthermore, MET/6AN reduced mitochondrial potential and induced oxidative stress and lipid peroxidation (TMRE, DCF, and C11-BODIPY, flow cytometry, $p<0.01$). Preliminary evidence suggests that lipid accumulation within droplets may act as a protective mechanism against peroxidation. Together, these findings indicate that MET/6AN co-treatment disrupts lipid metabolism and redox balance, uncovering a complex interplay between metabolic reprogramming and stress adaptation in glioblastoma cells. Further studies are required to clarify the contribution of lipid droplet dynamics to the therapeutic potential of this combination.

PLANTS

PL-13

EFFECT OF RHAMNOLIPIDS ON SOYBEAN–RHIZOBIUM NITROGEN FIXATION

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Legumes establish symbiotic relationships with rhizobacteria, leading to the formation of root nodules that enable the fixation of atmospheric nitrogen, a key nutrient for plant development, growth, and productivity. In addition, this symbiosis triggers systemic and immune responses that enhance plant tolerance to various biotic and abiotic stresses. Rhizobia and other soil bacteria can induce Systemic Resistance which, in addition to protecting plants against pathogens, also strengthens the symbiotic interaction. Rhamnolipids (RLs) are glycolipids produced by certain bacteria. They exhibit direct antimicrobial properties and, indirectly, activate the plant immune system, thereby protecting plants against pathogenic microbes. Their use as adjuvants or biopesticides is particularly attractive for sustainable agricultural practices due to their low toxicity and biodegradability. It has been proposed that RLs play a role in bacterial communication (*quorum sensing*), a key mechanism for the establishment of nodulation. Our working hypothesis is that RLs may enhance the interaction between soybean roots and rhizobia, contributing to the improvement of both the physiological and immunological status of the plant. As an experimental strategy, we carried out soybean seed pretreatments with RLs prior to inoculation with *Bradyrhizobium diazoefficiens*. Plants were subsequently transferred to hydroponic culture, and after 21 days, several parameters were

evaluated. Growth parameters included root and shoot length and weight, leaf area, and number and weight of nodules. Photosynthetic performance was assessed by measuring CO_2 assimilation and Photosystem II efficiency. In addition, nitrogen assimilation was analyzed through the quantification of ureide content in nodules and leaves. Among the parameters evaluated, a significant difference was observed only in ureide content. While its concentrations increased in leaves, it decreased in nodules, suggesting an enhanced nitrogen flux between the nodules and the aerial part of the plant. This shift could potentially lead to improved growth, higher yields, or greater tolerance to adverse conditions, such as abiotic stress as salinity. We consider that biotechnological strategies based on the application of RLs to seeds could optimize nitrogen fixation, reduce dependence on synthetic fertilizers, and improve plant resilience to abiotic stress, thereby offering promising alternatives for a more sustainable agriculture.

PL-14

CW-MBD PROTEINS AND THEIR ROLES IN THE REGULATION OF GENOMIC STABILITY IN ARABIDOPSIS PLANTS UNDER UV-B CONDITIONS

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DNA methylation, such as 5-methylcytosine, is a well-studied epigenetic mark that participates in establishing stable patterns of gene expression. There are different mechanisms by which DNA methylation inhibits gene expression. For example, DNA methylation can directly block the binding of transcriptional activators to DNA sequences. On the other hand, a different mechanism involves the interaction of methylated DNA with proteins capable of binding to it. One family of proteins capable of recognizing and binding these methyl groups are Methyl-CpG Binding (MBD) Proteins. This family is divided into different subfamilies, and our object of study is the CW-MBD family, which includes MBD1-4 and MBD12. The CW abbreviation refers to the conserved cysteine and tryptophan residues in a zinc-finger structure, the DNA-binding motif, located near the N-terminal. Recently, our group demostrated that Arabidopsis and maize plants deficient in CW-MBD proteins are less tolerant to different genotoxic conditions. Because plants are sessile organisms, they are exposed to various genotoxic agents, such as UV-B radiation, drought, and salinity. In particular, UV-B radiation damage DNA by generating pyrimidine cyclobutane dimers (CPDs) that distort the double helix, impairing different vital functions. In this work, we present the characterization of a *mbd1234* quadruple mutants grown under UV-B conditions that damage the DNA, together with its comparison with triple *mbd124* and *mbd234* triple mutants. Our results show that *mbd1234* quadruple mutants accumulate higher accumulation of CPDs in the DNA when exposed to UV-B radiation than triple mutants or WT plants. However, 1 day after UV-B exposure, *mbd1234* have a similar number of dead cells as the triple mutants, but lower number of dead cells than WT plants in the root meristematic zone. Interestingly, UV-B similarly affects cell proliferation in the root meristematic zone of WT plants and in the quadruple and in the triple mutants. Together, our results demostrate that CW-MBD proteins have important roles in maintaining genome stability under genotoxic stress conditions, and

some of these roles are non-redundant, such as during DNA damage repair, while others roles could be similarly accomplished by several CW-MBDs.

PL-15

SELECTIVE AUTOPHAGY VIA NBR1 MODULATES ABSCISIC ACID SIGNALING TO BALANCE GROWTH AND DEFENSE RESPONSES UNDER HIGH TEMPERATURE

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Autophagy is a conserved eukaryotic process that maintains cellular homeostasis and regulates stress responses and growth through selective degradation and recycling of cellular components. In plants, autophagy also contributes to immunity, balancing growth and defense. Cargo receptors such as NBR1 (Neighbour of Breast cancer 1) confer selectivity by recognizing specific substrates and delivering them to the autophagic machinery. High temperatures promote thermomorphogenesis, reshaping development by altering hormone signaling, but also increasing susceptibility to pathogens. In particular, the accumulation of abscisic acid (ABA) has been associated with enhanced pathogen susceptibility. We previously showed that infection by *Pseudomonas syringae* enhances autophagic flux and NBR1 turnover in *Arabidopsis thaliana* under high temperatures (29 °C). Interestingly, *nbr1-2* mutants grown at 29 °C were more susceptible to *P. syringae* pv. *maculicola* (*Psm*). Here, we investigated the role of NBR1 in growth and defense responses under high temperature. Physiological and growth traits associated with elevated temperature, including hormone levels, expression of hormone-related genes, petiole elongation, and photosynthetic pigments content, were analyzed in wild-type and *nbr1-2* plants infected with *Psm* and grown at 22 °C or 29 °C. Loss of NBR1 led to increased accumulation of chlorophylls and carotenoids, and marked changes in hormone-related gene expression at 29 °C. Notably, ABA biosynthesis and signaling were enhanced in *nbr1-2* mutants. Consistently, ABI5 protein accumulated in *nbr1-2* and physically interacted with NBR1, suggesting that NBR1 directs ABI5 turnover through autophagy. The associated changes in leaf petiole elongation and pigment concentration further support a role for NBR1 in modulating growth and stress responses during thermomorphogenesis. These findings identify NBR1 as a novel regulator of ABA signaling and plant defense under elevated temperatures, linking selective autophagy to hormonal crosstalk in stress adaptation.

PL-16

CHARACTERIZATION OF PLASTID-LOCALIZED IMMUNE RECEPTORS AS BIOTECHNOLOGICAL TOOLS TO ENHANCE DISEASE RESISTANCE IN TOBACCO AND SOYBEAN

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Plants defend themselves against a wide range of pathogens through innate, non-adaptive immunity mediated by immune receptors. Nucleotide-binding Leucine-rich repeat receptors (NLRs) are key components that recognize pathogen effectors and activate effector-triggered immunity, while closely related NLR-like proteins, such as members of the RPW8/HR family, also contribute to pathogen perception. Subcellular localization of NLRs is critical for their function, and plastids play a particularly relevant role during pathogen perception, as they are sites of reactive oxygen species (ROS) production, defense hormone biosynthesis, and retrograde signaling. Many pathogen effectors specifically target plastids, highlighting their importance in plant–pathogen interactions. Despite this, only two plastid-localized NLRs have been described to date—one identified in our laboratory—and none has been fully characterized. This gap limits our understanding of plastid immune signaling and its potential applications in crop improvement. In a screening for plastid-targeted proteins, we identified a novel N-terminal signal for plastid (or mitochondrial) targeting, predicted in several *Arabidopsis thaliana* NLRs, including the receptor BURNOUT1 (BNT1). BNT1 is required for resistance to both the aphid *Myzus persicae* and the bacterium *Pseudomonas syringae*. Additional candidate genes include the NLRs *AT5G18370* and AT5G38340, predicted to localize to chloroplasts and mitochondria, as well as the NLR-like genes *HR2* and *HR3*, which are likely plastidial and implicated in broad-spectrum resistance, even when expressed heterologously. As proof of concept of their biotechnological potential, *A. thaliana* plastid-predicted NLRs were evaluated through heterologous expression in model and crop plants. Subcellular localization and pathogen assays were performed in *Nicotiana benthamiana*, while functional resistance was tested in *Nicotiana tabacum* (tobacco) and *Glycine max* (soybean) using *Agrobacterium*-mediated transient transformation. Defense activation was evaluated both locally and systemically, providing experimental evidence for the functionality of plastid-targeted NLRs. Given the central role of plastids in plant defense and the limited knowledge of plastid-localized immune receptors, the identification and functional characterization of these genes represent a promising avenue to uncover novel defense mechanisms and to develop biotechnological strategies for improving crop resistance.

PL-17

TWEAKING PROLINE METABOLISM IN *MEDICAGO*

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Environmental stresses, such as drought, heat, and salinity, are the primary causes of lost crop productivity. Legumes of the genus *Medicago*, specifically alfalfa (*Medicago sativa*) and its close relative *Medicago truncatula*, are among the most important forage legumes globally for feeding livestock. As a result of climate change, drought periods have lengthened, and rainfall has become more erratic. Therefore, developing varieties with greater tolerance to environmental stress would significantly help the production of these crops. Proline (Pro) is an amino acid with suggested important functions in plants. In response to different types of environmental stress, plants accumulate Pro in their roots and leaves. This poster presents the

partial results of a doctoral thesis that proposes increasing the drought tolerance of both alfalfa and *M. truncatula* by enhancing their proline accumulation. We will use CRISPR/nCas9 to edit the P5CS2 gene in both *Medicago* species, which is expected to cause a greater Pro accumulation under stress and thereby increase their tolerance. We will then evaluate the performance of these plants under stress conditions and compare it to that of wild-type plants. Another outcome of this research will be P5CS2-KO plants, which will be useful for analyzing the functionality of Pro accumulation in these legumes under stress. We have already transformed alfalfa petioles with the base editing and KO constructs, and we are currently genotyping the transformed events. Subsequently, phenotyping of the transformation events will be performed under saline and drought stress conditions, and plants with the desired phenotype will be selected. To evaluate the genetic modifications in subsequent generations, and within the framework of this project, we also evaluated different conditions of light After quality, light intensity, and photoperiod to determine the optimal conditions for speed breeding both *Medicago* species, intending to significantly reduce their generation time. Here, we present preliminary results from experiments under different photoperiods (16/8, 20/4, 22/2 hours light/dark), light intensities (250, 450, 650 µmol.m-2.s-1), and light types (Blue-Red and Full-spectrum). For each condition, we evaluated flowering time, fruiting time, days to harvest, and number of seeds per fruit.

PL-18

MICRORNAS AS CENTRAL NODES IN PLANT DEFENSE AND ABIOTIC STRESS RESPONSES

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MicroRNAs (miRNAs) are key post-transcriptional regulators of master genes, such as transcription factors, that control plant development, tolerance to abiotic stress, and resistance to biotic stress. Moreover, several miRNAs have been described as regulators of multiple plant traits, including resistance to various stresses. Although individual miRNA sequences are not highly conserved across species, the mechanisms governing their biogenesis and activity, as well as many of their targets, are conserved. We evaluated the roles of two specific miRNAs in responses to both biotic and abiotic stress. Using mutants in the corresponding *MIR* genes, we found that these miRNAs modulate resistance/tolerance to pathogens, heat, and dark stress. RNA-seq and sRNA-seq analyses revealed that the abundance of these miRNAs is modulated under different stress conditions. We also examined the expression of their previously described mRNA targets, as well as additional targets predicted in silico. Because these miRNAs are not widely conserved among plants —including crops— identifying conserved targets and developing approaches to modulate their activity could provide a novel crop-breeding strategy to enhance tolerance to multiple stresses.

PL-19

GLYCOSIDE HYDROLASES FROM *STREPTOMYCES* SP. N2A MEDIATE PLANT–MICROBE INTERACTION, ENHANCING GROWTH AND DEFENSE IN *ARABIDOPSIS THALIANA*

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The use of plant growth-promoting rhizobacteria (PGPR) offers a promising strategy for sustainable agriculture, providing a biological alternative to traditional agrochemicals. In this context, our group previously isolated and characterized *Streptomyces* sp. N2A from soybean roots (*Glycine max* [L.] Merr.), demonstrating its ability to promote soybean growth and yield, as well as to confer protection against fungal phytopathogens. Subsequent studies revealed that *Streptomyces* sp. N2A also establishes beneficial interactions with other plants, including *Arabidopsis thaliana*, enhancing development and offering protection against bacterial pathogens. In the present work, we explored the potential role of glycoside hydrolases (GHs) encoded by *Streptomyces* N2A in mediating plant–microbe interactions. To this end, *A. thaliana* Col-0 seeds were treated with either the wild-type N2A strain or GH-deficient (cellulase-null) mutants, and grown under controlled conditions. Several developmental parameters were assessed, including number of leaves, rosette area, rosette and root dry weight, silique weight, flowering time, and stem height. Plants inoculated with the wild-type strain showed significant improvements in all parameters compared to non-inoculated controls ($p < 0.05$), confirming its growth-promoting activity. Conversely, at least two GH-null mutant strains failed to reproduce these effects, indicating that specific glycoside hydrolases are required for the promotion of plant growth. These results suggest that GHs may facilitate bacterial interaction with the plant root system, potentially through remodeling or recognition of cell wall components, which could be essential for successful colonization and signaling. In addition to growth promotion, we examined whether *Streptomyces* sp. N2A could enhance resistance to biotic stress. Inoculated *A. thaliana* plants exhibited increased tolerance to *Pseudomonas syringae* pv. tomato DC3000 infection, as evidenced by a reduction in disease symptoms. Evaluation of biocontrol behavior by GH-deficient strains is under analysis. This result will reinforce the idea that GHs may also contribute to defense priming mechanisms. Altogether, our findings provide evidence that GHs from *Streptomyces* sp. N2A could play a role both in plant growth stimulation and in defense induction. These insights deepen our understanding of beneficial plant–microbe interactions and support the development of microbial-based bioinputs for sustainable crop production.

PL-20

EFFECT OF ENVIRONMENTAL FACTORS ON DORMANCY DEPTH AND PHYSIOLOGY OF GRAPEVINE BUDS (CV. TANNAT)

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Grapevine buds (*Vitis vinifera* L.) undergo a seasonal state of dormancy in order to survive unfavorable conditions and synchronize growth with environmental cues. Although dormancy

transitions have been widely studied in perennial woody species from temperate climates, little is known about these dynamics in buds of the Tannat variety, the most important cultivar in Uruguay. Furthermore, in grapevine, the physiological and metabolic dynamics underlying these transitions remain poorly understood. Our three-year consecutive study on *V. vinifera* cv. Tannat, conducted in vineyards located in Canelones, Uruguay,
investigates the effect of seasonal changes on bud dormancy depth, physiology, and metabolism. During January and February, limited budburst was observed under forcing conditions. From May onwards, the time required for budburst progressively decreased. Similarly, cellular respiration reached its lowest values in April, suggesting that dormancy depth peaks prior to May. Bud and cane water content progressively decreased from January, reaching a minimum during the coldest months, followed by a subsequent increase until budburst. In addition, in non-dormant buds we investigated how temperature and light promote budburst by activating metabolism, with a particular focus on respiration and potential photosynthetic activity. Following the completion of this third year of sampling, we aim to conduct transcriptomic and metabolomic experiments in buds to identify the biochemical signals that play a key role in transitions of varying dormancy depth. This information will be especially relevant for developing eco-friendly alternatives, either chemical or management-based, to synchronize grapevine budburst and to prevent yield losses caused by premature budburst.

PL-21

STUDIES ON REDOX-BASED MECHANISMS REGULATING THE MITOTIC CELL CYCLE IN ARABIDOPSIS THALIANA.

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The final size of plant organs depends on both the number of cells produced in meristematic regions and the magnitude of cell expansion. Mitotic divisions in meristems determine cell number, making the precise regulation of the mitotic cell cycle (CCM) a key factor determining plant organ size and shape. After division, cells expand and differentiate to acquire their final size, shape, and function. The transcriptional control of cell cycle–associated genes is mediated by several transcription factors, among which MYB3R proteins are central regulators that activate G2/M-specific genes through binding to MSA promoter elements. We hypothesize that redox-dependent post-translational modifications of cysteine residues act as key regulators of cell cycle progression, linking the cellular redox state with mitotic processes. Glutaredoxins (GRXs), small thioredoxin-fold proteins that catalyze glutathione-dependent redox reactions, are strong candidates for this role. GRXs regulate cysteine oxidation states, modulate protein glutathionylation, and participate in oxidative stress responses. We analyzed the transcriptome of *Arabidopsis thaliana* cells at G2/M and identifying hundreds of genes enriched in this phase, including a GRX-coding gene. This finding suggests a potential role for this GRX in mitotic regulation. Current work focuses on characterizing this mitotic GRX at both transcriptional and functional levels, evaluating its regulation by MYB3R factors, and analyzing the phenotypic effects of altered GRX expression. Also, using several redox-sensors and laser scanning confocal microscopy we aim to analyze the redox status of the cells and their compartments

during the different phases of the cell cycle. Our study aims to elucidate the contribution of redox regulation to mitotic progression in plants, with a particular emphasis on the role of GRXs. Integrating transcriptional networks with redox-based post-translational control is expected to provide novel insights into how plant cells coordinate proliferation with environmental and metabolic cues, ultimately revealing new regulatory layers that shape organ growth and plant development.

PL-22

EXPLORING THE SUBCELLULAR LOCALIZATION OF P5CS1: A KEY ENZYME IN PROLINE ACCUMULATION

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Under abiotic stress conditions, the physiological and metabolic processes of plants are altered, including the biosynthesis and accumulation of proline. The P5CS1 enzyme, responsible for proline accumulation, has a cytoplasmic subcellular localization. However, fusions of this enzyme with GFP have shown controversy regarding the subcellular localization of P5CS1, as some authors have demonstrated that it could be localized to the chloroplast under osmotic stress, while others insist that its localization is strictly cytoplasmic. In this research, we performed cellular fractionation assays coupled with targeted proteomics to determine if the P5CS1 protein could be detected in chloroplasts as well as confocal microscopy (Zeiss LSM800)
in transgenic lines that express the protein fused to GFP (pUBC:P5CS1GFP and pP5CS1:P5CS1GFP), under control, salinity (150 mM NaCl, 3 days), and drought (10-12% field capacity) conditions. The characterization of these lines also includes analysis of proline accumulation and gene expression. This study will provide evidence on the possibility that under stress conditions, P5CS1 can relocalize to chloroplasts, either by chaperone mechanisms or an increase in chloroplast permeability, where it could balance chloroplastic NADP/NADPH levels and thus reduce photoinhibition.

PL-23

COMPARATIVE ANALYSIS OF THERMOMORPHOGENESIS AND AUTOPHAGY IN *ARABIDOPSIS THALIANA* AND *PHYSCOMITRIUM PATENS*

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High global temperatures associated with climate change challenge plant survival and demand developmental plasticity. Thermomorphogenesis, the growth remodeling triggered by mild heat, provides an evolutionary framework to investigate how conserved mechanisms shape adaptive responses. We performed a comparative analysis of thermomorphogenic

growth and the role of autophagy in two phylogenetically distant model species, the angiosperm *Arabidopsis thaliana* and the bryophyte *Physcomitrium patens*. Plants were grown at 22 °C under a 12:12 photoperiod and transferred to 22 °C (control) or 29 °C (thermomorphogenesis conditions). In Arabidopsis, we quantified leaf area, primary root length, number and length of lateral roots, leaf angle, and hypocotyl elongation. At the same time, in *P. patens,* we evaluated gametophytic traits, including colony area, developmental changes in chloronemata and caulonemata, the number of gametophores, phyllid expansion, and rhizoid length. In Arabidopsis, exposure to 29 °C triggered the typical thermomorphogenic response that facilitates heat dissipation and cooling, such as pronounced hypocotyl elongation and leaf hyponasty. In moss, exposure to 29 °C reduced colony area, enhanced protonemal branching, and increased gametophore production, revealing a profound remodeling of gametophytic development. By contrast, at 22 °C protonemata growth was favored, with colonies expanding mainly in 2 dimensions and producing fewer gametophores. Thus, mild heat appears to shift growth priorities from 2-dimensional expansion to 3-dimensional development. Comparative analysis of wild-type and autophagy-deficient mutants revealed differences in their response. Remarkably, exposure to 29 °C promoted autophagic flux in both species, detected using the GFP-ATG8a reporter. These results indicate that autophagy is a conserved regulatory hub connecting heat perception, developmental remodeling, and cellular homeostasis. In addition, our findings highlight autophagy as an ancient mechanism contributing to plant resilience under climate warming.

PL-24

TAILORING SOYBEAN RESILIENCE: NOVEL APPROACHES USING HD-ZIP I TFS FOR SOYBEAN STRESS RESILIENCE AND YIELD

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Soybean is a globally important crop, particularly in Argentina, yet its yield increases have lagged behind others like maize or wheat. This is largely due to its exclusion from the Green Revolution and its susceptibility to abiotic stresses, compounded by a breeding focus on oil and protein content. While optimized agricultural practices have driven a linear yield increase of ~1.50% annually since 1960, genetic modification offers a path to developing drought and heat-tolerant varieties. Transcription factors (TFs) are promising candidates for enhancing resilience. Our research focuses on *HaHB4* and *HaHB11*, divergent sunflower HD-Zip I TFs previously shown to increase yield and abiotic stress tolerance when ectopically expressed in *Arabidopsis* and other plant species. Preliminary lab results indicated that combining aerial and root parts from different transgenic genotypes can further improve resilience and yield. To corroborate this, we generated grafted *Arabidopsis* plants using various transgenic lines, observing enhanced seed yield. For instance, *HaHB4* transgenic roots combined with MIT aerial parts resulted in grafted plants yielding two to three times more than controls. To achieve stable chimeric plants, we isolated root- and shoot-specific promoters and transformed both *Arabidopsis* and soybean, validating expression via transcript levels and GUS histochemistry. Additionally, we employed a strategy using constructs to generate small RNAs derived from the studied TFs in soybean. Among these new plants, two genotypes showed enhanced tolerance to water deficit stress applied during the seedling stage compared with HB4 and wildtype soybeans. While promising, further studies are needed to confirm these improved

soybean varieties and elucidate the underlying molecular mechanisms of their enhanced tolerance.

PL-25

RHAMNOLIPID NANOEMULSIONS ENRICHED WITH ESSENTIAL OILS: DISINFECTANT AND PRIMING EFFECTS ON LEGUMINOUS PLANTS OF AGRICULTURAL RELEVANCE

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Common bean (*Phaseolus vulgaris*) is the third most consumed legume worldwide, and Argentina is its main exporter. Together with soybean, it represents a cornerstone of Argentine agriculture. However, legume production is strongly affected by climatic stresses and diseases caused by fungi, viruses, and bacteria. Among them, bacterial blight caused by *Xanthomonas spp*. and *Pseudomonas syringae pv. phaseolicola* represents one of the most damaging diseases. It manifests as yellow-green halo lesions on leaves, pods, stems, or petioles, leading to significant yield losses worldwide. Control is difficult, as no curative treatments are available and preventive measures rely mainly on chemical seed disinfection with fungicides and bactericides. Although effective, these compounds entail high economic costs, environmental contamination, risks to human health, and promote the selection of resistant pathogens. In search of sustainable alternatives, we developed nanoemulsions (NEs) based on rhamnolipids (RLs), amphiphilic biosurfactants with well-known antimicrobial properties, low ecotoxicity, and the ability to stimulate the innate immune system of plants. RLs also act as natural emulsion stabilizers, enabling the encapsulation of hydrophobic bioactive molecules such as essential oils (EOs). This encapsulation improves EOs stability, delivery, and efficacy. We prepared RLs-based NEs containing EOs from rue, thyme, and tea tree, and tested their activity both in model and crop plants. In *Arabidopsis thaliana*, foliar application of these formulations improved immunity against *Pseudomonas syringae pv. tomato DC3000* without detrimental effects on beneficial soil microorganisms or bioinoculants commonly used in agriculture. In beans, however, foliar pre-treatments with NEs did not visibly reduce symptoms caused by *Xanthomonas* infection, although RLs showed a direct inhibitory effect on its bacterial growth *in vitro*. Considering that the use of certified, disinfected, pathogen-free seeds is one of the most effective preventive strategies, we are currently evaluating the potential of RLs-based NEs as seed treatments (“seed dressings”). Specifically, we analyze their dual capacity to (i) act as disinfectants reducing seed-borne bacterial inoculum, and (ii) induce innate immune resistance mechanisms in legumes of agronomic importance. Preliminary results indicate that seed pre-treatments with NEs contribute to achieve clean seeds and reduce pathogen symptoms in young plants, without affecting the normal plant development. Our findings support the use of RLs-based NEs as safe, biodegradable, and economically viable biopesticides. These formulations represent an innovative alternative to synthetic pesticides, offering a sustainable tool to enhance plant health, reduce yield losses, and foster circular economy practices in legume production.

PL-26

PROTEOMIC ANALYSIS OF MINIMALLY PROCESSED RUBY PRINCE PEACH FRUIT EXPOSED TO GAMMA IRRADIATION

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Minimally processed fruits are convenient products highly valued by consumers as they retain their natural characteristics. However, operations such as peeling or cutting damage plant tissues, triggering deteriorative processes like increased metabolism, enzymatic and browning. To mitigate these effects, the application of gamma irradiation (GI), a non-thermal technology, has emerged as a promising research area. The objective of this work was to evaluate the effect on the proteome of a GI treatment on minimally processed 'Ruby Prince' peaches. Fruits were disinfected and cut into slices with skin. The slices were placed on plastic trays, packaged in low gas permeability films and either exposed to GI (dose: 0.2 kGy, sample T) or left untreated (control sample, C). The trays were stored at 4°C and evaluated at the beginning of the experiment (C0, T0) and after 7 days (C7, T7). Proteomic analysis was carried out by label-free quantitation-MS using four replicates for each sample. Differentially abundant proteins (DAPs) were identified using Perseus software. Proteins with a fold-change ($|FC| \geq 1.5$) and $p \leq 0.05$ were considered for the analysis. DAPs were functionally classified using MapMan v4. A total of 2771 different proteins were analyzed, of which 456 varied in C7 vs. C0, 134 changed in T0 vs. C0, 507 resulted differentially accumulated in T7 vs. T0 and 96 changed in abundance in T7 vs. C7. Only 16 DAPs were common between the C7 vs. C0 and T7 vs. T0 comparisons. Eighty-eight proteins were exclusively modulated in T7 vs. C7. Within this comparison, unknown was the most represented category, followed by protein metabolism (biosynthesis, homeostasis and modification). Interestingly, GI caused a decreased in 1-aminocyclopropane-1-carboxylate oxidase, enzyme involved in ethylene synthesis; suggesting a decrease in ethylene in GI in comparison with untreated peach slices which would help to preserve the samples from decay. On the other hand, increases in different isoforms of alcohol dehydrogenases, together with decreases in pyruvate dehydrogenase in T7 vs. C7 and T0 vs. C0 suggest an induction of fermentative metabolism in GI-treated slices. A significant fraction of the DAPs belonged to cell wall metabolism, revealing that GI modifies the cell wall. Furthermore, repression of enzymes related to carotenoid, phenylpropanoid, flavanones and anthocyanidins were observed in GI samples, which could influence both the colour of the treated samples as well their properties in terms of bioactive compounds. Overall, this study identified DAPs in GI-treated peaches and demonstrates the potential of proteomics as a powerful tool to investigate the molecular mechanisms underlying the effects of gamma irradiation, revealing changes in protein expression profiles that influence not only fruit quality but also shelf life.

PL-27

FUNCTIONAL INSIGHTS INTO PI3K COMPLEXES I AND II IN THE BRYOPHYTE *PHYSCOMITRIUM PATENS*

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Phosphatidylinositol-3-phosphate (PI3P) is a key regulatory lipid governing membrane dynamics and intracellular trafficking. In plants, PI3P is synthesized exclusively by type III PI3Ks, VPS34, which functions within two distinct complexes sharing three core subunits (VPS34, VPS15, ATG6), and a fourth defining subunit. This subunit is ATG14 in Complex I, which acts in the pathway of autophagy, and VPS38 in Complex II, which is involved in endocytosis. Although well-characterized in yeast and mammals, the composition and regulation of these complexes in plants remain poorly understood. Here, we characterized the different subunits of PI3K complexes I and II in the moss *Physcomitrium patens* using a bioinformatic approach. While the core subunits PpVPS34, PpATG6 and PpVPS15 are highly conserved compared to yeasts and humans, we found that PpATG14 and PpVPS38 display plant-specific features. Unlike human ATG14/BARKOR, plant ATG14 lacks a C-terminal BATS/ALPS domain but contains a disordered region ending in a hydrophobic helix (HH). Its hydropathic profile and amino acid composition differ from human ALPS but identical to the one found in *Nicotiana benthamiana* ATG14. On the other hand, PpVPS38 lacks the entire lipid-interacting C2 domain and the C-terminal regulatory region found in human UVRAG, suggesting a distinct regulatory mechanism. Using homologous recombination, we generated *atg14* knockout moss plants displaying canonical autophagy-deficient phenotypes. In addition, we obtained CRISPR-Cas9–edited *vps38* mutants carrying in-frame deletions which exhibited distinct phenotypes. A ΔGGL deletion in a conserved hinge region triggered premature senescence, a hallmark autophagy-deficient phenotype. In contrast, mutations in the C-terminal BARA2 domain resulted in gravitropism defects. *In silico* modeling indicates that a ΔGGL mutation disrupts PpVPS38-PpATG6 interaction, impairing autophagy, whereas BARA2 domain mutations prevent interaction with PpVPS15's WD40 domain, abrogating endocytosis. This mechanistic dissection might explain the uncoupled phenotypes. Our results suggest that in bryophytes, PI3K-CII has dual functionality, co-regulating autophagy and endocytosis through distinct PpVPS38 domains. The unique plant-specific structure of PpVPS38 highlights the separable contributions of PI3K-CII to central cellular pathways. Further studies, including genetic complementation, are required to fully corroborate these mechanistic insights.

PL-28

MOLECULAR MECHANISMS INVOLVED IN PARASITISM BY *RHIZOBIUM FAVELUKESII* STRAINS IN *MEDICAGO SATIVA*

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Alfalfa (*Medicago sativa*) is a perennial forage legume that forms a symbiosis with rhizobia. In Uruguay, it is inoculated with the *Ensifer meliloti* strain U143, a commercial inoculant recommended by the Department of Livestock, Agriculture, and Fisheries (MGAP). Although

alfalfa associated with specific rhizobia achieves good yields (approx. 210 kg of $N.ha^{-1}.year^{-1}$), the presence of inefficient or parasitic strains in some soils leads to suboptimal biomass production or the failure of the pasture. This group includes strains of the Oregon type, such as *Rhizobium favelukesii* ORY1, which are tolerant to acidic soils and parasitic to alfalfa. To characterize the parasitism of the *R. favelukesii* ORY1 strain, a pot trial was conducted by inoculating with this strain and using as controls, the efficient strain (*E. meliloti*), the legume with nitrogen, without nitrogen, and without inoculation. To identify the biological processes differentially modulated in alfalfa by the parasitic strain ORY1 relative to the efficient strain, proteomic assays were performed. Alfalfa was inoculated with *E. meliloti* strain U143 (efficient), with *Rhizobium favelukesii* strain ORY1 (parasitic), and an un-inoculated control. Root samples were taken at different times post-inoculation, 48h and 7 days, and root samples with nodules were taken at 21 days post-inoculation, with 4 biological replicates in each case. Soluble proteins were extracted and the proteins of *M. sativa*, *E. meliloti*, and *R. favelukesii* were analyzed by mass spectrometry. The differentially abundant proteins between the roots of the treatments were identified for each time, and an enrichment analysis of the differentially abundant proteins will determine the biological processes induced or repressed in the presence of inefficient strains. In addition, we will evaluate after 35 days, the accumulated biomass, phenotype, Green Index, and number of nodules and at 21 days post-inoculation, the nodule formation kinetics in tubes for, along with biochemical stress indicators (TBARS, proline) and photosynthetic rate. These results will contribute to a better understanding of the plant-microorganism interaction and will elucidate the molecular mechanisms involved in parasitism.

PL-29

GENOMIC CHARACTERIZATION OF URUGUAY-DEVELOPED SOYBEAN (*GLYCINE MAX*) VARIETIES

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Soybean (*Glycine max*) is the most important legume crop in Uruguay, where breeding programs have developed locally adapted cultivars with stable yields, particularly under early-season drought conditions. However, current genomic resources are limited, as a single reference genome is insufficient to capture the full genetic diversity of this species. To address these gaps, this study proposes the first comprehensive genomic characterization of three nationally developed soybean cultivars (Génesis 6301, SJC13621, and SJC13625). This study, framed within an international collaboration between Uruguay and Korea, aims to valorise local genetic resources and support the development of high-value bio-based products. Our goals are: (i) to optimize protocols for isolating high molecular weight DNA for single-molecule sequencing; (ii) to sequence, assemble, and annotate the genomes of the three cultivars, identifying SNPs and structural variants; (iii) to perform phenological and physiological characterizations under controlled drought conditions. The methodological strategy combines long-read sequencing (ONT) for resolving complex and repetitive genomic regions with short-read sequencing (Illumina) for polishing assemblies and variant calling. Comparative genomics will enable the construction of a pangenome, distinguishing core and accessory regions among

the cultivars. Finally, phenotypic evaluations under water stress will be integrated with genomic data, providing insights into molecular mechanisms underlying yield stability and drought tolerance. This integrative approach will not only generate valuable genomic resources for Uruguay but also establish a foundation for future multi-omics studies and the development of bio-inputs from locally relevant soybean varieties.

PL-30

FUNCTIONAL ANALYSIS OF THE EFFECTOR HaRXL45 AND ITS INTERACTION WITH TCP14 IN ARABIDOPSIS

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Plants possess innate defense mechanisms that enable them to resist many microorganisms. However, some pathogens have evolved strategies to evade or manipulate these defenses by secreting effector proteins, which are translocated into host cells and act on plant targets. A model case is the oomycete *Hyaloperonospora arabidopsidis* (Hpa), the causal agent of downy mildew in the model plant *Arabidopsis thaliana*. This pathogen delivers RxLR-type effectors that interfere with both immunity and plant development. The goal of this particular project is to unravel the molecular and physiological mechanisms by which an Hpa RxLR effector, HaRxL45, interacts with and manipulates the transcription factor TCP14, which plays dual roles in cell proliferation and in the regulation of salicylic acid–dependent defenses. Understanding this interaction will help identify if TCP14 is a susceptibility factor (SF) that could be modified by gene editing to enhance resistance without impairing development. Previous results indicate that expression *in planta* of two different versions of HaRxL45 (ES and EL) increase host susceptibility to bacterial pathogens in different *A. thaliana* ecotypes. We also observed that delivery of HaRxL45EL via *Pseudomonas syringae* (Pst) into DR5::GFP reporter plants increases the expression of GFP, indicating that this effector might be altering auxin responses. Auxins are hormones that play a central role in plant growth and development. The observed phenotypes could be due to the modulation of TCP14 activity, which participates from this hormonal pathway. We modeled *in silico* the interaction between both versions of HaRxL45 and TCP14 in order to generate hypothesis about how this effector might be modulating the activity of the transcription factor. We also generated *A. thaliana* transgenics over expressing GFP-tagged versions of both effectors and of the plant target (TCP14-RFP). Single transgenics (GFP-HaRxL45ES/EL) did not showed visible alterations in growth and development of leaves and flowers. Conversely, plants expressing TCP14-RFP showed an altered architecture of the floral stem, as previously described by other groups. Double transgenics of TCP14-RFP containing GFP-HaRxL45ES showed a severe decrease in the generation of seeds. We are currently evaluating the susceptibility of all these transgenics to Hpa and Pst at different growth stages (seedlings, adult plants). We will also perform chromatin immunoprecipitation (ChIP-qPCR) assays comparing TFP14-RFP lines with those also expressing the effector versions. Together, these tools will shed light on how Hpa effectors exploit the trade-off between growth and immunity in plants. Ultimately, this knowledge could contribute to the development of crops with more durable and sustainable resistance to biotrophic pathogens of agricultural relevance.

PL-31

TRANSCRIPTOMIC ANALYSIS OF TWO TOMATO ISOLINES IN RESPONSE TO THE FUNGUS *STEMPHYLIUM LYCOPERSICI*: RESISTANCE MECHANISMS MEDIATED BY THE *SM* LOCUS

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The tomato (*Solanum lycopersicum L.*) is a crop of great global importance and the second most important in Uruguay. Gray leaf spot, caused by fungi of the genus Stemphylium, is a devastating disease that causes significant losses in susceptible varieties. Within the framework of the INIA breeding program, tomato isolines with different levels of resistance to Stemphylium were generated by incorporating the *Sm* resistance locus. To identify the mechanisms associated with this resistance, the isolines were inoculated with a virulent isolate of *S. lycopersici* (UYSLS32). Clear symptoms were observed in the susceptible isoline 5 days post-inoculation (dpi), with progression to necrosis and chlorosis at 9 dpi. In contrast, the resistant isoline showed only small spots at 9 dpi and no significant symptoms at 5 dpi. The transcriptomes of both isolines were analyzed in untreated plants and at 5 and 9 dpi compared to a water-only control. In untreated plants, 320 differentially expressed genes (DEGs) were identified between the isolines. Gene ontology (GO) enrichment analysis revealed that, compared to the susceptible isoline, the resistant isoline showed an induction of DEGs in biological processes such as protein export from the nucleus, coumarin synthesis, response to biotic stimuli, response to stress, and jasmonic acid regulation. At 5 dpi, the resistant isoline showed an increase in the expression of 257 DEGs, while the susceptible showed 688 DEGs. In the resistant strain, DEGs were enriched in molecular functions such as antioxidant activity, catalytic activity, and abscisic acid binding, as well as in biological processes such as catalysis of cell wall components, detoxification, response to stress and biotic stimuli, and chitin catabolism. At 9 dpi, the resistant strain showed 136 induced DEGs, while the susceptible strain presented 384 induced DEGs. In the resistant strain, enrichment was observed in genes associated with hormone signaling pathways and signal transduction systems. On the other hand, the susceptible strain showed enrichment in molecular functions such as polysaccharide and chitin binding, oxidoreductase activity, protein tyrosine kinase activity, and transcription factors. These results demonstrate that the resistant strain presents activation of defense-related genes, which could explain the observed resistance to *S. lycopersici*. Confocal microscopy revealed alterations in the cell wall during infection, along with a differential accumulation of reactive oxygen species (H_2O_2 and O_2^-): in the resistant isoline, they were observed mainly in mesophyll cells, while in the susceptible one, they were detected in epidermal cells located in the lesion area.

PL-32

POLYAMINE AND HORMONAL CROSSTALK IN ANTHOCYANIN-DEFICIENT *Arabidopsis thaliana* MUTANTS UNDER IRON DEFICIENCY

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Iron (Fe) is essential for plant growth, but its low availability in alkaline soils hinders absorption, affecting agricultural production and crop nutritional value. This exacerbates Fe deficiency in the human diet, especially in regions where plants are the main dietary source of this mineral. Flavonoids perform multiple functions in plants, including UV protection, pollinators attraction and defense against pathogens and herbivores. In Arabidopsis, Fe uptake follows Strategy I, involving rhizosphere acidification by H+-ATPases, ferric reduction by FRO2, and transport into roots through IRT1, regulated by FIT and bHLH transcription factors. Previous studies showed that flavonoid-pathway mutants display opposite regulation of Fe-deficiency genes: *tt4* exhibits repression of *bHLH38*, *bHLH39*, *IRT1*, *FEP2*, *BTSL1* and *BHLH100*, while *tt19* shows induction of these, linking flavonoids to Fe homeostasis. Polyamines and phytohormones have emerged as key regulators of redox balance, cell division, and adaptive growth, but their interplay with flavonoids during Fe deficiency remains uncharacterized. We aim to analyze the interaction between polyamines and hormones in two representative mutants *tt4*, lacking flavonoids due to a chalcone synthase mutation, and *tt19*, defective in vacuolar anthocyanin transport. Plants grown for 10 days on MS agar were transferred for 5 days either to Hoagland medium with (Fe-sufficient) or without Fe (Fe-deficient), and 15-day-old seedlings were analyzed. Free polyamines (putrescine, spermidine, spermine) were extracted and separated by HPLC. Hormones (ACC, IAA, ABA, SA, JA) were extracted in aqueous ethanol and quantified by LC-MS/MS in MRM mode using calibration curves with pure standards. Results revealed that under control conditions *tt19* accumulated higher levels of putrescine and total polyamines, whereas *tt4* showed intermediate values. Under Fe deficiency, putrescine and spermidine strongly decreased in all genotypes, while spermine remained more stable; notably, *tt19* maintained higher spermine and total polyamines than Col-0 and *tt4*, suggesting a more efficient compensatory strategy. Hormonal profiles showed that *tt4* accumulated more ACC in control but reduced it under Fe–, while Col-0 and *tt19* increased ACC in Fe deficiency, consistent with the role of ethylene in activating Strategy I. Salicylic acid was strongly induced in Col-0 and *tt4* but attenuated in *tt19*. Jasmonic acid was highest in *tt19* in control but decreased in all lines under Fe deficiency. Abscisic acid decreased in all genotypes under Fe–, and auxin did not show major differences. Altogether, these results indicate that flavonoid deficiency reshapes polyamine and hormonal integration during Fe deficiency, reinforcing the concept that these metabolic networks converge to modulate iron homeostasis and providing new insights into the adaptive strategies of plants facing nutrient limitations.

STRUCTURAL BIOLOGY

SB-1

Single-Molecule Insights into Drug–DNA Interactions for Nanomedicine Design

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The development of nanovehicles for drug delivery requires encapsulating materials that are both functional and biocompatible. DNA has recently emerged as a promising candidate, offering biodegradability and responsiveness to small molecule binding, which may be exploited for the controlled assembly of drug-loaded nanostructures. A critical challenge is to understand how therapeutic drugs interact with DNA at the molecular level and whether such interactions induce compaction, a prerequisite for nanovehicle formation. Single-molecule analysis with optical tweezers provides a direct strategy to address this challenge. By monitoring changes in DNA mechanics upon drug binding, this approach allows us to identify whether a molecule promotes stabilization, elongation, or compaction of the double helix (mechanistic features directly linked to its potential for nanomedicine applications). In this study, we aimed to investigate the mechanical alterations induced by propranolol (PPL), a hydrophilic aromatic low molecular-weight drug (HALMD) on a single DNA molecule, in order to elucidate how direct drug-DNA interactions affect the structural and physicochemical properties of the nucleic acid at the molecular level. A single 10 kbp dsDNA molecule was tethered between two optically trapped beads and subjected to repeated stretching - relaxation cycles, both in the absence and presence of increasing concentrations of PPL. For each PPL concentration tested, between 3 and 5 independent DNA molecules were analyzed, with 10 to 30 cycles recorded per molecule. Experiments revealed two distinct binding regimes. At low concentrations (< 4 mM), PPL acted as an intercalator, as shown by increases in contour length (Lc, from 3.2 to 4.5 µm) and decreases in persistence length (Lp, from 59.9 to 15.1 nm) and stretching modulus (St, from 1225.3 to 220.3 pN). These changes occurred under equilibrium conditions, with near-complete overlap of extension and relaxation curves, indicating rapid drug binding. Fractional elongation measurements indicated that PPL binding deviated from classical hyperbolic behavior, exhibiting a sigmoidal profile suggestive of cooperative or complex binding mechanisms. At higher concentrations (> 4 mM), stick - release patterns emerged in force - distance curves, reflecting periodic force jumps consistent with DNA compaction. The curves displayed variability in both stick - release profiles and apparent contour lengths, indicative of higher - order molecular organization induced by PPL. This dual behavior, intercalation at low concentrations followed by compaction at higher concentrations, provides proof-of-concept that HALMDs can reorganize DNA into compacted structures. These findings highlight DNA's potential as a biodegradable encapsulating matrix and demonstrate how single-molecule biophysics can inform the rational design of DNA-based nanovehicles.

SIGNAL TRANSDUCTION

ST-2

PDK1 COMPLEXES: UNVEILING STRUCTURE AND ALLOSTERIC REGULATION

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Protein kinases function as essential ON–OFF switches in cells, and their dysregulation is linked to diseases such as cancer and diabetes. PDK1 is a master AGC kinase downstream of the PI3K signaling pathway, phosphorylating at least 23 AGC kinases in mammals, including Akt/PKB, S6K, SGK, PKC, and PRK isoforms. Orthologs of PDK1 are conserved across eukaryotic evolution, including in plants, yeasts, and protozoa. Our laboratory has applied chemical biology approaches to investigate PDK1 regulation. PDK1 engages most kinase substrates via a docking mechanism in which a hydrophobic motif (HM) of the substrate binds to PDK1´s regulatory site known as the PIF-pocket. We demonstrated a bidirectional allosteric mechanism within the kinase domain, involving communication between the active site and the PIF-pocket. Previously, we showed that small molecules binding the active site can modulate HM peptide interactions at the PIF-pocket, either enhancing or inhibiting them. For example, the PDK1 inhibitor GSK2334470, which binds at the ATP site, disrupts interactions with the polypeptide PIFtide, which corresponds to the C-terminal region of the PDK1 substrate PRK2. We now validate that it disrupts the interaction with the whole kinase partner PRK2. Most importantly, we had identified that the metabolite adenosine enhanced the interaction of PDK1 with PIFtide. We wanted to check if other metabolites could bind at PDK1´s ATP-binding site and modulate its complexes. We will present the results of a screening for metabolites that interact with PDK1. Currently, we are preparing high-quality PDK1 for crystallography studies to depict at a molecular level the binding site of these metabolites. Also, we are preparing PDK1–kinase substrate complexes suitable for cryo-EM studies. Finally, to complement the above biochemical data, we generated AlphaFold models of diverse PDK1 complexes. Altogether, we are building an integrative structure-dynamic model to understand the mode of action of the compounds on PDK1-substrate complexes. Our work aims to provide insights into the possible “reverse allosteric” regulation of protein kinase complexes by interaction with metabolites. Such mechanisms may play a role in the physiological modulation of protein complexes and guiding future structural and therapeutic strategies targeting multiprotein assemblies.

ST-3

CHARACTERIZING THE PKA CATALYTIC ISOFORM-SPECIFIC ASSOCIATED PROTEOMES IN QUIESCENT *SACCHAROMYCES CEREVISIAE* CELLS

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In response to nutrient scarcity, *Saccharomyces cerevisiae* cells arrest the cell cycle and enter a reversible quiescent state that allows them to survive and resume growth once nutrients become available again. Protein kinase A (PKA) signals nutrient availability and thereby regulates both quiescence establishment and growth. *S. cerevisiae* PKA is a tetrameric holoenzyme consisting of a regulatory subunit (Bcy1) dimer and two catalytic subunits (Tpk1, Tpk2 and Tpk3). During quiescence, Tpk1 and Bcy1 remain diffusely distributed throughout the cytoplasm, while Tpk2 and Tpk3 condense into foci that partially overlap with mRNPs, like PBs

(P-bodies) and SGs (stress granules). Here, we systematically identified the proteomes associated with Tpk1, Tpk2, and Tpk3 in quiescent yeasts using a nanoLC-MS/MS label free quantification approach (QExactive- Thermo Scientific). We found 99 proteins associated with Tpk1, 82 associated with Tpk2 and 25 associated with Tpk3. While most proteins identified were significantly enriched to a single Tpk isoform, a small number of unique and shared proteins were detected across datasets. Specifically, four proteins were identified exclusively in association with Tpk2, whereas one protein was exclusively linked to Tpk1; additionally, 22 proteins were shown to be similar throughout the three Tpks. In order to identify biological functions of proteins in complex with each Tpk, proteins were examined for possible Gene Ontology (GO) term overrepresentations. Those analysis revealed significant enrichments in terms associated with biological processes and cellular components specific to each Tpk protein sets: Tpk1 showed enrichments for TOR complex, the Seh1-associated complex and the snoRNA complex terms; Tpk2 showed enrichment for glycolysis and TCA cycle, cytoplasmic tRNA synthases and 48S translation initiation complex terms; while Tpk3 protein set showed enrichment for terms involved with proteasome, the ribosome, the 43S pre-initiation translation complex and stress granules. Comparison of the proteins identified in each dataset with previously reported stress-induced ribonucleoprotein complexes and with the Tpk- or Bcy1-associated proteome revealed no overlap. However, the quiescent Tpk3-associated proteome showed similarities to a dataset obtained under azide stress. Analysis of proteins uniquely associated with each Tpk revealed that 10-15% contained one or more PKA canonical phosphorylation motifs (RRxS/T). Our findings suggest that the subcellular localization of each Tpk isoform in quiescent cells is determined by a distinct protein interaction network, a mechanism that may contribute to the specificity of each PKA catalytic subunit.

ST-4

SPECIFICITY OF THE CAMP-PKA PATHWAY IN CELLULAR MEMORY OF ACQUIRED HEAT STRESS RESISTANCE IN *SACCHAROMYCES CEREVISIAE*

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In *S. cerevisiae*, cAMP-dependent protein kinase (PKA) is a tetramer composed of two regulatory subunits (Bcy1) and two catalytic subunits (Tpk1, Tpk2, Tpk3). A fundamental question is how cAMP signaling specificity is achieved and how cells ensure phosphorylation of correct substrates under different stimuli. The three Tpk isoforms perform both redundant and specific functions. One of the cellular strategies contributing to PKA signaling specificity is the differential gene expression of these isoforms. Environmental changes disrupt homeostasis, and yeasts cells can acquire tolerance to severe stress after an initial mild pretreatment with the same or a different stressor. This process, known as cellular memory of acquired resistance, is biotechnologically relevant. We previously demonstrated differential expression of Tpk isoforms under stress. Here we show that Tpk1 is strongly upregulated during thermal stress resistance, thereby promoting the formation of a holoenzyme preferentially enriched in this isoform. Therefore, we analyzed transcriptomic, proteomic, and phosphoproteomic changes in a Tpk1-only strain and in the wild type (WT) during thermotolerance recovery (37°C mild stress, 45°C severe stress, 25°C recovery). We identified 2,291 differentially expressed genes (DEGs) and 277 differentially expressed proteins (DEPs) in WT, and 1,701 DEGs and 237 DEPs in the Tpk1-only strain. Transcriptomic analysis revealed enrichment of GO terms related to stress responses, protein folding, cell cycle, cell wall

regulation, and translation, among others, in both strains. In the mutant, additional enrichments included trehalose biosynthesis, RNA splicing, and proteasome activity. Proteomic analysis showed enrichment for stress responses, protein folding, trehalose biosynthesis, carbohydrate metabolism, and telomere organization in both strains, along with strain-specific GO terms. However, regression analysis of transcript vs protein fold changes showed a poor correlation in both strains. This study was complemented with a phosphoproteomic analysis. We identified 164 phosphopeptides from 143 proteins in WT and 609 from 488 proteins in the mutant that showed altered phosphorylation under thermotolerance, with limited overlap between strains. PKA motif analysis highlighted R[S/T][S/L]S as the most represented in both strains, diverging from the canonical RRXS. These results suggest that PKA-mediated phosphorylation during thermotolerance involves both canonical and noncanonical sites, and demonstrate the specificity of the cAMP pathway under stress conditions. Our study reveals how coordinated changes in gene expression, protein abundance, and phosphorylation shape the cellular memory of acquired thermal stress resistance, determining the specificity of cAMP–PKA signaling.

ST-5

AI-ASSISTED EXPLORATION OF NOVEL SCAFFOLDS AIMED AT A TOPBP1–53BP1 INTERACTION SURFACE

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TopBP1 activates ATR via its BRCT4/5 tandem, which recognises phosphorylated 53BP1; disrupting this contact offers leverage over S-phase entry and genome stability. To achieve that goal, we created de novo inhibitors targeting the TopBP1–53BP1 interaction by implementing a three-step AI pipeline: RFDiffusion for backbone generation, proteinMPNN for sequence optimisation, and AlphaFold2 for fold validation. Screening 10k backbone–sequence pairs yielded 46 designs that surpassed stringent thresholds (predicted alignment error index < 10; mean pLDDT > 90). From this subset, we manually curated the data and selected PPI393, whose solvent-exposed surface complements the 53BP1 footprint on TopBP1. A synthetic gene produced milligram quantities of soluble PPI393 in E. coli, while the TopBP1 BRCT4/5 cassette was expressed in parallel. Co-expression and tandem purification yielded a homogeneous sample that, at 25 mM NaCl, eluted as a single heterodimeric peak on analytical size-exclusion chromatography, confirming complex formation, TopBP1–PPI393, at the intended site. When expressed in isolation, PPI393 self-associates and does not bind TopBP1–BRCT4/5. This result suggests that PPI393-dimerisation may mask its recognition loops under near-physiological conditions (150-250 mM NaCl). Current structure-prediction tools cannot yet pinpoint the dimer interface with confidence. Future work will aim to obtain crystal structures of the TopBP1-PPI393 heterocomplex and the PPI393 dimer to characterise the nature of these interactions further and improve the design of the de novo binder, which disrupts the dimer surface and stabilises heterocomplex binding at physiological salt concentrations. This tight integration of large-scale AI design with streamlined wet-lab validation delivers bespoke probes of checkpoint signalling and establishes a transferable route towards novel therapeutic leads.

ENZYMOLOGY

EN-2

ARGININE METABOLISM DEFINES THE INNATE IMMUNE PROFILE OF *ECHINOCOCCUS GRANULOSUS*

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Human echinococcosis is a chronic zoonotic parasitic disease caused by ingesting flatworm eggs of the *Echinococcus* genus, found in contaminated food, water, soil or through direct contact with stools from infected definitive hosts such as dogs, foxes, or other canids. Accidentally, domestic ungulates and humans ingest the eggs, and the resulting oncospheres develop into metacestodes or cysts in organs such as the liver and lungs. *Echinococcus*, an acoelomate animal of the Lophotrochozoa clade would have the potential to develop an innate immune response that remains unknown. In this work, based on BLAST searches and on conserved structure of oxygenase and reductase domains, we identified in *Echinococcus* genome and deposited in GenBank, a putative nitric oxide synthase gene (Eg-NOS, XXQ85382.1). The presence of the enzyme in metacestodes and protoscoleces was confirmed by in situ immunodetection, verifying its high level of endogenous expression and its punctiform expression pattern, suggesting a key hallmark of phagocytic-like cells in the parasite. Lipopolysaccharide (LPS), poly (I: C), and arginine acted as Eg-NOS activators, significantly increasing nitric oxide (NO) release in comparison with untreated parasites, measured by in situ histochemistry with the fluorescent probe DAF-FM-DA. The increase in NO levels was strongly reduced by specific competitive NOS inhibitors (L-NAME and NG-nitro-L-arginine) and calcium chelators indicating that Eg-NOS activity is calcium-dependent in parasitic tissues. These results were confirmed by measuring in vitro enzyme activity using Griess assay. On the other hand, arginine, a versatile amino acid that have a meaningful impact on immune cell behavior is involved in a crucial metabolic competition between NOS and arginases. The NOS can convert it to NO and citrulline or arginases can degrade it into ornithine and urea, highlighting its key role as a metabolic node that was evolutionarily selected to regulate immune response. We revealed by bioinformatic analysis the presence of at least three arginase genes in E. granulosus genome. We also detected intermediates of the tricarboxylic acid cycle, end products of fermentation and amino acids of the hydatid fluid (HF) and germinal cells (GCs) from metacestodes cultured under increasing concentration of arginine using ^{1}H Nuclear Magnetic Resonance (NMR). Ornithine could be identified in both GCs and HF, increasing proportionally its concentration to the arginine concentration. Furthermore, urea in HF was measured using a biochemical assay obtaining comparable results to those of ornithine. These enzymatic products confirm the existence of active arginases in the parasite. NOS activity in *E. granulosus* resembles phagocyte-like functions observed in higher organisms, suggesting that the parasite may use NO production to modulate host–parasite interactions and to mount defences against tissue-resident pathogens, including bacteria and viruses.

EN-3

BIOCHEMICAL CHARACTERIZATION OF TRIM7 B30.2 DOMAIN S-NITROSYLATION

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The ubiquitin conjugation process involves the sequential participation of three enzymes: the E1 activating enzyme, the E2 conjugating enzyme, and the E3 ubiquitin ligase. Of these, E3 ubiquitin ligases are responsible for direct recognition of the substrate protein and, therefore, are responsible for the specificity of the process. This makes them attractive targets for the development of therapeutic regulators. One of the largest E3 families is that of TRIM proteins, which are characterized by a conserved tripartite motif in their N-terminal region. TRIM7 is a member of this family that, in addition to the canonical TRIM motif, contains a C-terminal B30.2 domain. Dysregulated expression of TRIM7 is implicated in several types of cancer, viral pathogenesis, and certain metabolic disorders.
The B30.2 domain mediates the interaction of TRIM7 with its substrate proteins, including glycogenin (GN1), the enzyme responsible for the initiation of the de novo glycogen biosynthesis. In our laboratory, TRIM7 B30.2 domain mutants were generated and their interaction with GN1 was evaluated. The results showed that Cys501 mutation prevented the interaction, indicating that this residue is critical for substrate recognition and suggesting a potential site for post-translational regulation.
The regulatory mechanisms of TRIM7 remain poorly understood. As with many proteins, post-translational modifications could represent a relevant regulatory mechanism. Nitric oxide (NO), a free radical produced by macrophages during inflammatory processes, induces S-nitrosylation, a reversible modification involving the covalent binding of NO to cysteines, which can regulate protein function.
TRIM7 contains 18 cysteines, four of which are located in the B30.2 domain. Crystallographic studies of this domain revealed the oxidation of three cysteines in the presence of β-mercaptoethanol, including Cys501, which, as mentioned, is necessary for interaction with GN1. Based on this observation, we hypothesized that S-nitrosylation could be a mechanism of TRIM7 regulation, and to prove this, we began by studying this modification on the B30.2 domain of the E3 ligase. First, we analyzed whether the cysteines in this domain were accessible to the solvent and therefore potentially susceptible to S-nitrosylation. Then, S-nitrosylation assays were performed with S-nitrosocysteine and S-nitrosoglutathione. Finally, to determine whether this modification could constitute a regulatory mechanism of TRIM7, interaction assays were performed between the S-nitrosylated B30.2 domain and GN1. Here we present the results obtained from these studies.

NEUROSCIENCES

NS-4

DEVELOPMENT OF A RAC1 ACTIVITY ASSAY AN ITS APLICATION IN STRESS-INDUCED VULNERABILITY TO COCAINE DEPENDENCE MODEL

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The Rho family of GTPases is a family of small signaling G proteins that play a fundamental role in regulating intracellular signaling and the dynamics of the actin cytoskeleton. Their ability to alternate between an active state (GTP-bound) and an inactive state (GDP-bound) allows them to function as molecular switches, controlling multiple signaling pathways essential for cellular processes. Within this family, Rac1, a small protein (~21 kDa), stands out as an essential regulator of structural plasticity in the central nervous system, with a crucial role in the morphology of dendritic spines. Specifically, in brain regions associated with reward and memory, such as the nucleus accumbens, Rac1 activity is fundamental for learning and memory processes. Research in our lab has shown that chronic stress-induced behavioral sensitization to cocaine is mediated by a decrease in Rac1 activity. This finding was confirmed by observing that the expression of a constitutively active Rac1 mutant in the nucleus accumbens prevents behavioral sensitization to cocaine. However, the precise measurement of Rac1 activity in tissues and cell cultures is a challenge in research. One of the most common strategies to overcome this is the use of the p21-binding domain (PBD) of the Pak1 protein, also known as the CRIB domain ("Cdc42- and Rac-interactive binding"). This domain selectively and specifically binds to the active (GTP-bound) form of Rac1, allowing it to be differentiated from its inactive form. Existing commercial kits have significant limitations, such as high costs, specialized technical requirements, and limited accessibility for academic or research laboratories with limited resources. The objective of this work is to implement and standardize an affordable and reproducible experimental method for the detection and quantification of Rac1 RhoGTPase activity in tissues and cells. This kit, based on accessible molecular biology techniques, aims to provide an efficient research tool, and demonstrate its applicability in our experimental model of stress-induced cocaine addiction.

NS-5

CEREBELLAR NEURODEVELOPMENTAL DEFECTS IN A MOUSE MODEL OF MAG DEFICIENCY: IMPLICATIONS FOR AUTISM SPECTRUM DISORDER

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Myelin-associated glycoprotein (MAG) is a minor constituent of the nervous system, selectively expressed in the periaxonal layer of myelinated axons. MAG plays a multifaceted role, regulating axonal caliber, controlling the distribution of molecules at the nodes of Ranvier, promoting axon stability and neuronal survival against excitotoxicity, and modulating early postnatal apoptosis of motoneurons. Based on these established functions, we extended our studies to the developing cerebellum, a process that occurs primarily during the early postnatal period in rodents. Preliminary results from our group demonstrate that the absence of MAG expression in neonatal mice (MAG-null) correlates with altered cerebellar neurodevelopment and a behavioral phenotype associated with experimental models of Autism Spectrum Disorder (ASD), without moderate-to-severe motor impairments. Specifically, morphometric analysis of the cerebellum indicated a temporary increase in granule cells (GCs) at postnatal day 7 (P7), followed by increased neurodegeneration and GC death at P14-P21 in MAG-null

mice. Additionally, these mice showed an increased number of Purkinje cells (PCs) at all-time points tested, while the cells themselves displayed a dystrophic phenotype. Given that alterations in the cerebellum, particularly in PCs, have been described in several ASD models, in the present work we aimed to further characterize PC morphology and dendritic growth in adult MAG-null mice. We subjected cerebella from three-month-old wild-type (WT) and MAG-null mice to a modified Patro´s Golgi staining protocol to label individual Purkinje cells (PCs), in order to visualize the dendritic trees morphology. We also sectioned another subset of the cerebella for immunohistochemistry, using a specific PC marker (Calbindin) along with glutamatergic and GABAergic markers (VGLUT1/VGAT/GAD65-67) to detect potential circuitry alterations. Our results showed that in the absence of MAG, there was a significantly higher density of PCs that persisted into adulthood. Moreover, these cells exhibited reduced dendritic growth into the molecular layer and an altered ratio of excitatory to inhibitory connections. Sholl analysis revealed that PCs from MAG-null mice had an abnormal morphology, with a significant reduction in dendritic branching complexity and shorter dendrite length than those in WT mice. Future studies will focus on evaluating the functional consequences on cerebello-thalamo-cortical pathways using a tractography approach. Altogether, our data support the role of MAG as a critical modulator of postnatal cerebellar development, extend our knowledge about the impact of myelination on neurodevelopment during the early postnatal period, and suggest that MAG could be a novel factor contributing to the pathogenesis of ASDs.

NS-6

DUAL ROLE OF ALPHA-2-MACROGLOBULIN IN RETINAL NEUROTOXICITY, GLIOSIS, AND ANGIOGENESIS: IN VITRO AND IN VIVO STUDIES

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Proliferative retinopathies are among the leading causes of vision loss worldwide. Several studies have demonstrated that, in early stages, the neurovascular unit is affected. In recent years, the protein alpha-2-macroglobulin (α2M) has been proposed as a mediator of retinal neurotoxicity. In this study, we evaluated in an *in vivo* model the effect of α2M on the vascular and non-vascular retinal components, and explored its possible involvement in angiogenic processes using an *in vitro* model. To increase endogenous levels of α2M, C57/BL6 mice at postnatal day 12 (P12) received an intravitreal injection of 1 µL of 60 nM activated α2M (α2M*) or PBS (control group). At P17, a group of animals was sacrificed and retinal flatmounts were analyzed for vascular density and diameter. Confocal images were acquired with an Olympus FV 1200 microscope and quantified using the Vessel Analysis plugin in ImageJ. At P26, retinal function was assessed by flash electroretinogram (ERG), and expression of damage and glial stress markers such as glutamine synthetase (GS) and glial fibrillary acidic protein (GFAP) was analyzed by Western blot. For the tube formation assay, BAEC cells (1.5×10^4) were seeded in 96-well plates previously coated with 30 µL of Matrigel and incubated for 18 h (at 37 °C and 5% CO_2) in the presence or absence of 60 nM α2M*. Images were obtained using an Olympus CKX41 microscope and quantified with ImageJ software. Tubular structures and inhibition percentages (I%) were calculated as follows: I% = [1 − (total tube length of treatment/total tube length of control)] × 100. Intravitreal injection of α2M* at P12 in healthy retinas did not alter vascular density but showed a trend toward reduced vessel diameter at P17 ($p = 0.1415$), suggesting that α2M* may contribute to retinal vascular tree obliteration. Western blot

analyses revealed that α2M* treatment significantly increased GFAP protein expression compared to controls, indicating retinal glial activation (gliosis). Moreover, GS expression showed a marked decrease, reflecting alterations in glutamate detoxification metabolism. Regarding retinal function, the b-wave amplitude was significantly reduced ($p = 0.0462$) compared to controls. In the tube formation assay, preliminary results revealed that exogenous α2M* significantly increased the number of branching points ($p = 0.0357$) compared to control. Both experimental approaches demonstrate that α2M* may exert deleterious effects on retinal vascular, glial, and neuronal components, the latter two validating the role of α2M* as a mediator of neurotoxicity in the context of retinopathy.

NS-7

STUDY OF FGD6 PROTEIN AS REGULATOR OF NEURONAL ACTIN CYTOSKELETON DYNAMIC ORGANIZATION

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In the CNS, essential processes in neurons, such as morphology and synaptogenesis, involve remodelling of the actin cytoskeleton. These rearrangements are coordinated by Rho GTPases (RhoA, Rac1 and Cdc42) which act as switches cycling between active (GTP-bound) and inactive (GDP-bound) states mediated by GEFs and GAPs with specific location and activity. FGD6 belongs to a family of proteins with multidomain organization that may function as scaffold between plasma membrane and the actin cytoskeleton. Although FGD6 is expressed in neurons, its function is not established and the association with Cdc42 and Rac1 was recently described. The aim of this work is to study FGD6 functional relevance and its potential role regulating neuronal cytoskeleton. Our hypothesis is that in neurons, FGD6 modulates the activity of key Rho GTPases for actin cytoskeleton reorganization and therefore, several neuronal processes. *In silico* studies determined that FGD6 N-terminal region is intrinsically disordered, most likely to interact with other ordered domains/proteins, and contains several phosphorylation sites. We observed modulation of FGD6 expression during neuronal differentiation *in vitro*. FGD6 knockdown resulted in Rac1 basal activation, suggesting that FGD6 may negatively modulate this GTPase and, consequently, its downstream effector proteins. Rac1 activates a cascade of phospho/dephosphorylations involving PAK, LIMK and Cofilin, which leads to regulation of actin filaments turnover. ARP2/3 is a nucleator complex essential for actin filament branching. FGD6 knockdown resulted in no differences neither in Cofilin or LIMK1 phosphorylation nor in Arp3 levels. However, FGD6 decrease lead to a significant reduction in Arp2 protein levels (without affecting its mRNA), along with changes

in cell morphology and a decrease in F-actin staining, with no modification in total actin pool. Therefore, Rac1 hyperactivity, imbalance of Arp2/3 complex stoichiometry and F-actin disassembly may impact on proper neuronal function. FGD6 mutations are associated with macular degeneration, autism, and epilepsy. Fibroblasts from Self-limited Focal Epilepsy of Childhood (SFEC) patients displayed an altered actin cytoskeleton similar to our FGD6 knockdown experiments. We postulate that FGD6 mutation in SFEC may induce loss of protein function affecting the actin cytoskeleton and impairing neuronal activity previously reported in these patients. In conclusion our results may contribute to shed some light on the mechanism by which FGD6 modulates Rac1 signalling pathways, directly and/or indirectly, through factor(s) that interact(s) with different components along the axis, and to understand the potential involvement of FGD6 in the pathophysiology of neurological diseases such as SFEC.

NS-8

MOLECULAR MECHANISMS INVOLVED IN THE INTERNALIZATION OF STEM CELL DERIVED EXTRACELLULAR VESICLES BY NEURONS

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Extracellular vesicles (EVs) are nanovesicles released by all cell types, carrying a variety of cellular components including proteins, lipids and nucleic acids. They play a fundamental role in intercellular communication by transferring their cargo to other cells to influence their cellular activity in health and disease. In central nervous system (CNS) pathologies, EVs can cross the blood brain barrier, and evidence indicates that stem cell-derived EVs may promote CNS regeneration. However, the biological mechanisms regulating their uptake remains unclear. Previous results from our laboratory suggest that EVs secreted by human neural rosettes (hNR-EVs) derived from induced pluripotent stem cells induce a significant increase in neurite length in human and murine neurons. These biological effects are mediated by an unexpected neuroglial cargo of hNR-EVs: the major proteolipid protein (PLP). This is the major myelin transmembrane protein, capable of forming both cis- and trans-oligomers that contribute to myelin compaction and stability; however, its role in CNS-derived EVs remains unknown. Based on this background, the hypothesis of this study is that PLP associated with hNR-EVs may regulate their uptake by neurons. To evaluate hNR-EVs' internalization, we performed inhibition assays with specific antibodies directed to the N- and C-terminal domains of PLP and analysed hNR-EVs biological effects by immunofluorescence and confocal microscopy. Our results showed that blocking the N-terminal domain of PLP significantly decreases EVs' uptake and their biological effects in neurons. These findings indicate that PLP contributes, at least in part, to the neuronal internalization of EVs and may contribute to future studies focused on the development of EV-based tools for the targeted delivery of bioactive molecules that promote regeneration in CNS pathologies.

NS-9

MICROTUBULE DYNAMICS SHAPE NUCLEAR ARCHITECTURE AND HETEROCHROMATIN ORGANIZATION IN DEVELOPING HIPPOCAMPAL NEURONS

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During neuronal development, the nucleus undergoes major structural changes reflecting profound chromatin reorganization and gene expression remodeling. These processes are mediated not only by transcriptional and epigenetic factors but also by mechanostructural mechanisms linking the cytoskeleton to nuclear organization. In this context, the LINC (Linker of Nucleoskeleton and Cytoskeleton) complex, composed of SUN proteins (SUN1/2) in the inner nuclear membrane and Nesprins in the outer nuclear membrane, acts as a physical bridge between the cytoskeleton—particularly microtubules—and the nuclear lamina. The LINC complex has been implicated in spatial genome organization, mechanotransduction, and regulation of cell differentiation .Nuclear architecture actively contributes to gene regulation through the organization of large-scale chromatin domains, such as lamina-associated domains (LADs), which are typically enriched in repressive histone marks (H3K9me2/3, H3K27me3) and contribute to transcriptional silencing. Dynamic anchoring of these regions to the nuclear lamina, mediated by lamin A/C and lamina receptors, is thought to play a role in the silencing of developmental genes and may require active mechanisms to maintain domain organization during cell differentiation. However, in neurons, the mechanisms by which microtubules influence chromatin architecture and the epigenetic landscape remain poorly understood. To address this question, we used primary rat hippocampal neurons (E18) as a model system to study nuclear organization and gene expression during neuronal maturation. Microtubule dynamics were perturbed using nocodazole (depolymerization) or taxol at two concentrations—500 nM, which promotes microtubule depolymerization, and 3 nM, which induces microtubule stabilization—allowing us to probe opposite effects on the cytoskeleton. Cells were subsequently analyzed by immunofluorescence and confocal/super-resolution imaging to assess nuclear architecture and the distribution of histone marks H3K9me3, H3K27me3, and H3K4me3. STED nanoscopy revealed that histone marks are organized into epigenetic nanodomains, ranging from 40 to 200 nm of equivalent diameter. Early quantitative analyses suggest that microtubule perturbations may modulate the relative abundance and spatial distribution of these domains, potentially affecting the epigenetic regulation of gene expression during neuronal differentiation. Together, these results support a model in which microtubule dynamics contribute to the maintenance of nuclear architecture and heterochromatin organization in developing neurons. Our findings highlight a potential mechanistic link between cytoskeletal forces, the LINC complex, and the spatial regulation of chromatin during neuronal differentiation, opening new avenues for exploring how mechanical cues integrate with epigenetic programs to establish and maintain neuronal identity.

NS-10

ROLE OF THE TRANSCRIPTION FACTOR CREB3L1 IN CEREBRAL CORTEX DEVELOPMENT

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During the development of the mammalian cerebral cortex, multiple cellular events play a fundamental role in the division, differentiation, and subsequent migration of neuronal cells, key events that lead to neuronal maturation. Both external and internal cellular factors influence neurodevelopment. In terms of intrinsic molecular mechanisms, these include membrane expansion, cytoskeletal reorganization, and adaptation of the secretory pathway to maintain cellular homeostasis. In this context, the CREB3L1 transcription factor, a member of the CREB3 family, regulates proteins involved in the secretory pathway and tissue-specific genes. However, the function of CREB3L1 during neuronal differentiation and development remains unexplored. Previous findings from our lab, using rat hippocampal neurons, show that suppression of the CREB3L1 factor decreases neurite outgrowth, thereby impairing overall neuronal development. Based on these results, we investigated the role of CREB3L1 *in vivo* during the development of the cerebral cortex using *in utero* electroporation technique. Our findings indicate that CREB3L1 knockdown impairs neuronal differentiation by preventing the morphological transition of neurons from a multipolar to a bipolar phenotype. Taken together, the *in vitro* and *in vivo* studies suggest that CREB3L1 is a key regulator in nervous system development. Future studies will focus on identifying the molecular targets and biological processes regulated by CREB3L1, which are critical not only for neurodevelopment but also for understanding neurodegeneration.

NS-11

GABA RECEPTORS MODULATE VEGF IN RETINAL MÜLLER GLIAL CELLS

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GABA receptors, beyond eliciting hyperpolarizing responses in neurons, have been implicated in glial proliferation and migration. Their role in Müller glia (MGC) remains poorly characterized, although previous evidence links GABA signaling to intracellular Ca^{2+} increases, a key modulator of VEGF-A release. We investigated the effect of GABA receptor activation on VEGF-A production and secretion in primary mouse MGC cultures. Cells were exposed for 48 h to GABA and to selective agonists and antagonists of $GABA_A$ and $GABA_B$ receptors. VEGF-A expression and release were assessed by immunofluorescence, Western blot, RT-qPCR and ELISA. To probe the involvement of extracellular calcium and MAPK signaling, experiments were performed in calcium-free Ringer–Krebs solution and in the presence of the L-type calcium channel (LTCC) blocker nimodipine or the ERK kinase inhibitor FR180204. In MGC cultures, GABA increased intracellular VEGF-A immunofluorescence intensity while paradoxically reducing VEGF-A secretion. These effects were mediated via $GABA_A$ receptors. Removal of extracellular Ca^{2+}, LTCC blockade, or ERK1/2 inhibition prevented the GABA-induced modulation of VEGF-A. Regulation of VEGF-A by $GABA_A$ receptor signaling in MGC reveals a novel glial mechanism for controlling this angiogenic factor, with potential implications for retinal vascular homeostasis.

NS-12

AMYLOID DEPOSITION AND ASTROGLIOSIS LINKED TO IMPULSIVITY AND COGNITIVE DEFICITS IN A NEW APP KNOCK-IN MOUSE MODEL

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Modeling Alzheimer's disease (AD) *in vivo* remains a major challenge in neuroscience. Since the 1990s, several transgenic mouse models have been developed, providing valuable insights into amyloid-β (Aβ) pathology and potential therapeutic strategies. However, many of these models rely on the overexpression of APP or APP/presenilin-1 (PS1), which can lead to artifacts associated with protein overproduction or mislocalization. To address these limitations, single App knock-in lines carrying familial AD mutations have been developed. In this study, we characterize the behavioral and neuropathological traits of a new App NL-F Psen1P117L knock-in (APP KI) mouse strain. Homozygous APP KI and C57BL/6 wild-type (WT) mice were tested at 3 and 6 months of age in the open field, elevated plus maze, novel object recognition (NOR), and Y-maze tasks to assess locomotion, anxiety-like behavior, episodic memory, and working/spatial memory. At 6 months, APP KI mice exhibited a reduction in working memory, as evidenced by a lower mean time spent in the novel arm during the Y-maze test compared to WT mice. Despite exhibiting preserved locomotor activity, these mice showed increased entries into the novel arm with reduced exploration time, suggesting novelty-seeking/impulsivity and impaired sustained exploration. Additionally, APP KI mice demonstrated normal discrimination index scores in the NOR test, indicating intact episodic memory. However, they exhibited an increased number of interactions with both objects, reflecting attentional alterations or impulsivity. These behavioral changes correlated with moderate cortical Aβ deposition, minimal hippocampal pathology, and increased glial reactivity. Ongoing studies aim to further characterize this model. Together, our findings strengthen the link between amyloid and glial pathology and early behavioral alterations in this new-generation AD model, which avoids the confounds of APP overexpression. These alterations include not only cognitive impairments but also traits such as disinhibition, impulsivity, and a higher exploration rate, which have been observed in the early stages of the disease in patients. Our findings provide measurable features in this mouse model that are relevant for early diagnosis and for testing disease-modifying interventions.

NS-13

LRP1 LIGANDS MODULATE THE INFLAMMATORY RESPONSE IN MONONUCLEAR PHAGOCYTIC CELLS

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Age-related Macular Degeneration (AMD) is a leading cause of blindness worldwide in adults over 65 years. It is associated with aging, environmental and genetic-related risk factors. This disease involves progressive degeneration of photoreceptors in the macula, the retinal region responsible of central vision. Neovascular AMD is characterized by the accumulation of mononuclear phagocytic cells (MPCs), including resident microglia and monocyte-derived-macrophages, which establish a chronic inflammatory environment that promotes pathological choroidal neovascularization (CNV). The multifunctional, multiligand receptor LRP1 (Low-density lipoprotein receptor-related protein 1) is expressed in MPCs and It has been proposed as an anti-inflammatory receptor in other pathologies. Previous studies reported that a group of ligands, such as activated alpha-2-Macroglobulin (α_2M*) and SP16, promotes the anti-inflammatory functions. The objective of this study was to evaluate LRP1 ligands effect on MPCs under inflammatory conditions through *in vivo* and *in vitro* assays. *In vivo*, C57BL/6 mice were anesthetized, pupils were dilated and four argon laser photocoagulation burns were applied per eye with a slit lamp. Intravitreal injections of LRP1 ligands, α_2M* or SP16, were administered after laser. After four days, MPCs (CD11b+ F4/80+) were analysed by Flow cytometry. *In vitro*, BV2 microglial cells were pretreated with the same ligands 30 minutes before LPS stimulation (10 ng/ml, 8 hours). Supernatants were collected and samples processed to evaluate proinflammatory cytokines (TNFα) by Western blot and migration by Wound Healing assay. Also, bone marrow-derived macrophages (BMDM) were stimulated with BV2 supernatants 24 hours and their polarization and LRP1 surface expression was studied by Flow cytometry (CD86-FITC, CD206-APC.Cy7 and LRP1). MPCs infiltration increased at 4 days after laser in retina and RPE-choroid in CNV group. LRP1 ligands reduced the number of infiltrating cells in both tissues after intravitreal injections. In culture, BV2 cells pretreated with α_2M* or SP16 decreased TNFα protein expression and migration induced by LPS proinflammatory stimuli. Furthermore, supernatants from preconditioned BV2 cells enhanced the CD206+CD86- BMDM population. We also studied if LRP1 surface expression changes between experimental conditions. We observed that, from total LRP1+ BMDM, preconditioned BV2 promote a higher proportion of LRP1^{high} cells than LRP1^{low}, compared to BV2 supernatants with LPS stimuli without precondition. In conclusion, the results show that intreavitreal administration of LRP1 ligands reduced MPCs infiltration in the CNV mouse model. *In vitro* assays suggest that this process could be mediated by microglia, whose supernatants promote an anti-inflammatory profile in macrophages. Further studies are needed to understand the mechanism involved.

NS-14

CAV1-EARLY ENDOSOMES REGULATE TGFβ RECEPTOR TRAFFICKING IN NEURONAL DEVELOPMENT

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Transforming Growth Factor beta (TGFβ) signaling is essential for neuronal polarity and axonal growth. The regulation of endocytic pathways is critical for TGFβ signaling, given that TGβ

receptors (TβRs) use two independent endocytosis routes. The clathrin-dependent pathway promotes signaling from early endosomes (EEs), whereas the caveolae-dependent pathway leads to TβR ubiquitination and subsequent degradation. In HeLa cells, these pathways can converge in novel structures: endosomes positive for Early Endosomal Antigen 1 (EEA1) and Caveolin-1, referred to as Cav1-EEs. Our research aimed to characterize Cav1-EEs in neurons and determine whether these endosomes modulate TGFβ trafficking. Using STED super-resolution microscopy in developing hippocampal neurons, we identified populations of endosomes positive only for EEA1; only for Caveolin-1; and Cav1-EEs positive for both proteins simultaneously (Cav1-EEA1) located in the soma and neuritic process. We characterized Cav1-EEs throughout development and found them to be most abundant during the polarization and active growth stages (2-7 DIV). Notably, these vesicles showed a higher density in axons than in dendrites, suggesting a specific role in regulating axonal growth. To investigate the formation of Cav1-EEs, we treated neurons with Pitstop2, a pharmacological inhibitor of clathrin. This treatment significantly reduced the number and altered the distribution of EEA1-positive endosomes. However, the relative proportion of Cav1-EEs remained stable despite the decrease in the total EEA1 pool. This finding suggests a regulatory mechanism for the fusion of Cav1-containing vesicles to early endosomes, ensuring that Cav1-EE formation is preserved even when the clathrin endocytic route is impaired. We confirmed that in neurons, TGFβ receptors are also internalized into Cav1-EEs under endogenous conditions, and that the abundance of these vesicles increased after the addition of the TGFβ ligand. Immunofluorescence and colocalization assays revealed that Cav1-EEs predominantly colocalize with the E3 ubiquitin ligase Smurf2 and the recycling endosome marker Rab11. They also showed moderate interactions with the signaling mediators SARA and Smad2/3, and with the lysosome marker Lamp1. These findings suggest that Cav1-EEs function as multicomponent stations where endocytosed TGFβ receptors can be sorted for signaling, recycling, or degradation. Live-cell imaging confirmed the dynamic nature of Cav1-EEs, showing they form near the plasma membrane and exhibit distinct fusion rates in different neuronal compartments. In summary, this study provides the first direct evidence of Cav1-EEs in neurons and proposes that Cav1-EEA1 vesicles could act as a secondary sorting station that fine-tunes TGFβ receptor trafficking in the neuronal context.

NS-15

SARA AND SMURF2 MODULATION: A POTENTIAL PATHWAY FOR SEIZURE TRACKING IN EPILEPSY.

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The transforming growth factor β (TGFβ) plays a crucial role in epileptogenesis. Smad Anchor for Receptor Activation (SARA) modulates the TGFβ pathway during neuronal development. A recent study reported that the expression of SARA, pSmad3, and TGFβ is increased in the hippocampus and temporal cortex of pilocarpine-induced status epilepticus (SE) rats as well as in patients with temporal lobe epilepsy (TLE). One of the most frequent forms of epilepsy is TLE, which often presents with a high percentage of patients resistant to the drugs commonly used for its treatment. Moreover, the ubiquitin-proteasome system (UPS) plays a key role in regulating TGFβ signaling, and dysfunction of this complex has been proposed as a common pathological feature among brain disorders, including epilepsy. Smurf2 is an E3 ubiquitin ligase that contributes to a variety of physiological and pathological processes, regulates the stability and signaling of the TGFβ pathway, and catalyzes the degradation of TβRs. Recently, Smurf2 was also described in a cell line model to induce the proteasomal degradation of SARA, suggesting that both proteins influence the extent of the cellular response to TGFβ. To investigate this, we induced SE in 30-day-old rats with pilocarpine. After 7, 35, and 45 days (time points at which increased SARA expression has been reported in human patients with TLE), the animals were perfused, and 40 µm cryostat sections were obtained to evaluate the expression of SARA, Smurf2, and GFAP (glial fibrillary acidic protein) by immunofluorescence. In addition, the levels of these proteins were analyzed by Western blot in both SE-induced animals and healthy controls. Furthermore, we examined the expression of the same proteins in astrocyte cultures derived from epileptic and non-epileptic human brain tissue samples undergoing surgery. Our results revealed an increase in SARA expression in human TLE astrocytes and in brain tissue from SE animal models compared to controls, along with a significant decrease in Smurf2 levels in epileptic astrocytes. Notably, a strong co-localization between SARA and Smurf2 was observed in human astrocytes from patients undergoing surgery. In addition, elevated GFAP levels in the epileptic models suggest the presence of neuroinflammation or structural damage to the central nervous system, a hallmark of TLE. These findings suggest that Smurf2 dysfunction may play a critical role in the pathological accumulation of SARA, a process that could, in turn, exacerbate cellular signaling imbalances and facilitate the onset and progression of seizures. Taken together, this research highlights the urgent need to identify and validate novel therapeutic targets—particularly SARA and proteins associated with the ubiquitin-proteasome system (UPS)—with the ultimate goal of developing more effective treatment strategies for pharmacoresistant epilepsy.

NS-16

DYSREGULATION OF ENDOSOMAL RAB11-DERIVED EXTRACELLULAR VESICLES GENERATION IN AN IN VITRO MODEL OF ALZHEIMER'S DISEASE

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Amyloid-β (Aβ) aggregation plays a central role in the pathogenesis of Alzheimer's disease (AD). We previously reported that Aβ oligomers and fibrils increase APP and BACE1 levels in Rab11-positive endosomes, leading to intracellular accumulation of Aβ1-42 in human iPSC-derived neurons (HN-iPSCs). Here, we investigated how Aβ affects APP trafficking along the endocytic pathway and its impact on extracellular vesicles (EVs) biogenesis. Using pulse–chase assays and quantitative colocalization analyses, we found that Aβ enhances APP endocytosis

while reducing its recycling, resulting in APP accumulation within Rab11-positive recycling endosomes and impaired lysosomal targeting through a Gβγ-dependent signaling pathway. Super-resolution imaging revealed a striking enlargement of Rab11-positive endosomes in HN-iPSCs exposed to Aβ, an effect fully prevented by gallein (GAL), a selective Gβγ inhibitor. To examine whether APP accumulation in recycling endosomes is linked to changes in EVs secretion, we analyzed small EVs from the secretome of different developmental stages of rat cortical neurons treated with Aβ. At intermediate stages (10 DIV) Aβ exposure led to a significant increase in Rab11-, CD81-, and Syntenin-1-positive EVs, an effect completely abolished by GAL pre-treatment. In contrast, at later stages (15 DIV) Rab11- and Syntenin-1-positive EVs were significantly reduced, whereas CD81-positive EVs were increased. GAL pre-treatment only partially recovered Rab11-positive EVs number, suggesting that aging engages alternative, less GAL-sensitive mechanisms of EVs dysregulation. Interestingly, at 15 DIV the reduction in Rab11-positive EVs was accompanied by an increase in their mean size. This shift toward fewer but larger EVs closely parallels the endosomal enlargement observed in Aβ-treated neurons, supporting the idea that endosomal dysfunction alters EVs biogenesis and secretion dynamics. Together, our findings demonstrate that aggregated Aβ disrupts intracellular APP trafficking by enhancing endocytosis and impairing recycling, resulting in APP retention within Rab11-positive recycling endosomes and endosomal enlargement events driven by Gβγ signaling. These trafficking defects are accompanied by an age-dependent remodeling of EVs biogenesis and secretion, suggesting that neurons initially adjust their EVs output to compensate for endosomal pathology but progressively lose this adaptive capacity over time.

BIOTECHNOGY

BT-5

NANOEMULSION-BASED DELIVERY OF ETHANOLIC EXTRACTS FROM *TRAMETES VERSICOLOR* WITH ANTICANCER CYTOTOXIC ACTIVITY

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Cancer remains one of the leading causes of death worldwide and continues to represent a major public health challenge. Despite advances in treatment, conventional therapies such as chemotherapy and radiotherapy are often associated with high toxicity and significant adverse effects, which directly compromise patients' quality of life. This underscores the need for safer and more effective therapeutic alternatives, including the use of natural products with anticancer potential, which may hold promise as adjuvant therapies.

Trametes versicolor is a fungus widely used in traditional Asian medicine. Its importance lies in the diversity of bioactive metabolites it contains, particularly phenolic compounds, which have been linked to antioxidant, immunomodulatory, and antitumor activities. However, the instability and poor bioavailability of these compounds limit their direct application. To address this issue, nanotechnology offers an attractive strategy to protect, stabilize, and enhance the delivery of such metabolites.

In this study, we formulated nanoemulsions loaded with ethanolic extracts of *T. versicolor* (NemTV). These nanoemulsions exhibited reproducible and stable physicochemical properties, with an average particle size below 180 nm, a low polydispersity index, and a zeta potential of approximately –60 mV. In cytotoxicity assays (MTS), both the free extracts and NemTV significantly reduced the viability of murine melanoma cells (B16-F10) and human breast cancer cells (MDA-MB-231), whereas non-tumor murine lung cells (MLg) remained relatively unaffected. Notably, NemTV displayed lower IC50 values than the free extract: in B16-F10 WT cells, 47.04 µg/mL versus 57.28 µg/mL; in MDA-MB-231 cells, 150.3 µg/mL versus 155.8 µg/mL. In contrast, MLg cells showed the opposite trend (1274 µg/mL for NemTV vs. 481.4 µg/mL for the extract), suggesting an improved safety profile in non-cancerous cells.
We further assessed the effect of the formulations on three-dimensional gastric tumor spheroids derived from the SNU638 and SNU601 cell lines, where NemTV inhibited spheroid development by 89% and 92%, respectively. These findings are particularly relevant, as tumor spheroids more closely mimic the tumor microenvironment and provide a better approximation of *in vivo* conditions.
In conclusion, our results support *T. versicolor* as a valuable source of compounds with antitumor activity and highlight NemTV as an innovative, stable, and biocompatible formulation. While these results are promising, we acknowledge that they are based on in vitro studies, and further evaluation in preclinical models will be required to confirm efficacy and safety in more complex biological settings.

BT-6

CHARACTERIZATION OF BIOCHAR DERIVED FROM ALMOND HULL AND ITS ROLE ON TOMATO GROWTH

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Plant residues, particularly almond (*Prunusdulcis*) exocarp and endocarp by-products, represent a potential feedstock for the production of biochar, a carbon-rich solid material obtained through the pyrolysis of biomass under limited oxygen conditions. Biocharhas been increasingly recognized for its potential to enhance soil fertility, sequester carbon, mitigate greenhouse gas emissions, and adsorb or neutralize natural toxins, herbicides, and pesticides. Consequently, it is considered a promising amendment to improve soil quality in agricultural systems. This study assessed the potential of biochar derived from almond residues as a germination promoter in tomato (*Solanumlycopersicum*) under controlled conditions. Additionally, to warrant its safety, cytotoxicity and genotoxicity tests were carried out by *Allium cepa* bioassays. The biocharwas produced by carbonization at 350 °C. Its structural characteristics were analyzed by scanning electron microscopy (SEM), and its chemical composition was determined following standard protocols. To evaluate germination capacity, sterilized tomato seeds were placed on moistened plates containing different concentrations of biochar (0.1%, 0.5%, and 1% w/v), with distilled water as control. The plates were incubated at 25°C for 7 days. For the Allium test, six onion bulbs were exposed to four biochar solutions (0.05%, 0.1%, 0.5%, and 1% w/v), to a positive control (1% w/v hydrogen peroxide), and to a negative control (distilled water). The biochar obtained had the following composition: organic matter = 69.52 ± 0.46%, volatile solids = 64.53 ± 0.12%, carbon = 43.80 ± 0.12%, moisture =

5.90 ± 0.35%, and pH = 9.92 ± 0.06, and an electrical conductivity of 5.09 ± 0.05 dS/m. SEM analyses revealed low porosity. The high carbon and organic matter contents support its usefulness as a soil amendment, while the high proportion of volatile solids suggests the presence of readily available organic matter capable of releasing nutrients into the soil. Its low moisture content facilitates storage and handling, and its alkalinity combined with its relatively high electrical conductivity indicates potential benefits for acidic soils. The highest germination rate (97%) was achieved with 0.5% biochar, compared to 71% in the control. At this concentration, a significant increase in mean root length (4.52 ± 2.67 cm) was observed relative to the control (2.26 ± 2.40 cm) ($p < 0.05$). Nevertheless, evidence of cytotoxicity was detected in Allium roots at 1% biochar, as indicated by a marked reduction in root number, growth inhibition greater than 20%, and the presence of chromosomal aberrations (CA) such as anaphase bridges and laggard chromosomes. Lower concentrations exhibited neither cytotoxic nor genotoxic effects. Hydrogen peroxide (positive control) completely inhibited the mitotic index, with 100% of nuclei remaining in interphase. This study provides strong evidence that almond biowaste-derived biochar, when applied at appropriate concentrations, can serve as a valuable resource for sustainable agriculture. Its incorporation into agricultural practices could enhance crop performance, reduce reliance on chemical fertilizers and contribute to addressing the increasing problem of organic waste management.

BT-7

SECRETOME ANALYSIS OF *TRICHODERMA ASPERELLUM* GROWN IN A BY-PRODUCT-BASED MEDIUM FOR LIGNOCELLULOLYTIC ENZYME PRODUCTION

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The development of sustainable bioprocesses requires cost-effective strategies that valorise agro-industrial by-products within a circular economy. Lignocellulosic biomass is abundant but structurally resistant, requiring pretreatments to improve accessibility. Physicochemical methods are effective in disrupting its structure but often generate microbial inhibitors. Combining these approaches with enzymatic or biological treatments provides a more sustainable strategy, as biological systems not only enhance sugar release and reduce recalcitrance under mild conditions but can also degrade the inhibitors formed during physicochemical pretreatment, thereby improving overall process efficiency. In this study, we analysed the secretome of *Trichoderma asperellum*, selected for its metabolic versatility and tolerance to lignin-derived phenolic compounds, to design a cost-effective and efficient culture medium for enzyme production. A mineral medium with lactose (MML, control) was compared to this medium supplemented with liquid digestate and copper sulphate (MMLI). Liquid digestate, a by-product from biogas plants, provides nutrients, soluble phenolics, and residual polysaccharides that act as inducers of enzyme expression. Proteomic analysis by LC-MS/MS revealed a differential secretion profile between the two conditions. A total of 317 filtered proteins were identified, with principal component analysis revealing apparent clustering and separation between treatments. In MMLI, the secretion of a broad repertoire of hydrolases was induced, highlighting a coordinated enzymatic strategy that supports comprehensive degradation and utilization of biomass. Glucanases (A0A2T3ZAS8, A0A2T3YR50), cellulases (A0A2T3ZAP7), and β-glucosidases (A0A2T3YUC4) act on cellulose–β-glucans to release glucose, while hemicellulases such as β-mannosidase (A0A2T3YSU1), α-galactosidase

(A0A2T3Z3S2), and GH30 (A0A2T3Z8R1) liberate mannose and galactose. Pectin degradation by GH28 (A0A2T3YTH0) and pectinesterase (A0A2T3YYD8) provides galacturonic acid and arabinose, complemented by α-amylase (A0A2T3YUB0). Digestate supplementation proved to be more effective in inducing these enzymes than insoluble lignocellulosic materials, avoiding the operational limitations associated with solid or highly viscous fermentation media. The results confirm that *T. asperellum* combines high enzymatic diversity, secretion efficiency, and the ability to detoxify common inhibitors generated during physicochemical pretreatments, positioning it as a competitive alternative to industrial strains such as *T. reesei*. In conclusion, the design of a by-product-based culture medium not only reduces costs but also enhances the production of a broad and robust enzymatic cocktail, suitable for biological biomass pretreatment in biotechnological processes aimed at advancing the bioeconomy and waste valorization.

BT-8

DESIGN OF ANTIEVOLUTION AGENTS TO PREVENT THE ESTABLISHMENT OF CHRONIC INFECTIONS OF BACTERIAL PATHOGEN PSEUDOMONAS AERUGINOSA

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Antimicrobial resistance is one of the most critical threats to global health. In 2019, it was directly responsible for 1.27 million deaths, and by 2050 it is projected to cause over 10 million deaths annually. Among the six pathogens responsible for the highest mortality is Pseudomonas aeruginosa, an opportunistic bacterium that poses a severe risk to immunocompromised individuals. In cystic fibrosis patients, lung conditions favor P. aeruginosa in acquiring resistance to multiple antimicrobials. While early infections may be eradicated, chronic infections are much harder to treat and often require long-term bacterial suppression therapies to prevent severe lung damage. Developing new antibiotics is complex, costly, and time-consuming. An emerging strategy focuses on identifying factors involved in the acquisition of antimicrobial resistance and inhibiting their function. A promising pharmacological target is DNA polymerase IV (Pol IV), a low-fidelity enzyme involved in DNA synthesis. Our previous work demonstrated that genes associated with virulence and antibiotic resistance are hotspots for Pol IV-mediated mutagenesis in P. aeruginosa. In this study, we applied drug repurposing approaches to identify Pol IV inhibitors. A hybrid virtual screening strategy was implemented, starting with inhibitors of the human Pol IV homolog, Pol κ. Based on the structures of these Pol κ inhibitors, we identified potential binding sites on Pol IV through literature reports and computational modeling using two methods: P2Rank and DoGSiteScorer. Structurally similar molecules were then retrieved from open-access databases (DrugBank, ChEMBL, and COCONUT) using Morgan molecular fingerprints (radius = 2 Å, threshold = 0.3) and the Tanimoto coefficient. Candidate molecules were evaluated for their interaction with Pol IV binding sites through molecular docking, using the 2Vinardo scoring function. The most promising candidates were selected based on structural similarity and binding affinity for further testing through molecular dynamics simulations and assays to evaluate the inhibition of the acquisition of antibiotic resistance.

BT-9

PRODUCTION AND CHARACTERIZATION OF A RECOMBINANT ANTI-HER2 NANOBODY FOR APPLICATIONS IN TARGETED BREAST CANCER THERAPY

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The human epidermal growth factor receptor 2 (HER2) is a biomarker overexpressed in a subset of breast tumors characterized by aggressive phenotype and limited therapeutic response. HER2-positive breast cancer remains a clinical challenge due to resistance to conventional therapies and the need for more specific alternatives. Immunotoxins represent a promising strategy by combining a tumor-targeting domain with a cytotoxic domain; however, they often present limitations regarding stability, solubility, and yield. In this study, we designed an alternative strategy based on the modular assembly of two recombinant proteins using co-associating peptides from the p53 tetramerization domain. The cytotoxic domains usually correspond to potent bacterial toxins, while tumor specificity is conferred by an anti-HER2 nanobody (nanoHER2). In this context, we first set up the production of a recombinant camelid heavy-chain antibody domain (VHH) that targets HER2, with the aim of using it as a targeting module. The anti-HER2 VHH sequence was cloned into a pET bacterial expression vector and expressed in *Escherichia coli* by autoinduction method in optimized conditions. The nanoHER2 was obtained at high yields in soluble form and purification was carried out by immobilized metal (Co^{2+}) affinity chromatography (IMAC) resins followed by dialysis achieving 99% purity, as confirmed by SDS-PAGE and Western blot analysis. The biological activity of the recombinant nanoHER2 was evaluated by confocal immunofluorescence and flow cytometry in breast carcinoma cell lines with different HER2 expression levels, to determine both its specificity and antigen binding capacity. NanoHER2 showed strong selectivity to recognize HER2-positive cells (SK-BR-3), intermediate interaction with MDA-MB-453 cells, and minimal or no binding to HER2-negative cells (MCF-7), demonstrating nanomolar-range affinity. Considering that the tumor microenvironment is acidic, nanoHER2 stability was assessed under low-pH conditions, as well as after multiple freeze–thaw cycles, showing no significant loss of biological activity. These results demonstrate that the recombinant nanoHER2 antibody is properly expressed, folded and functional, providing a robust and reproducible platform with potential for developing modular immunotoxins assembled through co-associating peptides, for targeted therapies in HER2-positive breast cancer.

CB-9

EFFECT OF SERINE PROTEASE INHIBITOR RBMTI-6 ON HUMAN ALVEOLAR ADENOCARCINOMA CELLS

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Protease inhibitors have shown promising potential as therapeutic agents in oncology due to their ability to regulate proteolytic activity involved in tumor progression. rBmTI-6, a recombinant serine protease inhibitor belonging to the Kunitz-BPTI family, was cloned from the *Rhipicephalus microplus* tick. rBmTI-6 has three inhibitory domains and exhibits inhibitory activity against trypsin-like serine proteases (bovine trypsin and plasmin), with Ki values in the nM range. In previous work, it was demonstrated that rBmTI-6 is processed during its production, separating the original molecule into two parts: rBmTI-6d1 (domain 1) and rBmTI-6d2/3 (domains 2 and 3). The aim of this work was to evaluate the activity of rBmTI-6 (rBmTI-6d1 and rBmTI-6d2/3 combined) on A549 cells (human adenocarcinoma lung epithelial cells). rBmTI-6 was expressed in the *Pichia pastoris* system and purified using a trypsin-Sepharose affinity chromatography. A549 cells were incubated with different concentrations of active rBmTI-6 for 24h in DMEM medium with and without fetal bovine serum, at 37°C and 5% CO_2. Cytotoxicity was evaluated using MTT assays, followed by flow cytometry and western blotting for Caspase-3. The results demonstrated that rBmTI-6 at concentrations from 20 nM to 1000 nM decreased cell viability in a dose-dependent manner, which was potentiated under fetal bovine serum deprivation conditions. Flow cytometric analysis indicated the induction of apoptosis, which was corroborated by the Caspase-3 activation observed through western blotting assays. Additionally, microscopic observations showed morphological changes consistent with autophagy at higher doses. In MRC-5 fibroblasts, rBmTI-6 showed low cytotoxicity, demonstrating selectivity for tumor cells. These findings reinforce the role of serine protease inhibitors as promising candidates for therapeutic strategies against cancer.

CB-10

BIOSYNTHESIS OF SIALYLATED GLYCANS IN THE CELL NUCLEUS

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Protein glycosylation is one of the most common and important post-translational modifications. It is carried out by multiple glycosyltransferases and glycosidases that synthesize glycoconjugates in metabolic pathways controlled by substrate availability, enzyme activity, transcription levels, and the subcellular localization of the enzymes. This process occurs throughout the Golgi apparatus as well as in the cell nucleus. Two types of O-glycosylation have been described in the cell nucleus: O-GlcNAc and O-GalNAc biosynthesis, the latter recently described by our working group. O-GalNAc glycosylation is initiated by the covalent linkage of α-N-acetylgalactosamine (α-GalNAc) to Ser/Thr residues of proteins,

generating a structure known as Tn antigen (GalNAcα-O-Ser/Thr). This antigen is able to elongate in the cell nucleus, giving rise to the formation of the Core 1 or T antigen. These glycoconjugates present certain defined terminal sugars, such as fucose and sialic acid (Neu5Ac) residues, in glycosylated proteins. The glycan sialylation requires the presence of a sialic acid donor (CMP-Neu5Ac), which is synthesized in the cell nucleus. For the polymeric addition of sialic acid in the glycan biosynthesis the monosaccharide to occur, the presence of a sialyltransferase is required to catalyze the covalent attachment of an Neu5Ac, derived from the nucleotide sugar CMP-Neu5Ac to an acceptor, such as O-GalNAc, generating sialyl Tn antigen (Neu5Acα6GalNAcα-O-Ser/Thr) whereas sialyl T antigen acceptor (Galβ3GalNAcα-O-Ser/Thr) yields sialyl T antigen (Neu5Acα3Galβ3GalNAcα-O-Ser/Thr). There are reported 20 isoforms of sialyltransferases in human cells. This study is focused on the biosynthesis of O-glycan sialylation in the cell nucleus, to elucidate potential functions of sialylated nuclear glycans in cell physiology. In this study, we evaluated the subcellular localization of the sialyltransferase enzyme ST6GalNAc3 in different human cell lines such as HeLa (cervical carcinoma), SH-SY5Y (neuroblastoma), Caco-2 (colon cancer) and HEK293 (human kidney cells) by confocal microscopy. Here, significant nuclear localization was observed of ST6GalNAc3 in human cells. In accordance with the above, the enzymatic activity of sialyltransferase was measured in the nucleoplasm of these human cell lines. In addition, the presence of sialylated residues in the cell nucleus was evidenced by confocal microscopy. These results support the hypothesis that sialylated residues in nuclear proteins are biosynthesized, suggesting an important role in nuclear cell physiology.

CB-11

NEUROTROPHIN RECEPTOR P75^{NTR} INVOLVEMENT IN MACROPHAGE RESPONSES DURING WET AGE-RELATED MACULAR DEGENERATION

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Age-related macular degeneration is a prevalent ocular pathology affecting adults over 50 years of age, which can lead to severe visual impairment and blindness. In its neovascular stage, patients develop choroidal neovascularization (CNV), characterized by the formation of new vessels originating from the choroidal vasculature that disrupt Bruch's membrane and induce neuronal loss. Cellular infiltration plays a critical role in this process by modulating both inflammatory and pro-angiogenic responses. We previously demonstrated that the neurotrophin receptor p75 (p75^{NTR}) is expressed in phagocytic mononuclear cells in a murine CNV model, and that its deletion reduces neovessel formation and improves retinal function. In the present study, we investigated the cellular changes mediated by p75^{NTR} in macrophages via both ligand-dependent and ligand-independent mechanisms. To this end, we employed two complementary approaches: *in vivo* and *in vitro* studies. In the CNV model, 2-month-old mice were anesthetized, pupils dilated, and four retinal injuries were induced using a 532 nm wavelength photocoagulation laser via slit lamp. Four days post-laser, mice were sacrificed, and retinas and RPE-choroid tissues were processed separately. Both wild-type (WT) and p75^{NTR} knockout (KO) mice were included, and animals without CNV were employed as controls. *In vitro* studies were conducted using primary bone marrow-derived macrophages

(BMDMs) from WT and p75^{NTR} KO and RAW 264.7 cells, incubated with or without nerve growth factor (NGF) and its precursor pro-nerve growth factor (pro-NGF), as p75^{NTR} ligands. Also, macrophages were infected with adenovirus to over express p75^{NTR}. In our *in vivo* experiments we analysed mononuclear phagocytic cells in retinas and RPE choroids, as well as monocytes on blood peripheral samples. Four days after laser, increased levels of pro-NGF were detected in retinal homogenates from WT mice with CNV respect to no laser retinas. Flow cytometry revealed increased recruitment of phagocytic mononuclear cells in retina and RPE-choroid from WT CNV mice compared to p75NTR-KO CNV mice, with no significant changes in circulating monocyte counts between these groups. In addition, cell culture studies evidenced reduced CCR2 expression in p75^{NTR}-KO BMDM cells relative to WT BMDMs. In WT BMDMs, CCR2 levels remained unchanged upon pro-NGF stimulation but decreased in response to mature NGF. In addition, different p75^{NTR} expression levels were directly associated with morphological changes in macrophages of BMDM cells and Raw 264.7 cells infected with adenovirus that over express p75^{NTR}. In sum, our preliminary findings suggest that in macular degeneration, p75^{NTR} may mediate both ligand-dependent and -independent effects in macrophages, modulating their morphology and CCR2 expression.

CB-12

G4 VARIANTS DATABASE: GENERATING A TOOL FOR INDEXING GENOMIC VARIANTS WITHIN GUANINE QUADRUPLEX MOTIFS

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Advances in human genome sequencing over the past decades have shown that most of the variations among human populations consist of short insertions/deletions (indels) and single nucleotide variants (SNVs), which are associated with several diseases or human traits. Predominantly, SNVs are found in non-protein-coding sequences, including intergenic, intronic and untranslated regions, and can influence gene expression by overlapping with transcriptional or translational regulatory elements. Nevertheless, understanding the mechanisms by which SNVs in non-protein-coding sequences affect phenotype and predispose individuals to disease development remains a challenge in human genetics. Guanine quadruplexes (G4s) are a type of non-canonical nucleic acid structures largely described as transcriptional or translational regulatory elements. They are prevalent in regulatory regions and may be structurally and functionally affected by genetic variations. In this work, we used bioinformatics to build a database storing genomic information on SNVs that overlap with sequences presenting motifs with the potential to form G4s (SNV-PG4s). We performed a manual curation process by comparing the recovered sequences with several SNV-PG4s previously described and experimentally characterized in the literature. In addition, we incorporated as controls SNV-PG4s that we had previously identified through an alternative bioinformatics strategy and validated by *in vitro* biophysical assays, including circular dichroism spectroscopy, thermal melting assays and nuclear magnetic resonance spectroscopy, as well

as reporter genes assays in human cultured cells. With these experiments we determined the impact of SNVs on the structure and function of G4s present in the 5' UTRs and in the beginning of the coding sequence of selected cases for study. Our new bioinformatics tool will facilitate the identification of SNV-PG4s as possible causes of variation in gene expression and their use as potential alternative markers of susceptibility to human disease development, as well as novel therapeutic targets for drug design.

CB-13

MOLECULAR MECHANISMS UNDERLYING SYNAPTIC DYSFUNCTION IN ALZHEIMER'S DISEASE: A FOCUS ON RHOGTPASES AND THEIR INTERACTOME

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Synaptic dysfunction in Alzheimer's disease (AD), yet their underlying molecular mechanisms remain incompletely understood. Rho GTPases, key regulators of cytoskeleton dynamics have been proposed as central mediators in this process. However, most studies to date are inconclusive, and no clear consensus exists regarding their activation dynamics. In this study, we employed FRET biosensors to quantify, with high spatial and temporal resolution, the activity of RhoA, Rac1, and Cdc42 in primary hippocampal neurons following early and late exposure to Aβ oligomers. Our preliminary data show that, at 5 minutes post-treatment, RhoA and Rac1 activities are significantly elevated in dendrites, whereas Cdc42 activity decreases both 5 and 30 minutes while these effects were not observed in late exposure to oligomers, suggesting a transient and rapid effect. The signaling responses of Rho GTPases within cells are localized in terms of space, time, and duration, with several of them operating simultaneously depending on different stimuli and in each specific subcellular context. To further characterize how Aβ reshapes the interaction networks of RhoA, Rac1, and Cdc42, we will use TurboID proximity labeling to map changes in their interactomes under pathogenic conditions. This approach will enable the identification of novel pathways and molecular assemblies involved in the early synaptic dysfunction events in AD.

CB-14

DIFLUOROMETHYLORNITHINE (DFMO) SENSITIZES CHRONIC MYELOID LEUKEMIA CELLS TO IMATINIB BY INHIBITING PROTECTIVE AUTOPHAGY

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Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm driven by the BCR-ABL1 oncoprotein, for which tyrosine kinase inhibitors (TKIs) such as Imatinib (IMB) represent the

standard treatment. Despite their success, acquired resistance remains a major clinical challenge. Increasing evidence indicates that autophagy, a conserved lysosomal degradation pathway, provides a protective mechanism that allows leukemic cells to survive under therapeutic stress. In fact, IMB treatment paradoxically induces an autophagic response in CML cells, which contributes to survival and resistance. Therefore, targeting autophagy has emerged as a promising strategy to improve TKI efficacy. Difluoromethylornithine (DFMO) is an irreversible inhibitor of ornithine decarboxylase that blocks polyamine biosynthesis, reducing intracellular spermidine levels, a metabolite known to promote autophagy. Previous studies have shown that DFMO acts as a low-toxicity autophagy inhibitor, reducing autophagosome formation both in vitro and in vivo. In this work, we investigated the ability of DFMO to suppress IMB-induced autophagy and enhance therapeutic efficacy in CML cells. Using K562 cells, derived from a CML patient, as a model, we evaluated cell survival by Trypan Blue exclusion and PrestoBlue metabolic assays. Autophagic flux was assessed through LC3-II Western blotting, complemented by confocal imaging of LC3-RFP puncta and MDC staining. Our results demonstrate that IMB treatment (0.5µM) markedly increased LC3-II levels, confirming the induction of autophagy. DFMO treatment (1mM) significantly reduced basal LC3-II and abrogated the IMB-induced increase, restoring LC3-II/actin ratios to near control values. The LC3 profile with DFMO resembled wortmannin, consistent with inhibition at an early stage of autophagosome biogenesis, while bafilomycin induced LC3-II accumulation as expected for late-stage blockade. Confocal microscopy confirmed a reduction in LC3-positive vesicles in DFMO-treated cells. Importantly, DFMO alone did not compromise cell viability, confirming its low cytotoxicity. In contrast, while IMB alone reduced viability, the combination of DFMO and IMB resulted in a significantly greater loss of viability, highlighting a synergistic effect. Statistical analyses confirmed that DFMO potentiated the cytotoxicity of IMB beyond additive levels. Taken together, these findings indicate that DFMO functions as an early-stage inhibitor of protective autophagy in CML cells, preventing the adaptive response triggered by IMB and thereby sensitizing cells to TKI-induced death. Given that DFMO is FDA-approved for other indications, our results provide strong preclinical rationale for its repurposing as an adjuvant therapy in CML. Further work will extend these observations to IMB-resistant cell lines and assess the effects of DFMO on extracellular vesicle release and miRNA cargo, aiming to better understand its impact on intercellular communication and resistance transmission. Overall, this study identifies DFMO as a promising candidate to enhance TKI efficacy and overcome therapeutic resistance in CML.

CB-15

HYDROXYCHLOROQUINE EFFECTS ON PLACENTAL CELLS EXPOSED TO HYPOXIA: PRELIMINARY RESULTS

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The placenta is a transient but essential organ. Among its cell types, extravillous trophoblasts (EVTs) play a central role in placental attachment to the uterus, communication with maternal immune cells, and spiral artery remodelling to ensure oxygen (O_2) and nutrient supply to the fetus. This population is particularly affected in preeclampsia (PE), a frequent pathology that in Argentina affects ~12% of pregnancies. PE is characterized by hypertension, proteinuria, and edema, and can progress to eclampsia or HELLP syndrome, leading to maternal death.

Currently, no curative treatment exists; only low-dose aspirin is recommended before 16 weeks of pregnancy in women at risk. In this context, the immunomodulator hydroxychloroquine (HCQ) has emerged as a potential therapy, since women with systemic lupus erythematosus (SLE) treated with HCQ show lower PE risk, although its placental effects remain poorly understood. PE and SLE share features such as inflammation and endothelial dysfunction. In PE, inadequate spiral artery remodelling induces oxidative stress, placental damage, and release of antiangiogenic factors (e.g., soluble fms like tyrosine kinase 1: s-Flt-1) and proinflammatory cytokines (e.g., tumor necrosis factor-α: TNF-α), with concomitant reduction of vascular endothelial growth factor (VEGF). The resulting hypoxia stabilizes the hypoxia inducible factor HIF-1α, increases reactive oxygen species, and triggers mitochondrial dysfunction. Herein, we explored HCQ effects in HTR8/SVneo cells (EVT-derived cell line) exposed to hypoxia (1% O_2) versus 20% O_2. Two schemes were applied: -treatment (hypoxia followed by HCQ, to test if damage can be reverted), and -pretreatment (HCQ prior to hypoxia, to test protection). HCQ concentrations were chosen from the literature. Cell viability (resazurin), expression of HIF-1α, KLF6, s-Flt, VEGF, and β-catenin (Western blot/qRT-PCR), and mitochondrial features (immunofluorescence) were evaluated. Preliminary results indicate that HCQ does not affect cell viability. As expected, hypoxia stabilized HIF-1α, and interestingly HCQ (treatment and pretreatment) reduced HIF-1α and β-catenin protein levels, without affecting KLF6. Pretreatment decreased s-Flt and VEGF mRNA, while treatment increased s-Flt and reduced VEGF. TOM20 immunodetection showed that hypoxia decreased mitochondrial mass, and HCQ partially restored it. These findings suggest that HCQ modulates EVT responses at the molecular and cellular levels in a scheme-dependent manner and may exert beneficial effects for PE prevention or treatment.

CB-16

SATURATED FAT AND SUGAR PROMOTE BREAST CANCER MALIGNANCY VIA HIF-1A, FASN AND THE TUMOR MICROENVIRONMENT

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Breast cancer is the most frequent diagnosed cancer in women worldwide and remains a major cause of cancer-related mortality. In addition to genetic predispositions, increasing evidence highlights the influence of diet on tumor metabolism and progression. Population-based studies from Córdoba, Argentina, identified dietary patterns enriched in palmitic acid (PA) and sucrose as risk factors for breast cancer. However, the cellular and molecular mechanisms by which these nutrients affect tumor biology remain unclear. In this study, we evaluated the impact of high-palmitic acid, high-carbohydrate, and combined diets on breast tumor development in a murine model. Mice were divided into four dietary groups (n=20 each): CONTROL (6% corn oil + 30% sugar), HPOD (20% palm oil + 15% sugar), HCD (20% corn oil + 45% sugar) and HPCD (20% palm oil + 45% sugar). After 90 days on dietary treatment, mice were inoculated with murine breast adenocarcinoma cells (LM3: $1x10^6$ cells) and sacrificed 30 days later to analyze biochemical blood profile, tumor membrane composition, cancer growth

parameters (Ki67, necrosis, mitosis, infiltration, HIF-1α, FASN) and tumor microenvironment (α-SMA, F4/80, CD8 T cell). *In vitro*, LM3 cells were treated with PA (40μM), Fr (2.5mM) and the combination of both treatments (PA+Fr), to evaluate cell viability, proliferation, lipid profile, HIF-1α and FASN expression. Experiments were repeated at least three times and analyzed by ANOVA ($p<0.05$). We observed that the combined diet (HPCD) significantly accelerated tumor growth compared to the other diets. Biochemical analyses revealed in HPCD mice group, elevated circulating triglycerides and altered fatty acid profiles in tumor cell membranes. In this mice dietary group (HPCD) tumor histopathological examination demonstrated poorly differentiated adenocarcinomas with inflammatory infiltrates, while immunohistochemical analyses showed increased expression of HIF-1α and FASN. Also, at the microenvironmental level, tumors from this diet displayed enhanced α-SMA and F4/80 staining, indicating activation of cancer-associated fibroblasts and recruitment of pro-tumorigenic macrophages. *In vitro*, cell treatments with PA+Fr (40μM/2.5mM) reduced apoptosis and increased viability compared to the control samples. Also, this combination of nutrients increased FASN expression on LM3 cells. Collectively, these findings provide evidence demonstrating that saturated fats and simple sugars synergistically drive tumor progression by activating the HIF-1α/FASN axis and remodeling the tumor microenvironment. These nutrients mediated a crosstalk promoting lipid accumulation, cellular proliferation, and stromal/immune activation leading to an aggressive tumor phenotype. Our findings highlight the critical role of dietary patterns in breast cancer biology and open new evidence for nutri-epigenetic and metabolic interventions targeting the tumor microenvironment.

CB-17

CANCER RESEARCH IN CHALLENGING TIMES: HARNESSING FREE BIOINFORMATICS TOOLS FOR BIOMARKERS STUDIES

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The advances in omics and bioinformatics have revolutionized cancer research, enabling a better comprehension of molecular mechanisms, biomarker studies, precision medicine, and the development of public databases that compile abundant data on cancer patients. Our work is based on the utilization of this information and its analysis using bioinformatics tools to address specific questions for different cancer types. In the first place, we studied AKT expression in patients with lung adenocarcinoma (LUAD). Lung cancer is the main cause of cancer mortality worldwide, with LUAD being the most incident subtype. AKT gene regulates crucial functions for tumor development, such as cell survival and division, metabolism, migration, angiogenesis and immunomodulation. The PI3K/AKT/mTOR pathway is overactivated in several tumors, and this is associated with poor survival. AKT protein presents three isoforms: AKT1, which is mainly involved with apoptosis inhibition and tumor cell proliferation; AKT2, associated with metabolism, invasion and metastasis; and AKT3, related to the nervous system. Regarding this evidence, there is a need for a deeper understanding of the processes on which each isoform is particularly participating in cancer and its relevance for patients' prognosis. In our study, we focus on AKT2, exploring its impact in LUAD patients' survival and its specific role in tumor biology. In a second study, we used data from a cohort of patients with glioblastoma (GBM) for the screening of the genes that constitute the circadian clock machinery, in order to analyse their potential as prognosis biomarkers. GBM is the most

frequent and aggressive cancer of the central nervous system, with a median overall survival of about 15 months for patients under the standard treatment. Circadian disruption has been associated with pathologies such as cancer and metabolic disorders, and results from our laboratory show evidence that GBM cells conserve a functional clock and present differences in their response to chemotherapy across the circadian range. This suggests that the clock may control tumor growth in a temporal manner. The nuclear receptors RORs and REV-ERBs are clock genes that have been linked to the integration between the circadian system and metabolism, which is often reprogrammed in tumor cells. Many authors propose that these genes may have a relevant role in tumor progression. Our aim is to further investigate the impact of these clock genes and their relationship with cancer development and metabolic reprogramming. For these studies, data from patients included in the TCGA Pan-Cancer atlas were used and the analysis was made using different bioinformatics platforms, including cBioPortal, KMPlot, String and WebGestalt.

CB-18

IDENTIFICATION AND CHARACTERIZATION OF NUCLEIC ACID SEQUENCES INVOLVED IN MEIOTIC ALIGMENT AND RECOMBINATION

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Spermatogenesis is the process of sperm cell formation; these cells constitute what an organism will contribute to the next generation, making their proper development essential for the preservation and continuity of a species. Among the different stages of spermatogenesis, meiosis is one of the most critical. Meiosis is a cell division process that involves a round of DNA replication followed by two consecutive divisions (meiosis I and II), ultimately producing haploid cells. During prophase of the first meiotic division, two crucial events take place: the pairing and recombination of homologous chromosomes. Pairing is essential for maintaining the species chromosome number, while recombination represents a fundamental source of biodiversity in sexually reproducing organisms. Both processes are mediated, at least in part, by a protein structure unique to meiosis: the synaptonemal complex (SC). In mammals, eight proteins have been identified as constituents of SCs. However, the molecular mechanisms enabling SCs to mediate the recognition, pairing, and recombination of homologous chromosomes remain unknown, as does the potential involvement of specific DNA sequences in this process, and more so, their identity. It has been shown that certain structural SC proteins, such as SYCP3 and SYCP1, are capable of binding DNA, although the specific bound sequences remain unidentified. In the present study, we aimed to evaluate the existence of specific SC-interacting nucleic acid sequences and determine their identity, using chromatin immunoprecipitation (ChIP) with anti-SYCP1 and SYCP3 antibodies, followed by library preparation and high-throughput sequencing of the DNA bound to these proteins (ChIP-seq). Bioinformatic analyses showed an enrichment in repetitive sequences, particularly those of the L1 type (for LINE-1, Long Interspersed Nuclear Element-1), followed by repeats of the ERVK class (Endogenous Retrovirus-K), a type of endogenous retrovirus. The association of SC

components with this category of repeats has not been previously reported and may suggest a novel functionality for these sequences, which have often been regarded as "junk DNA". Although these findings are still preliminary, the characterization of the identified sequences will contribute to the elucidation of the molecular bases underlying the interaction between the SC and chromatin, as well as their associated processes, which, despite their essentiality for all sexually reproducing organisms, are still poorly understood so far.

CB-19

CHARACTERIZATION OF NOVEL SHORT LINEAR MOTIF (SLiM) VARIANT THAT MEDIATE MITOTIC FUNCTIONS OF POCKET PROTEINS

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The pocket proteins (PP) Rb, p107 y p130 regulate cell proliferation and differentiation by interacting with different cellular regulators. PPs control cell cycle progression at the G1/S checkpoint by binding to E2F transcription factors and blocking the transcription of S phase genes, but new mitotic roles have been proposed for which the molecular mechanisms are unknown. The central domain of PP harbors the E2F and LxCxE clefts, which mediate the interaction with two Short Linear Motifs (or SLiMs): the E2F and LxCxE SLiMs, present in cellular interactors. SLiMs are small segments of 5-16 amino acids present in disordered regions of proteins, and mediate dynamic interactions that are essential for signaling and regulate the assembly of protein complexes. SLiMs can be represented by regular expressions that reflect the variability admissible in their sequence, obtained from the analysis of conservation found in orthologous sequences. To identify new interactors that potentially mediate PP functions and contain SLiMs for pocket protein binding, our laboratory implemented two unbiased proteome-wide approaches: 1) a proteomic screening by peptide phage display and 2) a bioinformatic screen using SLiM regular expressions. The present project aims to validate three new candidates: KNL1, DDX24 and SPNDC employing an *in vitro* Coupling-Pull Down assay between peptides fused to GST (GST-peptide) and the Rb and p107 pocket proteins. The use of PP variants developed in our group which disrupt binding to the E2F and LxCxE clefts enabled mapping of the binding site for each candidate. Proteomic peptide phage display identified a non-canonical E2F motif in KNL1, an intrinsically disordered kinetochore-associated protein. Pull down confirmed KNL1 interaction with the E2F cleft of Rb with medium to low affinity. Additionally, two KNL1 motif mutants were generated to validate the non-canonical motif: one restored a canonical high-affinity motif, enhancing the interaction, while the other inactivated the motif, drastically reducing binding. The other two candidates harbor LxCxE motifs and were identified from bioinformatic screens: SPNDC is a chromatin-associated protein and DDX24 is an RNA helicase that regulates mRNA stability. We confirmed that SPNDC and DDX24 interact with the LxCxE cleft of Rb, with SPNDC showing medium to low and DDX24 showing high affinity. We also confirmed that DDX24 interacts with the p107 LxCxE cleft with high affinity. These findings expand the repertoire of pocket protein interactors and suggest that suboptimal motifs may modulate non-classical mitotic functions, offering new perspectives on their role in pocket protein-mediated interactions.

CB-20

REWIRING OF THE SECRETORY HUB DURING PLACENTAL DEVELOPMENT

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The placenta is a transient organ that sustains fetal growth, mediates maternal metabolic adaptation, and acts as a barrier to protect the fetus. Trophoblast differentiation is essential for placental development. Villous cytotrophoblasts (CTB) fuse to form the syncytiotrophoblast (STB), a multinucleated layer specialized in maternal–fetal exchange and in secreting hormones and other proteins essential for the initiation and maintenance of pregnancy. Because this process involves a marked increase in secretory demand, remodeling of the Golgi complex and activation of adaptive transcriptional programs are expected, but remain poorly explored. Members of the CREB3 transcription factor family (CREB3 and CREB3L1–4) are differentially expressed across tissues, where they perform distinct functions, including promoting cellular differentiation, regulating genes of the secretory pathway, and controlling the expression of tissue-specific proteins, which suggests they may play a central role in supporting trophoblast adaptation during differentiation. In this work, we used forskolin (FSK)-induced differentiation of choriocarcinoma derived-BeWo cells to monitor structural and molecular changes in the secretory pathway. Immunofluorescence analysis of cis- and medial-Golgi markers (GM130 and GalNAc-T2) revealed that FSK treatment triggers the redistribution of Golgi markers from a compact perinuclear position to scattered cytoplasmic particles. Quantitative image analysis showed a progressive increase in both the number of Golgi particles and their cumulative area during differentiation. Consistently, Western blot and qRT-PCR experiments demonstrated increased GM130 and GalNAc-T2 protein and mRNA levels. Furthermore, transcript profiling of the five CREB3 family members revealed the selective induction of CREB3L1 and CREB3L2, with peak expression at 6 h post-treatment, highlighting them as candidate regulators of trophoblast secretory adaptation. Together, these findings provide new evidence of secretory pathway remodeling during CTB-to-STB differentiation and support a role for CREB3L1/2 in coordinating this process. Understanding these mechanisms may contribute to elucidating the pathophysiological alterations underlying pregnancy disorders.

CB-21

UNBIASED PROTEOME-WIDE IDENTIFICATION OF NEW MOLECULAR INTERACTIONS OF POCKET PROTEINS

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Pocket proteins (PP) play key regulatory roles in cell proliferation and differentiation. The PP

family, which includes the Retinoblastoma protein (pRb) and its paralogs, p107 and p130, regulates cell cycle progression at the G1/S checkpoint by direct binding to E2F transcription factors and other cellular effectors. However, novel functions have recently been described for which there is no knowledge of the underlying molecular mechanisms. Short Linear Motifs (SLiMs) are 5 to 15-residue sequences located in intrinsically disordered regions that drive dynamic protein-protein interactions essential for signaling, making them strong candidates to explain novel PP functions. PPs use two SLiMs, the LxCxE and E2F motifs, to bind to E2F and other protein partners. The aim of this work is to discover SLiM-mediated interactions that regulate PP functions. For this, we carried out Proteomic Peptide Phage Display (ProP-PD) experiments using the central pocket domain of the PP family as bait and three libraries expressing 16 amino acid peptides that represent all intrinsically disordered regions of the human proteome. As a result, we obtained over 100 high quality hits that represent novel candidate SLiM-mediated interactors for pRb and p107. Known and novel interactors were found to contain E2F-like and LxCxE-like SLiMs. We selected the most interesting hits according to whether they had cell cycle related functions and/or nuclear localization. We optimized pull-down assays and cross-validated 18 of these novel candidates, mapping the binding sites on the PP by pull-down and Size-Exclusion Chromatography assays. Among them, a novel LxSxE motif present in E2F4, the best known interactor of p107, stands out. This protein was chosen to perform cellular experiments using a luminescence reconstitution assay (GPCA) and constructs carrying mutations to assess the contribution of both motifs (E2F and LxSxE) to the interaction between E2F4 and p107. Loss of the E2F motif drastically decreased the interaction, whereas the absence of the LxSxE motif reduced the interaction by 15-30% and affinity measurements confirmed its contribution to E2F4-p107 binding. Competition experiments suggest that the E2F4 LxSxE motif acts as a molecular switch that competes with Lin52 regulating DREAM Complex assembly. We also assessed novel cell cycle regulators such as the GTSE1 protein. Affinity measurements confirmed the presence of a high-affinity (Kd 1 µM) LxAxE motif variant in GTSE1, and a motif-disrupting mutant abrogated p107 binding in HEK293 cells. Our results validate the role of a new SLiM in E2F4 which regulates DREAM complex assembly and identifies novel regulators such as GTSE1. Our unbiased screen identifies novel PP interactors at a proteome-wide scale, contributing to elucidate SLiM-mediated interactions that regulate PP functions. Future work will validate further hits and explore their functions in physiological and cancer cellular models.

CB-22

ALTERATIONS IN CHOLESTEROL TRAFFIC IN ASTROCYTES INDUCED BY INFLAMMATORY MOLECULES AND THEIR RESCUE BY CANNABIDIOL THROUGH NON-TRANSCRIPTIONAL MECHANISMS

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The brain contains over 20% of the cholesterol found in mammalian bodies, where neurons require large amounts of cholesterol to maintain their complex morphology and facilitate synaptic transmission. Due to the presence of the blood-brain barrier, which prevents the entry of peripheral cholesterol, neuronal cholesterol is mainly synthesized by astrocytes and exported in the form of ApoE-cholesterol complexes, which are then imported by neurons through a process of endocytosis. Previous work in the laboratory has shown that the aging of hippocampal neurons is associated with a decrease in plasma membrane cholesterol levels, resulting in reduced synaptic function and memory loss. In addition, in a recent study by the

laboratory, we demonstrated that aged hippocampal astrocytes accumulate high levels of cholesterol in lysosomes, preventing the transport of cholesterol from astrocytes to neurons. Additionally, the intracellular accumulation of cholesterol observed in aged astrocytes has been shown to improve with cannabinoid treatment, via an unknown pathway independent of CB1, the main cannabinoid receptor in the hippocampus. In line with evidence indicating that the transcriptional profile of aged astrocytes is similar to that of neuroinflammatory reactive astrocytes, we found that reactive astrocytes reproduce the alterations observed in aging conditions, accumulating high levels of cholesterol in the lysosomal compartment, late endosomes, and lipid droplets. In addition, we observed that cholesterol-laden lysosomes acquire a perinuclear distribution, suggesting an alteration in lysosomal trafficking in these cells. Furthermore, we found that transport between reactive astrocytes and neurons was stimulated by cannabidiol (CBD) treatment through mechanisms that do not involve protein synthesis. Since inflammation is a common feature of neurodegenerative diseases and brain infections, this study provides relevant information about a new mechanism by which these inflammatory processes impact brain function and proposes a possible rescue strategy through CBD administration.

CB-23

CONTACT INHIBITION TRIGGERS AUTOPHAGIC DEGRADATION OF YTHDF PROTEINS TO FINE-TUNE SMOOTH MUSCLE CELL DIFFERENTIATION

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Contact inhibition is a fundamental process that leads to arrest of cell proliferation and maintains tissue homeostasis. Its dysregulation is a hallmark of cancer and contributes to other pathological conditions that present undesired cell-growth. Smooth muscle cells (SMCs) are one of the main cell types present in the arterial wall, where they play a central role in regulating vascular tone and function. SMCs present a remarkable phenotypic plasticity, being able to switch from a contractile-quiescent to a synthetic-proliferative state. These cell-state transitions, often called SMC "phenotypic switch", are involved in several pathologies like pulmonary hypertension and atherosclerosis, where SMCs behaviour has been compared to that of tumour cells. Molecular mechanisms underlying SMCs phenotypic switch are still being unravelled. N6-Methyladenosine (m6A), the most prevalent mRNA modification, has recently been linked to regulation of cell proliferation, differentiation, apoptosis and has a proven role in several types of cancers. This modification is recognized among others by a family of proteins that share the YT521-B homology (YTH) domain, which then control degradation or translation of bound mRNAs. While the downstream mechanisms involving these proteins are increasingly understood, less is known about their own regulation. Here we show that YTHDF proteins are downregulated at high cell density, but only in cells that exhibit contact-inhibition. Concurrently, we found that autophagy flux increases during the onset of contact inhibition in non-cancerous cells, a feature notably absent in cancer cell lines. We detected that the previously observed downregulation of YTHDF proteins is dependent exclusively on active protein degradation. This post-translational control is dependent on both the proteasome and autophagy. Mechanistically, we observed a direct interaction between YTHDF2 and the core

autophagy machinery proteins of the GABARAP/LC3 family. An in silico screen identified eight putative LC3 Interacting Regions (LIRs) in YTHDF2, several of which we validated in vitro, confirming a direct, motif-dependent interaction. Finally, we employed a combined transcriptomic and proteomic approach to dissect the contribution of YTHDF2 to smooth muscle contact-induced differentiation. This demonstrated that while removal of YTHDF2 is important for the progression of differentiation, premature downregulation of YTHDF2 produces aberrant, less contractile cells, with upregulation of markers of synthetic SMCs and of the interferon pathway. This work presents a novel relationship between contact-inhibition, autophagy and the m6A-epitranscriptome, with potentially profound implications for cancer and vascular disease. Our findings underscore that the timely regulation of m6A reader proteins is a critical checkpoint for ensuring correct cell-state transitions.

CB-24

ROLE OF NUCLEAR N-ACETYLLACTOSAMINE RESIDUES IN CELLULAR PHYSIOLOGY

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Among the post-translational modifications of proteins in human cells, one of the most frequent is O-glycosylation, which is fundamental to cell physiology and associated with various pathologies. In particular, O-GlcNAc-type glycosylation can occur in different subcellular regions, performing different regulatory functions. Recently, our working group observed that the elongation of this terminal (GlcNAcβ-O-Ser/Thr) can occur in the nucleus of human tumor cells when purified nuclei are incubated with UDP-Gal to generate the terminal N-acetyllactosamine (Galβ4-GlcNAcβ-O-Ser/Thr; LacNAc). However, its nuclear function remains unknown. To investigate this, a controlled glycosylation model is needed, and the CHO ldlD cell line is suitable because it lacks GALE epimerase, which is required to synthesis of UDP-Gal from UDP-glucose. However, the exogenous addition of galactose to the cell culture medium allows the biosynthesis of UDP-Gal to be recovered and, consequently, UDP-Gal-dependent glycans such as LacNAc terminals are synthesized. The aim of this study is to investigate the nuclear significance of galactose terminals of glycans such as LacNAc in relation to changes in the cell phenotype associated to cell protein levels. CHO ldlD cells cultured in the presence or absence of galactose showed an increase in LacNAc terminals in the cell nucleus. This nuclear increase in terminal LacNAc was observed in both whole cells and purified nuclei using confocal microscopy. Subsequently, we evaluated changes in LacNAc terminal levels on certain nuclear proteins in the experimental model CHO ldlD ± Gal using sandwich ELISA. We quantified LacNAc terminals in nuclear proteins such as Lamina B1, Histone H3, and RNA pol II, where an increase in terminal LacNAc was observed in Lamina B1. These results allowed us to identify at least one glycosylated protein associated with the organization of the nuclear envelope that plays an important role in gene expression regulation. This finding suggests that nuclear LacNAc residues have a potential regulatory role in nuclear architecture and protein expression. To evaluate this property, the relative abundances of total cellular proteins from CHO ldlD cells with low- and high-level of nuclear LacNAc were compared. The results of the proteomic analysis show that 2 proteins increased and 26 decreased their relative protein levels. Here, it was observed that there are regulated proteins that are part of different metabolic pathways such as ATP-binding or nucleotide binding. We are currently evaluating the mRNA levels of several target genes by qPCR to determine whether the observed regulation occurs at the transcriptional or translational level.

These results support the hypothesis that the presence of terminal LacNAc in the cell nucleus is associated to regulatory processes of protein expression.

CB-25

TARGETED SUMOYLATION TRIGGERS ANTIGENIC VARIATION IN *Trypanosoma brucei*

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SUMOylation is a post-translational modification in which the ubiquitin-like protein SUMO is covalently attached to lysine residues of target proteins, altering their interaction properties. This process requires the sequential action of an activating enzyme (E1), a conjugating enzyme (E2), and in some cases, a ligase (E3).
In our laboratory, we use Trypanosoma brucei as a model system. The surface of this parasite is densely coated with a single variable surface glycoprotein (VSG), whose periodic replacement enables immune evasion through antigenic variation. For a VSG gene to be transcribed, it must reside in one of ~20 telomeric expression sites (ES). Transcription occurs within the extranucleolar Expression Site Body (ESB), a nuclear compartment that harbors RNA polymerase I (RNAPI), the enzyme responsible for VSG expression.
The active ES is characterized by a strong accumulation of SUMOylated proteins in a distinct nuclear structure termed the Highly SUMOylated Focus (HSF). The HSF colocalizes with the active VSG promoter and the RNAPI extranucleolar focus, but it is absent from silent ESs. Several additional condensates associated with the ESB have been described in T. brucei, each linked to processes such as splicing or transcriptional activation. SUMOylation of key factors appears essential for their assembly or localization within these condensates.
To test whether SUMOylation can drive the formation of condensates that activate silent VSG loci, we adapted the lac operator–repressor system. We engineered bloodstream-form T. brucei carrying LacO repeats upstream of a silent ES, and inducibly expressing a fusion between LacI and the SUMO-conjugating enzyme E2 (LacI–E2). This strategy directs E2 activity specifically to the LacO-tagged silent ES.
Immunofluorescence analysis revealed that recruitment of LacI–E2 to a silent ES triggers activation of VSG expression. After induction, parasites switched VSGs at a frequency that increased over time and reached levels four orders of magnitude higher than in control lines. These findings demonstrate that targeted SUMOylation at the promoter of a silent ES is sufficient to activate VSG transcription and drive the replacement of the parasite's surface coat.

CB-26

FUNCTIONAL CIRCADIAN CLOCKS REGULATE CELLULAR SENSITIVITY TO PHOTODYNAMIC THERAPY THROUGH PHOTOSENSITIZER UPTAKE AND REDOX STATUS

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Glioblastoma (GBM) is the most aggressive and deadliest tumor of the central nervous system, originating from glial cells and characterized by its infiltrative capacity and rapid progression. The current treatment follows the Stupp protocol, which involves surgical removal of the tumor mass followed by chemo and radiotherapy. Despite treatment, a patient with GBM usually survives around 14 months, highlighting the critical need for new therapeutic strategies. Photodynamic therapy (PDT) represents a promising approach that combines a photosensitizer (PS), light at the excitation wavelength of the PS, and oxygen. Individually harmless, these components together generate reactive oxygen species, hydroxyl radicals, and singlet oxygen, creating a redox imbalance in cells that leads to cell death. Emerging research has shown that the effectiveness of cancer treatments can be affected by the biological clock inherent in each cell, which coordinates multiple metabolic functions. Disruptions in genes associated with this molecular clock have been linked to the development of various cancers, including GBM. Given the complex link between the biological clock and cancer cell growth, combining chronopharmacology with existing treatments like PDT could improve therapy outcomes. In this study, we explore the novel combination of PDT and chronotherapy in GBM cells, using PSs from the phthalocyanine family (ZnPc and ClAlPc) and 5-aminolevulinic acid (5-ALA), the latter being an FDA-approved PS precursor. Initial characterization of clock genes *Bmal1* and *Per1* confirmed the presence of a functional molecular clock in GBM cells. Phototoxicity assays in dexamethasone-synchronized cells, with photosensitizers (PS) administered at different post-synchronization times, revealed a time-dependent variation in cell viability after PDT. These findings suggest that the timing of PS administration has a critical influence on PDT efficacy. Periodicity analysis using mathematical algorithms (JTK, ARS, and RAIN) showed that each PS displayed a distinct oscillatory profile. Uptake assays with ZnPc further revealed a phase shift compared to cell viability, suggesting decreased PS incorporation during periods of increased resistance. Finally, evaluating cellular redox status through ROS and NRF2 measurements indicates increased detoxification activity at times associated with greater resistance to 5-ALA PDT. This study represents a pioneering effort to explore the potential of combining PDT and chronopharmacology in the treatment of glioblastoma, offering hope for an improved prognosis for those affected by this devastating tumor.

CB-27

BIOCOMPATIBILITY STUDY OF PLYETHYLENGLYCOL-COATED IRON OXIDE NANOPARTICLES IN HUMAN BLOOD COMPONENTS

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Nanoparticles are used in a wide range of applications, including diagnostics, drug delivery, and the development of targeted therapies. Because of their small size, they can interact with

biological molecules at the cellular and molecular levels, opening new possibilities for the treatment and prevention of diseases. In previous work, we synthesized and characterized polyethylene glycol-coated iron oxide nanoparticles (MAG@PEG), which exhibit magnetic properties along with favorable size, shape, and hydrodynamic diameter, making them suitable nanocarriers for biomedical applications. Based on *in vitro* cytotoxicity studies, we demonstrated that MAG@PEG is a safe nanosystem. Since intravenous administration is the most efficient route to deliver magnetic nanoparticles to target tissues, in this study we further investigated their hemocompatibility by evaluating the hemostatic and thrombogenic effects of MAG@PEG in human blood. All experiments were carried out using blood or plasma from volunteers who provided informed consent. For hemolysis assays, anticoagulated human blood was incubated with different MAG@PEG concentrations for one hour at 37°C, and hemoglobin release from red blood cells was spectrophotometrically measured at 540 nm. Results showed hemolysis rates below 2% under all tested conditions compared with the control, indicating that the nanosystem is non-hemolytic. Additionally, optical microscopy of blood smears from each condition showed that red blood cells maintained normal morphology across all samples. Conversely, incubation of whole non-anticoagulated blood with MAG@PEG shortened clot formation time. H&E-stained histological sections of the resulting clots revealed platelets and a fibrin network associated with nanoparticles. Coagulation factor analyses, including activated partial thromboplastin time (APTT), fibrinogen, thrombin, and thromboplastin in human plasma, showed no significant differences between treated and control conditions. However, at higher nanoparticle concentrations, APTT was prolonged, suggesting that the intrinsic coagulation pathway might be affected. Altogether, these results indicate that the MAG@PEG nanosystem exhibits good hemocompatibility, with negligible hemolytic activity and only a moderate impact on the intrinsic coagulation pathway at high concentrations, supporting their potential for safe intravenous use in biomedical applications.

CB-28

EISOSOMES PLAY A ROLE IN THE BIOLOGY OF THE CYSTEINE-RICH PALMITOYLATED DOMAIN (CYSPD) PROTEIN FAMILY IN YEAST

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The cysteine-rich palmitoylated domain (CYSPD) protein family, a group of small proteins conserved across eukaryotes, is poorly understood functionally. These proteins are anchored to the plasma membrane via palmitoylation. In yeast, the family includes Cpp1, Cpp2, Cpp3, and YDL012C. To uncover their function, we used proximity labeling with TurboID followed by mass spectrometry to characterize the interactome (or "proxitome") of these proteins. STRING analysis of the identified proteins revealed interactions with key components of several biological processes, including eisosome assembly, cell wall organization, endocytosis, and vesicular transport. The interactions with eisosome components were particularly notable, involving proteins like Pil1, Lsp1, Sur7, Eis1, Seg1, Mdg1, and Ygr130c. To investigate this connection further, we examined the localization of GFP-tagged Cpp1 in yeast strains lacking the essential eisosome genes PIL1, LSP1, and SUR7. We observed a significant shift in Cpp1's localization. In wild-type cells, Cpp1 showed a homogeneous plasma membrane distribution polarized towards the daughter cell. However, in the mutant strains, it adopted a distinct punctate, submembranous pattern. This localization change was specific to Cpp1, as control

proteins like Sso1 (a transmembrane protein) and Ras2 (a lipidated protein) did not exhibit this behavior. Additionally, some of these Cpp1 puncta in the pil1Δ mutant colocalized with early endosomes labeled with the FM4-64 dye, suggesting that the endocytosis of Cpp1 is impaired in these strains. Together, our findings provide the first evidence for a functional link between eisosomes and the CYSPD protein family, specifically implicating eisosomes in the proper localization and endocytic regulation of these proteins in yeast.

CB-29

GLIAL STRESS AND α2-MACROGLOBULIN/LRP-1 SYSTEM UNDER METABOLIC STIMULI IN MIO-M1 CELLS

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Metabolic syndrome (MS), is a complex disorder of metabolism, characterized by obesity, hypertension, hyperglycemia, insulin resistance and dyslipidemia, and it is considered a global epidemic with high socioeconomic cost. MS affects different organs including the retina, triggering retinopathy. This retinal disease is characterized by vascular, glial and neuronal damage. Retinopathy has been considered as vasculopathy; however, there is limited knowledge about the neuronal and glial damage that takes place even before the vascular alterations. This highlights the need to study cellular and molecular mechanisms involved in early stages of retinopathy, which could lead to new therapeutic targets. Over the last twenty years, our group has been studying the role of a broad-spectrum protease inhibitor, α_2-Macroglobulin (α_2M), in ischemic-induced retinopathies. Previous results showed α2M and LRP-1 protein expression in Müller glial cells (MGCs) and retinal neurons, primarily ganglion cells, as well as increased retinal protein expression of α2M in MS mice. Since MGCs are essential for retinal microenvironment homeostasis, the integrity of the Blood-Retinal Barrier (BRB) and neuron thriving, this study aimed to evaluate glial stress and the expression of α2M and LRP-1 in MIO-M1 cell culture under metabolic stimuli, including high concentrations of glucose (HG) or modified low-density lipoproteins (LDL) such as highly-oxidized and glycated LDL (HOG-LDL), aggregated (aggLDL), or oxydazed (oxLDL). In this opportunity, MIO-M1 cells with 80% confluence were serum-deprived for 30 minutes and then exposed to HG 30 mM or aggregated low-density lipoproteins (aggLDL, 50–100 µg/mL) for 8 or 24 h. Protein expression of glial stress (GFAP), antioxidant (HO-1), and α2M/LRP-1 was analyzed by western blot. One-way ANOVA followed by Bonferroni post-test was applied ($p \leq 0.05$). Results showed an increase in GFAP protein expression (p= 0.0008), a decrease in HO-1 expression (p= 0.04), without modifying LRP-1 levels, MIO-M1 lysates stimulated with HG 30 mM for 24 h. Meanwhile, aggLDL-stimuli at a concentration of 100 µg/mL upregulated GFAP at 8 h (p= 0.003), while reducing HO-1 (p= 0.02) and LRP-1 (p= 0.0006) expression at 24 h, in MIO-M1 lysates. Although α2M was expressed in MIO-M1 cells, its levels remained unchanged under all of the conditions evaluated in this approach. These findings demonstrate glial reactivity and impaired antioxidant response in MGC under metabolic stressors. Even though α2M expression was not altered, aggLDL-induced LRP-1 downregulation highlights the importance of investigating the α2M/LRP-1 system in MGCs exposed to adverse metabolic stimuli.

CB-30

NUCLEAR β-GALACTOSIDASE ACTIVITY IS INCREASED IN HUMAN SENESCENT CELLS

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Cell nucleus houses the genome and coordinates key processes such as DNA replication, repair, segregation, and gene expression. These regulated events involve a complex network of proteins whose activity is frequently fine-tuned by post-translational modifications (PTMs). Among them, protein glycosylation, the covalent addition of carbohydrates to proteins, is one of the most relevant. Biosynthesis of O-glycans as GlcNAcβ-O-Ser/Thr, GalNAcα-O-Ser/Thr and Galβ-3GalNAcα-O-Ser/Thr (core 1 glycan) take place in the cell nucleus. Evidence for this includes the presence of donor and acceptor substrates of glycosyltransferases, several glycosyltransferases and products of O-GalNAc glycosylation exposing β-Gal residues in human cell nuclei. Biological functions of nuclear glycoproteins have shown to be controlled by glycosyltransferases that yield the PTMs and glycosidases that process the terminal glycans as part of a dynamic process. Increased β-galactosidase activity is a hallmark of senescent cells, where this biomarker is described as senescence-associated β-galactosidase (SA-β-Gal) activity. Interested in the biosynthesis, function and processing cycle of nuclear glycans, we began investigating glycosidases located in cell nucleus. Our previous results have shown solid evidence of β-galactosidase activity in human cell nuclei by using different catalytic assays. Confocal microscopy allowed us to appreciate the nuclear localization of Glb1 (human β-galactosidase), an enzyme responsible for the hydrolysis of β-galactose residues. In the present work we aim to evaluate the nuclear β-galactosidase characteristics in human senescent cells. For that purpose, human U87MG glioblastoma cells were assayed, and senescence induction was carried by incubation with 300 mM galactose during 7 days. Senescence-induced cells showed higher positivity SA-β-Gal stain by cytochemistry, validating the experimental model previously described. Using a catalytic assay, we measured increased β-galactosidase activity in the nucleoplasm of senescent cells. Consistently, confocal microscopy revealed higher nuclear localization of Glb1 in these cells. Overall, these findings highlight the potential significance of nuclear β-galactosidase activity in senescent cells, a process critically involved in cell aging and multiple human pathologies such as cancer and neurodegenerative diseases.

CB-31

THERAPEUTIC POTENTIAL OF MIRNAS IN CHRONIC KNEE OSTEOARTHRITIS

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Knee osteoarthritis (KOA) is a multifactorial joint disorder characterized by chronic inflammation, extracellular matrix (ECM) degradation, and chondrocyte hypertrophy, leading to progressive cartilage loss and joint dysfunction. It affects over 300 million individuals worldwide and remains a major unmet clinical need, as no disease-modifying drugs have been approved to date. MicroRNAs (miRs), have emerged as central regulators of cartilage homeostasis and inflammatory signaling, offering the potential to reprogram dysfunctional

chondrocytes and restore tissue integrity. Here, we aim to functionally validate the therapeutic potential of miRs in OA by establishing a human-derived biologically relevant model of chondrocytes under inflammatory stress. Primarily, we perform a clinical study (NCT-05416255) to search for relevants miRs in the pathophysiology of the disease. MiR-140a-5p, miR-92a-3p, miR-222, miR-204 and miR-31 were identified as potential candidates for KOA therapeutic strategies. We established a biologically relevant *ex-vivo* model isolating human articular chondrocytes (HAC) from grade 4 KOA patients after total arthroplasties. After enzymatic isolation, HAC culture was characterized by flow cytometry. Data indicates a high expression of CD105, CD90 and CD73 (54%, 95.5% and 98%, respectively), markers associated with chondrogenic progenitor cells, a high presence of CD44 (96%), fundamental to cartilage homeostasis and a moderate expression of podoplanin (39.5%), a hypertrophic marker. HACs were negative for markers associated with myeloid and hematopoietic lineages such as CD45, CD34, CD11b, CD19 and HLA-DR. To better represent the disease, we simulate the inflammatory environment using high-IL6 synovial fluid (SF) from patients with severe KOA. In this context, HACs cultured in the presence of SF express a dysregulated metabolic profile evidenced by an increase in the expression of pyruvate dehydrogenase and hexokinase II ($p<0.05$), key enzymes associated with the pathology. In order to evaluate miR-140a-5p and miR-92a-3p as therapeutic candidates to restore chondrocyte function, SF-treated HACs were transfected with its mimics. Subsequently, the regulatory potential of each miR was analyzed using Western blot, qPCR, and immunofluorescence. Overall, the selected miRs were found to exert a regulatory effect on key molecules in OA. Specifically, with both miR-92 and miR-140, a significant increase in the expression of aggrecan, collagen II and a decrease in the expression of ADAMTS4 and collagen I was observed ($p<0.05$), promoting mechanisms associated with the regeneration of the ECM. Likewise, an increase in SOX9 expression ($p<0.05$) was observed. Sox9 is a transcription factor associated with the activation of the chondrogenic cell program. In summary, our findings underscore the promise of miRs as a novel class of therapeutics agents for KOA and open broader perspectives for addressing chronic musculoskeletal pathologies where regenerative options remain limited.

CB-32

LIGHT-INDUCED FORMATION OF STRESS GRANULES AND PROCESSING BODIES EXHIBITS CIRCADIAN OSCILLATIONS IN MURINE CONE CELLS

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Circadian rhythms are ~24-hour oscillations regulated by biological clocks that control multiple cellular functions. The retina has an autonomous circadian system that adjusts its physiology to daily variations in light intensity and spectrum, thereby regulating its response to light-induced damage. We have demonstrated that Processing Bodies (PBs) and Stress Granules (SGs)— RNA–protein biocondensates involved in stress responses—undergo circadian oscillations in cultured cells, modulating their abundance according to circadian phase. Importantly, SGs are associated with protection against cell death, as they can inhibit apoptosis. Furthermore, we have identified the presence of SGs in the retina of Wistar rats exposed to constant LED light. Based on this, we hypothesize that the circadian modulation of SGs and PBs serve as a protective mechanism against light damage during the day. First, we establish the appropriate light stimulus to induce SG and PB formation without causing

excessive cell death. To that end, murine cone cells (661w) were exposed to white LED light (~4500 lux) for durations ranging from 1 to 5 hours. We used immunocytochemistry (ICC) and fluorescence microscopy to analyze SG and PB number and size, alongside MTT and Alamar Blue assays for cell viability. 1h of exposure was sufficient to induce SG and PB formation (a ~26% increase compared to dark control) and, though it affects the cell metabolism, it does not cause cellular death. Next, we studied if the response to this stimulus varies with time. 661w cells were synchronized with dexamethasone and maintained in darkness until its irradiation every 4 hours interval between 8 to 52 hours post-synchronization. We observed that the number and size of both SGs and PBs oscillate in a circadian manner, as well as PB signal intensity, giving credit to our hypothesis. Future studies will explore if this phenomenon replicates *in vivo*.

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EISOSOMES AND PHENOTYPIC HETEROGENEITY DETERMINE THE REPLICATIVE LIFESPAN IN YEAST

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Eisosomes are plasma membrane nanodomains organized by Pil1 and Lsp1 proteins scaffolds, serving as reservoirs for multiple transporters, signaling molecules and proteins of still unknown functions. We are interested in understanding eisosomes' role in cell lifespan using *Saccharomyces cerevisiae* as a model. Replicative lifespan (RLS) is the number of daughter cells a mother yeast cell can produce before death. Traditionally, RLS is measured by manual dissection of mother cells from daughter cells, a technique that is labor-intensive, error-prone and requires several weeks of manual dissections. To overcome these limitations, we implemented a microfluidic single-cell analysis system coupled with a time-lapse bright-field and fluorescence microscopy, enabling real-time monitoring of hundreds of *S. cerevisiae* cells for more than 96 h under controlled nutrient conditions. We assessed both wild-type (WT) and eisosome-mutant strains. We found that the disassembly of eisosomes exhibited an extended RLS, underscoring the structural relevance of eisosomes in lifespan regulation. Furthermore, these devices enabled us to assess phenotypic changes that occurred during aging and correlate them with different lifespan trajectories. We observed that WT yeast daughter cells exhibited either elongated (mode 1) or rounded (mode 2) morphologies during the later stages of their lifespan, leading to significant variations in their mother's longevity. Our analysis revealed that the lifespan extension observed upon eisosome disassembly appears to be independent of the prevalence of morphological modes 1 and 2, indicating that the effect of eisosome organization on RLS is not directly linked to these aging-associated phenotypic states. In addition, we monitored eisosomes using a Pil1-mNeonGreen fusion protein which allowed us to analyze Pil1 dynamics in individual cells from birth until death by fluorescence microscopy. Our results highlight the power of microfluidic devices to accelerate lifespan studies and integrate physiological, morphological, and genetic data at the single-cell level. This work provides new insights into plasma membrane nanodomain function and their contribution to the complexity of cellular aging.

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